Review of Indicators for Development of Nutrient Numeric Endpoints in California Estuaries

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Review of Indicators for Development of Nutrient Numeric Endpoints in California Estuaries

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Executive Summary

California State Water Resources Control Board (SWRCB) is developing nutrient water quality objectives for the State's surface waters, using an approach known as the Nutrient Numeric Endpoint (NNE) framework. The NNE establishes a suite of numeric endpoints based on the <u>ecological response of an aquatic waterbody to nutrient over-enrichment</u> (eutrophication, e.g., algal biomass, dissolved oxygen). In addition to numeric endpoints for response indicators, the NNE framework must include models that link the response indicators to nutrient loads and other management controls. The NNE framework is intended to serve as numeric *guidance* to translate *narrative* water quality objectives. The NNE framework is currently under development for estuaries, with selection of appropriate ecological response indicators as the first step in developing an NNE assessment framework. The purpose of this document is to review literature supporting the use of a variety of candidate ecological response indicators, recommend a suite of indicators which met review criteria, identify data gaps and recommend next steps. Note that this review does not include data gaps relevant to the development of NNE load-response models for estuaries. Recommended NNE indicators specifically for San Francisco Bay were reviewed by McKee et al. (2011).

Recommended NNE Indicators

The <u>NNE assessment framework</u> is the structured set of decision rules that helps to classify the waterbody in categories from minimally to very disturbed, in order to determine if a waterbody is meeting beneficial uses, or to establish TMDL numeric targets. Development of an assessment framework begins by choosing <u>response indicators</u>, which were reviewed using four criteria: 1) strong linkages to beneficial uses; 2) well-vetted means of measurement; 3) ability to model the relationship between the indicator, nutrient loads and other management controls; and 4) acceptable signal to noise ratio for eutrophication assessment. Two types of indicators were designated. <u>Primary indicators</u> are those which met all evaluation criteria and for which regulatory endpoints should be developed. <u>Supporting indicators</u> fell short of meeting evaluation criteria, but may be used as supporting lines of evidence, though establishment of NNE endpoints for these indicators is not envisioned in the near term. The use of primary and supporting indicators is consistent with the freshwater streams and lakes NNE framework.

Appropriate indicators vary by estuarine class as well as habitat type. For the purposes of designating NNE indicators, California estuarine classes aggregated into two main groups according to the status of the tidal exchange with the coastal ocean: 1) "open" to surface water tidal exchange and 2) "closed" to surface water tidal exchange. Estuarine classes that fall entirely into this "open" category include enclosed bays as well as perennially tidal lagoons and river mouth estuaries. In addition, intermittently tidal lagoons and river mouths that open at least once per year may be assessed using indicators applicable to this category when the mouth of the estuary is open to surface water tidal exchange. Estuaries that are "closed" to surface water tidal exchange include intermittently and ephemerally tidal lagoons and river mouth estuaries <u>when the mouth is closed</u>. Indicators also vary by three main estuarine habitat types: 1) unvegetated subtidal, 2) seagrass and brackish SAV, and 3) intertidal flats.

The review found four types of indicator met all evaluation criteria and are designated as primary (see summary table below): dissolved oxygen, phytoplankton biomass and productivity, and cyanobacterial abundance and toxin concentration (all subtidal habitats), macroalgal biomass and cover (fine-grained intertidal and seagrass habitats). Other indicators evaluated met three or fewer of the review criteria and designated as supporting indicators: phytoplankton assemblage, HAB cell counts and toxin concentration, urea and ammonium, light attenuation (all subtidal), light attenuation and epiphyte load (seagrass/brackish SAV), macrobenthic taxonomic composition and biomass (subtidal <18 ppt) and sediment TOC:TN:TP:TS and degree of pyritization (fined grain intertidal and shallow subtidal).

Summary of Recommended Primary and Secondary Indicators by Ocean Inlet Status and Habitat Type

Ocean Inlet Status	Habitat	Primary Indicators	Supporting Indicators
Open	All Subtidal Habitat	Phytoplankton biomass and productivity Cyanobacteria cell counts and toxin concentration[1] Dissolved oxygen	Water column nutrient concentrations and forms[2] (C, N, P, Si) Phytoplankton assemblages HAB species cell count and toxin concentrations Macrobenthic taxonomic composition, abundance & biomass Sediment C, N, P, S, particle size (and ratios therein) and degree of pyritization
	Seagrass and Brackish SAV Habitat	Phytoplankton biomass and productivity Macroalgal biomass & cover	Light attenuation, suspended sediment conc. or turbidity Seagrass areal distribution,% cover, density Epiphyte load Brackish SAV areal distribution,% cover, biomass
	Intertidal Flats	Macroalgal biomass and cover[3]	Sediment % OC, N, P, S, particle size, degree of pyritization Microphytobenthic taxonomic composition, benthic chl a
Closed	All Subtidal Habitat	Phytoplankton biomass and productivity Cyanobacteria cell counts and toxin concentration Dissolved oxygen Rafting or floating macroalgae biomass and % cover	Phytoplankton assemblages, including HAB species cell count and toxin concentrations Sediment C, N, P, S, particle size (and ratios therein) and degree of pyritization Microphytobenthos taxonomic composition and benthic chl a biomass Water column nutrient concentrations and forms[2] (C, N, P, Si)
	Brackish SAV	Phytoplankton biomass and productivity Macroalgal biomass & cover Dissolved oxygen	Light attenuation, suspended sediment conc. Epiphyte load Brackish SAV areal distribution, % cover, biomass

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[1] Note that cyanobacteria cell counts and toxin concentrations are included for polyhaline and euhaline habitats in an attempt to capture effects of cyanobacteria blooms transported from freshwater and oligohaline environments.
[2] Forms referred to relative distribution of dissolved inorganic, dissolved organic, and particulate forms of nutrients, including urea and ammonium
[3] Not an ideal indicator for sandy intertidal flats. Recommend the inclusion of microphytobenthos, though factors controlling biomass not understood and little known about taxonomy as an indicator of disturbance gradient.

Data Gaps and Recommended Next Steps

Development of an NNE assessment framework for California estuaries begins by specifying how primary and supporting indicators would be used as multiple lines of evidence to diagnose adverse effects of eutrophication. This report identifies the data gaps and recommended next steps to use the identified primary and supporting indicators in development of an assessment protocol to assess eutrophication. Assessment frameworks would need to be created for habitat types identified in this review, with some differences specified by estuarine inlet status (closed or open). **Note that no attempt is made to neither prioritize nor reduce/eliminate "next steps" in any habitat types, despite acknowledged limitation in available resources. The NNE technical team assumes this prioritization and focusing of resources would be done by the SWRCB, with advice from its advisory groups.**

Dissolved Oxygen - All Subtidal Habitat in Open and Closed Estuaries

All six coastal Regional Boards have numeric dissolved oxygen objectives applicable to estuaries. However, there is generally a lack of consistency among RWQCBs in their approach. This lack of consistency resulted in the review of science supporting estuarine dissolved oxygen objectives for California estuaries (excluding San Francisco Bay) with the goal of developing a consistent approach statewide that protects specific designated uses and aquatic habitats. This study is already funded and the technical report summarizing the findings of the literature review and data synthesis will be available in June of 2011. *Data gaps and recommended next steps are identified in this document.*

Phytoplankton and Water Column Nutrient Indicators - Unvegetated Subtidal Habitat in Open Estuaries

Within the realm of phytoplankton indicators, biomass, productivity, cyanobacterial cell counts and toxin concentration are designated as primary indicators for all subtidal habitats. To establish numeric thresholds for these primary indicators, a number of data gaps and next steps must be addressed (see table below). For phytoplankton biomass and productivity, there is a large amount of experience and studies that exist globally, but a lack of data exists for most California estuaries, with the exception of San Francisco Bay (see review by McKee et al. 2011), where a water quality data set of nearly 40 years exists. It is recommended that a working group of experts be assembled to develop an assessment framework for biomass and productivity that takes into account the high spatial and temporal variability of phytoplankton, using San Francisco Bay as a "test case." For cyanobacteria cell counts and toxins concentrations, guidelines exist to establish NNE endpoints in fresh habitats, based on human and faunal exposure to toxin concentrations. The applicability of these endpoints should be examined for translation to estuarine habitats.

Summary of Data Gaps and Recommended Next Steps for Phytoplankton and Water Column Nutrient Indicators in Unvegetated Subtidal Habitat

Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Phytoplankton biomass and productivity	Primary indicator	Wealth of experience and studies exists globally, but lack of data for most California estuaries and lack of specific studies to establish thresholds. Precise thresholds may vary from estuary to estuary, depending on co-factors.	Recommend development of a white paper and a series of expert workshops to develop NNE assessment framework for phytoplankton biomass, productivity, taxonomic composition/assemblages, abundance and/or harmful algal bloom toxin concentrations in "open" and "closed" estuaries. Include review of relevant thresholds for nutrient stoichiometry as relevant for "closed" estuaries.	No work under-taken
Cyanobacteria cell count and toxin conc.	Primary indicator	Data and precedent exist to establish NNE thresholds.		
Nutrient stoichiometry	Supporting indicator	Lack of data in California estuaries on use of nutrient stoichiometry to predict cyanobacteria dominance in oligohaline to mesohaline habitats.		
Ammonium	Supporting	Ammonium inhibition of nitrate uptake by diatoms document, although importance of this effects vis-à-vis other controls on production and species dominance not well understood	Future investigations on utility of ammonium as an indicator should be focused first on San Francisco (SF) Bay, where debate on ammonium is a priority issue. Formulate a working group of scientists to synthesize available data on factors known to control primary productivity in different regions in SF Bay, develop consensus on relative importance of ammonium inhibition of phytoplankton blooms, and evaluate potential ammonium endpoints (see McKee et al. 2011 for further details).	No work undertaken
Phytoplankton assemblages, HAB species cell count, toxin conc.	Supporting indicator	Controls on phytoplankton assemblages, euhaline and marine HAB bloom occurrence and toxin production not well understood	Include as indicator in monitoring program and support basic research to increase understanding of drivers.	Not applicable
Urea	Supporting	Lack of data on urea concentrations in estuaries		

Phytoplankton, Macroalgae, Epiphyte Load and Light Attenuation in Seagrass Habitats

For seagrass habitats, macroalgal biomass and cover and phytoplankton biomass are designated as primary indicators, while light attenuation and epiphyte load are designated as supporting indicators (see table below). Development of an assessment framework for seagrass based on these indicators will require addressing the following studies: 1) identify thresholds associated with adverse effects of macroalgal biomass and cover on seagrass growth, 2) collect data on light requirements of California seagrass and determine combinations of phytoplankton biomass and turbidity that result in light attenuation beyond levels of tolerance of seagrass, and 3) assemble a workshop of experts to construct assessment framework for seagrass habitat that uses macroalgae, phytoplankton, epiphyte load in a multiple lines of evidence fashion. Studies to identify thresholds associated with adverse effects of macroalgal biomass and cover are funded and will begin the summer 2011.

Data gaps and recommended next steps for development of an NNE assessment framework for seagrass habitat

Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Macroalgal biomass and cover	Primary indicator	Data lacking on dose and response of macroalgal biomass on seagrass growth	Conduct experiments on biomass, cover and duration of macroalgae that results in reduced seagrass growth. Survey ranges of biomass, duration and cover associated with macroalgae on seagrass	Funded. Study to begin summer 2011
Phytoplankton biomass	Primary indicator	Data lacking on light requirements for California seagrass	Determine light requirements for California seagrass and survey range of epiphyte loads on seagrass beds. Develop assessment framework as a function of light attenuation, macroalgal biomass and epiphyte load	No work undertaken
Light attenuation	Supporting indicator			
Epiphyte load	Supporting indicator	Scientific foundation exists, but epiphyte load difficult to quantify		

Macroalgae, Sediment C:N:P:S Ratio, Degree of Pyritization and Microphytobenthos on Intertidal Flats

Discussion of data gaps in intertidal flat habitat in "open" estuaries distinguishes between fine-grained (mud flats) and course grained (sand-flats) habitat types. In mud flats of "open" estuaries, macroalgal biomass and percent cover are the primary NNE indicators. In these habitat types, data are lacking on the thresholds of effects of macroalgae on benthic infauna as well as documentation of the range of duration of biomass and cover associated with macroalgae on intertidal flats. To address these data gaps, recommended next steps include: 1) conducting experiments and field surveys to address these data gaps, and 2) synthesis of these data into an assessment framework. These studies are funded and underway, with an assessment framework anticipated in July 2013. In sand flats, use of macroalgae as an indicator is questionable, as it is more common to see high biomass of microphytobenthos in eutrophic conditions. Therefore, in "open" estuaries dominated by sandy intertidal flats, as is the case in

river mouth estuaries, a more important indicator may be microphytobenthos biomass and taxonomic composition. Since little is known about controls on microphytobenthos, it recommended that research be supported to improve understanding of appropriate indicators of eutrophication in river mouth estuaries in the "open" condition.

Data gaps and recommended next steps for development of an NNE assessment framework for intertidal flats in "open" estuaries

Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Macroalgal biomass and cover	Primary	Data lacking on dose and response of macroalgal biomass on benthic infauna in intertidal flats	Conduct experiments on biomass, cover and duration of macroalgae that results in reduced diversity and abundance of benthic infauna in tandem with sediment C:N:P:S and degree of pyritization. Survey ranges of biomass, duration and cover associated with macroalgae on seagrass	Funded and study in progress
Sediment C:N:P:S and degree of pyritization	Supporting indicator	Data lacking on the sensitivity of this indicator vis-à-vis primary producers		
Microphytobenthos biomass and taxonomic composition	Supporting	Data lacking effects of eutrophication on biomass and taxonomic composition across gradients of particle size and salinity	Conduct field studies that document change in biomass and taxonomic composition of microphytobenthos along disturbance gradient in sandy intertidal flats and shallow subtidal habitat of "open" estuaries.	No work undertaken

Macrobenthos Biomass, Taxonomic Composition, and Abundance, Sediment C:N:P:S and Degree of Pyritization in Subtidal Habitats >18 ppt

In subtidal habitats of "open" estuaries with salinities greater than 18 ppt, macrobenthos biomass, taxonomic composition and abundance may provide additional information on eutrophication. As macrobenthos taxonomic composition and sediment %C and %N are already being used in the assessment of sediment quality objectives, the addition of macrobenthic biomass and sediment sulfur and degree of pyritization represents an attempt to enhance information collected through the SWRCB's Sediment Quality Objective (SQO)protocol to assess effects of eutrophication (see table below). Recommended next steps includes: 1) analysis of existing regional monitoring datasets for useful taxonomic indicators of eutrophication; and 2) conduct a pilot study in a future regional monitoring program study to test the utility of including biomass, sediment C:N:P:S ratios, and degree of pyritization as a standard part of this protocol.

Data gaps and recommended next steps for use of macrobenthos and indicators of sediment organic matter accumulation in "open" estuaries with salinities >18 ppt

Indicator	Design-ation	Status of Science	Recommended Next Steps	Status of work
Macrobenthos taxonomic composition, abundance, biomass	Supporting	Lack of data on the degree to which macrobenthos biomass, in combination with taxonomic composition and abundance, may provide specific diagnosis of eutrophication and how this would differ by salinity regime.	Analyze existing regional monitoring datasets for taxonomic indicators of eutrophication Conduct pilot study in future regional monitoring program study to test utility of including biomass in macrobenthos assessment protocol.	No work undertaken
Sediment C:N:P:S and degree of pyritization	Supporting	Lack of understanding of the sensitivity of sediment C:N:P:S ratio or degree of pyritization in diagnosing eutrophication	Analyze existing regional monitoring datasets for utility of C:N:P:S or degree of pyritization Include indicator in pilot study (polyhaline-euhaline) or field studies (oligohaline-mesohaline) to determine sensitivity and utility for NNE framework Include as indicator in experiments on effects of macroalgae on benthic infauna on intertidal flats (see below)	No work undertaken

Phytoplankton, Macroalgae and Epiphyte Load in Vegetated (Brackish SAV) and Unvegetated Subtidal Habitats of "Closed" Estuaries

In intermittently and ephemerally tidal estuaries during a "closed" tidal inlet condition, primary NNE indicators include macroalgal biomass and cover, phytoplankton biomass, cyanobacterial cell counts and toxin concentrations. The table below gives a summary of data gaps and recommended next steps for these indicators both vegetated (brackish SAV) and unvegetated subtidal habitats.

For unvegetated subtidal habitat in closed estuaries, phytoplankton grow under lentic conditions similar to that of freshwater lakes. Little data is available on the concentrations of phytoplankton biomass and speciation in California estuaries in this condition. However, it is recommended that the numeric endpoints for the California lakes NNE (phytoplankton biomass, cyanobacteria cell counts and toxin concentrations) be evaluated for applicability to unvegetated subtidal habitats.

Floating or rafting mats of macroalgae can have a significant effect on other primary producers in "closed" estuaries. No data or studies are available to document what levels of floating algae result in adverse effects. To address these data gaps, two types of studies are recommended: 1) modeling or experiments to document thresholds of effects of floating or rafting macroalgae on microphytobenthos; and 2) field studies or experiments that documents linkage between macroalgae, phytoplankton, microphytobenthos, dissolved oxygen and pelagic invertebrates as a function of nutrient loading and other co-factors.

Data gaps and recommended next steps for development of an NNE assessment framework for unvegetated and vegetated (brackish SAV habitat) in closed estuaries

Habitat Type	Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Unvegetated Oligohaline to Mesohaline Habitat	Macroalgal biomass and cover	Primary	Lack of data on thresholds of effects of macroalgal biomass/cover associated with effects on dissolved oxygen, microphytobenthos and pelagic invertebrates	Modeling studies and/or experiments to investigate linkage between macroalgae biomass/cover and dissolved oxygen, microphytobenthos and pelagic invertebrates	No work undertaken
	Cyanobacteria cell count and toxin concentrations	Primary	Studies exist to establish thresholds for freshwater lakes.	Evaluate applicability of freshwater lakes NNE thresholds and WHO guidelines for "closed" estuaries	No work undertaken
	Phytoplankton biomass	Primary			
	Microphytobenthos biomass and taxonomic composition	Supporting	Lack of information on controls on biomass and taxonomic composition	Conduct experiments on degree to which floating macroalgae, phytoplankton and epiphyte loads adversely affect brackish SAV and microphytobenthos	No work undertaken
Brackish SAV	Macroalgal biomass and cover	Primary indicator	Data lacking on response of canopy-forming brackish SAV to factors that result in greater water column light attenuation: floating macroalgae, phytoplankton biomass and epiphyte load.		
	Phytoplankton biomass	Primary indicator		Conduct field studies documenting biomass, areal extent and % cover of brackish SAV relative to gradients of nutrient loading	
	Light attenuation	Supporting indicator			
	Epiphyte load	Supporting indicator			

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1. Introduction

Martha Sutula

1.1 Introduction, Background, Purpose of Document, and Organization

Eutrophication of estuaries and coastal waters is a global environmental issue, with demonstrated links between anthropogenic changes in watersheds, increased nutrient loading to coastal waters, harmful algal blooms, hypoxia, and impacts on aquatic food webs (Valiela, Foreman et al. 1992; Kamer and Stein 2003). These ecological impacts of eutrophication of coastal areas can have far-reaching consequences, including fish-kills and lowered fishery production (Glasgow and Burkholder 2000), loss or degradation of seagrass and kelp beds (Twilley 1985; Burkholder, Noga et al. 1992; McGlathery 2001), smothering of bivalves and other benthic organisms (Rabalais and Harper 1992), nuisance odors, and impacts on human and marine mammal health from increased frequency and extent of harmful algal blooms and poor water quality (Bates, et et al. 1989; Bates, DeFreitas et al. 1991; Trainer, Hickey et al. 2002). These modifications have significant economic and social costs (Turner, Qureshi et al. 1998). According to EPA, eutrophication is one of the top three leading causes of impairments of the nation's waters (US EPA 2001). Scientifically-based state water quality objectives and tools that relate these criteria to management controls are needed to diagnose adverse effects from eutrophication.

In California, the impacts of nutrient loading on estuaries and coastal waters have not been well monitored (Bricker, Clement et al. 1999), with the notable exception of San Francisco Bay where there has been research and ongoing publication by a number of authors (Cloern 1982; Cloern et al. 1985; Cloern 1991; Cloern 1996, 1999). Without management actions to reduce anthropogenic nutrient loads and other factors controlling eutrophication, symptoms are expected to develop or worsen in the majority of systems, in part due to projected population increases along the coastal areas. Scientifically-based statewide water quality objectives and tools that relate these objectives to management controls are needed to prevent eutrophication from occurring and to provide targets for restoration or mitigation of systems where adverse effects of eutrophication have already occurred.

The US EPA initiated the National Nutrient Management Strategy in 1998 to begin addressing the pervasive impacts of excessive nutrient loading to both fresh and marine waters (Wayland 1998). A primary goal of the strategy was to develop numeric nutrient criteria to measure the progress of the management strategy. The US EPA issued a series of technical guidance manuals for the development of nutrient criteria. Initial national guidance on nutrient criteria development advocated the use of a statistical approach to establish thresholds based on the nutrient concentrations in surface waters (US EPA 1998). In this approach, reference conditions were based on 25th percentiles of all nutrient concentration data including a comparison of reference condition for the aggregate ecoregion versus the subecoregions. These 25th percentile concentrations were characterized as criteria recommendations that could be used to protect waters against nutrient over-enrichment. The "Nutrient Criteria Technical guidance Manual: Estuarine and Coastal Waters" was released by US EPA in 2001.

Several studies have demonstrated the shortcomings of using ambient nutrient concentration criteria alone to predict eutrophication, in streams (Welch et al. 1989, Fevold 1998, Chetelat et al. 1999, Heiskary and Markus 2001, Dodds et al. 2002) and estuaries (Cloern 2001, Dettman et al. 2001, Kennison et al. 2003). Use of ambient, surface water nutrient concentrations is generally not effective for assessing eutrophication and the subsequent impact on beneficial use because ambient concentrations reflect the biological processing that has already occurred. In addition, biological response to nutrients (e.g., algal productivity) depends on a variety of mitigating factors such as basin morphology and substrate characteristics, tidal energy, stratification, temperature, light availability, biological community structure, and seed populations. Thus high concentrations are not an obligatory indicator of eutrophication and low concentrations do not necessarily indicate absence of eutrophication.

Given these problems, in 1999 the US EPA Region 9 and the California State Water Resources Control Board (SWRCB) chose an alternative approach to developing nutrient objectives (US EPA 2006). This approach, known as the Nutrient Numeric Endpoint (NNE) framework, establishes a suite of numeric endpoints based on the ecological response of an aquatic waterbody to nutrient over-enrichment (eutrophication, e.g., algal biomass, dissolved oxygen). It was suggested that numeric endpoints, if successfully developed, would serve as *guidanc*e to translate *narrative* water quality objectives (State of California's term for water quality criteria) for nutrients and biostimulatory substances. A key component of the NNE framework is the availability or development of stressor- response tools that link the ecological response indicators with nutrient loads and other potential management controls for TMDL development and implementation.

The California NNE framework was first developed for streams and lakes (US EPA 2006) and is currently under development for estuaries. A scientific framework has been presented to support the development of numeric endpoints for a suite of biological response indicators and highlight data gaps and research recommendations for their development (US EPA 2007). A subsequent document articulated a broad work plan to address data gaps, develop numeric endpoints, and support the efficient and cost-effective development of stressor-response TMDL tools (US EPA 2008). The SWRCB has initiated a project to implement this work plan for California estuaries, which includes a review of scientific literature on candidate indicators to diagnose eutrophication in estuaries, with the intent of developing an NNE assessment framework for estuaries,

The purpose of this document is to provide a thorough review of the candidate indicators of eutrophication in estuaries, with the goal of identifying indicators suitable for inclusion in the NNE assessment framework, data to support the selection of numeric endpoints, data gaps and research needs, and next steps.

1.2 Document Organization

The document is organized as follows:

Chapter 1: Introduction, Purpose and Organization of Document, Important Definitions

Chapter 2: NNE Conceptual Approach, Key definitions, Estuarine Classes and Major Habitats, General Conceptual Models, and Candidate E-NNE Indicators

Chapter 3: Macroalgae

Chapter 4: Phytoplankton

Chapter 5: Submerged Aquatic Vegetation

Chapter 6: Sediment and Water Column Physiochemical Response Indicators

Chapter 7: Benthic Macroinvertebrates

Chapter 8: Synthesis of Findings for Development of the E-NNE Assessment Framework

1.3 Important Definitions

For those outside the regulatory world, distinction between terms like "criteria," "standards", "objectives," and "endpoints" can be confusing. The purpose of this section is to provide definitions of the terms that are linked closely to how the NNE framework will be implemented.

Eutrophication: Eutrophication is defined as the acceleration of the delivery, in situ production of organic matter, and accumulation of organic matter (Nixon 1995). One main cause of eutrophication in estuaries is nutrient over enrichment (nitrogen, phosphorus and silica). However, other factors influence primary producer growth and the build-up of nutrient concentrations, and hence modify (or buffer) the response of a system to increased nutrient loads (hereto referred to as **co-factors**). These co-factors include hydrologic residence times, mixing characteristics, water temperature, light climate, grazing pressure and, in some cases, coastal upwelling.

Indicator: A characteristic of an ecosystem that is related to, or derived from, a measure of biotic or abiotic variable, that can provide quantitative information on ecological condition, structure and/or function. With respect to the water quality objectives, indicators are the ecological parameters for which narrative or numeric objectives are developed.

Water Quality Standards: Water quality standards are the foundation of the water quality-based control program mandated by the Clean Water Act. Water Quality Standards define the goals for a waterbody by designating its uses, setting criteria to protect those uses, and establishing provisions to protect water quality from pollutants. A water quality standard consists of three basic elements:

1. **Designated uses** of the water body (e.g., recreation, water supply, aquatic life, agriculture; Table 1.1),

2. **Water quality criteria** to protect designated uses (numeric pollutant concentrations and narrative requirements), and

3. **Antidegradation policy** to maintain and protect existing uses and high quality waters.

Water Quality Criteria: Section 303 of the Clean Water Act gives the States and authorized Tribes power to adopt water quality criteria with sufficient coverage of parameters and of adequate stringency to protect designated uses. In adopting criteria, States and Tribes may:

- Adopt the criteria that US EPA publishes under §304(a) of the Clean Water Act;

- Modify the §304(a) criteria to reflect site-specific conditions; or

- Adopt criteria based on other scientifically-defensible methods.

The State of California's water criteria are implemented as "water quality objectives," as defined in the Water Code (of the Porter Cologne Act; for further explanation, see below).

States and Tribes typically adopt both **numeric** and **narrative** criteria. **Numeric** criteria are quantitative. **Narrative** criteria lack specific numeric targets but define a targeted condition that must be achieved.

Section 303(c)(2)(B) of the Clean Water Act requires States and authorized Tribes to adopt numeric criteria for priority toxic pollutants for which the Agency has published §304(a) criteria. In addition to narrative and numeric (chemical-specific) criteria, other types of water quality criteria include:

- Biological criteria: a description of the desired biological condition of the aquatic community, for example, based on the numbers and kinds of organisms expected to be present in a water body.

- Nutrient criteria: a means to protect against nutrient over-enrichment and cultural eutrophication.

- Sediment criteria: a description of conditions that will avoid adverse effects of contaminated and uncontaminated sediments.

Water Quality Objectives: The Water Code (Porter-Cologne Act) provides that each Regional Water Quality Control Board shall establish water quality objectives for the waters of the state i.e., (ground and surface waters) which, in the Regional Board's judgment, are necessary for the reasonable protection of beneficial uses and for the prevention of nuisance. The State of California typically adopts both **numeric** and **narrative** objectives. **Numeric** objectives are quantitative. **Narrative** objectives present general descriptions of water quality that must be attained through pollutant control measures. Narrative objectives are also often a basis for the development of numerical objectives.

Numeric Endpoint: Within the context of the NNE framework, numeric endpoints are thresholds that define the magnitude of an indicator that is considered protective of ecological health. These numeric endpoints serve as guidance to Regional Boards in translating narrative nutrient or biostimulatory substance water quality objectives. They are called "numeric endpoints" rather than "numeric objectives" to distinguish the difference with respect to SWRCB policy. Objectives are promulgated through a public process and incorporated into basin plans. Numeric endpoints are guidance that can evolve over time without the need to go through a formal standards development process.

Table 1.1. Definition of estuarine beneficial uses applicable to selection of E-NNE indicators.

Marine Habitat (MAR) - Uses of water that support marine ecosystems including, but not limited to, preservation or enhancement of marine habitats, vegetation such as kelp, fish, shellfish, or wildlife (e.g., marine mammals, shorebirds).

Estuarine Habitat (EST) - Uses of water that support estuarine ecosystems including, but not limited to, preservation or enhancement of estuarine habitats, vegetation, fish, shellfish, or wildlife (e.g., estuarine mammals, waterfowl, shorebirds).

Cold Freshwater Habitat (COLD) - Uses of water that support cold water ecosystems including, but not limited to, preservation or enhancement of aquatic habitats, vegetation, fish or wildlife, including invertebrates.

Warm Freshwater Habitat (WARM) - Uses of water that support warm water ecosystems including, but not limited to, preservation or enhancement of aquatic habitats, vegetation, fish or wildlife, including invertebrates.

Wildlife Habitat (WILD) - Uses of water that support terrestrial ecosystems including, but not limited to, preservation and enhancement of terrestrial habitats, vegetation, wildlife (e.g., mammals, birds, reptiles, amphibians, invertebrates), or wildlife water and food sources.

Rare, Threatened, or Endangered Species (RARE) - Uses of water that support habitats necessary, at least in part, for the survival and successful maintenance of plant or animal species established under state or federal law as rare, threatened or endangered.

Spawning, Reproduction, and/or Early Development (SPWN) - Uses of water that support high quality aquatic habitats suitable for reproduction and early development of fish. This use is applicable only for the protection of anadromous fish.

Migration of Aquatic Organisms (MIGR) - Uses of water that support habitats necessary for migration, acclimatization between fresh and salt water, or other temporary activities by aquatic organisms, such as anadromous fish

Commercial and Sport Fishing (COMM) - Uses of water for commercial or recreational collection of fish, shellfish, or other organisms including, but not limited to, uses involving organisms intended for human consumption or bait purposes.

Shellfish Harvesting (SHELL) - Uses of water that support habitats suitable for the collection of filter-feeding shellfish (e.g., clams, oysters and mussels) for human consumption, commercial, or sport purposes.

Aquaculture (AQUA) - Uses of water for aquaculture or mariculture operations including, but not limited to, propagation, cultivation, maintenance, or harvesting of aquatic plants and animals for human consumption or bait purposes.

Contact Water Recreation (REC-1) - Uses of water for recreational activities involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, swimming, wading, water-skiing, skin and SCUBA diving, surfing, white water activities, fishing, or use of natural hot springs.

Non-contact Water Recreation (REC-2) – Uses of water for recreational activities involving proximity to water, but not normally involving body contact with water, where ingestion of water is reasonably possible. These uses include, but are not limited to, picnicking, sunbathing, hiking, beachcombing, camping, boating, tidepool and marine life study, hunting, sightseeing, or aesthetic enjoyment in conjunction with the above activities.

2. Context for Review of Candidate Indicators: California's Approach to Developing Nutrient Water Quality Objectives in Estuaries

Martha Sutula

2.1 NNE Conceptual Approach

The SWRCB staff strategy for developing nutrient objectives for the State of California is to develop a narrative nutrient water quality objective, with numeric guidance that serves to interpret that narrative objective. The Nutrient Numeric Endpoints (NNE) framework, consisting of a diagnostic assessment protocol and load-response models, would be used as numeric guidance to interpret the narrative nutrient objective. The narrative objective and NNE guidance would be incorporated by default into the Basin Plans of the Regional Water Quality Control Boards. The purpose of developing NNEs for California estuaries is to provide the State Water Resources Control Board and the Regional Water Quality Control Boards with a scientifically-defensible framework that can serve as guidance for adopting water quality objectives for nutrients.

2.1.1 Approaches to Setting Nutrient Objectives

Nutrient objectives are scientifically challenging. Nutrients are required to support life, but assessment of how much is "too much" is not straightforward. Typical paradigms used to set thresholds for toxic contaminants do not apply, in part because adverse effects of nutrient over enrichment are visible at orders of magnitude below recognized toxicity thresholds for ammonium and nitrate.

US EPA guidance on nutrient objective development generally recommends three means to set nutrient criteria (EPA 2001): 1) reference approach, 2) empirical stress-response approach, and 3) cause-effect approach. The reference waterbody approach involves characterization of the distributions of nutrient in "minimally disturbed" waterbodies. Nutrient concentrations are chosen at some statistical percentile of those reference waterbodies. The empirical stress-response approach involves establishing statistical relationships between the causal or stressor (in this case nutrient concentrations or loads) and the ecological response (changes in algal or aquatic plant biomass or community structure, changes in sediment or water chemistry (e.g., dissolved oxygen, pH). The cause-effect approach involves identifying the ecological responses of concern and mechanistically modeling the linkage back to nutrient loads and other co-factors controlling response (e.g., hydrology, grazers, denitrification, etc.).

SWRCB staff and USEPA Region 9 staff evaluated these three approaches for setting nutrient objectives in California waterbodies and determined that, while it may choose to ultimately incorporate some elements of all approaches into California's strategy for setting nutrient objectives, it would rely most heavily on the cause-effect approach. There were several reasons for this. First, the cause-effect approach has a more direct linkage with beneficial uses and is generally thought to lend itself to a more precise diagnosis of adverse effects. Second, the alternative approaches require a tremendous amount

of data not currently available in such a large state. Third, the reference approach is particularly problematic because it automatically relegates a certain percentage of the reference sites to an "impaired" status. In addition, for many waterbody types, minimally disturbed reference sites are largely unavailable. Fourth, statistical stress-response relationships can be spurious, or have lots of unexplained variability (i.e., poor precision). This poor precision is translated to a larger margin of safety required (more conservative limits) for load allocations and permit limits. While waterbody typology, to some degree, can assist in explaining some of this variability, it cannot completely remove the concern. Thus, while simpler than the cause-effect approach, the empirical stress-response will result in more false negative and false positive determinations of adverse effects, and in the end will be more costly to the public.

For estuaries, reliance on the cause-effect approach is strongly suggested, because in the majority of circumstances, the reference or empirical stress-response approaches are simply untenable. Estuaries within California are highly variable in how they respond to nutrient loading due to differences in physiographic setting, salinity regime, frequency and timing of freshwater flows, magnitude of tidal forcing, sediment load, stratification, residence time, denitrification, etc. This combination of "co-factors" results in differences in the dominant primary producer communities (i.e., phytoplankton, macroalgae, benthic algae, submerged aquatic vegetation, emergent macrophytes). It also creates variability in the pathways that control how nutrients cycle within the estuary. At times, these co-factors can play a larger role in mitigating estuarine response to nutrient loads or concentrations, blurring or completely obscuring a simple prediction of primary productivity limited by nutrients (e.g., Figure 2.1). For example, in many lagoonal estuaries, benthic algal blooms can act to reduce surface water concentrations of nutrients to non-detectable levels. Thus while the estuary may be in a clearly impacted state, it would appear to meet N and P ambient water quality objectives. In estuaries such as San Francisco Bay, synthesis of existing data by Cloern and Dugdale (2010) have clearly shown that ambient nutrient concentrations do not correlate with measures of primary productivity, in part because of important co-factors that override simple nutrient limitation of primary production.

2.1.2 Key Tenets of the NNE Approach

The NNE framework for California waterbodies is basely largely on the cause-effect approach. The framework has three organizing principals (Creager et al. 2005):

1. *Ecological response indicators provide a more direct risk-based linkage to beneficial uses than nutrient concentrations or loads alone. <u>Thus the NNE framework is based on the diagnosis of eutrophication or other adverse effects and its consequences rather than nutrient over enrichment.</u>*

   Except in some cases, such as unionized ammonium causing toxicity, nutrients themselves do not impair beneficial uses. Rather, ecological response to nutrient loading causes adverse effects that impair uses. Instead of setting objectives solely in terms of nutrient concentrations, it is preferable to use an analysis that takes into account the risk of impairment of these uses. The NNE framework needs to target information on ecological response indicators such as dissolved oxygen, surface water phytoplankton and harmful algal bloom (HAB) biomass (e.g., chlorophyll-

a, water clarity), macroalgal biomass and percent cover, benthic algal biomass (sediment chlorophyll-a) and submerged aquatic vegetation (SAV) density and percent cover, and aesthetics (e.g., foul odors, unsightliness). These ecological response indicators provide a more direct risk-based linkage to beneficial uses than the ambient nutrient concentrations or nutrient loads. Given this approach, it is critical that tools be developed that link the response indicators back to nutrient loads and other co-factors and management controls (hydrology, etc.).

2. *A weight of evidence approach with multiple indicators will produce a more robust assessment of eutrophication.*

   When possible, the use of multiple indicators in a "weight of evidence" approach provides a more robust means to assess ecological condition and determine impairment. This approach is similar to the multimetric index approach, which defines an array of metrics or measures that individually provide limited information on biological status, but when integrated, functions as an overall indicator of biological condition (Karr and Chu 1999).

3. *Use of models to convert response indicators to site-specific nutrient loads or concentrations.*

   A key premise of the NNE framework is the use of models to convert numeric endpoints, based on ecological response indicators, to site- specific nutrient load goals appropriate for assessment, permitting, and TMDLs. A key feature of these models is that they account for site-specific co-factors, such as light availability, temperature, and hydrology that modify the ecological response of a system to nutrients.

Thus the intent of the NNE framework is to control excess nutrient loads to levels such that the risk or probability of impairing the designated uses is limited to a low level. If the nutrients present – regardless of actual magnitude – have a low probability of impairing uses, then water quality standards can be considered met.

2.2 How Response Indicators would be Used: Development of a Diagnostic Assessment Framework

Within the regulatory context, waterbody assessments are made in order to determine compliance with permits, TMDL implementation plans, or to make determination of whether the waterbody is meeting beneficial uses or impaired, as an example, for nutrients. In this context, a diagnostic assessment framework is the structured set of decision rules and guidance for interpretation that helps to classify the waterbody in categories of minimally disturbed (fully sustaining beneficial uses) to moderately disturbed (still sustaining beneficial uses, but functions reduced), to very disturbed (clearly not meeting beneficial uses). Although scientists can provide a lot of guidance and data synthesis to illustrate how the assessment framework could be formed, ultimately the decision of what levels to set thresholds that separate the categories (e.g., minimally versus moderately and very disturbed) is a policy decision. These thresholds are what are referred to as "nutrient numeric endpoints."

Development of the diagnostic assessment framework begins by choosing indicators that would be measured and used to determine waterbody status. It is important to distinguish between three types of indicators for an NNE assessment framework:

1. Primary indicators
2. Supporting indicators
3. Co-factor indicators required for data interpretation

Primary indicators are those for which regulatory endpoints could be developed. Designation of these indicators as "primary" implies a higher level of confidence in these indicators to be used to make an assessment of adverse effects, based on a wealth of experience and knowledge about how this indicator captures and represents ecological response. Primary indicators are those which are considered to meet all explicit criteria (see Section 2.5) established to evaluate candidate NNE indicators.

Supporting indicators are those which could be collected to provide supporting lines of evidence. These indicators may have met many, though not all evaluation criteria, but are consider important because they are commonly used to assess eutrophication in scientific studies. Establishment of NNE endpoints for these endpoints is not anticipated, though use of the indicator as supporting evidence over time may increase confidence and cause it to be promoted to "primary."

Finally, co-factors are indicators that could be part of a routine monitoring program and important for data interpretation and trends analysis, but not used explicitly to make a diagnosis.

2.3 Process to Develop a NNE Diagnostic Framework for Estuaries

The process to develop a diagnostic assessment framework for estuaries has several discrete steps, enumerated below. These steps are likely to be iterative as data gaps are identified and addressed.

- Agree on target population of estuaries

- Agree on criteria for indicator selection and generation of a master list of candidate indicators

- Develop conceptual models for each candidate indicator which explicitly shows linkages to estuarine beneficial uses and management controls on eutrophication.

- Review of existing science to portray conceptual models linking the indicator to beneficial uses, support endpoint development for each candidate indicator and develop specific recommendations whether to retain or drop each indicator and, if retained, identify data gaps and required studies to arrive at endpoint development.

- Conduct analysis of existing data and/or new research required for endpoint development

- Develop diagnostic assessment framework and numeric endpoints for specific indicators.

- Peer review

To begin, a master list of candidate indicators were generated for the E-NNE assessment framework and a list of minimum criteria with which to evaluate the suitability of candidate indicators were

development. These criteria were used to evaluate the potential utility of each candidate NNE indicator and thus form a common thread among each indicator group.

2.4   Criteria for Selection of E-NNE Indicators

The following criteria were used in the reviews of existing science to evaluate the utility of each indicator for the E-NNE assessment framework.

Indicators Should:

- Have a clear link to beneficial uses

- Have a predictive relationship with causal factors such as nutrient concentrations/loads and other factors known to regulate response to eutrophication (hydrology, etc.). This relationship could be empirical (modeled as a statistical relationship between load/concentration and response or modeled mechanistically through tools such as a simple spreadsheet or dynamic simulation models)

- Have a scientifically sound and practical measurement process

- Must be able to show a trend either towards increasing or/and decreasing eutrophication with an acceptable signal: noise ratio

It would be beneficial if indicators also:

- Were easy to understand to a non-technical audience (unambiguous)

- Provide early warning of emerging problems

- Were adaptable for use at a range of spatial scales

- Can be used to diagnose multiple causative factors, not necessarily just eutrophication

- Show detectable trends in both directions (improving or degrading)

2.5   Context for Indicator Selection: Estuarine Classes and Major Habitat Types

Discussion of E-NNE candidate indicators requires mention of estuarine classes and key habitat types. The approximately 400 estuaries found in the State of California are highly variable in terms of physiographic setting, salinity regime, frequency and timing of freshwater flows, magnitude of tidal forcing, sediment load, stratification, residence time, etc (Largier in prep). This combination of factors results in differences in the dominant primary producer communities (i.e., phytoplankton, macroalgae, benthic algae, submerged aquatic vegetation, emergent macrophytes). It also creates variability in the pathways for nutrient cycling within estuaries. As a result of these differences, estuaries are expected to be variable in how they respond to nutrient loading. Partitioning this apparent natural variability into classes will improve the E-NNE framework by: 1) clearly defining relevant response indicators by

estuarine class and 2) providing framework expectations (reference) for these indicators among estuaries of the same class. It also standardizes vocabulary to discuss basic types of estuaries.

Classification approaches can by driven by conceptual, empirical or statistical approaches. A preliminary classification of California estuaries, based on a conceptual approach modeled after the Coastal Marine Ecological Classification Standard (CMECS; Madden et al. 2005). The preliminary classes are given in Table 2.1.

Table 2.1. Preliminary classification of California estuaries. Definitions of these terms are given in Appendix 1.

GEOFORM	SEASONALITY OF OCEAN INLET OPENING
Enclosed Bay	Perennial
Lagoon	Perennial
	Intermittent
	Ephemeral
River mouth	Perennial
	Intermittent

Among the three geoforms (Figure 2.1), enclosed bays are well flushed with a strong tidal prism and dominated by shallow or deepwater subtidal habitat. The inlet mouth is not restricted and is perennially open to tidal exchange. In contrast, lagoons have restricted tidal inlets, are dominated by shallow subtidal and intertidal habitat and have a long residence time due to the restricted width of the mouth. The inlet can be open or closed, perennially (all year round), intermittently (open at least once per year) or ephemeral (opens infrequently, usually every several years or not known recently to open).

Figure 2.1. Examples of three major estuarine geoforms in California: enclosed bay (left), lagoon (center) and river mouth estuary (right).

Among all classes, four key habitat types can be found across a basic elevation gradient (Figure 2.2): emergent marsh, intertidal flats and unvegetated shallow subtidal, intertidal and subtidal aquatic beds, and deepwater subtidal habitat. Of these four habitat types, emergent marsh is generally considered to be the least sensitive to eutrophication, due to high rates of denitrification, increased of oxygenation of

sediments within the rooted zone of marsh plants, and daily exposure to air and sunlight in the high intertidal zone increases the decomposition of organic matter (Day et al. 1989). For this reason, the review focused on candidate NNE indicators in the unvegetated intertidal and subtidal habitats.
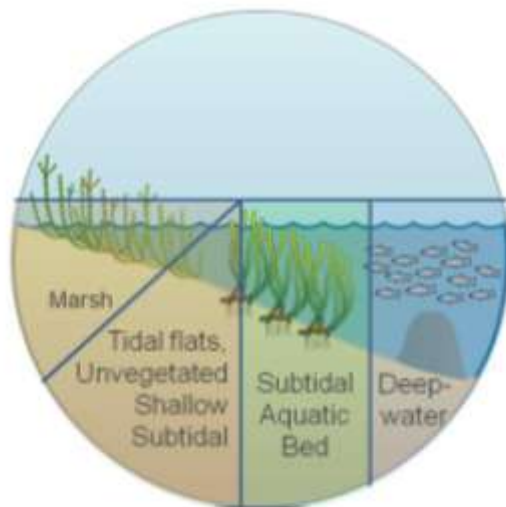
Figure 2.2. Graphic of major habitat types found across an elevational gradient in all estuarine classes.

Among these three major habitat types, the appropriate NNE indicators will differ. For example, four major aquatic primary producer groups (phytoplankton, macroalgae, microphytobenthos, and submerged aquatic vegetation) exist in estuaries (Figure 2.3). Across a cross-sectional or elevational gradient within an estuary (high intertidal to deepwater subtidal), the relative dominance of each of these primary producer groups is controlled by a suite of factors that vary with respect to the basic physiological requirements of each group and present environmental constraints to their stability, growth and reproduction (Day et al. 1989). These factors include: 1) light, 2) water depth, 3) temperature, 4) desiccation, 5) water velocities and turbulence, 6) nutrient and organic matter availability, 7) grazing by consumers. The interplay of these factors controls the presence and relative dominance of primary producer groups within estuaries and across estuarine classes.

These four primary producers tend to distribute themselves in predictable patterns across this elevational gradient (Table 2.2). In turbid or deepwater subtidal habitats, particularly in wave dominated environments, phytoplankton tends to be the dominant primary producer, or co-dominant with microphytobenthos in deepwater habitats with high water clarity (Day et al. 1989, Wetzel 2001). As depths decrease towards the shallow subtidal zone and particularly in macrotidal estuaries, microphytobenthos, submerged aquatic vegetation (SAV), and macroalgae that are attached to sediment are at a competitive advantage over phytoplankton, which can be easily flushed out during a tidal cycle. Depending on water residence time, nutrient availability, substrate, etc. phytoplankton, SAV, microphytobenthos, and macroalgae can co-dominate in shallow subtidal habitat (>10 m in depth). In intertidal flats, macroalgae and microphytobenthos are generally the dominant primary producers.
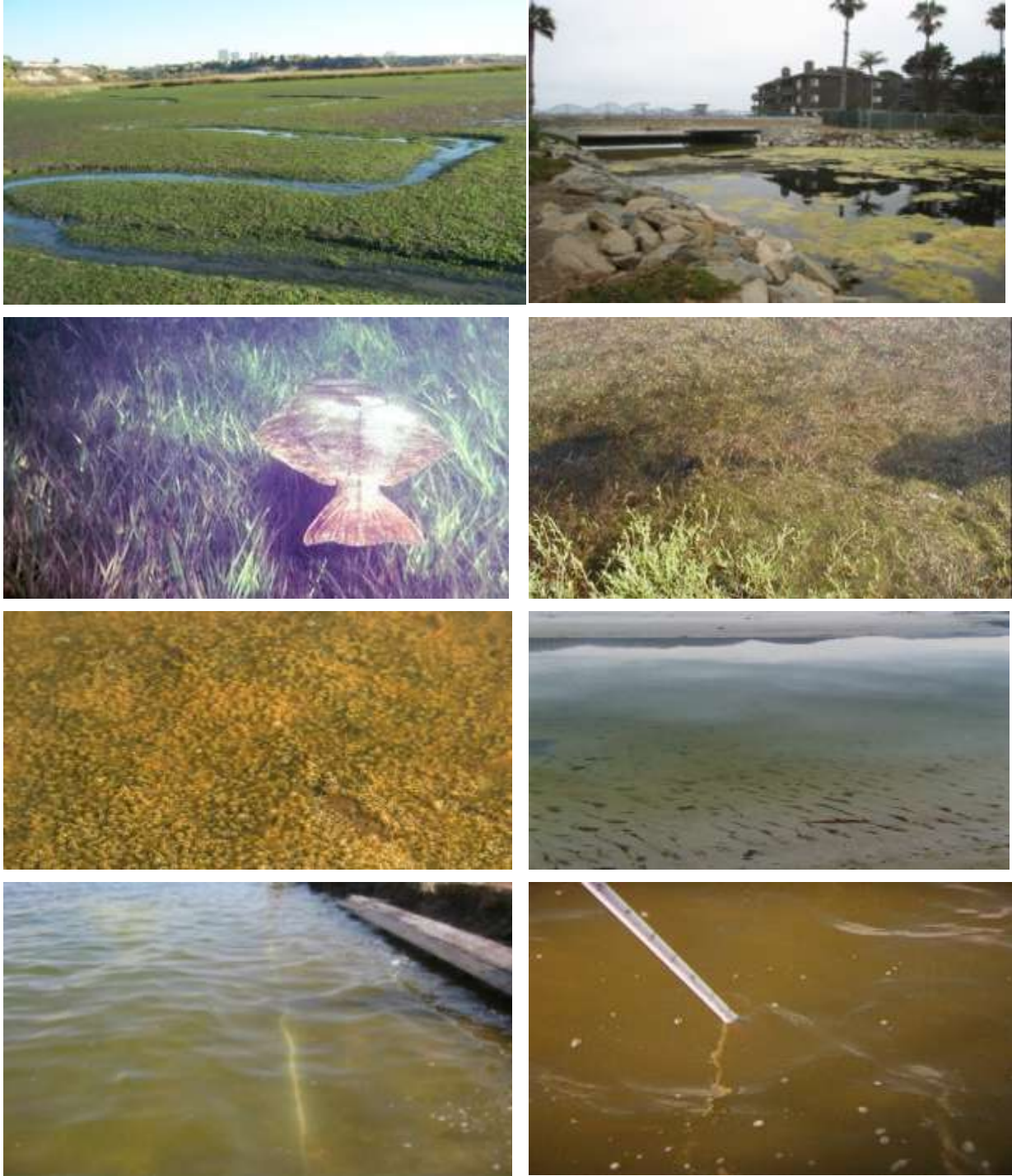
Figure 2.3. Examples of four major primary producer groups found in tidal flats, shallow and deepwater habitat types in estuaries: macroalgae on tidal flats (top left) and floating macroalgae in a closed lagoon (top right), seagrass (second panel, left), and Ruppia sp., a type of brackish water SAV (second panel, right), microphytobenthos (third panel left and right) and phytoplankton (bottom panel left and right).

Table 2.2. Dominant primary producer groups present in California estuaries as a function of water depth and salinity range.

Depth	Dominant Primary Producers
Intertidal	Macroalgae Microphytobenthos Seagrass (intertidal Central & No.Calif.)
Shallow subtidal (<10 m)	Macroalgae Microphytobenthos Brackish water SAV and Seagrass Phytoplankton
Deep or light limited subtidal (>=10 m)	Microphytobenthos Phytoplankton Drift or Floating Macroalgae (in oligohaline habitats)

2.6   Conceptual Models and Candidate Ecological Response Indicators

Eutrophication is defined as the acceleration of the delivery, in situ production of organic matter, and accumulation of organic matter within an aquatic ecosystem (Howarth 1988, Nixon 1995, Cloern 2001). One of the main causes of eutrophication in estuaries is nutrient over enrichment (nitrogen, phosphorus and silica). Other factors influence primary producer growth and nutrient availability, and hence modify (or buffer) the response of a system to increased nutrient loads (referred to as **co-factors**). These co-factors include hydrologic residence times, mixing characteristics, water temperature, light climate, grazing pressure and, in some cases, coastal upwelling (Figure 2.4). A simple conceptual model of estuarine ecological response to eutrophication can be described (Figure 2.4). The increased nutrient loads and alterations in co-factors can result in:

1. Changes to aquatic primary producers,
2. Altered water and sediment biogeochemistry, and
3. Altered community structure of secondary (invertebrates) and tertiary consumers (fish, birds, mammals).

This cascade of effects has a direct effect on the ecosystem services and beneficial uses an estuary provides (Table 1.1), including reduced:

*   Habitat for aquatic life (including EST, MAR, WILD)
*   Protection of biodiversity including rare, threatened and endangered species and migratory and spawning habitat (RARE, SPWN, MIGR)
*   Productivity of commercial and recreational fisheries (SHELL, COMM, AQUA).
*   Good aesthetics and lack of odors (REC2)
*   Maintenance of good water quality (REC1, COMM, AQUA, SHELL)
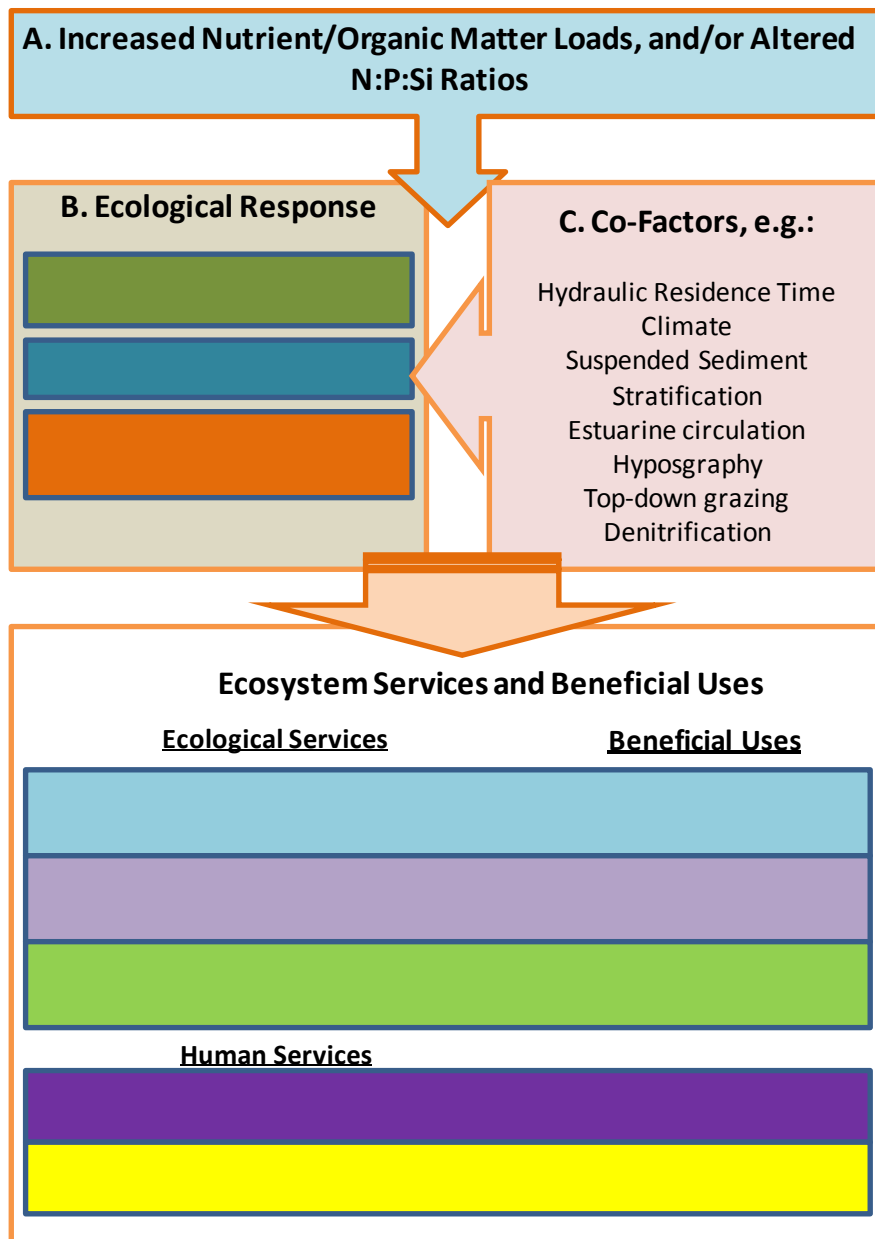
Figure 2.4. Conceptual framework of linkage of nutrient loading (A), ecological response (B), which includes altered primary producers, sediment and water biogeochemistry, and secondary & tertiary consumers), co-factors modulating response (C), and altered ecological services and beneficial uses (D).

The three identified components of the ecological response to eutrophication (Figure 2.4 component (B); Figure 2.5) can be used as an organizing framework within which to list and review possible indicators for the E-NNE. Each component is further explained below, along with a list of corresponding indicators under consideration for the E-NNE framework.

Ecological Response Indicator Groups

Altered Primary Producers	Altered Sediment & Water Biogeochemistry	Altered Consumer Communities
Macroalgal Mats	Benthic Metabolism	Invertebrates
Phytoplankton	Water Clarity	Fish
Microphytobenthos	Nitrogen Cycling	Birds
Submerged Aquatic Vegetation	Organic Matter Accumulation	Mammals
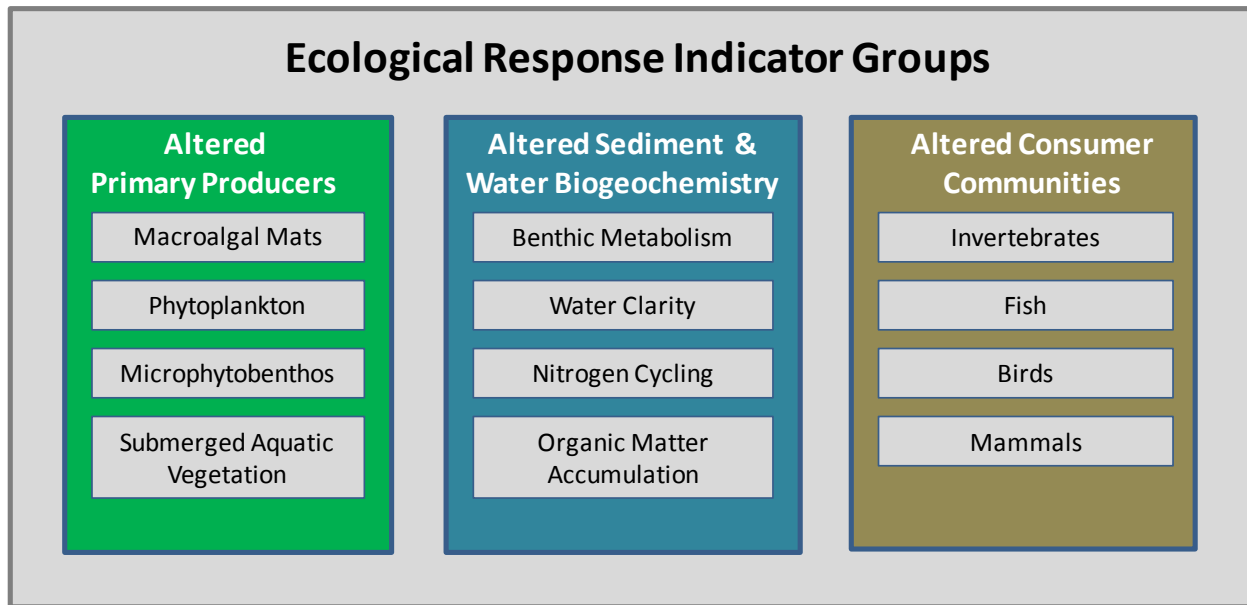
Figure 2.5. Ecological indicator groups, which include altered primary producers, sediment and water biogeochemistry, and secondary & tertiary consumers. OM=sediment organic matter accumulation.

2.6.1 Changes in Aquatic Primary Producer (APP) Community Structure

As an estuary becomes increasing eutrophic, predictable changes occur with respect the types and relative abundance of the primary producer communities, as depicted in Figure 2.6. Estuaries in a "minimally disturbed" condition are typically dominated by primary producers tolerant of low nutrient conditions, such as microphytobenthos (benthic microalgae), seagrasses, or, in deep or turbid estuaries, a high diversity of phytoplankton at relatively low biomass. As nutrient availability increases, the growth of epiphytic micro-, macroalgae as well as opportunistic ephemeral macroalgae is favored in shallow subtidal estuaries. In deep or turbid estuaries, phytoplankton biomass increases, favoring nutrient tolerant and often, harmful algal bloom species that can produce toxins harmful to marine life and humans (Fong et al. 1993 Valiela et al. 1997, Viaroli et al. 2008). In the extreme end of the eutrophication gradient, macroalgae and cyanobacterial mats dominate intertidal and shallow subtidal habitat, while in deepwater or turbid habitat, cyanobacteria and/or picoplankton blooms can dominate, causing dystrophy.

(a) Intertidal flats

(b) Shallow subtidal (unvegetated and aquatic beds)

(c) Deepwater (<10 m) and turbid subtidal

* Depends on water residence time

+ Mediated by herbivory
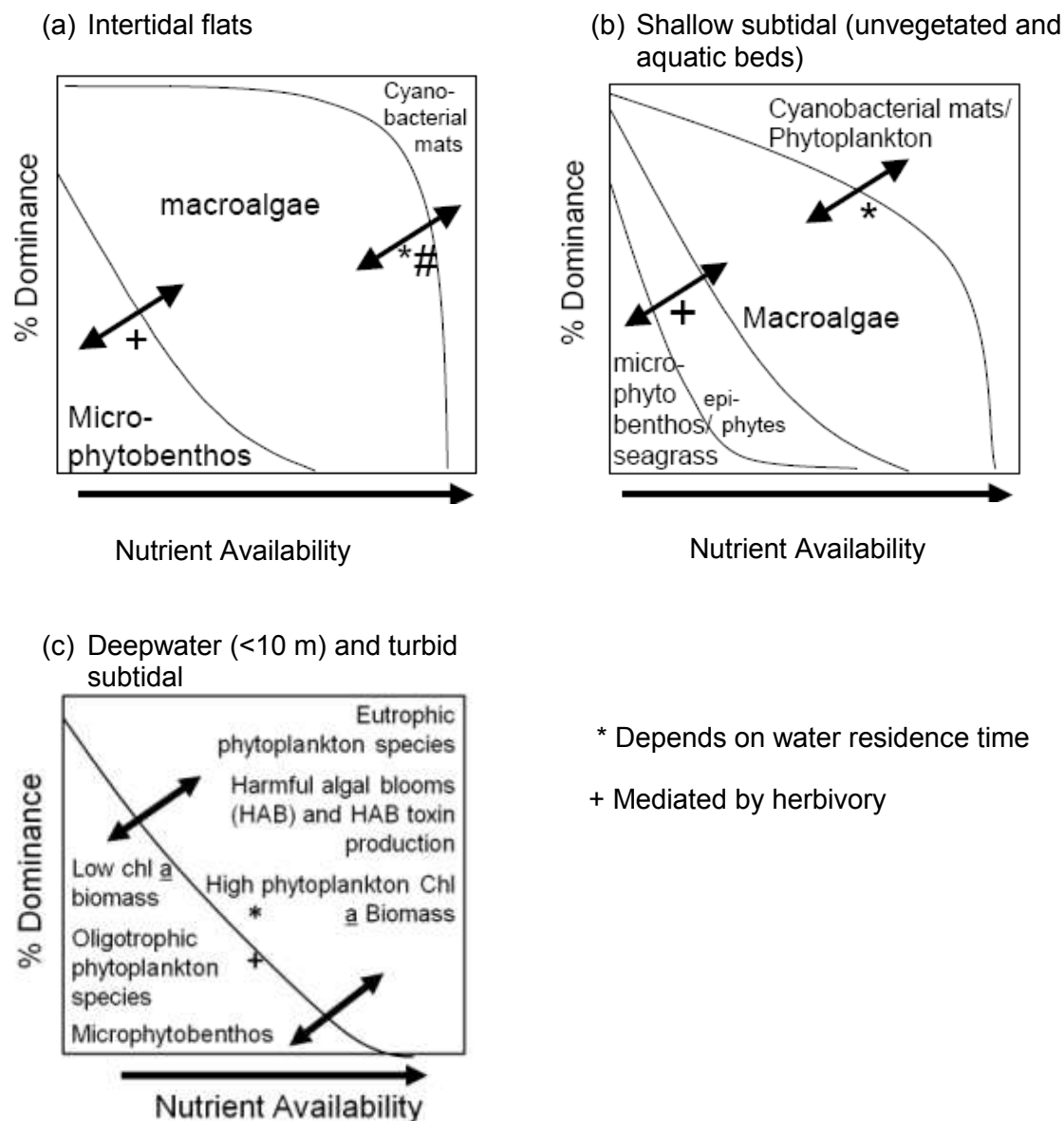
Figure 2.6. Conceptual model of relationship between nutrient availability and relative dominance of primary producers in California estuaries by major habitat type: intertidal flats (a), shallow subtidal (b), and deepwater or turbid subtidal (c).

These changes along a gradient of increasing nutrient availability provide the basis for selecting one or more primary producers as indicators for the E-NNE framework. The precise indicators that will be relevant are dependent on the habitat type and estuarine class. Table 2.3 lists the indicator groups and specific indicators under evaluation for the E-NNE framework. Literature used to evaluate these indicators is summarized in Chapters 3-7.

Table 2.3. List of primary producer indicator groups and specific indicators reviewed as candidate indicators for the E-NNE.

Primary Producer Indicator Group	Indicator or Metric
Macroalgae	Percent Cover
	Biomass
Phytoplankton	Chlorophyll a concentration
	Taxonomic composition
	Harmful algal bloom species abundance
	Harmful algal bloom species -- toxin concentration
Seagrass and Brackish Water Submerged Aquatic Vegetation	SAV Aerial Distribution
	SAV Taxonomic Composition
	SAV Biomass
	SAV Density
	Epiphyte Load on SAV
	Macroalgal Biomass/Cover on SAV
	Water Column Chlorophyll a
	Water Column Light Attenuation

Earlier on in the process, microphytobenthos (MPB) was excluded from the list of candidate indicators. MPB are important primary producers found in intertidal mudflats, sandbanks and subtidal sediments worldwide (Kromkamp et al. 2006). Although MPB photosynthetic activity is restricted to the photic zone (top 1-3mm) of the sediment, they contribute organic matter through photosynthetic activity and have been found to be particularly important in shallow estuarine habitats where they can account for up to 50% of total benthic primary production (Underwood and Kromkamp 1999). MPB are complex communities of benthic microalgae comprised of multiple taxonomic groups including unicellular benthic diatoms (phylum Bacillariophyta), filamentous cyanobacteria (Cyanophyta), chlorophytes (Chlorophyta), dinoflagellates (Dinophyta), euglenoids (Euglenophyta), and cryptophytes (Cryptophyta) as well as other photosynthetic bacteria (MacIntyre 1996). MPB was removed as a candidate NNE indicator because of the complete lack of information MPB taxonomy and factors controlling the biomass, and because it was assumed that macroalgae would be a suitable indicator for diagnosing eutrophication in environments where macroalgae and MPB co-dominate.

2.6.2 Altered Water and Sediment Chemistry (Biogeochemical Cycling)

As the process of eutrophication progresses, elevated live and dead aquatic primary producer (APP) biomass provide an elevated supply of labile organic matter, setting off a cascade of altered biogeochemical cycling in the sediments and surface waters. These effects include increased respiration in the sediments and surface waters, increased extent, frequency and duration of hypoxia, and increased concentrations of sediment pore water ammonium, sulfide, increasing the potential for toxicity to benthic organisms (D'Avanzo and Kremer 1994, Nixon 1995, Diaz 2001, Howarth et al. 2002). The efficiency of nitrogen and carbon cycling decreases, fueling increased organic matter accumulation in the sediments.

With respect to review of candidate E-NNE indicators, changes in biogeochemical cycling in estuarine sediments and surface waters due to eutrophication can be broken down into six general categories (Table 2.4) each having a set of discrete candidate indicators:

- o Changes in water column ammonia, urea, and N:P, which can direct effects on the community composition of certain primary producers

- o Changes in water clarity, due to increased suspended live and dead biomass

- o Altered concentrations of dissolved oxygen

- o Altered rates of benthic metabolism, which capture the relative rates of carbon production and respiration within a system

- o Increased sediment organic matter accumulation and sediment redox status, which is the rate at which organic matter is accumulates within sediments and the effects it has on sediment oxidation reduction reactions

- o Altered rates of nutrient cycling, which can be defined as the rates of in key transformation mechanisms for nitrogen, phosphorus

Table 2.4. Table of candidate water column and sediment chemistry indicators reviewed for the E-NNE framework (Sutula et al. 2011).

Indicator Group	Indicator or Metric
Nutrients	Ammonia
	Urea
	N:P Ratio
Water Clarity	Secchi Depth
	Kd (Light extinction)
	Turbidity
Dissolved Oxygen	Dissolved Oxygen Conc.
	Biological or Chemical Oxygen Demand
	Sediment oxygen demand
Benthic Metabolism	Benthic production: respiration ratio
	Benthic TCO2 flux
Organic Matter Accumulation and Sediment Redox Status	Sediment %OC, %N, and %P
	Sediment C:N: P ratio
	Sediment TOC:TS and degree of pyritization
Nitrogen Cycling	Denitrification Efficiency

2.6.3 Altered Community Composition of Secondary and Tertiary Consumers

Poor habitat quality and altered abundance of primary producers causes shifts in the secondary consumers (benthic infaunal, epifauna and pelagic invertebrates) that are directly impacted by alterations in primary producer community structure and degradation in water and sediment chemistry. Higher level consumers, such as fish, birds, mammals, and other invertebrates that prey upon these secondary consumers (referred to here as tertiary consumers), experience reduced food availability and quality, reduce reproductive success, increased stress and disease, and increased mortality.

While secondary and tertiary consumers are closely linked to ecosystem services and beneficial uses (Figure 2.4), use of these organisms as indicators for the E-NNE framework is problematic because organism and population measures of health are impacted by a variety of different stressors in a complex environment which is not easy to model. Within the group of secondary and tertiary consumers, benthic macroinvertebrates are the sole taxonomic group recommended pursuing for possible inclusion as an E-NNE indicator in some key habitat types and estuarine classes.

Because invertebrates that live in or on sediments are exposed to environmental stressors on an ongoing basis, the benthic life present at a particular location often provides a good indicator of sediment habitat quality. Benthic community composition can be impacted by contamination, eutrophication as well as natural variations in habitat and physical disturbance. The State of California has been developing a benthic response index (BRI) for bays and estuaries with salinities of 18 ppt or greater. Benthic indices apply standard mathematical formulas to data on the number and diversity of benthic organisms at a particular location to find a score that rates the disturbance of the community. This provides a simple means for communicating complex ecological data to environmental managers. The BRI is a component of the SWRCB's sediment quality objectives (www.waterboards.ca.gov/water_issues/programs/bptcp/sediment.shtml), which establishes numeric endpoints for sediment quality due to toxic contaminants.

3. Suitability of Macroalgae as an Indicator of Eutrophication: A Review of Existing Science

Peggy Fong, Lauri Green, and Rachel Kennison

3.1 Introduction

Macroalgae are an ancient group of single to multicellular primary producers found in all aquatic ecosystems. They provide the same ecological functions as vascular plants in terrestrial ecosystems, but lack the structural tissues characteristic of plants. They are important primary producers in intertidal and shallow subtidal estuaries, providing food and refuge for invertebrates, juvenile fish, crabs and other species. However, some species of macroalgae thrive in nutrient-enriched waters, outcompeting other primary producers. For this reason, macroalgae have been proven to be useful indicators of eutrophication in estuaries. Blooms of macroalgae are stimulated by high nitrogen (N) and phosphorus (P) loading, (Raffaelli et al. 1989, Valiela et al. 1992, Peckol and Rivers 1995, Pihl et al. 1999, Krause-Jensen et al. 2007). Estuarine ecosystems have been subjected to increased frequencies and magnitudes of harmful macroalgal blooms, outcompeting seagrasses and other primary producers and resulting in hypoxia, reduced biodiversity, fish and invertebrate mortality, altered food webs and energy flow, and disruption of biogeochemical cycling (Sfriso et al. 1987; Valiela et al. 1992, 1997; Coon 1998; Young et al. 1998; Raffaelli et al. 1989; Bolam et al. 2000).

The purpose of this review is to summarize existing information on macroalgae and its suitability as an indicator of eutrophication in California estuaries, utilizing the criteria specified in Chapter 2.

3.2 General Ecology of Macroalgae

Marine macroalgae form an important component of productive and highly diverse ecosystems in estuaries worldwide and in moderate abundances provide vital ecosystem services. Members of this functional rather than phylogenetic group are from two Kingdoms and span at least four major Divisions (Rhodophyta, Phaeophyta, Chlorophyta, and Cyanophyta). Structurally, macroalgae include such diverse forms as simple chains of prokaryotic cells, multinucleate single cells over a meter in length, and giant kelps over 45m in length with complex internal structures analogous to vascular plants. The overall percentage of total oceanic net primary productivity attributed to macroalgae ranges from 4.8 - 5.9% (calculated from Duarte and Cebrian 1996). Although relatively low compared to oceanic (81.1%) and coastal (8.5%) phytoplankton productivity, on a global basis macroalgae rank third most productive. However, local macroalgal productivity is comparable to some of the most productive terrestrial ecosystems such as tropical forests (Mann 1982) and is especially important in estuaries subject to high nutrient supplies.

Mechanisms that control net production of macroalgae are the same as for other primary producers: geographic limits for growth are set by temperature and light and for removal by grazing and physical

disturbance. Within these geographical limits, biomass accumulation is controlled by many interacting biotic and abiotic factors including light quantity and quality, water motion, intra- and inter-specific competition, herbivory, and physical disturbance. However, in most places where macroalgae proliferate and dominate estuarine communities in temperate zones, this dominance is a function of nutrient, usually nitrogen (N), availability (for reviews see Howarth and Marino 2006, Valiela et al. 1997, Vitousek et al. 1997, Downing et al. 1999).

Although macroalgae of all Divisions and functional forms have been known to form nuisance blooms, the overwhelming majority are red (Rhodophyta) or green (Chlorophyta) algae with very simple body forms (thalli) and relatively rapid turnover times (life spans) of weeks to months. In California estuaries where macroalgae bloom, with few exceptions these blooms are formed by members of the green algae in the genus Ulva. One exception is Tomales Bay, where red algae in the genus Gracilariopsis as well as Ulva comprise blooms (Huntington and Boyer 2008b). All species of Ulva have very simple thallus morphologies. They are 1 - 2 cell layers thick; some are flatten or sheet-like (e.g., *Ulva expansa*) while others form hollow tubes (e.g., Ulva intestinalis, formerly Enteromorpha intestinalis). All cells are photosynthetic and capable of both sexual and asexual reproduction (Lee 2008). Sexual reproduction is prolific and occurs approximately every 2 weeks, timed to lunar cycles. Asexual reproduction can also be very prolific, both through formation of actively swimming zoospores and by fragmentation of adult thalli. Simple morphologies with high rates of reproduction are some of the traits that account for the widespread distribution and high abundance of these bloom-forming macroalgae.

All species of Ulva that dominate in estuaries undergo an ontogenetic shift in habitats usage (Kennison 2008) that makes them particularly successful in estuarine environments. Both zygotes (sexual reproduction) and zoospores (asexual reproduction) require hard substrate or mud to settle onto in order to grow into adult thalli. Thus, early stages of the life cycle are tied to this benthic habitat, restricting their distribution to intertidal or shallow subtidal regions where sufficient light penetrates. However, once the thallus reaches a critical size, which depends on local current velocities (Kennison 2008), it detaches from the benthos and forms floating mats (Astill and Lavery 2001, Cummins et al. 2004, Kopecky and Dunton 2006). These mats are no longer restricted to intertidal or shallow subtidal regions; rather, they accumulate into floating rafts and can grow in virtually any portion of the estuary where the current transports them. For example, in Hog Island Bay, Thomsen et al. (2006) found biomass of *Ulva spp*. occurred throughout all habitats, but reached highest abundance in the deeper lagoon area (3m), indicating that the mobility of the algae allowed them to occupy many different locations in the bay. By detaching and moving as adult mats, estuarine algae can relocate to lower energy, deeper, subtidal areas taking advantage of the whole euphotic zone and facilitating distribution throughout the entire estuary (Bell and Hall 1997, Ceccherelli and Piazzi 2001, Berglund et al. 2003, Thomsen and McGlathery 2005, Thomsen et al. 2006, Biber 2007).

Opportunistic green algae in the genus *Ulva* have many additional physiological traits that allow them to dominate in a dynamic estuarine environment. These include rapid nutrient uptake and growth rates (Fujita 1985, Pedersen 1994, Lartigue and Sherman 2005, Naldi and Viaroli 2002) and a high tolerance for a wide range of temperature (Fong and Zedler 1993) and salinities (Young et al. 1987, Edwards et al. 1987, Kamer and Fong 2000). In an uptake experiment exposing *Ulva expansa* and *U. intestinalis* from

California estuaries to low, medium and high NO_3^- pulses, both species of algae exhibited a high affinity for NO_3^- across all N concentrations (Figure 3.1; Kennison 2008). In addition, increased nitrogen supply enhances tolerance of these bloom-forming algae to a variety of environmental extremes. For example, high nutrient supply can ameliorate the negative effects of lowered salinity. In a laboratory microcosm experiment, growth of algae subjected to lowered salinity and ambient nutrients were suppressed by 50% while algae in the same salinity but higher nutrients were suppressed by only 15% (Kamer and Fong 2000). This trait is especially adaptive, as high pulses of nitrogen from the watershed are always associated with lower salinity.
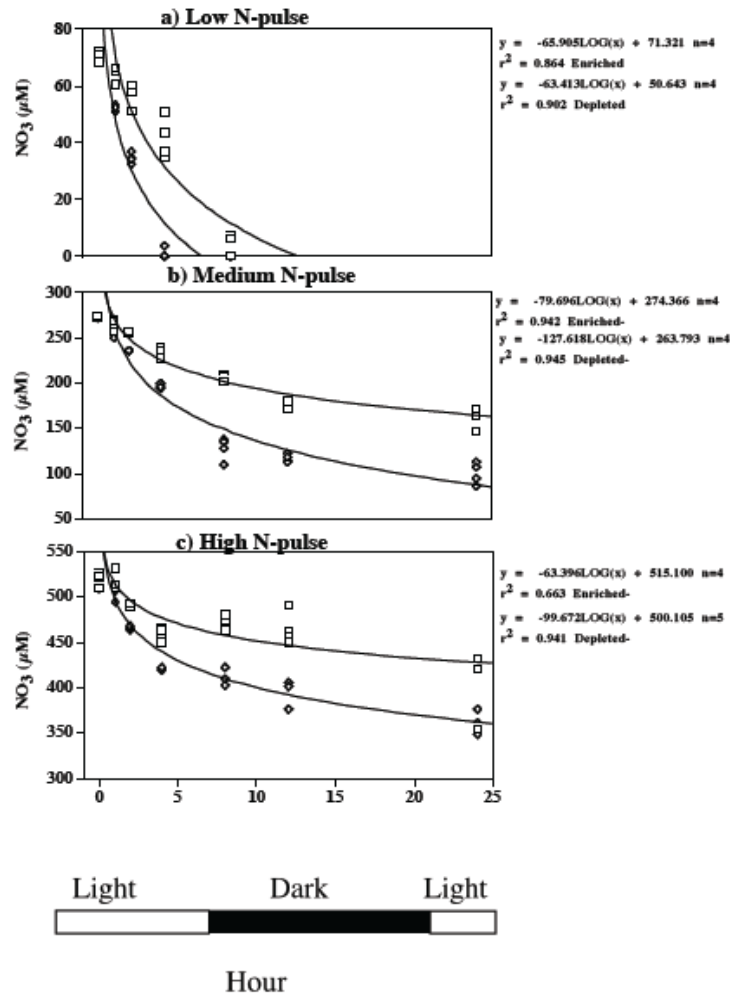
Figure 3.1. Depletion of water column NO3 in: low initial NO3 (a), medium initial NO3 (b), and high initial NO3 for U (c). Expansa with enriched (squares) and depleted (diamonds) initial tissue N content. Regression analysis of the data for water nutrient concentrations generates p-values of <0.0001 for all regression lines. High concentration, enriched, n=3, for all other treatments, n=4 for each time interval. Periods of daylight (clear) and darkness (solid) are shown below x-axis. Scales for y-axis differ.

Common bloom-forming species of *Ulva* have been used as biological indicators of nutrient supplies in estuaries. They are good indicators because of their ability to rapidly take up large pulses of inorganic nitrogen (Fujita 1985, Pedersen 1994, Lotze and Schramm 2000, Runcie et al. 2003) and store it for future growth (Fujita 1985, Bjornsater and Wheeler 1990, Fong et al. 1994, Pedersen and Borum 1997, Lotze and Schramm 2000, Naldi and Viaroli 2002). Thus, tissue nutrients in macroalgae integrate nutrient supplies over time (Wilson 1994). This is especially important in Mediterranean systems, where nutrient supply and availability can be variable due to pulses of nutrients that are delivered by runoff from seasonal storms in the wet season as well as during periodic discharges of sewage and agricultural waste in both the wet and dry seasons (Zedler 1996). Therefore, traditional water column nutrient sampling methods may miss pulsed nutrient signals, and not provide an accurate estimate of nutrient enrichment. With the combination of a high affinity for nitrogen and ability to store nutrients, macroalgal tissue nutrient status can be used as a biological indicator (Harrocks et al. 1995, Fong et al. 1998, Costanzo et al. 2000, Huntington and Boyer 2008b) to determine nutrient availability.

The effects of nutrient loading rate on macroalgal distribution and biomass accumulation are heavily influenced by the hydrological connection to the ocean of each estuary. Temporal patterns in these hydrological connections are so important in determining which primary producer group dominates in each estuary that they form the basis for estuarine classification (Chapter 2). Due to the ability of most bloom species to shift habitat usage from benthic to floating stages, macroalgae are able to occupy all estuarine habitats by rafting in surface waters or depositing on subtidal or intertidal sediments (Figure 3.2) . Biomass accumulation, however, is linked to nutrient supply. Thus, low abundances of macroalgae may co-occur in low nutrient systems with subtidal and intertidal seagrasses and the microphytobenthos (benthic microalgal community). It is only as nutrient loads increase that proliferation of macroalgae has negative impacts on other producer groups across all estuarine classes.

In estuaries with perennially open connections to ocean, the effects of macroalgae on other producers, water and sediment physiochemical parameters, and rates of metabolism most likely decrease with water depth, though this relationship has rarely been quantified (see Olynarnik 2008 for a partial test). However, it is reasonable to assume that intertidal and shallow subtidal areas are far more affected by macroalgal mats than deepwater habitat (>10 m), simply because the biomass to water volume ratio is so much lower and flushing so much greater. In addition, some producer communities, like seagrass beds, may be more vulnerable to deposition of macroalgae mats than others (e.g., Hauxwell et al. 2001). However, the effects of macroalgae on the intertidal and subtidal microalgal community in estuaries have been less well-studied (see Sundbäck and McGlathery 2005).
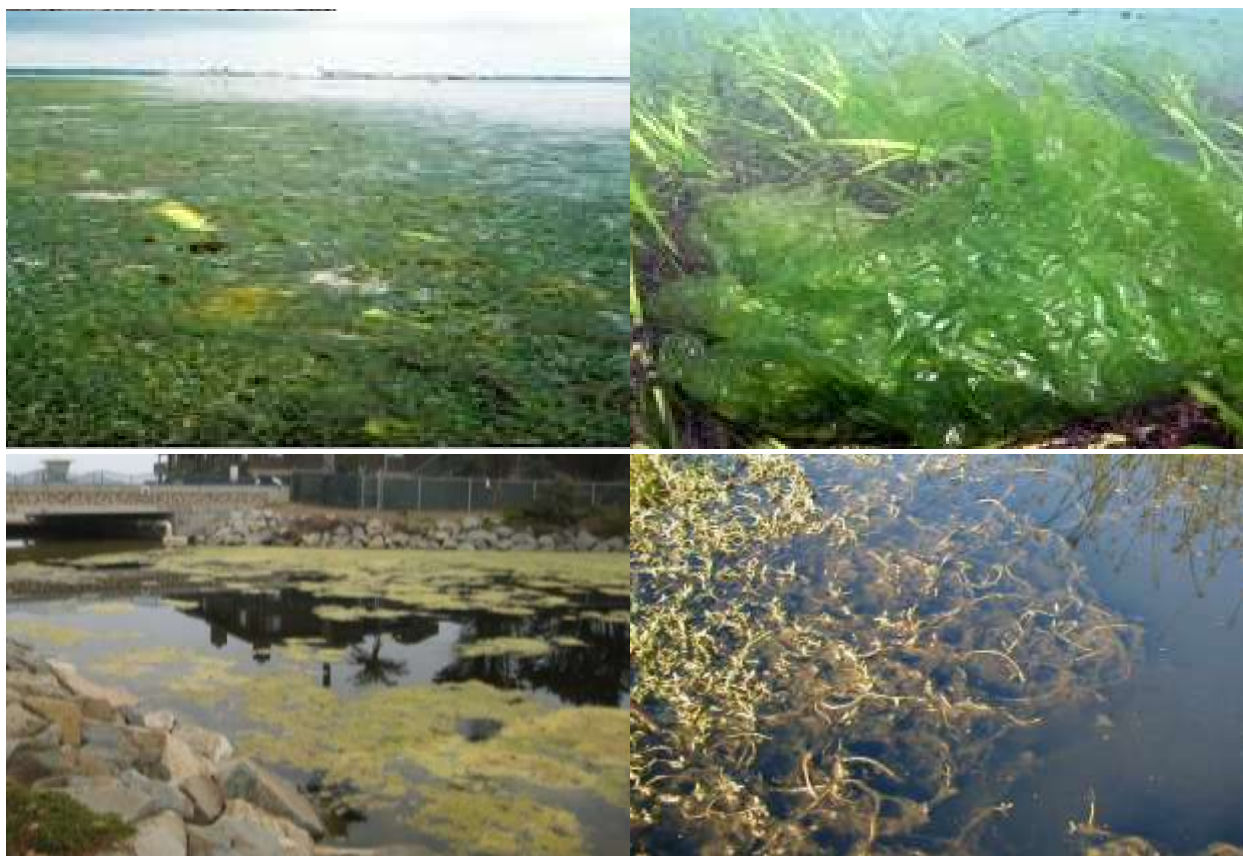
Figure 3.2. Examples of types of habitats in which macroalgae can occupy a dominant role among other primary producers: mats on intertidal flats (upper left), rafting mats on seagrass (upper right), floating mats in closed river mouth estuary (lower left), rafting mats intercalated with Ruppia in a closed lagoon (lower right).

3.2   Ecological Effects of Increased Abundance of Macroalgae and Relationship to Ecosystem Services and Beneficial Uses

3.3.1   General Conceptual Model of Effects of Macroalgae on Other Primary Producer Groups in Californian Estuaries

Macroalgae are important members of the primary producer community in shallow soft-sediment systems worldwide where light penetrates to large areas of the benthos. They are present in all estuarine geoforms, but their relative abundance is, at least in part, proportional to the amount of suitable habitat (see Chapter 2) and nutrient supply. In oligotrophic systems, macroalgae are a component of the primary producer community, but are generally not dominant (Figure 3.3). Rather, in shallow subtidal and intertidal portions of these estuaries, benthic communities may be dominated by the microphytobenthos (MBP), an assemblage of diatoms, dinoflagelllates, cyanobacteria, and sporling green macroalgae living on the sediment surface that can contribute up to 50% of the primary production in an estuary (Underwood and Kromkamp 1999). In larger, well-flushed California estuaries,

especially in northern California, shallow subtidal portions are often dominated by the seagrass *Zostera marina* (see Chapter 6). However, this "minimally disturbed" state has not been well characterized for California estuaries because of the extensive hydromodification and increased contaminant loads to the State's estuaries.
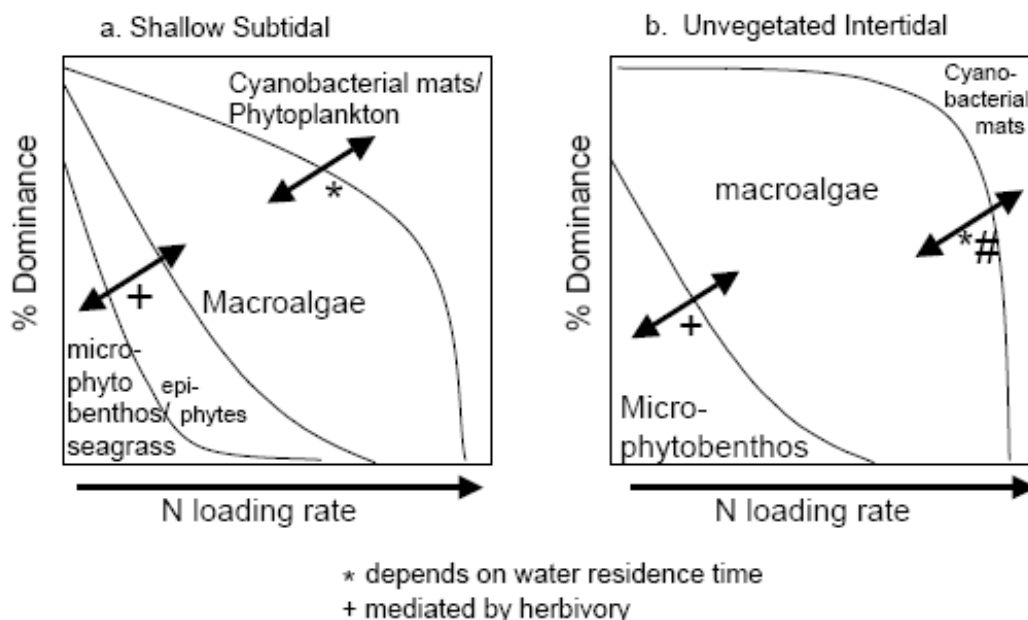
Figure 3.3. Conceptual model of the relationships between N loading rate and the community composition of primary producers in shallow subtidal (a) and unvegetated intertidal habitat in California estuaries (b).

As nutrient availability increases, it has been well-documented in many parts of the world that blooms of green or red macroalgae become dominant in shallow subtidal and intertidal estuaries and lagoons, replacing seagrass or MPB (Figure 3.2; e.g., Sfriso et al. 1987, 1992; Raffaelli et al. 1989; Valiela et al. 1992, 1997; Geertz-Hansen et al. 1993; Peckol et al. 1994; Marcomini et al. 1995; Page et al. 1995; Hernández et al. 1997; Hauxwell et al. 1998; Kamer et al. 2001). This process is referred to as a "phase shift." While the phase shift from seagrass to macroalgae is well documented elsewhere, there is only anecdotal evidence of shifts in California, due to lack of historic monitoring data. In Tomales Bay (northern California), where seagrass communities are relatively common and appear healthy, Huntington and Boyer (2008a) hypothesized that the recent rapid proliferation of macroalgae may represent an initiation of a phase shift in this system. Thus, these systems should be monitored carefully for signs of loss. In contrast, in southern California, it is likely that loss of seagrass occurred in the past and was associated with loss of suitable habitat (hydromodification, excessive sedimentation, dredging, etc.) and eutrophication. However, any seagrass loss that may have occurred pre-dated scientific documentation in this region, so is highly uncertain. At present, only a handful of estuaries in southern California support remnant seagrass populations (Stewart 1991, Merkel et al. 2009). We hypothesize that shifts from MPB to macroalgae were also common, and may still be occurring; however, studies of this transition in California estuaries are lacking (see Chapter 5). What is certain is that, at present, the

dominant primary producers in eutrophic estuaries of California are green macroalgae in the genus *Ulva*. For example, in southern California, every estuary that has been surveyed is dominated either continuously or episodically by blooms of Ulva expansa and Ulva intestinalis (Kennison et al. 2003).

There is mounting evidence that another phase shift to cyanobacterial mats, or more rarely in California, to phytoplankton, may occur if nutrient loading continues to rise in estuarine systems presently dominated by macroalgal blooms (Figure 3.3). The driving mechanism is self-shading and senescence of macroalgal mats once they reach a critical density (Peckol et al. 1994, Krause-Jensen et al. 1999); decomposition of these mats leads to release of nutrients to the water for use by other primary producers. In subtidal estuaries with low to moderate tidal influence, or those that are intermittently or ephemerally tidal, seasonal blooms of macroalgae are succeeded by blooms of phytoplankton during closed conditions (e.g., Sutula et al. 2009). This has occurred in estuaries along the East Coast of the U.S. and in Europe (Duarte 1995, Valiela et al. 1997) and the speed of the transition to phytoplankton-dominance is thought to be dependent on water residence time (Valiela et al. 2000). In California, however, the ultimate dominance by phytoplankton may be limited to deeper or more turbid systems (See Chapter 6). Rather, in California, where many estuaries are well-flushed and extremely shallow with large areas of intertidal flats, macroalgae may be replaced by cyanobacterial mats. Results of the only *in situ* enrichment experiment on the West Coast of the United States documented this final ecosystem transition along an eutrophication gradient to benthic cyanobacterial mats (Armitage and Fong 2004) rather than to phytoplankton. Mats of cyanobacteria were found to be unpalatable and toxic to the dominant herbivores (Armitage and Fong 2004). In Southern California, the shift from MPB to macroalgae to cyanobacteria mats has been documented to occur seasonally in both perennially and intermittently tidal estuaries, with peak macroalgal biomass in late spring and summer, shifting to cyanobacterial mats in the fall (P. Fong, unpublished data).

3.3.2   Effects of Epiphytic and Rafting Macroalgae on Seagrass

Macroalgae are an integral component of the seagrass community that can proliferate in subtidal portions of perennially tidal estuaries, where they can be attached to the benthos, grow epiphytically (attached to plants) on seagrass blades (Figure 3.4), or form drifting mats. However, in seagrass systems with low nutrient availability and intact herbivore populations, epiphyte biomass accumulation is modest. In low nutrient systems, drift macroalgae are also present in low abundance, but are ecologically important as they may provide protection from predation (Salovius et al. 2005), aid in dispersal of invertebrates and fishes (Holmquist 1994), and ameliorate desiccation for plants in the upper intertidal zone (Penhale and Smith 1977).

As biomass of epiphytic algae increases, there is correlative and experimental evidence that effects on diversity and abundance of seagrasses become strongly negative. Negative effects include reduction in the quantity and quality of light for photosynthesis, limitation of nutrient and gas exchange across blades, and an increase in drag resulting in loss of blades or shoots (Borowitzka and Lethbridge 1989). A recent review concluded that the dominant effect of heavy epiphyte cover appears to be decreased growth and survival of seagrasses due to competition for light (Nelson 2009). The review also revealed that this light reduction effect is extremely variable. For example, estimates from laboratory

experiments measuring light attenuation with maximum epiphyte density on seagrass blades from 20 different studies found reduction of light to seagrass blades varied from 30 up to ~100%. Further, there are few direct measurements of the effect of epiphytes on seagrass photosynthesis, so the link from reduced light to reduced productivity of seagrass blades is missing (Nelson 2009). Finally, extrapolation of laboratory results to prediction of effects on rooted plants in a complex community is tenuous, at best. Because epiphytes are also affected by in situ light levels, the overall effect of epiphyte shading is likely to be determined by complex interactions. In one case, in a field experiment where the seagrass community was shaded in situ with shade cloth, epiphytes proved more sensitive to reduced light than seagrass due to seagrass' greater internal energy stores (Neveraukas 1988). Despite the numerous uncertainties, however, the guidance document for management of seagrasses for Chesapeake Bay (U.S. EPA 2003) acknowledges the importance of light reduction by seagrass epiphytes and uses the percent of light at the surface of a seagrass blade (epiphytes + water column attenuation) to establish a minimum light level required for persistence of seagrass.

There are many studies that correlate water column nutrient concentrations with field distribution of epiphytes, which suggests that nutrient enrichment increases epiphyte accumulation (for a review see Nelson 2009). Overall, this review found the strongest relationships between nutrients and epiphytes were found where nutrient enrichment was from a strong point source, such as sewage outflows (Silberstein 1986, Neveraukas 1987) septic tanks, and bird rookeries (Tomasko and Lapointe 1991, Frankovich and Fourqurean 1997). Other studies at larger scales found only weak correlations between nutrient concentrations and epiphyte loads (Frankovich and Fourqurean 1997, Tomasko et al. 1996). Also, Hanisak (2001) pointed out that grazers play an important role in determining epiphyte biomass accumulation, and must be considered along with nutrients.

Figure 3.4. Epiphytes on seagrasses. Epiphytes can be comprised of algae such as Ulva spp.

There are numerous mesocosm and laboratory microcosm experiments relating nutrient addition to epiphyte accumulation. Nelson (2009) summarized 18 key studies, but concluded that the extreme variation in mesocosm size, flow rate, nutrient addition regimes, and presence/absence of different grazers between experimental systems made drawing any generalizations difficult. While there was an overall positive response of epiphytes to increased nutrient supplies in many studies, both the epiphyte and seagrass response varied greatly. Importantly, the overall response to nutrient addition was often blooms of phytoplankton or drift macroalgae rather than epiphytes, causing complex interactions among these competing primary producers. The only experimental study on the West Coast of the US linked increased nutrients with increased epiphytes and subsequent decreased seagrass leaf growth (Williams and Ruckelshaus 1993). All of these results argue that some index that summarizes light attenuation at the blade surface from phytoplankton, epiphytes, and drift macroalgae may be a more appropriate indicator for seagrass than any single indicator group.

Evidence exists that rafts of drift macroalgae may have negative effects on seagrass. Negative effects are often attributed to "suffocation" (den Hartog and Poldermen 1975), which may be caused by either prevention of sediment oxygenation by direct contact with the water column or reduction in oxygen transport to the roots by the lacunae (Young 2009). An early study also recognized that floating rafts of drift macroalgae in a subtropical bay in Texas shaded large areas of the seagrass bed causing light limitation (Cowper 1978). Young (2009) reviewed 48 field studies that examined relationships between macroalgal abundance and the distribution and abundance of seagrass. Results varied from "no effect" in ~10% of the studies to complete replacement of seagrass by macroalgae in ~45% of the studies. Although these studies were correlative, and did not study causation, seagrass loss was often attributed to anoxia and build up of toxic substances such as hydrogen sulfide. The most thorough set of field studies of seagrass loss to date were conducted in Waquoit Bay, Massachusetts, as part of a Long Term Ecological Research Project. Valiela et al. (1992) quantified sources and effects of nutrients, tracing terrestrial sources through the groundwater, into the Bay, and into macroalgal blooms that correlated to a decrease in seagrass. Lyons et al. (1995) developed a linear relationship between nitrogen loading and macroalgal biomass and a concurrent exponential decline of seagrass. Further, Hauxwell et al. (2003) showed a linear increase in macroalgal canopy height and an exponential decrease in eelgrass shoot density and bed area as nitrogen loads increased. However, these studies emphasized that the relationship between nutrients and eelgrass was indirect, mediated by drift macroalgae as well as epiphytes and phytoplankton reducing light availability.

There have been few field surveys examining the relationship between macroalgae and eelgrass on the Pacific Coast of the U.S. While large masses of green macroalgae have been noted to occur within seagrass beds for many years (e.g., Phillips 1984), there has been only one study that found negative effects (Kentula and McIntire 1986). In this study in Oregon, there was a decrease in shoot primary productivity in midsummer concurrent with lower light and a bloom of *Ulva prolifera*. Instead of blocking light, however, the authors attributed the negative effects to drift algae entangling in the blades and uprooting plants and epiphytic algae increasing sedimentation and burying seagrass. In contrast, neither Kentula and DeWitt (2003) in Yaquina Bay nor Thom et al. (2003) in Coos Bay found any

evidence of negative effects of green macroalgae on seagrass, though both sets of authors expressed concern at possible future effects.

There is some evidence from mesocosm and field experiments that drift macroalgae have negative effects on seagrass, though these effects may be mediated by other producer forms. In an early field experiment, Harlin and Thorne-Miller (1981) found that addition of NH_4 stimulated both seagrass and *Ulva* while NO_3 only stimulated growth of *Ulva*, and speculated that growth of algae limited adjacent seagrasses. A mesocosm experiment demonstrated that the negative effects of warm water temperature on eelgrass were exacerbated by increased inorganic nutrients, which, in combination with warm water, enhanced growth of macroalgae (Bintz et al. 2003). Total system responses to nutrient addition, however, may be complex. For example, while nutrients consistently stimulated growth of some form of algae, it was impossible to predict if phytoplankton, epiphytes or macroalgae would dominate, even in replicate experimental units of one mesocosm experiment (Short et al. 1995). In contrast, Taylor et al. (1995) found that different forms of algae bloomed at different nutrient loads. However, none of these mesocosm studies made a direct link between biomass of macroalgae and eelgrass.

There are a few key field experiments that have linked macroalgal biomass with seagrass declines. Hauxwell et al. (2001) replicated an experiment controlling macroalgal mat thickness in two estuaries with a 6-fold difference in nitrogen loading rate and an almost 5-fold difference in natural macroalgal mat thickness. They established a critical macroalgal mat thickness of 9-12 cm at which eelgrass declines in shoot density by over 90% (Figure 3.5). However, mat thickness was never related to macroalgal biomass in this study. As macroagal biomass is by far the most common measure of abundance, this limits our ability to relate this study to other studies or other estuaries.

On the Pacific Coast, three field experiments manipulating macroalgae in seagrass beds have been conducted. In Yaquina Bay estuary, Oregon, Sullivan (unpublished but presented in Young 2009) found that macroalgae added to enclosures decreased eelgrass shoot density by an order of magnitude compared to removal treatments. However, the macroalgal "dose" was not specified in this report. Nelson and Lee (2001) removed Ulvaria blooms from eelgrass beds in Washington State, and found that loss of eelgrass during the summer bloom was greatly reduced. In addition, in removal plots, shoot density was a function of the amount of algae removed (Figure 3.6). In a field experiment encompassing 4 summers in Bodega Bay Harbor, Olyarnik (2008) only found negative effects of Ulva in 2006, when a very large bloom caused reduction in eelgrass shoot density in all but the Ulva removal treatments. Unfortunately, the control treatments in this experiment showed significant artifacts of the enclosures themselves across the entire experimental duration, suggesting the cages themselves were affecting seagrass shoot density; thus these data cannot be used in consideration of a macroalgal endpoint. Finally, Huntington and Boyer (2008a) manipulated biomass of Gracilariopsis in caging experiments in Tomales Bay, and found that the highest abundance of algae found in the field surveys (1.7 kg ww m^{-2}) reduced eelgrass shoot density by ~50% after only 3 months (Figure 3.7). This key study confirmed that a threshold for negative effects occurred somewhere between 325 and 1700 g ww m^{-2}. It is essential that we repeat this key experiment in estuaries with a variety of conditions that occur across California, and with finer resolution among treatments.
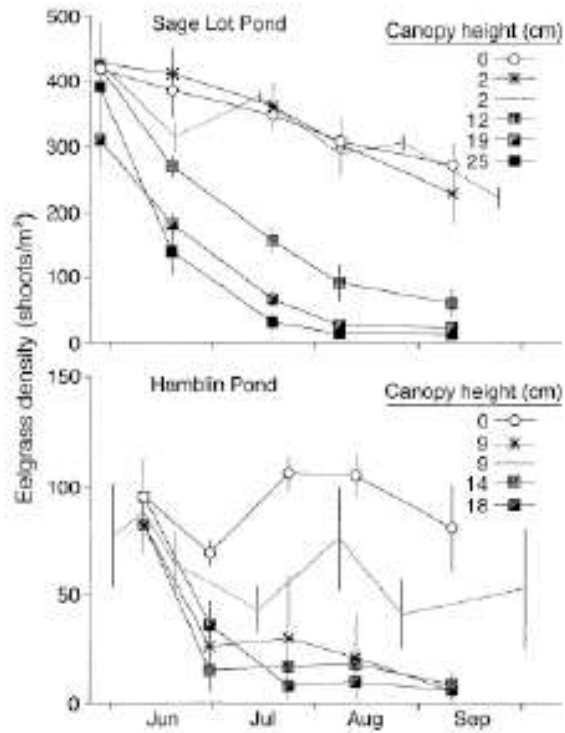
Figure 3.5. Density of eelgrass shoots in enclosures containing different canopy heights of macroalgae (solid lines) and in unencolsed plots (dotted lines without symbols) during summer 1998. Data are means ± SE (Hauxwell et al. 2001).
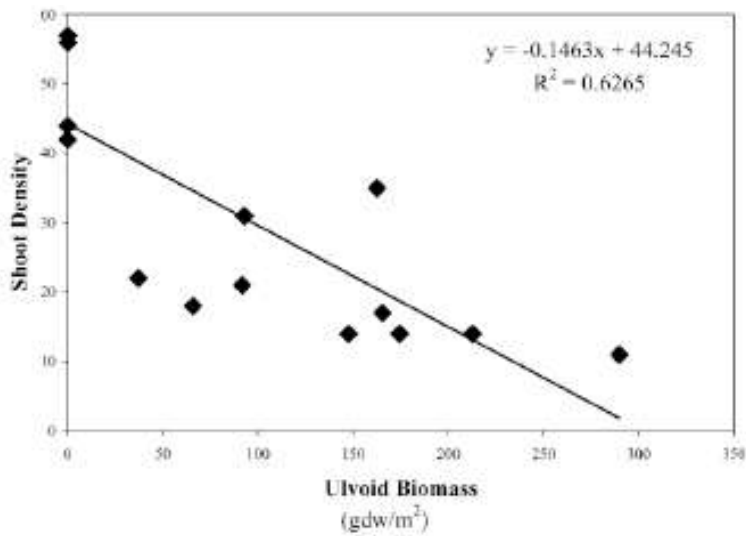
Figure 3.6. Relationship between Ulvaria biomass and Zostera shoot density (Nelson and Lee 2001).
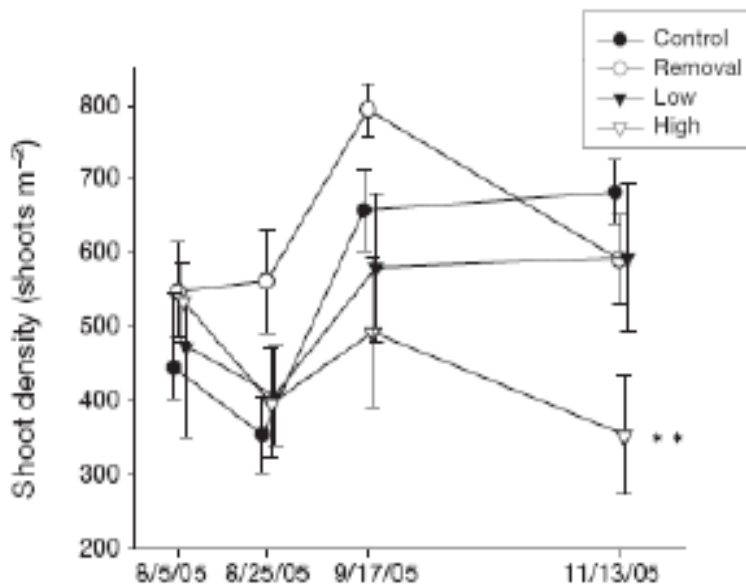
Figure 3.7. Zostera marina shoot density in an enclosure experiment in Tomales Bay over 3 months (n=6, means±SE). ** significant at p <0.01. Treatments were control (no manipulation), 0 (removal), 325 kg ww m^{-2} (low), and 1.7 kg ww m^{-2} (high) representing the range of biomass observed during field surveys (Huntington and Boyer 2008).

3.3.3   Relationship between Macroalgae and Invertebrates in Intertidal Flats

There is a plethora of research that demonstrate a negative relationship between macroalgal biomass accumulation in mats deposited on intertidal and subtidal sediments and the health of sediment-associated invertebrate communities (for a summary see Table 3.1). Effects can be described as a function of the magnitude (mat thickness, biomass, and/or percent cover) and duration of algal mats. Authors have investigated measures of benthic epifauna and infaunal community structure response including: species richness, abundance, diversity (combines richness and abundance), and biomass.

Effects of Magnitude and Duration of Macroalgal Blooms

Research conducted in estuaries around the world that vary with respect to climate, hydrology, nutrient loading and benthic community structure suggests that low abundances of macroalgae have positive effects on benthic faunal communities, but that excessive accumulations of macroalgae and/or prolonged cover have strongly negative effects. While results suggest there may be a tipping point or threshold above which macroalgal mats begin to exert negative effects on benthic communities, these thresholds have yet to be established for most estuarine ecosystems (see Green 2010).

Table 3.1. Summary of observed effects of macroalgal blooms on infauna and resident epifauna on intertidal flats.

Location	Source	Treatment Level / Observed Abundance	Duration	Observed	Comments
Baltic Sea	Norko and Bonsdorf 1996)	2 kg ww m^{-2}	34 days	Reduced abundance of most macrobenthic invertebrates but not all	Single treatment level –single algal application
Australia	Cummins et al. 2004	4.5 kg ww m^{-2}	12 weeks	Reduced macrobenthos species abundance	Single treatment level -single algal application
Portugal	Cardoso et al. (2004)	0.3 kg ww m^{-2} no effect 3 kg ww m^{-2} adverse effect	4 weeks	Reduced macrobenthos species abundance species specific response	Multi treatment levels -single algal application
California	Green (2010)	0.5 cm no adverse effect after 8 wks 1.5 cm adverse effect after 4 wks 3.0 cm adverse effect after 2 wks	2-8 weeks	Increased biomass reduced surface deposit feeders and increased subsurface deposit feeders	Multi treatment levels - maintained algal treatment level biweekly
Scotland	Hull (1987)	3 kg ww m^{-2} adverse effects were species specific	22 weeks	After 10 weeks many species higher under mats some surface deposit feeders decreased while some subsurface feeders increased. After 22 weeks patterns similar	Multi treatment levels -single algal application
Scotland	Raffaelli (2000)	0 kg ww m^{-2} after 10 weeks increase is species specific. 3 kg ww m^{-2} after 10 weeks adverse effects are species specific Equivalent abundances of both species in all treatments after 22 weeks	22 weeks	High abundances result in increase of subsurface deposit feeders, decrease in surface deposit feeders after 10 weeks.	Multi treatment levels-single algal application
Sweden	Osterling & Pihl (2001)	1.2 kg ww m^{-2} adverse effect on all taxa after 21 days Adverse effect on some taxa after 36 days	36 days	Initially all macrofauna were negatively affected by macroalgae. After 36 days subsurface detritivores and carnivores positively affected	Single treatment level -single algal application
California	Everett (1991)	~6 kg ww m^{-2} adverse effects after 2 months and six months	6 months	Clams and shrimp abundance increased in plots where macroalgae was removed	Removal experiment
Scotland	Bolam et al. (2000)	~ 1 kg ww m^{-2} species specific effects after 6 and 20 weeks	20 weeks	Surface deposit feeders negatively affected, subsurface feeders positively affected after 6 weeks effects persisted through 20 weeks	Single treatment level -single algal application
England	Jones and Pinn (2006)	Adverse effects >70% cover	Mat duration not recorded	Species diversity declined when % cover increased from 5-70% in one month	Correlation. Low cover did not always = high diversity
Sweden	Pihl et al. 1995	Some negative effects with 1% cover, greatest effects >30% cover	Mat duration not recorded	Crabs negatively affected by moderate and high percent cover	Correlation. 1- Day sampling events. Cover duration unknown
Baltic Sea	Lauringson & Kotta (2006)	No clear relationship with mat depth and infaunal abundance	Mat duration not recorded	Herbivores more prominent within mats, detritivores more prominent in sediment	Correlation. Subtidal

Researchers working in bays with different initial benthic diversity demonstrated that effects of macroalgal abundance may vary with respect to nutrient loading. Sanchez-Moyano et al. (2001) conducted their work in the Bay of Algeciras which had relatively low nutrient loading and high benthic diversity. They correlated increased infaunal and epifaunal diversity with low abundances (0.1 kg ww m^{-2}) of macroalgae while high abundances (2.5 kg ww m^{-2}) were related to low benthic diversity. Conversely Lauringson and Kotta (2006) conducted their research in the Baltic Sea, which had considerably higher nutrient loading and much lower overall benthic diversity than the previous study. While Lauringson and Kotta (2006) did not find a correlation between increased macroalgal biomass, which peaked at approximately 14 kg ww m^{-2}, and a decline of infaunal species richness, this could have been due to the already low baseline diversity of infauna in this highly eutrophic system. Thus, it is critical to assess effects of macroalgal abundance in lagoons that vary with respect to nutrient loading. Moreover, only controlled field studies that manipulate macroalgal abundance can show causal relationships between macroalgal abundance and changes to benthic diversity.

In two separate manipulative experiments in the Baltic Sea, Norkko and Bonsdorff (1996) added approximately 2 kg ww m^{-2} and Cummins et al. (2004) added approximately 4.5 kg ww m^{-2} of macroalgae in a one-time addition to benthic plots and measured infaunal responses after approximately four weeks. Both studies found that macroalgal additions resulted in a significant loss of infaunal and epifaunal species richness compared to no algae controls. Unfortunately these studies had only a single algal density, and presence/absence studies do not elucidate at what biomass macroalgae begins to cause negative effects. For example, without varying macroalgal abundance across a wider range of treatments, it is unknown if negative effects on benthic diversity would have occurred had the researchers used lower abundances. Cardoso et al. (2004) compared responses of infauna to varied macroalgal abundance in portion of estuaries that differed with respect to eutrophication and found species specific responses to macroalgae varied depending on the level of eutrophication. They found species in the noneutrophic site often had stronger negative responses to elevated macroalgae than species in the eutrophic site. In general, many infaunal species were unaffected by biomasses of 0.3 kg ww/m^2 but 3 kg ww/m^2 resulted in significant declines in infaunal abundance. These experimental results highlight that eutrophication and macroalgal presence affect the community composition of benthic macrofauna.

The only study that directly tested the effects of both duration and magnitude of macroalgal blooms on infaunal communities was conducted in Mugu Lagoon in southern California (Green 2010). This experiment was also unique as algal mats were maintained at the "dose" levels over the entire 8 weeks of the experiment, a situation that commonly occurs in California estuaries (Green 2010). Although total macrofaunal abundances were not different across mat treatments (Figure 3.8), macroalgal mat thickness had a strong negative effect on surface feeding macrofauna (spionid polychaetes) while increasing the abundance of capitellid polychaetes, macrofauna typically found in benthos enriched with organic matter((Cohen and Pechenik 1999). Negative effects intensified over the duration the benthos was covered and showed that a tipping point from positive to negative effects occurred at an abundance between than 3 and 12 kg ww m^{-2} in summer (Figure 3.9). In this study, elevated abundances of

macroalgae were shown to cause rapid changes in the abundance of macrobenthic groups, and that duration of cover was important as well. For example, spionid polychaetes declined by approximately two thirds after four weeks under 3 kg ww m^{-2}. However, 12 kg ww m^{-2} nearly eliminated the spionid polychaete abundance after just two weeks of cover. These changes in infaunal community structure may have significant impacts on food webs as spionid tube worms are easily consumed by invertebrate and vertebrate predators (Reise 1977; Virnstein 1979) while capitellid polychaetes are visually more difficult to forage upon and are found at greater depths inaccessible to most predators (Reise 1977; Virnstein 1979).

Although this study established that a threshold exists, the lack of treatments between 3 and 12 kg ww m^{-2} leaves a wide gap and further refinement of this threshold point is needed. Additional studies are planned in California to repeat this experiment in other estuaries along gradients of sediment organic matter accumulation (L. Green, personal communication). This work will provide preliminary data with which to set endpoints; additional work will be needed to provide validation of this work over a larger range of environments.
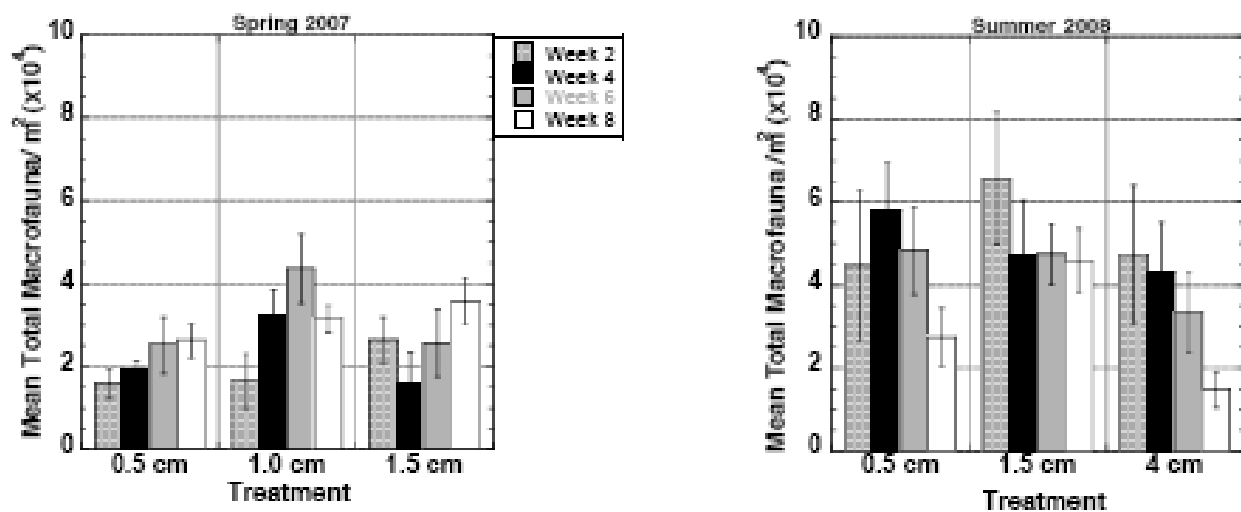
Figure 3.8. Total macrofauna were approximately twice as abundant in summer than spring, but were not affected by macroalgal mat depth in either season. Mat depths of 0.5 cm = 1 kg ww/m^2, 1.0 cm = 2 kg ww m^{-2}, 1.5 cm = 3 kg ww m^{-2}, and 4 cm = 12 kg ww m^{-2} (Green 2010).
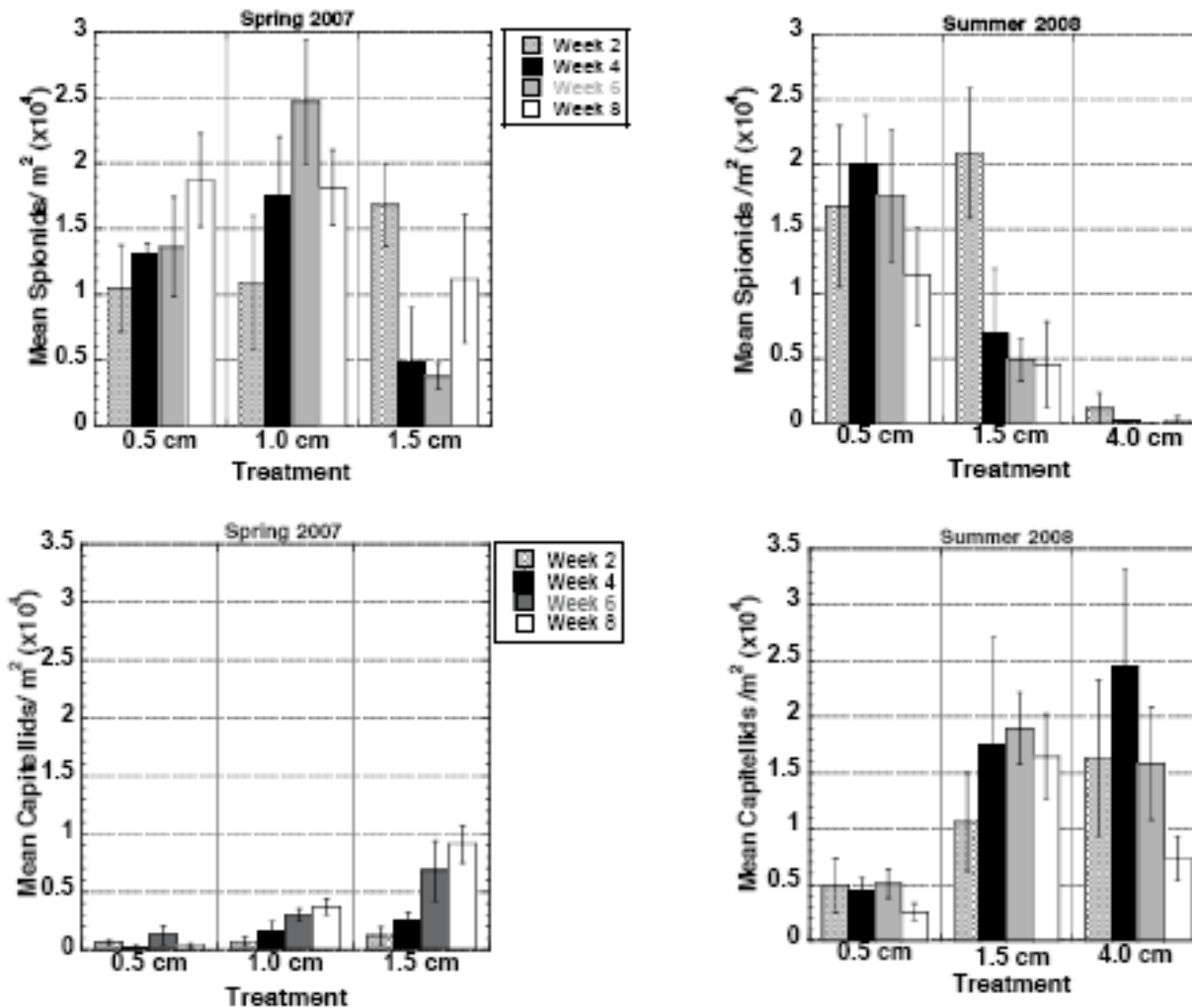
Figure 3.9. Benthic infaunal abundance as a function of macroalgal mat depths, where 0.5 cm = 1 kg ww m^{-2}, 1.0 cm = 2 kg ww m^{-2}, 1.5 cm = 3 kg ww m^{-2}, and 4 cm = 12 kg ww m^{-2}. Spionid polychaetes (top panel) decreased in abundance over time in both spring and summer under the higher treatments. Capitellid polychaete abundances (bottom panel) were more than double in summer than spring and increased with thicker macroalgal mats (Green 2010).

Field surveys of percent cover of macroalgae and infaunal diversity and abundance support these experimental findings. Diversity and biomass of epifauna was shown to increase with macroalgae until macroalgae covered 50% of the benthos. However, by ninety percent macroalgal cover there were marked decreases in diversity and biomass of invertebrates and fish (Pihl, Isaksson et al. 1995). Kotta & Orav (2001) also reported a decline in the biomass of infauna with a proportional increase in macroalgal cover. Jones & Pinn (2006) found that after a month of approximately 75% macroalgal cover, all species in the sediment declined and many organisms started migrating out of the sediment and moving into the mats.

There are a few studies that suggest it is important to consider the duration that macroalgae cover a given area of sediment as this may have strong effects on the magnitude of the effect on the benthic community. In a field experiment, Green (2010) found changes in infaunal abundance and diversity may occur within two weeks of continual cover by macroalgal mats. The rapidity of response, however, was macroalgal "dose" dependent, demonstrating the importance of considering both magnitude and duration together. Other studies found changes in infaunal community structure over longer macroalgal exposure periods. Hull (1987) found that abundance of many infauna and epifauna increased with higher doses of macroalgae after 10 weeks with only the highest macroalgal doses resulting in declines of some species. However, after 22 weeks of continuous cover, all mats, regardless of density, reduced sediment oxygen availability such that only the most tolerant polychaetes were still elevated in plots with a high macroalgal dose. This shows that even low macroalgal biomass may negatively affect benthic communities, given enough time. Bolam & Fernandes (2002) also found that duration of macroalgal cover affected infaunal community structure. After four weeks, plots with macroalgae had higher abundances and diversity than plots lacking macroalgae. However, after 20 weeks of cover, plots lost diversity and were dominated by polychaete species known to be tolerant of harsh conditions. Unfortunately, in the Bolam and Fernandes (2002) and Hull (1987) studies there were only two sampling points. Bolam and Fernandes (2002) took cores for infauna after 4 and 20 weeks while Hull (1987) reported sampling at 10 and 22 weeks. With such wide gaps between sampling times, it is impossible to know the time scale of the effect of mats of differing magnitude. Experiments with more frequent sampling events are essential to refine these time scales.

There is an overall lack of information about the effects of continual macroalgal mat coverage on infauna, as in most studies treatments were not maintained during the course of the experiment (Hull 1987; Raffaelli, Limia et al. 1991; Bolam and Fernandes 2002; Cummins, Roberts et al. 2004; Rossi, Forster et al. 2007). Rather, these experiments modeled a single macroalgal recruitment or depositional event. Only one study (Green 2010) maintained experimental treatments during the 8-week duration of the experiment, and thus modeled the effects of continuous cover. This experimental approach was based on field measures of continual macroalgal coverage of tidal flats for up to 5 months at Mugu Lagoon in southern California (Green 2010). These field data suggest that results of experiments using single, pulsed events of macroalgal deposition or recruitment may not apply to estuaries in California. More experiments using continuous cover of macroalgae are needed.

Causal Mechanisms for Decline in Invertebrates

Many studies suggest a causal mechanism for the adverse effects of macroalgal mats on sediment invertebrates. Labile organic matter associated with algal mats stimulates the bacterial communities in sediments, increasing benthic oxygen demand (Sfriso et al. 1987, Lavery and McComb 1991), decreasing sediment redox potential (Cardoso et al. 2004), and increasing rates of sulfate reduction. This in turn creates shallow zones of sediment anoxia and elevated pore water ammonia and sulfide concentrations (Gianmarco, Azzoni et al. 1997; Kristiansen, Kristensen et al. 2002), often times just under the algal mat, in the zone of surface deposit feeders (Dauer, Maybury et al. 1981; Hentschel 1996). Green (2010) found that pore water sulfide concentrations were significantly higher in mats of 1.5 cm or greater after 8 weeks (Figure 3.10).
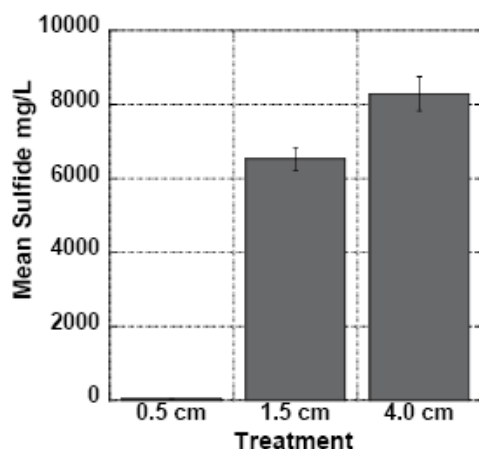
Figure 3.10. Concentrations of pore water sulfide in top 0-4 cm of sediment, showing significantly higher sulfide under mats > 1.5 cm than mats of 0.5 cm (Green 2010).

Surface feeding macrofauna such as spionid polychaetes are more susceptible to sulfide poisoning (Llanso 1991) than subsurface burrowing capitellid polychaetes (Cuomo 1985; Hargrave, Holmer et al. 2008). However, most research has related water column sulfide concentrations with invertebrate survival (Caldwell 1975; Llanso 1991; Miron and Kristensen 1993; Gamenick, Jahn et al. 1996), which may not correlate to infaunal exposure since sulfide oxidizes quickly in the overlying water (Hines, Faganeli et al. 1997; Hargrave, Holmer et al. 2008)). In one of the few studies relating pore water sulfide concentrations and surface deposit feeder populations, Magni et al. (2005) found a correlation between lower sulfide concentrations and higher spionid polychaete abundances. Another study showed steep declines in benthic diversity associated with increased pore water sulfide concentrations and resulted in a community depauperate in surface deposit feeders (Brooks and Mahnken 2003). Similarly, Green (2010) showed that high concentrations (>6000mg L^{-1}) of pore water sulfide found under dense mats caused the infaunal community to shift from spionids toward oligochaetes and capitellids, which are tolerant of high sulfide concentrations. In field surveys, oligochaetes, benthic indicators of pollution (Chapman 2001), were collected alive from sediment with nearly 1000 mM pore water sulfide (Giere, Preusse et al. 1999) while capitellids, known to be tolerant of sulfide, thrived in 4000 mM pore water concentrations (Brooks and Mahnken 2003).

Other Factors that Control Effects of Macroalgae on Sediment Invertebrates

The effects of macroalgae on invertebrates should vary seasonally as well as spatially across estuarine classes and habitats. Lower macroalgal abundances in spring followed by peaks in summer are common in estuaries worldwide (e.g. Sfriso, Marcomini et al. 1987; Murias, Cabral et al. 1996; Kamer, Boyle et al. 2001). Green (2010) found total macrofaunal counts in spring were approximately half of those found in

summer suggesting that seasonality plays an important role in their abundance (Figure 3.8). This may be due to increased food availability for detritivores (Williams 1984; Thiel and Watling 1998; Rossi 2007) during the summer. On the other hand it may be due to the expansive cover of macroalgal mats on tidal flats in summer, which may reduce water current speeds over the benthos (Escartin and Aubrey 1995) and increase macrofaunal recruitment (Salovius and Kraufvelin 2004).

Tidal influence plays a large role in the abundance of macroalgae, as well as the abundance, diversity and community composition of infauna and epifauna in estuaries. Overall, increased flushing results in decreased stress to organisms, principally through improved oxygenation and removal of toxic metabolites (ammonia and sulfide). In addition, estuaries with strong currents can flush out macroalgal mats (Thomsen and McGlathery 2006) reducing negative effects of macroalgae such as hypoxia that tend to occur when tidal exchange is weak (Viaroli, Bartoli et al. 1995). Thus macroalgal effects on invertebrates may be intensified in poorly flushed estuaries.

Other site-specific factors (climate, tidal elevation, sediment grain size and organic matter content, chemical contaminants, sedimentation rate, salinity, current velocity, dispersal strategies, etc.) can often govern suitable habitat for and cumulative stress to invertebrates. For example, organisms living in the intertidal experience more stress because physical factors tend to fluctuate more widely in intertidal habitats than in the subtidal (Woodin 1974). Peckol and Baxter (1986) found higher infaunal abundances at lower intertidal elevations but also increased mortality due to sedimentation. Tidal exposure affects abundance and diversity of infauna but also recruitment (Peckol and Baxter 1986). Most invertebrate colonists rely on transport via the bed load or rafts of vegetation (Moseman, Levin et al. 2004). Drift mats of macroalgae which can distribute quickly during the tidal cycle (Astill and Lavery 2001) are used by many intertidal and subtidal invertebrate species as a mechanism of dispersal (Holmquist 1994; Arroyo, Aarino et al. 2006; Lauringson and Kotta 2006). Other factors that may affect the dose response relationship between macroalgae and infaunal and epifaunal abundances are climate, hydrology, grain size, salinity and turbidity (see Chapter 7 for additional details).

3.3.3   Effects of Floating or Rafting Macroalgal Blooms in Intermittently Closed and Open Lakes and Lagoons

Intermittently and ephemerally tidal estuaries, which in the literature of Mediterreanan estuaries are referred to as "Intermittently closed and open lakes and lagoons (ICOLL)", are distinguished by their degree of surface water exchange with the ocean. Estuaries in this category are nearly or completely closed for some part of the year (intermittently tidal), or in some cases, open irregularly every several years (ephemerally tidal) or not known in recent history to open. With prolonged mouth closure, these estuaries become vulnerable to nutrient overenrichment and eutrophication because of increased hydraulic residence time. These conditions clearly put these systems at risk for extensive macroalgal blooms and their effects on other primary producers and consumers.

In Mediterranean ICOLL estuaries around the world, the literature documents a range of dominant primary producers, from phytoplankton (Knoppers et al. 1991, Perissinotto et al. 2000, Froneman 2004, Oczkowski and Nixon 2010), seagrass (both eelgrass, and brackish SAV (see Chapter 6)) and their

epiphytes (Lukatelich et al. 1987, Viaroli et al. 1997, Silva and Asmus 2001), and macroalgae (Whitfield 1988, Cummins et al. 2004, Odebrecht et al. 2010, Kjerfve 1986). When open, ICOLL estuaries tend to be dominated by intertidal habitat, well flushed, with intermediate to marine salinity ranges (Sutula et al. 2004, CLEAP 2009) and support primary producers in proportions typically found in the intertidal habitats of other perennially tidal river mouths and lagoons (macroalgal mats and MPB). When closed however, water levels rise and salinities can range from fresh to hypersaline, and dominant primary producers can greatly vary from estuary to estuary. As ICOLL estuaries experience mouth closure, dramatic changes in physicochemical variables (salinity, turbidity, light) may occur over very short time frames and can also greatly vary from year to year, depending on the timing and magnitude of freshwater inputs relative to mouth closure (Sutula et al. 2004). Froneman (2002) found that high variability in turbidity, temperature and salinity resulted from breaching and overtopping events in the Kasouga estuary in South Africa, and implied that structure and function of the estuary was directly related to variability in these physicochemical variables.

Within California, ICOLL estuaries are the most numerous of all estuarine classes in the state and can include both river mouth and lagoonal estuaries (M. Sutula, personal comm.). Notably, no comprehensive statewide studies have been published to date documenting patterns in primary producers and their response to eutrophication. One regional study of six Central Coast ICOLL estuaries documented phytoplankton biomass and community structure and SAV and macroalgal % cover over a two year period (CLEAP 2006). In two of the five (Aptos and San Lorenzo lagoons), primary producers were generally dominated by phytoplankton and macroalgae. Three other estuaries were co-dominated by brackish SAV and macroalgae (Scott, Laguna and Soquel Lagoons). In southern California, in a regional study that included eleven intermittently and ephemerally tidal estuaries, ten of eleven were dominated by floating and rafting macroalgae and *Ruppia spp*. in various proportions, while only one was dominated by phytoplankton (K. McLaughin, pers. comm.). Seasonally, microphytobenthos consistently dominated in the early spring, with a transition to dominance by macroalgae in late spring through the fall. Among all these systems, macroalgae appear to be a consistent component and may more easily lend itself to an assessment of eutrophication than other primary producers.

The combination of increased nutrients and low or no flushing in these systems creates a situation whereby drift and floating macroalgal mats can have maximum impact, as they are relegated to movement within the system and are limited in their ability to be flushed out (Whitfield 1988). With the exception of a handful of studies, little work has been done to investigate the effects of macroalgal biomass or drift algae, although several authors studying ICOLL's have suggested their impact could be extremely important (Knoppers et al. 1991, Herrera-Silveira and Morales-Ojeda 2010, Mutchler et al. 2010). When investigating the effects of mouth closure in Swartvlei Estuary in South Africa, an estuary that is closed 7 months out of the year, Whitfield (1988) found that in winter, when the mouth was normally closed, *Zostera capensis* beds were covered in Enteromorpha sp. (now Ulva). The algae then detached, forming mats that moved throughout the estuary when the mouth was open. Cummins et al. (2004) experimentally manipulated drift algal biomass on seagrass beds and found huge declines in macrophyte biomass as well as infaunal communities in enclosures with algae in the Tuggerah Lakes estuary, New South Wales, Australia, where tidal exchange is <1%. Elevated biomass of floating

macroalgal mats of *Ulva spp.*, *Cladophora spp.*, and *Rhizoclonium riparium* were found in the estuarine habitat of Patos Lagoon in southern Brazil (Odebrecht et al. 2010), but the effect of the mats on the estuarine community was not measured. Cenotes found in the Yucatan Peninsula, Mexico, that have a seawater source from submarine groundwater discharge were found to be covered with thick *Cladphora sp.* mats, but biomass was not quantified (Mutchler et al. 2010).

Conceptually, the biomass and percent cover of floating or drifting macroalgae in closed ICOLLs could have a multitude of adverse effects, including: 1) shading effects on MPB and brackish water SAV, 2) overproduction of organic matter, leading to water column hypoxia, production of sulfide in sediments, and poor water quality conditions due to an overabundance of heterotrophic bacteria, and 3) changes in richness and relative abundance of primary producers, with adverse effects on higher trophic levels (invertebrates, fish and birds).

There are various studies that support the association of macroalgae with decreased water quality in ICOLLs. Chronic hypoxia was observed coincident with extremely high abundances of filamentous algae in Loma Alta Slough (southern California; McLaughlin et al. 2010a). Epiphyte "slime" comprised of diatoms on *Ruppia maritima* leaves and on the sediments was found to keep sulfide production high in a brackish lagoon in France (Viaroli et al. 1997), suggesting that epiphytic loads of macroalgae might also contribute to sulfide toxicity and destabilize the macrophyte communities (Burkholder et al. 1994). Nighttime hypoxia and extensive fish kills were observed in Buena Vista Lagoon (McLaughlin et al. 2010b), a system that is maintained in a permanently closed state by a weir. Local residents claim these fish kills occur every summer when temperatures are high (Fong, pers. comm.). Drift algae may be a useful habitat for infaunal communities; however, if immobilized on sediments or seagrass in the dry season, macroalgae may create hypoxic conditions further disturbing both the infaunal and macrofaunal communities. In systems intermittently or permanently closed, where salinities tend to be uniformly low, species richness may be even more vulnerable to impact by floating algal mats, including negative effects such as hypoxia that tend to occur when tidal exchange is weak (Viaroli, Bartoli et al. 1995).

Floating or drifting mats of algae may also cause reduction of light availability to other primary producers (see previous sections and Chapter 7), eventually outcompeting and dominating these other groups for light and resources. Floating mats of macroalgae often begin as epiphytes on seagrass leaves before detaching (Wood 1969). Experiments investigating the effects of epiphyte growth on *Ruppia maritima* in low salinity (max. 10-12 ppt.) regions of the Chesapeake Bay showed that in enriched conditions (loading rates ≥ 60 µmol N L^{-1} wk^{-1}), epiphytes outcompeted phytoplankton and the combination of epiphytic growth and reduction of light negatively affected photosynthetic capability of seagrass. The effects of epiphytes on seagrass primary productivity and growth, as well as the ontogenetic shift from epiphytes to drifting mats need to be quantified in these systems where their growth may be facilitated by excessive nutrients. Data are currently not available that describe the effects of reduced light levels to benthic diatoms and brackish SAV due to floating or drifting mats of algae. Macrocosm experiments and field studies are required to understand the impacts of the magnitude and duration of floating or drift mats on other primary producers.

Direct effects of macroalgae on consumers such as invertebrates, fish and birds have also not been well quantified. While the shift in co-dominance of macroalgae, SAV, phytoplankton and MPB to dominance by macroalgae would expect to have similar trophic level effects as in open estuaries (see Section 3.x), no studies have directly documented these linkages in ICOLL systems. The studies of macroalgal effects on benthic infauna in sediments of tidal estuaries (summarized in Table 3.1) would only apply to ICOLL estuaries when the mouth is open and would typically not be considered the "critical condition." Studies have shown that ICOLL estuaries are typically lower in faunal diversity than open estuaries (Roy et al. 2001) because the high variability in physiochemical characteristics makes it a challenging place to live. Presumably the effects of an overabundance of macroalgae would serve to further stress fauna that are already challenged by natural conditions. Improved understanding of macroinvertebrate community structure in California ICOLLs and relationship to nutrient and eutrophication during closed condition is a key data gap in establishing NNEs for this class of estuaries.

Overall, there is a major knowledge gap of California's ICOLL systems in general, but specifically with respect to impacts of macroalgae and drift mats as indicators of the health of these potentially eutrophic systems, and clearly much more work needs to be done to make any recommendations for their use as indicators. Two types of studies are recommended:

Regional or statewide studies of ICOLL estuaries in California, documenting relationships between nutrient loads, water column and sediment nutrient inventories, primary producer community structure over time (seasonally for at minimum one year); one such study has been conducted in southern California (Bight '08 Regional Monitoring Program) and should be replicated in some form throughout the state.

Laboratory mesocosm or field studies documenting direct effects of floating or rafting mats of macroalgae on ecosystem condition or functions, including biomass and/or community composition of other primary producers and effects on macroinvertebrates.

3.3.4   Summary of Ecosystem Response to Increased Macroalgal Abundance and Effects on Ecosystem Services and Beneficial Uses

Excessive macroalgal blooms have a variety of negative effects on estuaries including: 1) increasing frequency of water column and sediment hypoxia and heightening heterotrophic bacterial activity, resulting in poor water quality and increased frequency of diseases, 2) alteration of biogeochemical cycling, more rapid nutrient regeneration (Tubbs and Tubbs 1980; Raffaelli, Limia et al. 1991; Wennhage and Pihl 1994; Bolam, Fernades et al. 2000), 3) shading or smothering of seagrass, shellfish beds and other important habitats (Nelson 2009, Young 2009), 4) decreased recruitment and survival of benthic invertebrates and reduced carrying capacities for fishes and shorebirds (e.g.Raffaelli 1999; Thomsen and McGlathery 2006; Nezlin, Kamer et al. 2009), 5) poor aesthetics and an increase in odors relating to the decomposition of organic matter and increased sulfide production, and 6) subsequent changes in both trophic and community structure of invertebrates, birds and fishes (Raffaelli et al. 1989, 1991; Bolam et al. 2000). Cumulatively, these adverse effects result in a reduction in recreational use of estuarine waters (REC1 and REC2), poor water column and benthic habitat quality for estuarine (EST) and marine

(MAR) aquatic species, direct impacts to populations of threatened and endangered (RARE), migratory (MIGR) and spawning (SPAWN) birds, fish and mammals, and reduction in the economic value of commercial and sports fisheries, aquaculture, and shellfish harvesting (COMM, AQUA).

Fish and invertebrate kills as a result of lowered dissolved oxygen may occur when estuaries are stressed by mats of macroalgae, especially in conjunction with high temperatures and high cloud cover (D'Avanzo and Kremer 1994). In addition to oxygen stress by respiring macroalgae, fish may be physically affected by drift mats. For example, cod foraging efficiency was drastically reduced with Ulva intestinalis cover of 70-80% (Pihl, Isaksson et al. 1995). Macroalgae may also affect piscine recruitment. Wennhage & Pihl (1994) found that juvenile flat fish preferred to recruit to bare sand compared to plots with dense macroalgal cover. They speculated that macroalgae invokes stress in juvenile fish through declines in dissolved oxygen and also restricts prey availability. However, drifting macroalgae are not the only form of algae that may result in the loss of fish and invertebrates. Increases in epiphytic algae on seagrass can result in dramatic reductions in the abundance and biomass of epibenthic invertebrates and fish (Isaksson and Pihl 1992). Thus ephemeral macroalgae may cause deleterious declines in both recreational and commercial fish stocks (Raffaelli 1999).

Field studies show that inverse correlations exist between the density of macroalgae and numbers of shorebirds. Cabral et al. (1999) made the claim, "In a long-term perspective, an increase of dense and contiguous macroalgal mats, covering large areas of the intertidal flats, may affect directly or indirectly all wader species in the Mondego estuary." Green (2010) showed that the presence of macroalgae changed foraging behavior from pecking to probing in Sandpipers and Marbled Godwits, suggesting that macroalgae hindered foraging by obscuring visual cues or physically interfering with foraging effort. Further, if macroalgal blooms reduce prey intake rates by shorebirds, then shorebirds with less flexible diets may be more negatively affected than generalist foragers that feed on a wide range of prey species. Green (2010) found avoidance of mats based on foraging ecology. For example, shorebirds that are generalist foragers, such as Least and Western Sandpipers and Willets, foraged on macroalgal mats and bare sediment equally. In contrast, shorebirds with more specific dietary requirements such as Marbled Godwits often avoided mats while foraging. In another study, Cabral et al. (1999) observed that Dunlin, a bird species with more restricted diets, tended to avoid dense mats. These studies suggest that as macroalgal blooms become more prevalent in estuaries, specialist species of birds may suffer losses in population numbers.

3.4 Relationship of Macroalgae to Increased Nutrient Availability

There is overwhelming evidence that blooms of macroalgae are stimulated by high nutrient loading, particularly of nitrogen (N) and phosphorus (P) (e.g., Raffaelli et al. 1989, Valiela et al. 1992, Peckol and Rivers 1995, Pihl et al. 1999, Krause-Jensen et al. 2007). Historically, field surveys have established that in areas where the major supplies of nutrients are from rivers, biomass of bloom-forming macroalgae may be predicted by water column and sediment nutrient concentrations that reflect nutrient availability (Sfriso et al. 1987, 1992, Menendez and Comin 2000, Thybo-Christinsen et al. 1993, Flindt et al. 1997, Raffaelli et al. 1989, Peckol et al. 1994, Pihl et al. 1996, Nelson et al. 2003). This was true for Carpinteria Estuary in southern California, where higher macroalgal biomass was found at the head of

the estuary in winter and was related to watershed sources of nitrate. In this estuary, there was a simple relationship between water column nutrient concentration and macroalgal biomass, most likely because nutrient sources during the wet season were largely riverine (Kennison 2008).

Recent studies have shown that estimates of nutrient loading that include all possible sources as well as physical removal (flushing) and biological processes are accurate and generalizable predictors of macroalgal biomass. Nutrient sources can include inputs from terrestrial runoff, groundwater (Valiela et al. 1992), aerial deposition (Paerl 1997), and remobilization from the sediments to surface waters (Trimmer et al. 2000). In one of the best examples of this approach, Fox et al. (2008) compared three sub-estuaries of Waquoit Bay, Massachusetts, with different nitrogen loads and found the magnitude of macroalgal standing stock was predicted by total nitrogen load over a six-year period. Notably, this level of detail of the relationship between nutrient loading and producer biomass has been quantified in only a few systems (Valiela et al. 1992, 1997; Hauxwell et al. 1998; Conley et al. 2000; Fox et al. 2008), as it is an expensive and time-consuming process. Measures of nutrient removal to the ocean via tidal flushing are also key to ensure accurate predictions. For example, in Mugu Lagoon (Calleguas Creek arm), southern California, water column nutrient concentrations were always high but algal biomass always low due to low hydraulic residence time and scouring of sediments (Kennison 2008). Finally, biological processing such as nutrient uptake and algal growth, internal nutrient cycling, and grazing (see Schramm 1999 for review) must be taken into account to improve the predictive capability of any model. For example, longer residence times that allow more biological uptake and resultant macroalgal growth may result in lower water column nutrient concentrations and more proliferation of macroalgae as found in Mugu (West arm), Tijuana Estuary, and Upper Newport Bay in southern California (Kennison 2008). Additional work is needed to understand conditions in which phase shifts from microphytobenthos to macroalgae occur, including quantifying rates of uptake and release of nutrients from macroalgae and seasonal storage and release of inorganic nutrients in sediments, in order to parameterize dynamic simulation models of estuarine water quality and biological response to nutrient loads.

3.5   Review of Macroalgal Candidate Indicators

A suite of methods to assess the extent and impact of macroalgae in estuaries has been developed, centered on measures of taxonomic composition, biomass and cover. Of these, taxonomic composition is not a particularly useful indicator of eutrophication because the taxonomic composition of macroalgae in California estuaries is limited to 1 or 2 species and the presence of these species alone does not indicate eutrophication. Many bloom species, including those in California, are natural members of estuarine communities with world-wide distributions (Scalan et al. 2007). Similarly, the absence of other, perhaps more sensitive, members of the community cannot be used as an indicator of eutrophication. Estuaries are generally subject to fluctuation in salinity, and this alone many result in overall low diversity or seasonal losses of species. Therefore this review concentrates on biomass and percent cover of macroalgae.

3.5.1 Biomass

Biomass is one of the key measures of macroalgal abundance that can be linked to eutrophication (for an example, see Valiela et al. 1997), and for which methods are fairly well standardized in the scientific literature. There are no remote methods for quantifying biomass, all methods involve physically getting to the places where you need to sample; therefore, all of the methods are relatively labor-intensive. In addition, it is a non-trivial endeavor to walk across many muddy substrates to collect the algae for biomass estimation, so minimizing sample sizes is optimal. Thus, in order to increase the ability to generalize at the same time as reducing the number of samples needed, sampling for biomass often involves some sort of stratified random choice of plots, typically by laying out a transect (a metered tape) and randomly selecting points along the transect to collect biomass. Stratification can be used if there are known differences in patterns of biomass or variability in biomass among different "strata" (e.g., water depth). Once points are chosen, biomass of algae is quantitatively collected from a known area. To accomplish this, a quadrat (a rigid frame of any shape, usually square, that delineates a known area) is placed in a pre-determined orientation along the transect. Choices of orientation are chosen a priori and can include to the left, to the right, or bisecting the transect line. Optimum size of quadrats will vary depending on the density and patchiness of the algae. In general, a 0.5 x 0.5 m quadrat has proven adequate (Boyle et al. 2004, Kennison 2008).

Methods will vary if sampling macroalgae on a tidal flat versus floating or rafting algae in subtidal habitats, but in general collecting algal biomass samples from the field relies heavily on being able to see the algae on the benthos. Thus, sampling is optimal at low tide for intertidal zones when tidal flats are exposed, which limits the time available for sampling to low tides of a sufficient magnitude. For subtidal areas in tidally flushed estuaries, sampling can be done while snorkeling or on SCUBA depending on the water depth and clarity. For perennially or intermittently closed estuaries, water quality and visibility is often too poor for these techniques. In these systems, a known volume of water must be contained (PVC pipes or bottom-less buckets can be used) and then searched manually for algal biomass or filtered through netting of an appropriate mesh size. This makes sampling of these systems even more labor-intensive, and dependant on sufficient on water quality that body contact is safe.

Once algae are collected, there are different techniques to measure biomass that vary in their cost-effectiveness, resolution, and acceptance by the scientific community (Table 3.2). By far, the most common method is to place the collected algae from each quadrat on ice in the dark in sealable plastic bags and return it to the lab for processing. One key to accurate and replicable measures of biomass is rigorously removing intercalated mud, associated fauna, and entangled debris (Boyle et al. 2004, Kennison 2008). The relative importance of these non-algal components of mats varies widely and depends on many things such as age of the mat, where it has rafted and accumulated debris, and both the substrate type and the duration of where it may have been deposited. Although the variability is immense and unpredictable, mats are often comprised of over 50% non-algal material by weight (Fong, unpublished data). After cleaning the algae there are two methods for quantifying abundance. To measure wet weight, perhaps the most common metric used, cleaned algae from each quadrat is placed into nylon bags, spun in a salad spinner for 1 minute to remove a consistent amount of water, and weighed on a scale. The algae can then be placed in a drying oven and dried until constant weight for a

measure of dry weight. This technique has the advantages of high resolution and wide acceptance in the scientific community. A less-used technique is to quantify the "biovolume" of the algae by measuring the volume of water it displaces in a graduated cylinder. This technique gives information that is slightly different than biomass and may be valuable when considering the effect of algae at the community level. However, it is rarely used in isolation, but may complement biomass measures.

Table 3.2. Common methods for estimating biomass of macroalgae in estuarine habitats. These methods are for processing and weighing collected biomass after stratified random collections.

Method	Measure	Cost	Labor Intensive	Differ by Habitat	Scientific Acceptance	Resolution	Has Promise Alone or in Tandem
Clean/ spin/weigh	Wet and dry weight	Medium	High	Yes	High	High	Alone
Graduated cylinder	Biovolume	Medium	High	Yes	High	High	In tandem
Mat depth	Depth	Low	Medium	Yes	Medium	Unknown	In tandem
Uncleaned grab	Wet weight	Low	Medium	Yes	Low	Low	In tandem

Because cleaning the algae in the lab is so labor intensive, two methods have been developed that eliminate this step. One is to measure mat depth in situ using a ruler. Mat depth is a potentially important measure of the impact of macroalgae on seagrass (Hauxwell et al. 2001) and benthic invertebrates (Green 2010). However, at present there is little data that relates mat depth to standard measures of biomass and the resolution is largely unknown (see Green 2010), making this method better used in tandem with accepted measures. The second less labor-intensive biomass estimate is grab samples. Instead of bagging the samples and bringing them to the lab, they are wet weighed in the field using a hanging spring scale (fish scale). The resolution in these samples is low, because they contain unknown quantities of water, mud and debris, and therefore the acceptance of this technique among scientists is low.

Although it is rarely evaluated, frequency of sampling for any of these techniques is most likely highly system specific. In highly seasonal climates like the UK, a single or a few sampling times in summer may be adequate (Scanlan 2007), while in Mediterranean climates like southern California, maximum biomass can occur in any season depending on watershed development (Kennison 2008), and sampling should be at least quarterly until any replicable seasonal patterns emerge. Although biomass measures can show great spatial and temporal variability, this is most likely an accurate representation of the extremely patchy distribution of macroalgal mats.

Overall, biomass is a powerful and accurate indicator of macroalgal abundance and potential for impact on estuaries. It is widely used and accepted, and its measurement can be quite accurate and replicable. The tradeoff is, however, that is it very labor-intensive. If used in tandem with other measures, such as percent cover, this measure shows great promise as an indicator.

3.5.2 Percent Cover

Measures of the percent of the benthos covered by algal mats are a second approach that have been used effectively to link algal abundance to eutrophication (Boyle et al. 2004, Scanlan 2007). Measures of cover include both in situ and remote approaches and vary widely in their precision and ability to capture spatial variability (Table 3.3). The first two methods, transects/quadrats and photography/digitizing, are in situ field approaches and historically have been the most commonly used techniques. For these, field procedures for choosing stratified random points are identical to those described for biomass (above) and are often conducted side-by-side with estimates of biomass. Instead of having a quadrat that is an open square as for biomass, the quadrat is usually strung with fine fishing line to create intersections (usually 30-100 intersection points). The presence/absence of algae directly under each intersection is noted, and percent cover calculated from these data. In the second method a known area of the benthos is photographed using a framer that limits parallax. Photographs may be quantified using digitizing programs such as Image-J, freeware from NIH that calculates percent cover. This technique takes less time in the field and is more accurate than visual estimates; however, it is considerably more time consuming in the lab. Both techniques are labor intensive and their usefulness varies among habitats in the same way as described for biomass. As for biomass, they are also limited in spatial scale and therefore must be repeated at several locations for an adequate representation of cover at larger scales.

Table 3.3. Common methods for estimating percent cover of macroalgae in estuarine habitats, including subtidal, intertidal, and brackish closed systems.

Method	Cost	Labor Intensive	Differ by Habitat	Scientific Acceptance	Resolution	Has Promise Alone or in Tandem
Transects & quadrats	Low	Yes	Yes	High	High	In tandem
Photography & Digitizing	Low	Yes	Yes	High	High	In tandem
Telescope surveys	Low	No	Subtidal difficult	Medium	High	In tandem
Hovercraft	Medium	No	Subtidal difficult	High	Medium	In tandem
Aerial photography	High	Yes	Subtidal difficult	Medium	Low	In tandem
Compact airborne spectral imager (CASI)	High	Yes	No	High	High	In tandem
Satellite	Medium	Yes	Subtidal difficult	Medium	Low	In tandem

At the intermediate spatial scale, Green (2010) adapted a telescope survey method from Nedwell et al. (2002) where large areas of intertidal flats are scanned "remotely" from adjacent higher ground. When intertidal sites are completely exposed, they are scanned with a spotting scope or binoculars fitted with a cross hair eyepiece for one minute and the presence or absence of macroalgae recorded for 60 points.

These visual raster scans of each site are performed at a constant speed using a timer that beeps once per second. At the sound, the presence or absence of algae at that moment under the cross-hair is recorded into a digital voice recorder for later transcription. The number of points that were positive for the presence of macroalgae is divided by 60 and used to generate percent cover measurements. This has been found to be both relatively quick and cost effective for large (~2500 m^2 or 0.25 ha) areas of intertidal flats (Green 2010); additional work is needed to compare results of using a spotting scope with transect-based sampling. One limitation is that only floating rafts in subtidal areas can be surveyed using this technique, and it is impossible to know what portion of the algae is floating.

For methods that can be used at the scale of the whole estuary, such as aerial photography and remote sensing, the costs can be considerable (Nezlin et al. 2007, Scanlan 2007). All have the advantage of reduced labor in the field, but costs are driven up by the use of technology that is expensive and requires considerable expertise. At present, most of these techniques are limited to intertidal regions and several require clear skies coupled with daytime low tides, a combination that can be rare along the California coast. However, advances in this technology are ongoing, so their use in the future will most certainly expand.

The key limitation of using any technique to measure percent cover is that there is limited ability to relate this to biomass, which has a known relationship with eutrophication (Valiela et al. 1997). For example, 100% cover of a visible yet thin and still attached mat of algae that may weigh only grams per square meter will be counted the same as 100% cover of a 12 kilogram per square meter raft deposited on a tidal flat. The former tidal flat is most likely in a healthy state, while the latter has been demonstrated to have strong negative effects. As an example, in Carpinteria Salt Marsh, where both biomass and cover were measured in the same plots (Figure 3.12 a,b), an average of 70% cover in the Middle site in Feb 2002 weighed ~250 g wet wt m^{-2}, while approximately the same cover in the Head site in Feb 2002 weighed over 1500 g wet wt m^{-2}. Clearly, these represent very different mats types that relate to very different levels of eutrophication. The former may represent a natural seasonal event while the latter has known negative effects on higher trophic levels, including invertebrates and birds (Green 2010).

In summary, while measures of algal cover are generally faster and easier to accomplish, they should not be done in isolation. Ground-truthing with in situ biomass estimates is a key complement to measures of percent cover, and should always be done in tandem.

3.5.3   Existing Assessment Frameworks for Macroalgae

With the adoption of the Water Framework Directive (WDF 2000), the European Union has been working to assess the ecological condition of its waterbodies. Work has been ongoing to develop an assessment framework for eutrophication, based on biological as well as chemical indicators.   Scanlan et al. (2007) proposed an assessment framework to diagnose eutrophication.

The Scanlan et al. (2007) assessment framework utilizes both macroalgal cover and biomass in a multiple lines of evidence approach (Figure 3.11). Both biomass and cover are required to make a

diagnosis, because the measurement of just one indicator in isolation could be misleading. For example, an estuary may have low biomass (a positive indicator for estuarine health) but high macroalgal cover (a negative indicator for estuarine health) resulting in a moderate impact to the ecosystem. On the other hand, high macroalgal biomass may be recorded locally, but be mediated by low percent cover over the whole estuary.

Figure 3.11. Proposed assessment framework to diagnose eutrophication using macroalgae for macroalgae in intertidal and shallow subtidal habitat for the European Water Directive Framework (Scanlan et al. 2007). Biomass is in wet weight.

The framework uses biomass and percent cover to classify an area within an estuary into one of five categories: High, Good, Moderate, Poor, and Bad. Each of these categories was defined as a deviation from a reference or pristine condition. They used a combination of data and expert opinion to generate their categories and assign threshold values between categories, emphasizing that more work was needed, especially to differentiate between moderate, poor, and bad conditions. Scanlan et al. (2007) emphasized that the proposed threshold values must be validated by examining multiple ecological indicators across the eutrophication gradient.

The Scanlan et al. (2007) assessment framework provides a good conceptual model for how to incorporate both biomass and cover into a diagnostic tool and as such is a good starting point for California. However, several caveats should be considered. First, the assessment framework does not explicitly incorporate duration of mat presence into the framework, a factor that we have determined to be important through in situ experiments and published literature (Hull 1987; Balducci, Sfriso et al. 2001; Osterling and Pihl 2001; Bolam and Fernandes 2002). Second, Scanlan et al. (2007) did not clearly

specify the geographic scope of these specific thresholds for macroalgal biomass and percent cover. Countries within the European Union span the range from Artic to Mediterranean climates and it is unreasonable to think that, given differences in water temperatures across large area, that some differences in the thresholds for biomass and cover are not warranted. Third, while reasonable, the thresholds are based on best professional judgment with little citation of the actual data used to derive the thresholds. Additional work would need to be conducted to develop an appropriate macroalgal assessment framework for California estuaries.

3.6 Summary and Recommendations

Overall, the combination of macroalgal biomass and percent cover appears to be a good candidate for the NNE framework in estuaries, for the following reasons:

- Macroalgae may be useful indicators in all of our estuary types and habitats due to their ubiquitous distribution and strong association with nutrient enrichment and subsequent eutrophication. Macroalgal biomass and cover, though spatially and temporally patchy, can be used to detect a trend in either increasing or decreasing eutrophication.

- In perennially tidal estuaries, elevated levels of macroalgal mats are associated with declines in water quality, seagrass abundance, and diversity and abundance of sediment invertebrates. This results in adverse effects to estuarine birds, fish and other invertebrates and subsequently affects EST, MAR, COMM, SHELL, AQUA, MIGR, SPWN beneficial uses. Less is specifically documented about the impacts of elevated macroalgal biomass in closed brackish water estuaries.

- Macroalgae have well-established, albeit complex relationship with external and internal nutrient loads.

- Macroalgal biomass and cover can be measured through a well-established field-based method.

- Some precedent exists for the use of macroalgal biomass and percent cover in an assessment framework to assess eutrophication in estuaries in the European Union.

Given this recommendation to move forward with macroalgae as an NNE indicator, it is important to identify critical data gaps that must be addressed in order to develop a macroalgal endpoint for the E-NNE. These data gaps and next steps can be broken up by the three habitat types in which macroalgae are a dominant primary producer: 1) intertidal flats in "open" estuaries, 2) seagrass beds in "open" estuaries, and 3) rafting mats in "closed" estuaries.

3.6.1 Intertidal Flats in "Open" Estuaries

For the effects of macroalgae on tidal flats in estuaries "open" to surface water tidal influence: Green (2010) produced experimental data showing effects of duration and variation in magnitudes of macroalgal mat abundance on sediment invertebrate community structure at Mugu Lagoon, an estuary at the extreme end of the disturbance gradient. These experiments should be repeated in less-disturbed estuaries and an attempt should be made to characterize the macroalgae-benthic infauna dose-response relationship across a variety of environmental conditions including sediment organic matter, grain size, climate and hydrology and across various regions of the state. Note that this data gap has been partially funded by the SWRCB through caging experiments in a northern and southern California estuary and surveys of algal biomass and thickness in 8-10 estuaries statewide. This work is anticipated to be completed in March 2012. Additional caging experiments could be repeated over time to gather additional resolution on the importance of environmental gradients and validate this preliminary work. Generate additional information needed to develop an assessment framework for macroalgae. This includes: 1) Compiling existing data on macroalgal mat biomass and cover where they exist throughout the state, 2) Relating experimental measure (mat thickness) to more commonly measured biomass; these data are currently available for southern California through the Bight '08 Eutrophication Assessment and 3) Determining how to integrate macroalgal biomass/thickness and cover into an assessment protocol (SOP); this involves conducting field surveys to document the range of macroalgal biomass and cover across a wide variety of estuaries across the state.

3.6.2 Seagrass Habitat

For seagrasses, a combination of epiphyte load, chlorophyll- a (+ turbidity), macroalgal biomass/cover and light penetration show some promise as indicators of eutrophication (see Chapter 6 for more detailed discussion). Huntington and Boyer (2008a) showed that experimental enclosures in Tomales Bay containing high macroalgal loads (high "dose" was 1.7 kg ww m^{-2} mean maximum in a field survey) had significantly lower *Z. marina* shoot densities and growth rates after only 3 months of cover and concluded that light limitation was the primary mechanism for decreases in shoot density. Experiments that develop quantitative relationships between macroalgal abundance (as biomass and thickness) and specific impacts on eelgrass (e.g., Hauxwell (2001) and Huntington and Boyer (2008)) are essential to establish numeric thresholds for effects of macroalgae on seagrass. At present, we have very low resolution around the threshold for macroalgal effects on seagrass as all of these studies had wide gaps between treatme*nts* with no effects and those having strong negative effects. Further, experiments must be replicated in water bodies with different characteristics, such as water flow, temperature, and turbidity.

A thorough understanding of the effects of epiphytes on reducing light to seagrass is essential for evaluating overall impacts of nutrients on seagrass. However, despite considerable research, our knowledge of this key relationship is still limited. We are missing a direct link between reduced light and changes in primary productivity of seagrass. In addition, little is known about effects of epiphytes on seagrass communities in the field. The few studies that exist suggest it is complex, as phytoplankton, epiphytes, macroalgae and seagrass all compete for light and nutrients. Mesocosm experiments have

demonstrated this complexity, as different algal groups may dominate in replicate experimental units with seemingly identical starting conditions. The only mesocosm study on the West Coast showed that addition of nitrogen stimulated epiphytes and increased epiphytes subsequently decreased seagrass growth. More studies of this nature, including field experiments, are essential.

3.6.3   Rafting Mats in Closed Bar-built Estuaries

Poor understanding exists of the relative abundance and community structure of macroalgae versus other aquatic primary producers versus aquatic macroinvertebrates in ICOLL estuaries across the state; furthermore, there are no existing studies on the effects of rafting mats on all primary producer groups or epiphytes on brackish SAV in closed estuarine communities in California. Determining the threshold below which mats are advantageous and above which they become deleterious to overall ecosystem health needs to be established by quantifying community structure of consumer groups within ICOLLS. Ongoing Bight '08 Eutrophication Assessment in 11 ICOLL estuaries and completed TMDL studies in Loma Alta Slough and Buena Vista Lagoon is characterizing seasonal changes in primary producer biomass and percent cover relative to nutrient loads and availability in southern California, but an ambient survey looking at similar parameters as well as water column invertebrates is needed in ICOLLs throughout the rest of the State.

As with data gaps for subtidal seagrass habitats, the effects of rafting mats and epiphytes on brackish water SAV (*Ruppia maritima)* need to be understood in brackish/closed systems. First, these SAV habitats need to be surveyed and quantified as to their distribution and productivity in ICOLL estuaries. Second, experiments addressing competition for light and nutrients between SAV, phytoplankton and epiphytes are essential. Third, knowledge about what environmental conditions (water flow, light, and nutrients) create the transition from epiphyte to algal mat would be helpful in determining which life stage has the most impact on ecosystem function. Additionally, experiments that quantify release of metabolites (ammonia and sulfide) by decomposing epiphytes and macroalgae and their effect on macrophytes as well as benthic infauna would assist us in establishing the dose response of mats on seagrass habitats in these systems. If algal mats are floating, but with limited movement, and decompose in situ, altering the flux of nutrients in the sediment-water interface, a thorough investigation of biogeochemical processes such as nitrification and denitrification rates, and the extent N and P may be stored in the sediments, seagrass or algal tissue is also necessary.

3.8 References

Abbott, I. and G. Hollenberg. 1976. Marine Algae of California. Stanford University Press, Stanford California.

Aldridge, J. N. and M. Trimmer. 2009. Modelling the distribution and growth of 'problem' green seaweed in the Medway estuary, UK. Hydrobiologia 629:107-122.

Allen, J. 1992. Benthic invertebrates living in macroalgal mats on intertidal mudflats of Elkhorn Slough, California. Masters. Moss Landing Marine Laboratories at San Jose State University.

Ambrose, R.F and D.J. Meffert. 1999. Fish-assemblage dynamics in Malibu Lagoon, a small, hydrologically altered estuary in southern California. Wetlands. 19 (2):327-340.

Argese, E., G. Cogoni, L. Zaggia, R. Zonta and R. Pini. 1992. Study on redox state and grain size of sediments in a mud flat of the Venice Lagoon. Environmental Geology and Water Sciences. 20 (1): 35-42.

Arroyo, N., K. Aarino, and E. Bonsdorff. 2006. Drifting algae as a means of re-colonizing defaunated sediments in the Baltic Sea. A short-term microcosm study. Hydrobiologia **554**:83-95.

Astill, H. and P. S. Lavery. 2001. The dynamics of unattached benthic macroalgal accumulations in the Swan-Canning Estuary. Hydrological Processes 15:2387-2399.

Balducci, C., A. Sfriso, and B. Pavoni. 2001. Macrofauna impact on *Ulva rigida* C. Ag. production and relationship with environmental variables in the lagoon of Venice. Marine Environmental Research **52** 27-49.

Bell, S.S. and M.O. Hall. 1997. Drift macroalgal abundance in seagrass beds: investigating large-scale associations with physical and biotic attributes, Marine Ecology Progress Series 147 (1997), pp. 277–283.

Berglund J., J. Mattila, O. Ronnberg, J. Heikkila and E. Bonsdorff. 2003. Seasonal and inter-annual variation in occurrence and biomass of rooted macrophytes and drift algae in shallow bays. Estuarine, Coastal and Shelf Science. 56:1167–1175.

Biber, P.D. 2007. Transport and persistence of drifting macroalgae (Rhodophyta) are strongly influenced by flow velocity and substratum complexity in tropical seagrass habitats. Marine Ecology Progress Series. Vol. 343: 115–122.

Bintz, J.C., S.W. Nixon, B.A. Buckley, and S.L. Granger. 2003. Impacts of temperature and nutrients on coastal lagoon plant communities. Estuaries 26:765-776.

Björnsäter, B.R. and Wheeler, P.A. 1990. Effect of nitrogen and phosphorus supply on growth and tissue composition of *Ulva fenestrata* on *Enteromorpha intestinalis* (Ulvales: Chlorophyta). Journal of Phycology. 26:603-11.

Bolam, S. G. and T. F. Fernandes. 2002. The effects of macroalgal cover on the spatial distribution of macrobenthic invertebrates: the effect of macroalgal morphology. Hydrobiologia 475/476:437-448.

Bolam, S., T. Fernades, P. Read, and D. Raffaelli. 2000. Effects of macroalgal mats on intertidal sandflats: an experimental study. Journal of Experimental Marine Biology & Ecology 249:123-137.

Bona, F. 2006. Effect of seaweed proliferation on benthic habitat quality assessed by Sediment Profile Imaging. Journal of Marine Systems 62:142-151.

Borowitzka, M.A. and R.C. Lethbridge. 1989. Seagrass Epiphytes. pp. 458-499. In A.W.D. Larkum, A.J. McComb and S.A. Shepherd (eds). Biology of Seagrasses: A treatise on the biology of seagrasses with special reference to the Australian region. Elsevier, Amsterdam. pp. 841.

Boyer, K.E., J.C. Callaway and J.B. Zedler. 2000. Evaluating the progress of restored cordgrass (*Spartina foliosa*) marshes: belowground biomass and tissue nitrogen. Estuaries. 23 (5): 711-721.

Boyle, K. A., K. Kamer, and P. Fong. 2004. Spatial and temporal patterns in sediment and water column nutrients in a eutrophic southern California estuary. Estuaries **27**:378-388.

Brooks, K. and C. Mahnken. 2003. Interactions of Atlantic salmon in the Pacific northwest environment II. Organic wastes. Fisheries Research **62**:255-293.

Brun, F.G., J.J. Vergara, G. Navarro, I. Hernandez, and J.L. Perez-Llorens. 2003b. Growth, carbon allocation and proteolytic activity in the seagrass *Zostera noltii* shaded by *Ulva* canopies. Functional Plant Biology 30:551-560

Burkholder, J.M., H.B. Glasgow, Jr. and J.E. Cooke. 1994. Comparative effects of water-column nnitrate enrichment on eelgrass *Zostera marina*, shoalgrass *Haodule wrightii*, and widgeongrass *Ruppia maritima*. Marine Ecology Progress Series. 105:121-138.

Busse, L., J. Simpson, S. Cooper, K. Kamer and E. Stein. 2003. A Survey of Algae and Nutrients in the Malibu Creek Watershed. Southern California Coastal Water Research Project. Technical Report 412.

Cabral, J., M. Pardal, R. Lopes, T. Murias, and J. Marques. 1999. The impact of macroalgal blooms on the use of the intertidal area and feeding behavior of waders (Charadrii) in the Mondego estuary (west Portugal). Acta Oecologica 20:417-427.

Caffrey, J. M., H. Neil, and B. Ward. 2002. Biogeochemical processes in a small California estuary. 1. Benthic fluxes and pore water consitutents reflect high nutrient freshwater inputs. Marine Ecology Progress Series 233:39-53.

Caldwell, R. 1975. Hydrogen sulfide effects on selected larval and adult marine invertebrates.*in* U. D. o. Interior, editor. Water Resources Research Institute.

Cardoso, P. G., M. A. Pardal, D. Raffaelli, A. Baeta, and J. C. Marques. 2004. Macroinvertebrate response to different species of macroalgal mats and the role of disturbance history. Journal of Experimental Marine Biology and Ecology 308:207-220.

Ceccherellia, G. and L. Piazzi. 2001. Dispersal of *Caulerpa racemosa* fragments in the Mediterranean: lack of detachment time effect on establishment. Botanica Marina Vol. 44, 2001, pp. 209-213.

Chapman, P. 2001. Utility and relevance of aquatic oligochaetes in Ecological Risk Assessment. Hydrobiologia 463:149-169.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series. 210:223–253.

Cohen, R. and J. Pechenik. 1999. Relationship between sediment organic content, metamorphosis, and post larval performance in the deposit-feeding polychaete *Capitella* sp. I. Journal of Experimental Marine Biology & Ecology **240**:1-18.

Cohen, T., S. S. Que Hee and R.F. Ambrose. 2001. Trace metals in fish and invertebrates of three California coastal wetlands. Marine Pollution Bulletin. 42: 224-232.

Conley, D.J., H. Kaas, F. Mohlenberg, B. Rasmussen and J. Windolf. 2000. Characteristics of Danish estuaries. Estuaries. 23: 820-837.

Cornwell, T.R. Fisher, P.M. Gilbert, J.D. Hagy, L.W. Harding, E.D. Houde, D.G., Kimmel, W.D. Miller, R.I.E. Newell, M.R. Roman, E.M. Smith and J.C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Marine Ecology Progress Series. 303: 1-29.

Costanzo, S.D., M.J. O'Donohue and W.C. Dennison. 2000. *Gracilaria edulis* (Rhodophyta) as a biological indicator of pulsed nutrients in oligotrophic waters. Journal of Phycology 36: 680–685.

Cowper, S.W. 1978. The drift algae community of seagrass beds in Redfish Bay, Texas. Contribution to Marine Science 21: 125-132

Cummins, S. P., D. E. Roberts, and K. D. Zimmerman. 2004. Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary. Marine Ecology Progress Series 266:77-87.

Cuomo, M. 1985. Sulphide as a larval settlement cue for *Capitella sp I*. Biogeochemistry 1:169-181

Dauer, D. M., C. A. Maybury, and R. M. Ewing. 1981. Feeding behavior and general ecology of several spionid polychaetes from the Chesapeake Bay. Journal of Experimental Marine Biology & Ecology 54:21-38.

D'Avanzo, C. and J. Kremer. 1994. Diel oxygen dynamics and anoxic events in an eutrophic estuary. Estuaries 17:131-139.

Day, J.W., J. Rybczyk, F. Scaron, A. Rismondo, D. Are and G. Cecconi. 1999. Soil accretionary dynamics, sea-level rise and the survival of wetlands in Venice Lagoon: A field and modeling approach. Estuarine, Coastal and Shelf Science 49: 607-628.

Diaz, R. J. 2001. Overview of Hypoxia around the World. Journal of Environmental Quality 30: 275-281.

Diez, I., A. Santolaria, and J. Gorostiaga. 2003. The relationship of environmental factors to the structure and distribution of subtidal seaweed vegetation of the western Basque coast (N Spain). Estuarine Coastal & Shelf Science 56:1041-1054.

Drake, P. and A. Arias. 1996. The effect of epibenthic predators and macroalgal cover on the benthic macroinvertebrate community of a shallow lagoon in the Bay of Cadiz (SW Spain). Hydrobiologia 333:165-180.

Edwards, D.M., R.H. Reed, J.A. Chudek, R. Foster and W.D.P. Stewart. 1987. Organic solute accumulation in osmotically-stressed *Enteromorpha intestinalis*. Marine Biology. 95: 583-592.

Erwin, R.M., D.R. Cahoon, D.J. Prosser, G.M. Sanders and P. Hensel. 2006. Surface elevation dynamics in vegetated *Spartina* marshes versus unvegetated tidal ponds along the Mid-Atlantic coast, USA, with implications to waterbirds. Estuaries and Coasts 29: 96-106.

Escartin, J. and D.G. Aubrey. 1995. Flow structure and dispersion within algal mats. Estuarine Coastal & Shelf Science 40:451-472.

Flindt, M.R., L. Kamp-Nielsen, J.C. Marques, M.A. Pardal, M. Bocci, G. Bendoricchio, J. Salomonsen, S.N. Nielsen, and S.E. Jorgensen. 1997. Description of the three shallow estuaries: Mondego River (Portugal), Roskilde Fjord (Denmark), and the lagoon of Venice (Italy). Ecological Modeling 102:17-31.

Flindt, M.R., M.S. Pardal, A.I. Lillebo, I. Martins and J.C. Marques. 1999. Nutrient cycling and plant dynamics in estuaries: a brief review. Oecologica. 20: 237-248.

Fong P., K.E. Boyer and J.B. Zedler. 1998. Developing an indicator of nutrient enrichment in coastal estuaries and lagoons using tissue nitrogen content of the opportunistic alga, *Enteromorpha intestinalis* (L. Link). Journal of Experimental Marine Biology and Ecology 231: 63–79.

Fong, P. and J.B. Zedler. 1993. Temperature and light effects on the seasonal succession of algal communities in shallow coastal lagoons. Journal of Experimental Marine Biology and Ecology 171: 259-72.

Fong, P. and J.B. Zedler. 2000. Sources, sinks, and fluxes of nutrients (N+P) in a small highly modified urban estuary in southern California. Urban Ecosystems. 4:125-144.

Fong, P. and R.L. Kennison. 2010. Phase shifts, alternative stable states, and the status of southern California lagoons. In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. pgs. 227-251.

Fong, P., J.J. Fong and C.R. Fong. 2004. Growth, nutrient storage, and release of dissolved organic nitrogen by *Enteromorpha intestinalis* in response to pulses of nitrogen and phosphorus. Aquatic Botany. 78:83-95.

Fong, P., R.M. Donohoe and J.B. Zedler. 1994. Nutrient concentration in tissue of the macroalga *Enteromorpha* as a function of nutrient history: An experimental evaluation using field microcosms. Marine Ecology Progress Series. 106:273-81.

Forster, R. and B. Jesus. 2006. Field spectroscopy of estuarine intertidal habitats. International Journal of Remote Sensing 27:3657-3669.

Fox, S.E., E. Stieve, I. Valiela, J. Hauxwell and J. McClelland. 2008. Macrophyte abundance in Waquoit Bay: Effects of land-derived nitrogen loads on seasonal and multi-year biomass patterns. Estuaries and Coasts. 31:532-541.

Frankovish, T.A. and J.W. Fourquean. 1997. Seagrass epiphyte loads along a nutrient availibility gradient, Florida Bay, USA. Marine Ecology Progress Series 159:37-50.

Franz, D. R. and I. Friedman. 2002. Effects of a macroalgal mat (*Ulva lactuca*) on estuarine sand flat copepods: An experimental study. Journal of Experimental Marine Biology & Ecology **271**:209-226.

French, J. 2006. Tidal marsh sedimentation and resilience to environmental change: exploratory modeling of tidal, sea-level and sediment supply forcing in predominantly allochthonous systems. Marine Geology 235:119-136.

French, K., S. Roberston, and M. O'Donnell. 2004. Differences in invertebrate infaunal assemblages of constructed and natural tidal flats in New South Wales Australia. Estuarine Coastal & Shelf Science 61:173-183.

Froneman, P.W. 2002. Seasonal variations in selected physico-chemical and biological variables in the temporarily open/closed Kasouga estuary (South Africa). African Journal of Aquatic Science. 27:117-123.

Froneman, P.W. 2004. Zooplankton community structure and biomass in a southern African temporarily open/closed estuary. Estuarine, Coastal and Shelf Science. 60:125-132.

Fujita, R.M. 1985. The role of nitrogen status in regulating transient ammonium uptake and nitrogen storage by macroalgae. Journal of Experimental Marine Biology and Ecology. 92: 283-301.

Galbraith, H., R. Jones, R. park, J. Clough, S. Herrod-Julius, B. Harrington and G. Page. 2002. Global climate change and sea level rise: potential losses of intertidal habitat for shorebirds. Waterbirds 25(2): 173-183.

Gamenick, I., A. Jahn, K. Vopel, and O. Giere. 1996. Hypoxia and sulfide as structuring factors in a macrozoobenthic community on the Baltic Sea shore: colonization studies and tolerance experiments. Marine Ecology Progress Series 144:73-85.

Gaughan, D.J. and I.C. Potter. 1995. Composition, distribuition and seasonal abundance of zooplankton in a shallow, seasonally closed estuary in temperate Australia. Estuarine, Coastal and Shelf Science. 41:117-135.

Gaylord, B. and M.W. Denny. 1997. Flow and flexibility: effects of size, shape and stiffness in determining wave forces on the stipitate kelps *Eisenia arborea* and *Pterygophora californica*. Journal of Experimental Biology. 200: 3141– 3164.

Gianmarco, G., R. Azzoni, M. Bartoli, and P. Viaroli. 1997. Seasonal variations of sulfate reduction rates, sulphur pools and iron availability in the sediment of a dystrophic lagoon (Sacca Di Goro, Italy). Water, Air, & Soil Pollution 99:363-371.

Giere, O., J. Preusse, and N. Dublier. 1999. *Tubificoides benedii* (Tubificidae, Oligochaeta)- a pioneer in hypoxic and sulfidic environments. An overview of adaptive pathways. Hydrobiologia 406:235-241.

Granhag, L.M., A. I. Larsson and P.R. Jonsson. 2007. Algal spore settlement and germling removal as a function of flow speed. Marine Ecology Progress Series. 344: 63-69.

Hargrave, B. T., M. Holmer, and C. P. Newcombe. 2008. Towards classification of organic enrichment in marine sediments based on biogechemical indicators. Marine Pollution Bulletin 56:810-824.

Harlin, M.M. and B. Thorne-Miller. 1981. Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. Marine Biology 65:221-229.

Hauxwell, J. Cebrian, J., Furlong, C., and I. Valiela. 2001. Macroalgal canopies contribute to eelgrass (Zostera marina) decline in temperate estuarine ecosystems. Ecology 82: 1007-1022.

Hauxwell, J., J. Cebrian, and I. Valiela. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. Marine Ecology Progress Series 247:59-73.

Hauxwell, J., J. McClelland, P. Behr and I. Valiela. 1998. Relative importance of grazing and nutrient controls of macroalgal biomass in three temperate shallow estuaries. Estuaries. 21:347-360.

Hentschel, B. 1996. Ontogenic changes in particle-size selection by deposit-feeding spionid polychaetes: the influences of palp size on particle contact. Journal of Experimental Marine Biology & Ecology 206:1-24.

Hernandez, I., G. Peralta, J. L. Perez-Llorens, J. J. Vergara and F. X. Niell. 1997. Biomass and dynamics of growth *Ulva* species in Palmones River estuary. Journal of Phycology. 33: 764-772.

Herrera-Silveira, J.A. and S.M. Morales-Ojeda. Subtropical Karstic Coastal Lagoon assessment, Southeast Mexico: The Yucatan Peninsula Case. In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. pgs. 307-333.

Higgins, S., R. Hecky, and S. Guildford. 2008. The collapse of benthic macroalgal blooms in response to shelf-shading. Freshwater Biology 53:2557-2572.

Hines, M., J. Faganeli, and R. Planinc. 1997. Sedimentary anerobic microbial biogeochemistry in the Gulf of Trieste, northern Adriatic Sea: Influences of bottom water oxygen depletion. Biogeochemistry 39:65-86.

Holmquist, J. G. 1994. Benthic macroalgae as a dispersal mechanism for fauna: influence of a marine tumbleweed. Journal of Experimental Marine Biology & Ecology 180:235-251.

Hopkinson, C.S., A.E. Giblin, J.Tucker and R.H. Garritt. 1999. Benthic metabolism and nutrient cycling along an estuarine salinity gradient. Estuaries. 22: 863-881.

Horrocks JL, G.R. Stewart GR and W.C. Dennison. 1995. Tissue nutrient content of *Gracilariopsis* spp. (Rhodophyta) and water quality along an estuarine gradient. Marine Freshwater Research 46: 975–983.

Howarth, R. W. 1988. Nutrient limitation of primary production in marine ecosystems. Annual Review of Ecology and Systematics 19: 89-110.

Howarth, R. W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. Estuaries 25: 656-676.

Hull, S. C. 1987. Macroalgal mats and species abundance: a field experiment. Estuarine, Coastal and Shelf Science 25:519-532.

Huntington, B.E. and K. Boyer. 2008a. Effects of red macroalgal (*Gracilariopsis sp.*) abundance on eelgrass *Zostera marina* in Tomales Bay, California, USA. Marine Ecology Progress Series 367:133-142.

Huntington, B.E. and K. Boyer. 2008b. Evaluating patterns of nitrogen supply using macroalgal tissue content and stable isotopic signatures in Tomales Bay, CA. Environmental Bioindicators. 3:180-192.

Isaksson, I. and L. Pihl. 1992. Structural changes in benthic macrovegetation and associated epibenthic faunal communities. Netherlands Journal of Sea Research 30:131-140.

Johnson, L.E. and S.H. Brawley. 1998. Dispersal and recruitment of a canopy-forming intertidal alga: the relative roles of propagule availability and post-settlement processes. Oecologia. 117: 517– 526.

Jones, M. and E. Pinn. 2006. The impact of a macroalgal mat on benthic biodiversity in Poole Harbour. Marine Pollution Bulletin 53:63-71.

Josselyn, M. and J. West. 1985. The distribution and temporal dynamics of the estuarine macroalgal community of San Fransisco Bay. Hydrobiologia 129:139-152.

Kamer, K. and P. Fong. 2000. A fluctuating salinity regime mitigates the negative effect of reduced salinity on the estuarine macroalga, *Enteromorpha intestinalis* (L.) link. Journal of Experimental Marine Biology and Ecology. 254:53-69.

Kamer. K., K. Boyle and P. Fong. 2001. Macroalgal bloom dynamics in a highly eutrophic southern California estuary. Estuaries 24:623-635

Kemp, W.M., W.R. Boynton, J.E. Adolf, D.F. Boesch, W.C. Boicourt, G. Brush, J.C., Cornwell, T.R. Fisher, P.M. Gilbert, J.D. Hagy, L.W. Harding, E.D. Houde, D.G., Kimmel, W.D. Miller, R.I.E. Newell, M.R. Roman, E.M. Smith and J.C. Stevenson. 2005. Eutrophication of Chesapeake Bay: historical trends and ecological interactions. Marine Ecology Progress Series. 303: 1-29

Kennison, R.L. 2008. Evaluating ecosystem function of nutrient retention and recycling in excessively eutrophic estuaries. Ph.D. dissertation. University of California, Los Angeles, California.

Kennison, R.L., K. Kamer and P. Fong. 2003. Nutrient dynamics and macroalgal blooms: A comparison of five southern California estuaries. Southern California Coastal Water Research Project. Technical Report 419.

Kentula, M.E. and C.D. McIntire. 1986. The autecology and production dynamics of eelgrass (*Zostera marina* L.) in Netarts Bay, Oregon. Estuaries 9:188-199.

Kentula, M.E. and T.H. DeWitt. 2003. Abundance of seagrass (*Zostera marina* L.) and macroalgae in relation to the salinity-temperature gradient in Yaquina Bay, Oregon, USA. Estuaries 26:1130-1141

Kjerfve, B. 1986. Comparative Oceanography of Coastal Lagoons. In: Estaurine variability ed. D.A. Wolfe. Academic Press. New York. Pgs. 63-81.

Knoppers, B., B. Kjerfve and J. Carmouze. 1991. Trophic state and water turn-over time in six choked coastal lagoons in Brazil. Biogeochemistry. 14:149-166.

Kopecky, A.L. and K.H. Dunton. 2006. Variability in drift macroalgal abundance in relation to biotic and abiotic factors in two seagrass dominated estuaries in the western Gulf of Mexico. Estuaries and Coasts. 29: 617-629.

Kotta, J. and H. Orav. 2001. Role of benthic macroalgae in regulating macrozoobenthic assemblages in the Vainameri (north-eastern Baltic Sea). Ann. Zool. Fennici 38:163-171.

Krause-Jensen, D., J. Carstensen and K. Dahl. 2007. Total and opportunistic algal cover in relation to environmental variables. Marine Pollution Bulletin. 55: 114-125.

Krause-Jensen, D., K. McGlathery, S. Rysgaard, and P. B. Christensen. 1996. Production within dense mats of the filamentous macroalga *Chaetomorpha linum* in relation to light and nutrient availability. Marine Ecology-Progress Series 134:207-216.

Kristiansen, K., E. Kristensen, and M. Jensen. 2002. The influence of water column hypoxia on the behaviour of manganese and iron in sandy coastal marine sediment. Estuarine Coastal & Shelf Science 55:645-654.

Kwak, T. and J. Zedler. 1997. Food Web analysis of southern California coastal wetlands using multiple stable isotopes. Oecologia 110:262-277.

Langis, R., M. Zalejko and J. B. Zedler. 1991. Nitrogen assessments in a constructed and a natural marsh of San Diego Bay. Ecological Applications 1:40-51.

Lartigue, J. and T.D. Sherman. 2005. Response of *Enteromorpha* sp. (Chlorophyceae) to a nitrate pulse: nitrate uptake, inorganic nitrogen storage and nitrate reductase activity. Marine Ecology Progress Series. 292:147-57.

Lauringson, V. and J. Kotta. 2006. Influence of the thin drift algal mats on the distribution of macrozoobenthos in Koiguste Bay, NE Baltic Sea. Hydrobiologia 554: 97-105

Lavery, P. S. and A. J. McComb. 1991. Macroalgal-Sediment Nutrient Interactions and Their Importance to Macroalgal Nutrition in a Eutrophic Estuary. Estuarine Coastal & Shelf Science 32:281-296.

Lewis, L. and T. Kelly. 2001. A short-term study of the effects of algal mats on the distribution and the behavioural ecology of estuarine birds. Bird Study 48:354-360.

Llanso, R. 1991. Tolerance of low dissolved oxygen and hydrogen sulfide by the polychaete *Streblospio benedicti* (Webster). Journal of Experimental Marine Biology and Ecology 153:165-178.

Lotze, H. K. and W. Schramm. 2000. Ecophysiological traits explain species dominance patterns in macroalgal blooms. Journal of Phycology. 36:287-95.

Lotze, H. K., B. Worm and U. Sommer. 2000. Propagule banks, herbivory and nutrient supply control population development and dominance patterns in macroalgal blooms. Oikos. 89: 56-58.

Lotze, H.K. and B. Worm. 2000. Variable and complementary effects of herbivores on different life stages of bloom-forming macroalgae. Marine Ecology Progress Series 200:167-175.

Lukatelich, R.J., N.J. Schofield and A.J. McComb. 1987. Nutrient loading and macrophytes growth in Wilson Inlet, a bar-built southwester Australian estuary. Estuarine, Coastal and Shelf Science. 24:141-165.

Lyons, J., J. Ahern, J. McClelland, and I. Valiela. 1995. Macrophyte abundance in Waquoit Bay estuaries subjectto different nutrient loads and the potential role of fringing salt marsh in groundwater nitrogen interception. Biological Bulletin 189:255-2256

Magni, P., S. Micheletti, D. Casu, A. Floris, G. Giordani, A. Petrov, G. Falco, and A. Castelli. 2005. Relationships between chemical characteristics of sediment and macrofaunal communities in the Cabras Lagoon (Western Mediterranean, Italy). Hydrobiologia 550:105-119.

Marcomini, A., A. Sfriso, B. Pavioni and H. H. Orio. 1995. Eutrophication of the Lagoon of Venice: Nutrient loads and exchanges. In: Eutrophic Shallow Estuaries and Lagoons ed. A.J. McComb, CRC Press, Boca Raton, Florida.

Martins I., M. Pardal, A. Lillebo, M. Flindt and J. Marques. 2001. Hydrodynamics as a major factor controlling the occurrence of green macroalgal blooms in a eutrophic estuary: A case study on the influence of precipitation and river management. Estuarine Coastal & Shelf Science 52:165-177

McGlathery, K.J., K. Sundback and I. Anderson. 2007. Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter. Marine Ecology Progress Series. 348: 1-18.

McLusky, D. S., S. C. Hull and M. Elliot. 1993. Variations in the intertidal and subtidal macrofauna and sediments along a salinity gradient in the upper Forth Estuary. Netherlands Journal of Aquatic Ecology 27:101-109.

Menendez, M. and F.A. Comin. 2000. Spring and summer proliferation of floating macroalgae in a Mediterranean coastal lagoon (Tancada Lagoon, Ebro Delta, NE Spain). Esturies, Coast and Shelf Science. 51:215-226.

Milligan, K.L.D. and R.E. DeWreede. 2000. Variations in holdfast attachment mechanics with developmental stage, substratum type, season, and wave-exposure for the intertidal kelp species *Hedophyllum sessile* (C. Agardh) Setchell. Journal of Experimental Marine Biology and Ecology. 254: 189-209.

Milligan, K.L.D. and R.E. DeWreede. 2004. Morphological variations do not effectively reduce drag forces at high wave-exposure for the macroalgal species, *Hedophyllum sessile* (Laminariales, Phaeophyta). Phycologia. 43: 236-244.

Miron, G. and E. Kristensen. 1993. Factors influencing the distribution of nereid polychaetes: the sulfide aspect. Marine Ecology Progress Series 93:143-153.

Moorhead, K.K. and M.M. Brinson. 1995. Response of wetlands to rising sea level in the lower coastal plain of North Carolina. Ecological Applications 5: 261-271.

Moseman, S., L. Levin, C. Currin, and C. Forder. 2004. Colonization, succession, and nutrition of macrobenthic assemblages in a restored wetland at Tijuana Estuary, California. Estuarine Coastal & Shelf Science 60:755-770.

Multcher, R. R.F. Mooney, S. Wallace, L. Podsim, S. Fredriksen and K.H. Dunton. 2010. Origins and Fate of Inorganic Nitrogen from Land to Coastal Ocean on the Yucatan Peninsula, Mexico. In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. pgs. 283-305.

Murias, T., J. Cabral, J. C. Marques and J. D. Goss-Custard. 1996. Short-term effects of intertidal macroalgal blooms on the macrohabitat selection and feeding behaviour of wading birds in the Mondego Estuary (West Portugal). Estuarine Coastal & Shelf Science 43:677-688.

Najjar, R.G., H.A. Walker, P.J. Anderson, E.J. Barron, R.J. Bord, J.R. Gibson, V.S. Kennedy, C.G. Knight, J.P. Megonigal, R.E. O'Conner, C.D. Polsky, N.P. Psuty, B. A. Richards, L.G. Sorenson, E.M Steele, R. S.

Swanson. 2000. The potential impacts of climate change on the mid-Atlantic coastal region. Climate Research 14:219-233.

Naldi, M. and P. Viaroli. 2002. Nitrate uptake and storage in the seaweed *Ulva rigida* C. Agardh in relation to nitrate availability and thallus nitrate content in a eutrophic coastal lagoon (Sacca di Goro, Po River Delta, Italy). Journal of Experimental Marine Biology and Ecology. 269:65-83.

Nedwell, D.B., A.S. Sage and G.J.C Underwood. 2002. Rapid assessment of macroalgal cover on intertidal sediments in a nutrified estuary. The Science of the Total Environment. 285: 97-105.

Neira, C. and M. Rackemann. 1996. Black spots produced by buried macroalgae in intertidal sandy sediments of the Wadden Sea: effects on the meiobenthos. Journal of Sea Research 36:153-170.

Neira, F.J. and I.C. Potter. 1992. The ichthyoplankton of a seasonally closed estuary in temperate Australia. Does an extended period of opening influence species composition? Journal of Fish Biology. 41:935-953.

Nelson, T.A. and A. Lee. 2001. A manipulative experiment demonstartes that blooms of the macroalga *Ulvaria obscura* can reduce eelgrass shoot density. Aquatic Botony 71:149-154.

Nelson, T.A., A.V. Nelson and M. Tjoelker. 2003. Seasonal and spatial patterns of ''green tides'' (ulvoid algal blooms) and related water quality parameters in the coastal waters of Washington State, USA. Botanica Marina. 46: 263–275.

Nelson, W.G. 2009. The interaction of epiphytes with seagrasses under nutrient enrichment. IN: Seagrasses and Protective Criteria: A review and assessment of research Status. Office of Research and Development, National Health and Environmental Effects Research Laboratory, EPA/600/R-09/050

Nevereauskas, V.P. 1987. Accumulation of periphyton biomass on artificial substrates deployed near sewage sludge outfall in South Australia. Estuarine, Coastal, and Shelf Science 25:509-519.

Nevereauskas, V.P. 1988. Response of a *Posidonia* community to prolonged reduction in light. Aquatic Botony 31: 361-366.

Nezlin, N., K. Kamer, and E. Stein. 2007. Application of color infrared aerial photography to assess macroalgal distribution in an eutrophic estuary, Upper Newport Bay, CA. Estuaries and Coasts 30:855-868.

Nezlin, N., K. Kamer, J. Hyde, and E. Stein. 2009. Dissolved oxygen dynamics in a eutrophic estuary, Upper Newport Bay, California. Estuarine, Coastal and Shelf Science 82:139-151.

Nixon, S. W. 1995. Coastal Marine Eutrophication: A definition, social causes, and future concerns. Ophelia 41: 199-219.

Nixon, S.W., J.W. Ammerma, L.P. Atkinson, V.M. Berounsky, G. Billen, W.C. Biocourt, W.R. Boynton, T.M. Church, D.M. DiToro, R. Elmgren, J.H. Garber, AE. Giblin, R.A. Jahnke, N.J.P. Owens, M.E.Q. Pilson and

S.P. Seitzinger. 1996. The fate of nitrogen and phosphorus at the land-sea margin of the North Atlantic Ocean. Biogeochemistry. 35: 141-180.

Nordby, C.S. and J.B. Zedler. 1991. Responses of fish ad macrobenthic assemblages to hydrologic disturbances in Tijuana Estuary and Los Penasquitos Lagoon, California. Estuaries. 14 (1):80-93.

Norkko, A. and E. Bonsdorff. 1996. Rapid zoobenthic community responses to accumulations of drifting algae. Marine Ecology Progress Series 131:143-157.

Norkko, J., E. Bonsdorf, and A. Norkko. 2000. Drifting algal mats as an alternative habitat for benthic invertebrates: Species specific responses to a transient resource. Journal of Experimental Marine Biology and Ecology 248:79-104.

Nozais, C., R. Perissinotto and G. Tita. 2005. Seasonal dynamics of meiofauna in a south African temporarily open/closed estuary (Mdloti Estuary, Indian Ocean). Estuarine, Coastal and Shelf Science. 62:325-338.

Oczkowski, A.J. and S.W. Nixon. 2010. Lagoons of the Nile Delta. In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. pgs. 253-282.

Odebrecht, C., P.C. Abreu, C.E. Bemvenuti, M. Copertino, J.H. Muelbert, J.P. Vierira and U. Seeliger. 2010. The Patos Lagoon Estuary, Southern Brazil: Biotic Responses to Natural and Anthropogenic Impacts in the Last Decades (1979-2008). In: Coastal Lagoons Critical Habitats of Environmental Change ed. M.J. Kennish and H.W. Paerl, CRC Press, Boca Raton, Florida. pgs. 433-455.

Osterling, M. and L. Pihl. 2001. Effects of filamentous green algal mats on benthic macrofaunal functional feeding groups. Journal of Experimental Marine Biology and Ecology. 263: 159-183.

Paerl, H. W. 1997. Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnology and Oceanography. 42: 1154-1165.

Peckol, P. and D. Baxter. 1986. Population dynamics of the Onuphid polychaete *Diopatra cuprea* (Bosc) along a tidal exposure gradient. Estuarine Coastal & Shelf Science 22:371-377.

Peckol, P. and J.S. Rivers. 1995. Physiological responses of the opportunistic macroalgae *Cladophora vagabunda* (L.) van den Hoek and *Gracelaria tikvahiae* (McLachlan) to environmental disturbances associated with eutrophication. Journal of Experimental Marine Biology and Ecology 190:1-16.

Peckol, P., B. DeMeo-Anderson, J. Rivers, I. Valiela, M. Maldonado and J. Yates. 1994. Growth, nutrient uptake capacities and tissue constituents of the macroalgae *Cladophora vagabunda* and *Gracilaria tikvahiae* related to site-specific nitrogen loading rates. Marine Biology 121: 175-185.

Pedersen, M.F.1994. Transient ammonium uptake in the macroalga *Ulva lactuca* (Chlorophyta): nature, regulation, and the consequences for choice of measuring technique. Journal of Phycology. 30: 980-86.

Penhale, P. and W.O. Smith, Jr. 1977. Excretion of dissolved organic carbon by eelgrass (*Zostra marina*) and its epiphytes. Limnology and Oceanography 22:400-407.

Perissinotto, R., D.R. Walker, P. Webb, T.H. Wooldridge and R. Bally. 2000. Relationships between zoo- and phytoplankton in a warm-temperate, semi-permanently closed estuary, South Africa. Estuarine, Coastal and Shelf Science. 51:1-11.

Phillips, R.C. 1984. The Ecology of Eelgrass Meadows in the Pacific Northwest: A Community Profile. U.S. Fish and Wildlife Service, FWS/OBS-84/24, 85 pp.

Pihl, L., A. Svenson, P-O. Moksnes and H. Wennhage. 1999. Distribution of green algal mats throughout shallow soft bottoms of the Swedish Skagerrak archipelago in relation to nutrient sources and wave exposure. Journal of Sea Research 41:281-294.

Pihl, L., G. Magnusson, I. Isaksson and I. Wallentinus. 1996. Distribution and growth dynamics of ephemeral macroalgae in shallow bays on the Swedish west coast. Journal of Sea Research. 35: 169-180.

Pihl, L., I. Isaksson, H. Wennhage, and P. Moksnes. 1995. Recent increase of filamentous algae in shallow Swedish bays: effects on the community structure of epibenthic fauna and fish. Netherlands Journal of Aquatic Ecology 29:349-358.

Raffaelli, D. 1999. Nutrient enrichment and trophic organisation in an estuarine food web. Acta Oecologica 20:449-461.

Raffaelli, D., J. Limia, S. Hull, and S. Pont. 1991. Interactions between the amphipod *Corophium volutator* and macroalgal mats on estuarine mudflats. Journal of the Marine Biological Association of the United Kingdom 71:899-908.

Raffaelli, D., S. Hull and H. Milne. 1989. Long-term changes in nutrients, weed mats and shorebirds in an estuarine system. Cahiers de Biologie Marine. 30:259-270.

Raffaelli, D.G., J.A. Raven, and L.J. Poole. 1998. Ecological impacts of green macroalgal blooms. Oceanography and Marine Biology: and Annual Review 36:97-125.

Reise, K. 1977. Predator exclusion experiments in an intertidal mudflat. Helgol Marine Research 30:263-271.

Rossi, F. 2007. Recycle of buried macroalgal detritus in sediments: use of dual-labelling experiments in the field. Marine Biology 150:1073-1081.

Rossi, F., R. Forster, F. Montserrat, M. Ponti, A. Terlizzi, T. Ysebaert, and J. Middleburg. 2007. Human trampling on intertidal mudflats: effects on macrofauna biodiversity and population dynamics of bivalves. Marine Biology 151:2077-2090.

Roy, P.S., R.J Williams, A.R. Jones, I. Yassini, P.J. Gibbs, B. Coates, R.J. West, P.R. Scanes, J.P. Hudson and S. Nichol. 2001. Structure and function of South-east Australian estuaries. Estuarine, Coastal and Shelf Science. 53:351-384.

Rudnicki, R.M. 1986. Dynamics of macroalgae in Tijuana Estuary: Response to simulated wastewater addition. Masters Thesis. San Diego State University.

Runcie, J.W., R.J. Ritchie and A.W.D. Larkum. 2003. Uptake kinetics and assimilation of inorganic nitrogen by *Catenella nipae* and *Ulva lactuca.* Aquatic Botany. 76:155-74.

Salovius, S. and P. Kraufvelin. 2004. The filamentous green alga *Cladophora glomerata* as habitat for littoral macrofauna in the northern Baltic sea. Ophelia **58**:65-78.

Sanchez-Moyano, J., F. Estacio, E. Garcia, and J. Garcia-Gomez. 2001. Effect of the vegetative cycle of *Caulerpa prolifera* on the spatio-temporal variation of invertebrate macrofauna. Aquatic Botany 70: 163-174.

Scanlan, C. M., J. Foden, E. Wells, and M. A. Best. 2007. The monitoring of opportunistic macroalgal blooms for the water framework directive. Marine Pollution Bulletin 55:162-171.

Schaadt, T. 2005. Patterns and causes of variability in the cover, biomass, and total abundance of *Ulva spp.* in Elkhorn Slough, California. Masters. California State University Monterey Bay, Monterey

Schramm, W. 1999. Factors influencing seaweed responses to eutrophication: some results from EU-project EUMAC. Journal of Applied Phycology. 11: 69-78.

Sfriso A., B. Pavoni, A. Marcomini and A.A. Orio. 1992. Macroalgae, nutrient cycles, and pollutants in the lagoon of Venice. Estuaries 15:517–528.

Sfriso, A. and A. Marcomini. 1997. Macrophyte production in a shallow coastal lagoon. Part I: Coupling with chemico-physico parameters and nutrient concentrations in waters. Marine Environmental Research 44:351-375.

Sfriso, A., A. Marcomini and B. Pavoni. 1987. Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice Lagoon Italy. Marine Environmental Research. 22: 297-312.

Short, F.T. and D.M. Burdick. 1996. Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts. Estuaries 19:730-739.

Short, F.T., D.M. Burdick, and J.E. Kaldy III. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. Limnology and Oceanography 40:740-749.

Silberstein, K.A., W. Chiffings, and A.J. McCombs. 1986. The loss of seagrass in Cockburn Sound, Western Australia. III. The effect of epiphytes on productivity of *Posidonia australis* Hook. F. Aquatic Botany 24:355-371.

Stenzel, L., H. Huber, and G. Page. 1976. Feeding behavior and diet of the long-billed curlew and willet. The Wilson Bulletin 88:314-332.

Sundbäck, K. and K McGlathery. 2005. Interactions between benthic macroalgal and microalgal mats, P/ 7-29, In E. Kristnesen, R.R. Haese, and J.E. Kostka (eds), Interactions Between Macro- and Microorganisms in marein Sediments, Vol 60, AGU, Washington, D.C.

Sundback, K., L. Carlson, C. Nilsson, B. Jonsson, A. Wulff and S. Odmark. 1996. Response of benthic microbial mats to drifting green algal mats. Aquatic Microbial Ecology. 10(2): 95-208.

Sutula, M., K. Kamer and J. Cable. 2004. Sediments as a Non-Point Source of Nutrients to Malibu Lagoon, California (USA). Southern California Coastal Water Research Project. Technical Report 441.

Taylor, D.I., S.W. Nixon, S.L. Granger, B.A. Buckley, J.P. McMahon, and H.-J. Lin. 1995. Responses of coastal lagoon plant communities to different forms of nutrient enrichment- a mesocosm experiment. Aquatic Botany 52: 19-34.

Teixeira da Silva, E. and M.L. Asmus. 2001. A dynamic simulation model of the widgeon grass Ruppia maritime and its epiphytes in the estuary of the Patos Lagoon, RS, Brazil. Ecological Modelling. 137:161-179.

Thiel, M. and L. Watling. 1998. Effects of green algal mats on infaunal colonization of a New England mud flat-long lasting but highly localized effects. Hydrobiologia 375/376:177-189.

Thom, R.M., A.B. Borde, S. Rumrill, D.L. Woodruff, G.D. Williams, J.A. Couthard, and S.L. Sargeant. 2003. Factors influencing spatial and annual variability in eelgrass (*Zostera marina* L.) meadows in Willapa Bay, Washington, and Coos Bay Oregon, estuaries. Estuaries 26:1117-1129.

Thomsen, M. S. and K. McGlathery. 2006. Effects of accumulations of sediments and drift algae on recruitment of sessile organsims associated with oyster reefs. Journal of Experimental Marine Biology and Ecology 328:22-34.

Thomsen, M.S. and K. McGlathery. 2005. Facilitation of macroalgae by the sedimentary tube forming polychaete *Diopatra cuprea*. Estuarine, Coastal and Shelf Science. 62:63–73.

Thomsen, M.S., K.J. McGlathery and A.C. Tyler. 2006. Macroalgal distribution patterns in a shallow, soft-bottom lagoon, with emphasis on the nonnative *Gracilaria vermiculophylla* and *Codium fragile*. Estuaries and Coasts. 29 (3): 465-473.

Thybo-Christensen, M., Rasmussen, M.B. and T.H. Blackburn. 1993. Nutrient fluxes and growth of *Cladophora sericea* in a shallow Danish bay. Marine Ecology Progress Series.100: 273-281.

Tomasko, D.A. and B.E. Lapointe. 1991. Productivity and biomass of *Thalassia testudinum* as related to water column nutrient availability and epiphyte levels: field observations and experimental studies. Marine Ecology Progress Series 75:9-17.

Tomasko, D.A., C.J. Dawes, and M.O. Hall. 1996. The effects of Anthropogenic enrichment on turtle grass (*Thalassia testudinum*) in Sarasota Bay, Florida. Estuaries 19:448-456.

Trimmer, M., D. B. Nedwell, D. B. Sivyer and S. J. Malcolm. 2000. Seasonal organic mineralisation and denitrification in intertidal sediments and their relationship to the abundance of *Enteromorpha* sp. and *Ulva* sp. Marine Ecology Progress Series. 203: 67-80.

Tubbs, C. and J. Tubbs. 1980. Wader and Shelduck feeding distribution in Langstone Harbour, Hampshire. Bird Study 27:239-248.

Twilley, R.R., W.M. Kemp, K.W. Staver, J.C. Stevenson and W.R. Boynton. 1985. Nutrient enrichment of estuarine submersed vascular plant communities. 1. Algal growth and effects on production of plants and associated communities. Marine Ecology Progress Series. 23:179-191.

U.S. EPA. 2003. Ambient water quality criteria for dissolved oxygen, water clarity and chlorophyll *a* for the Chesapeake Bay and its tidal tributaries. U.S. EPA, Office of Water and Office of Science and Technology. EPA 900-R-03-002.

Underwood, G. J. C. and J. Kromkamp. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. Page 306 in D. B. Nedwell and D. G. Raffaell, editors. Advances in ecological research estuaries. Academic Press, London

Valiela, I. K. Foreman, M. LaMontagne, D. Hersh, J. Costa, P. Peckol, B. DeMeo-Anderson, C. D'Avanzo, M. Babione, C.-H. Sham, J. Brawley, and K. Lajtha. 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichment in Waquoit Bay, Massachusetts. Estuaries 15:443-457.

Valiela, I., J. McClelland, J. Hauxwell, P.J. Behr, D. Hirsch, and K. Foreman. 1997. Macroalgal blooms in shallow estuaries: controls and ecophysiological and ecosystem consequences. Limnology and Oceanography 42:1105-1118.

van Hulzen, J. B., J. van Soelen, P. M. J. Herman, and T. J. Bouma. 2006. The significance of spatial and temporal patterns of algal mat deposition in structuring salt marsh vegetation. Journal of Vegetation Science 17:291-298.

Viaroli, P., M. Baroli, I.Fumagalli and G. Giordani. 1997. Relationship between benthic fluxes and macrophyte cover in a shallow brackish lagoon. Water, Air and Soil Pollution. 99:533-540.

Viaroli, P., M. Bartoli, C. Bondavalli, and M. Naldi. 1995. Oxygen fluxes and dystrophy in a coastal lagoon colonized by *Ulva rigida* (Sacca Di Goro, Po River Delta, Northern Italy). Fresenius Environmental Bulletin 4:381-386.

Viaroli, P., M. Bartoli, R. Azzoni, G. Giordani, C. Mucchino, M. Naldi, D. Nizzoli and L. Taje. 2005. Nutrient and iron limitation to *Ulva* blooms in a eutrophic coastal lagoon (Sacca di Goro, Italy). Hydrobiologia. 550:57-71

Villares, R. and A. Carballeira. 2003. Seasonal variation in the concentrations of nutrients in two green macroalgae and nutrient levels in sediments in the Ri' as Baixas (NW Spain). Estuarine Coastal and Shelf Sciences 58: 887-900.

Virnstein, R. 1979. Predation on estuarine infauna: response patterns of component species. Estuaries **2**:69-86.

Wells, E., M. Wilkinson, P. Wood, and C. Scanlan. 2007. The use of macroalgal species richness and composition on intertidal rocky shores in the assessment of the ecological quality under the European Water Framework Directive. Marine Pollution Bulletin **55**:151-161.

Wennhage, H. and L. Pihl. 1994. Substratum selection by juvenile plaice (Pleuronectes platessa L.): Impact of benthic microalgae and filamentous macroalgae. Netherlands Journal of Sea Research 32:343-351.

Wetzel MA, Weber A, Giere O (2002) Re-colonization of anoxic/sulfidic sediments by marine nematodes after experimental removal of macroalgal cover. Marine Biology 141:679-689

Whigham, D. 1999. Ecological issues related to wetland preservation, restoration, creation and assesment. The Science of the Total Environment 240:31-40.

Whitfield, A.K. 1988. The role of tides in redistributing macrodetrital aggregates within the Swartvlei estuary. Coastal and Estuarine Research Federation. 11 (3):152-159.

Wijnen, H.J. and J.P. Bakker. 2001. Long-term surface elevation change in salt marshes: a prediction of marsh response to future sea-level rise. Estuarine, Coastal and Shelf Science 52: 381-390.

Wilkes, R. 2004. A desk and field study of biomass estimation techniques for monitoring green tides in the Irish environment. Pages 1-42 IN: E. P. Agency and E. R. T. D. a. I. Programme, editors., Galway, Ireland.

Williams, S. L. 1984. Decomposition of the Tropical Macroalga Caulerpa-Cupressoides Field and Laboratory Studies. Journal of Experimental Marine Biology & Ecology 80:109-124.

Williams, S.L. and M.H. Ruckelshaus. 1993. Effects of nitrogen availability and herbvory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74: 904-918.

Wilson, J.G. 1994. The role of bioindicators in estuarine management. Estuaries. 17: 94-101.

Windom, H.L., L.F. Niencheski and R.G. Smith, Jr. 1999. Biogeochemistry of nutrients and trace metals in the estuarine region of the Patos Lagoon (Brazil). Estuarine, Coastal and Shelf Science. 48:113-123.

Wood, E. J. F. 1959. Some aspects of the ecology of Lake Macquarie, N.S.W., with regard to an alleged depletion of fish. VI. Plant communities and their significance. Australian Journal of Marine Freshwater Research. 10:322-344.

Woodin, S. A. 1974. Polychaete abundance patterns in a marine soft-sediment environment: importance of biological interactions. Ecological Monographs **44**:171-187.

Young, A.J., J.C. Collins and G. Russell. 1987. Ectypic variation in the osmotic responses of *Enteromorpha intestinalis* (L.) Link. Journal of Experimental Botany. 38: 1309-1324.

Young, D.R. 2009. Macroalgal interaction with the seagrasses *Zostera marina* and *Thalassia testudinum*. IN: Seagrasses and Protective Criteria: A review and assessment of research Status. Office of Research and Development, National Health and Environmental Effects Research Laboratory, EPA/600/R-09/050

Zedler, J. B. 1996. Coastal mitigation in southern California: The need for regional restoration strategy. Ecological Applications 6:84-93.

Zedler, J.B. 1996. Tidal wetland restoration: a scientific perspective and southern California focus. La Jolla, CA: California Sea Grant College System, University of California.

4. Suitability of Phytoplankton as Indicators of Estuarine Eutrophication

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4.1 Introduction

Phytoplankton are microscopic, single-celled photosynthetic organisms are ubiquitous in freshwater and marine ecosystems (Valiela 1995). The photosynthetic microalgae form the base of many food chains and thus are the primary producers that provide organic matter and energy to higher trophic levels in aquatic habitats. Phytoplankton dominate primary productivity in many types of estuaries and play a major role in the nutrient, carbon and oxygen cycling in surface waters (Nixon 1986). They have fast growth rates (their doubling time can be less than a day) and they rapidly respond to both chemical (nutrients, toxicants) and physical (light, temperature, turbulence) factors over a wide range of concentrations and intensities (Paerl et al. 2005). Changes in the total biomass (as measured by chlorophyll-a), or in community structure (species or taxonomic richness, relative abundance) over spatial and temporal scales often precede larger-scale and long-term changes in the larger functioning of the ecosystem as a whole, including shifts in metabolism, oxygen, food webs structure, fisheries and habitat quality (Paerl et al. 2005).

Phytoplankton possess many characteristics that make them potentially useful as indicators of eutrophication in estuaries. They can be useful and sensitive indicators of nutrient availability since their growth rates are rapid, across a wide range of nutrient concentrations and can be measured using an array of standard techniques (Paerl et al 2007). Water column chlorophyll-a is often used as a proxy for phytoplankton community biomass and is a particularly common metric. It is easily measured using a variety of fairly simple techniques, and has been used as biological response indictor for eutrophication in many systems, including estuaries throughout the globe. Phytoplankton community structure can also provide information related to the timing, duration and magnitude of nutrient enrichment in estuaries (Paerl et al. 2005).

This review is a summary of information about the suitability of phytoplankton as an indicator of eutrophication in California estuaries, utilizing the criteria specified in Chapter 2.

4.2 General Ecology of Phytoplankton

The term "phytoplankton" refers to the collection of single-celled microalgae suspended in waters of aquatic habitats (Day et al. 1989). Most phytoplankton cells are too small to be individually observed without a microscope. However, when present in high enough numbers, they may appear as a green, yellow, red or brown coloration of the water due to the presence of photosynthetic pigments within their cells. Phytoplankton are "autotrophs," meaning they are capable of synthesizing their own food by producing organic molecules from organic and inorganic nutrients via either photosynthesis (fueled by light energy) or chemosynthesis (fueled by chemical energy). Phytoplankton account for roughly half of

the oxygen produced by all plant life on the planet and provide carbon that is the basis for the majority of oceanic, estuarine and terrestrial aquatic food webs (Thurman 1997). The concept of "productivity" is an important aspect of phytoplankton ecology. Primary production is the fixation of organic material from carbon dioxide (CO_2) and water (H_2O). The rate at which this process occurs is productivity. Organic molecules produced, typically glucose or other sugars, are used to synthesize proteins, complex carbohydrates, lipids, and nucleic acids, and other complex compounds, or be respired to perform work. The consumption of primary producers (e.g. algae, macrophytes, etc.) by other organisms, such as invertebrates and fish, transfers phytoplankton production (and the energy stored within) through the food web, supporting productive higher trophic levels such as fisheries, and a vital ecosystem in general. Phytoplankton gross primary production (GPP) is a measure of the rate at which a specified amount of phytoplankton biomass captures and stores chemical energy. Some fraction of this acquired energy is expended for cellular respiration and maintenance of existing tissues (i.e., "growth respiration" and "maintenance respiration"). The remaining fixed energy represents an increase in the biomass of the plankton, referred to as net primary production (NPP).

NPP = GPP - respiration

When light, nutrient availability and other factors are favorable for phytoplankton growth and reproduction, cells can rapidly multiply and accumulate, a phenomenon referred to as an "algal bloom," or simply "bloom". Phytoplankton blooms are generally quantified by the abundance of cells of each species or taxonomic group or by their total biomass, measured as the amount of carbon or chlorophyll per unit volume of water. Typically blooms are dominated by a small number of phytoplankton species that proliferate when conditions are ideal for their growth.

Algal blooms are a natural phenomenon and part of natural cycles of aquatic ecology. However, some algal blooms are anthropogenically induced or enhanced and have negative impacts on the environment, human health and the economy. These are termed "harmful algal blooms" or HABs. Several aspects of blooms can be noxious to various ecosystem components including mechanical (gill irritation), physical (viscosity and gelatinous barriers), anoxia, the production of toxins and allelopathic deterrents to grazers (Smayda 1997). HABs in coastal waters are increasing in frequency and intensity globally (Smayda 1990, Hallegraeff 1993, Anderson et al. 2002, Glibert et al. 2005a, Glibert et al. 2008), attributed to increased urban nutrient loading, changes in agriculture practices and fertilizer use, increasing aquaculture, overfishing, ballast water discharge and climate change in general (Hallegraeff 2004; Glibert et al. 2005a,b). The increase in loading of nutrients to coastal systems is considered the most serious of these factors on the global scale (Smayda 1990, Anderson et al. 2002, Glibert et al. 2005b).

4.2.1   Importance of Phytoplankton Relative to Other Estuarine Aquatic Primary Producers

There are five major aquatic primary producer groups in estuaries: phytoplankton, macroalgae, microphytobenthos, submerged aquatic vegetation, and emergent macrophytes (see Figure 2.3, Chapter 2). Within an estuary, the relative dominance of each producer group is controlled by factors that favor the physiological requirements of each group or present constraints to their survival (Day et al. 1989)

and these factors vary along a depth gradient from high intertidal to deepwater subtidal. These factors include: 1) light, 2) water depth, 3) temperature, 4) desiccation, 5) water velocity and turbulence, 6) nutrients, 7) organic matter, and 8) grazing by consumers. The interplay of these factors controls the presence and composition of primary producer groups within estuaries and across different types of estuaries.

Phytoplankton require light for photosynthesis and therefore are typically limited to the upper, photic or lighted portion of the water column. In shallower parts of estuaries, where light can reach the bottom, benthic microalgae and macroalgae attached to the bottom generally become more dominant. This is because fixed, attached plant forms have a competitive advantage over phytoplankton, which can be easily flushed out of the estuary by strong river flow and tidal transport, particularly in macrotidal estuaries. Tidal resuspension of bottom sediments can also increase turbidity in the water column, further restricting phytoplankton production in these waters (Monbet 1992). Thus phytoplankton biomass is generally low in meso- and macro-tidal systems (e.g., deltas, estuaries and tidal creeks with mean tidal range >2 m) and benthic macrophytes are commensurately more dominant. In shallow habitats, phytoplankton can be found in codominance with rooted vascular plants, macroalgae and microphytobenthos. In deepwater subtidal habitats, particularly in wave dominated environments, phytoplankton tends to be the dominant primary producer, or co-dominant with microphytobenthos in deepwater habitats with high water clarity (Day et al. 1989, Wetzel 2001). With increasing depth, the advantage of phytoplankton over benthic producers increases, as planktonic forms remain suspended in the water column, with more access to light than autotrophs fixed on the bottom.

4.2.2   Composition of Estuarine Phytoplankton in California Estuaries

The composition of phytoplankton is generally described by size class, species, or taxonomic composition. Size class is nominally defined as microplankton (>20 µm), nanoplankton (<20 µm) and picoplankton (< 2 µm) (Dussart 1965). Nanoplankton are generally most abundant in coastal waters by number, and microplankton typically dominate by biomass (Day et al. 1989). Microplankton are less efficient than nanoplankton in taking up nutrients at low concentrations and therefore bloom when nutrient concentrations are high. They are vulnerable to sedimentation and transport out of the euphotic zone by advection, and must be able to grow and reproduce rapidly when conditions are conducive.

An important feature of phytoplankton communities in aquatic habitats is the presence of several algal species simultaneously. In some cases, one species dominates, though more often two or more species co-dominate. The species composition of phytoplankton within an estuary is dependent on environmental factors including salinity, ratios of macro- and micro-nutrients, turbidity, turbulence and depth. Species abundance varies spatially, seasonally and vertically depending on physiological requirements relative to the physical, chemical and biological constraints that control reproduction, growth and loss by advection, death and grazing.

Dominant species can change spatially within a waterbody, seasonally and even vertically as physiological requirements and constraints (physical, chemical and biological condition) that control

growth, reproduction and removal (die-off and grazing) change. This leads to the spatial and temporal variability observed in phytoplankton taxonomic composition and biomass observed in estuaries (Day et al. 1989).

The major taxonomic groups of phytoplankton include prokaryotic cyanobacteria and eukaryotic algae (e.g. diatoms, Dinoflagellates, Cryptophytes, Chlorophytes (green algae), and Dhrysophytes (golden brown algae). Generally, the dominant groups of phytoplankton in estuaries are diatoms and dinoflagellates, but cyanobacteria can also be prevalent under certain conditions. Diatoms are considered high quality food for consumers such as zooplankton, filter feeders, benthic invertebrates and larval fish (Richardson 1997, Paerl et al. 2007).

In brackish water reaches of upper estuaries, the size class distribution of phytoplankton can indicate the trophic status of the waters. Smaller species tend to be found in oligotrophic environments, and larger size classes such as diatoms are more prevalent in eutrophic regions (Wetzel 2001). This pattern is largely based on the efficiency of nutrient uptake at low concentrations by small cells with high surface area to volume ratios. In nutrient sufficient waters, the efficiency of uptake is not as important to survival and large cells with low surface to volume ratios tend to be favored. These large cells are able to maintain their high cell volumes and biomasses in a high nutrient environment while they are at a competitive disadvantage in low nutrient environments.

To date, with the exception of the extensive amount of phytoplankton work done in the San Francisco Bay there exists little peer-reviewed literature regarding phytoplankton community structure in California enclosed bays and estuaries. In San Francisco Bay, Cloern and Dufford (2005) detected 500 distinct phytoplankton taxa, of which 396 could be identified to species from a decade of monitoring data from the San Francisco Bay. The San Francisco Bay phytoplankton community was dominated by a small number of species in nearly 600 samples. Diatoms contributed 81% to cumulative biomass in all samples. Dinoflagellates (Pyrrophyta) and cryptophytes contributed 11 and 5%, respectively, to cumulative biomass. Other divisions (Chlorophyta, Cyanophyta, Chrysophyta, and Euglenophyta) were minor components of overall biomass when evaluating decadal trends of the phytoplankton community of the San Francisco Bay, although their contributions were important in some individual samples. Despite the persistent nutrient loading of San Francisco Bay, few HABs have occurred recently, apparently because nutrient enriched turbid conditions in the estuary favor larger celled diatoms associated with new production as opposed to regenerated nutrients (Cloern 1996, Ning et al. 2000). However, there have been occasional historical blooms (see Cloern et al. 1994 referenced in Cloern 1996), and recently cyanobacteria and dinoflagellate blooms have been documented. For example, blooms of the cyanobacteria Microcystis aeruginosa have been occurring in the late summer/autumn in the northern reaches of the Bay since 1999 (Lehman et al. 2005), the radiophyte *Heterosigma akashiwo* created a red tide in the Central Bay in summer 2002 (Herndon et al. 2003), the dinoflagellate Akashiwo *sanguinea* caused a red tide in the Central and South Bay areas during September 2004 (Cloern et al. 2005a). For a complete review of phytoplankton in SF Bay, see McKee et al. (2011), Cloern and Dugdale (2010) and Cloern et al. (2005a).

Generally speaking, less is known about phytoplankton community composition in intermittently or ephemerally tidal estuaries in the "closed" condition, when water depths increase with the formation of a sandbar at the mouth, typically during summertime low-flow conditions. In a two year study of five ICOLL estuaries in Central California, over 137 species were identified diatoms as the most common phytoplankton taxonomic group identified (CLEAP 2008). Among these estuaries, the most common phytoplankton species based on total cell abundance were *Merismopedia warmingiana* (Cyanophyte), *Cryptomonas sp*. (Cryptomonad), and *Planophila laetevirens* (Chlorophyte). The most common individual taxa in the lagoons based on biovolume were *Cryptomonas sp.* (Cryptomonad), *Cystodinium sp.* (Dinoflagellate), and *Cryptomonas marssonii* (Cryptomonad), the latter which are typically a good food source for zooplankton communities due to the relatively elevated fatty acid content of this phytoplankton. In lagoons with higher nutrient availability phytoplankton communities in most instances had a very low diversity (<0.2 Simpsons Index of Diversity) and were dominated by a few dinoflagellate species such as *Gynmnodium fuscum*. This species is associated with toxic red tides on the east coast, English Channel and Gulf of Mexico (Paerl 1988). In Southern California, a one-time survey of 12 intermittently tidal and ephemerally tidal coastal lakes and lagoons in 2009 indicated that cyanobacteria were the dominant taxonomic group, with the majority dominated by the harmful algal species *Microsystis spp*. and microcystin toxin was detected in the majority of sites (Magrun, unpublished data).

4.2.3 Factors Controlling Variability in Phytoplankton Biomass, Productivity and Composition

Phytoplankton biomass, productivity, taxonomic composition, and relative abundance are known to vary from estuary to estuary and, within an estuary, both spatially and temporally (Cloern and Jassby 2008, Cloern and Jassby 2010). Spatio-temporal variability can be attributed to two factors: 1) those controlling the synthesis of new biomass (light availability, water temperature, and available nutrients) (e.g. Cloern 1999) and 2) those that control the fate of cells once produced (turbulence, grazing, mortality, loss to coastal ocean, settling, etc.; Cloern and Dugdale 2010). These factors regulate phytoplankton assemblages at the scale of the individual cell to the overall community assemblage.

For phytoplankton to photosynthesize, they require light and therefore must occupy the well-lit surface layer (termed the "euphotic zone") of an aquatic waterbody. Light is one of the most important variables controlling rates of phytoplankton photosynthesis. Light radiation in the range of wavelengths from 400-700 nm, referred to photosynthetically active radiation (PAR), provides the dominant source of energy for autotrophic organisms. Light is reflected, adsorbed, and refracted by dissolved and suspended substances in the water and by water itself. Phytoplankton itself diminishes available light and is a component of the turbidity of surface waters, causing self-shading and reducing light available to other primary producers. The response of phytoplankton photosynthesis to light (the photosynthesis-irradiance or P-I curve) is both plastic, based on conditions and light history, and it is species-specific, with a range of adaptation to both very high irradiance levels and low light. These optimal light ranges control to some extent the relative dominance of species and the amount of biomass produced. Phytoplankton often exhibit some degree of photo-inhibition of photosynthesis at high light levels and thus maximum chlorophyll may occur at some depth below the surface (Day et al. 1989). Photosynthesis decreases with depth as light decreases, so as vertical mixing transports cells below euphotic depth

reduces the rate of photosynthesis and production. For this reason, estuaries that are stratified and confine vertical transport to an upper mixed layer are often more favorable for phytoplankton production (and blooms) than well-mixed estuaries.

Photosynthesis requires a basic suite of macro-nutrients (nitrogen, phosphorus, and in some cases silicate) and micro-nutrients (Cu, Mo, Fe, Mn, Ca, and vitamins such as B-12, etc.). In general, phytoplankton take up macro-nutrients in proportions of 106 molecules of carbon (C), 16 molecules of nitrogen (N), 15 molecules of silica (Si) and one molecule of phosphorus (P) in proportions related to the photosynthetic synthesis of organic carbon, otherwise known as the Redfield ratio (Redfield 1934). Thus ratio of the nutrients available in addition to the quantity of nutrients supplied affects the timing, frequency and magnitude of algal production. Dissolved inorganic N and P are most often preferred, but many species assemblages utilize dissolved organic forms of nutrients, particularly in oligotrophic estuaries (ref). When the concentration of one or more resources is present in less than saturated concentrations, that substance can become limiting to production (Monod 1942). The efficiency at which a particular phytoplankton species can uptake nutrients is concentration-dependent and cell size dependent. Smaller species typically found in oligotrophic habitats typically have higher surface-to-volume ratios making it easier to thrive in low nutrient environments. Larger species typically found in coastal waters and estuaries have lower surface-to volume ratios and thrive in high nutrient environments.

Uptake of nutrients by phytoplankton is regulated by temperature, by controlling metabolic rate and enzyme-mediated reactions within the cell. Temperature response curves for photosynthesis and growth are similar for most algal species studied (Eppley 1972), with a rapid decline in production at temperatures in excess of and below the optimal range. Temperature exerts a selective effect on species with different optima, and species distribution can reflect in part an adaptation to particular temperatures. Temperature also controls rates of biogeochemical processes in the system as a whole, for example, mediating the rate of microbial decomposition of organic matter and sediment diagenetic processes, influencing rates of nutrient remineralization and nutrient availability (Kennedy 1982).

The interaction of nutrient availability, temperature and light is important as the ultimate control on photosynthesis; the overall rate of any chemical reaction proceeds at the rate of the slowest or limiting step (Leibig's Law of Limiting Factors). Transient periods occur when more than one factor may be limiting, though replenishment rates differ, so one factor eventually becomes limiting. If a bloom is dominated by a single species, then determination of limiting factors can be relatively straightforward; if multiple species are involved, then limiting factors may be a complex due to differences between species in optimal light, temperature, nutrient ratios, as well as other factors such as niche diversification. Even under scenarios of high nutrient loading, phytoplankton productivity may be limited by low light or temperatures. Thus, in seasonally variable coastal estuarine systems the availability of the limiting nutrient (typically N) can change dramatically due to the complexity of interacting physiographic characteristics such as wind mixing, tidal mixing, air temperatures, etc. In addition, coastal estuaries can be extremely spatially heterogeneous in morphology, geology, solar exposure, circulation, salinity etc resulting in significant spatial variability in phytoplankton biomass and composition at any point in time.

Beyond factors limiting photosynthesis and factors constraining the environmental tolerances of particular species (salinity, pH, alkalinity), the biomass of the phytoplankton community is also controlled by: 1) mortality (natural, disease or virus induced); 2) grazing losses; 3) turbulent mixing by tide and wind-induced motions in the water column; 4) sinking and deposition of phytoplankton biomass on the bottom sediments; 5) resuspension of bottom-deposited microalgae by tidal currents and wind waves; and 6) export from the system. Thus, the net phytoplankton biomass at a point in time is potentially influenced by a variety of inputs and losses. In perennially tidal estuaries, these processes are highly variable and can account for extreme fluctuations in phytoplankton biomass over tidal cycles and spatially within the estuary. In intermittently and ephemerally tidal estuaries in "closed" condition, phytoplankton populations can be more stable and thus resemble typical variability observed in lacustrine environments. Coupled hydrodynamic – ecological models have been developed to predict phytoplankton production, biomass and transport when site-specific factors can be accounted for (e.g., Cerco 1995, Cerco and Moore 2001, Madden et al. 2008, Sheng 2008).

Grazing by zooplankton, invertebrates (e.g., filter feeders), and planktivorous fish can have a significant influence on temporal and spatial phytoplankton community dynamics. Zooplankton grazers and benthic filter feeders may consume significant amounts of phytoplankton biomass and primary productivity in some estuaries. For example, Li and Smayda (1998) reported that, over long time periods (1973-1990), the phytoplankton biomass in Narragansett Bay, Rhode Island, was controlled by zooplankton grazing. Lewitus et al. (1998) found that microzooplankton grazing during the summer months in the North Inlet Estuary, South Carolina, was an important regulator of phytoplankton biomass. In Fourleague Bay, Louisiana, Dagg (1985) estimated zooplankton grazing rates that were nearly equal to the phytoplankton standing stock in this estuary. Therefore, the standing stock of phytoplankton in the estuary can be conceptualized as the product of a dynamic balance between bottom- up and top-down control mechanisms. Filter feeders such as mussels, clams or oysters are among the most efficient grazers of phytoplankton (Day et al 1989). In South San Francisco Bay, the invasive Asian clam (*Corbicula flumenia*) significantly controls phytoplankton biomass and can impede bloom formation, despite high nutrient loading (Cloern 2001). Historical records indicate a causal linkage between the decline in annual average chlorophyll-a concentration and the arrival of the Asian clam. However, each spring neap tidal cycles are coupled with low wind conditions that stratify the water column, decoupling the grazers from the surface waters and the South San Francisco Bay experiences extensive spring phytoplankton blooms (Cloern 2001). Estuaries with established filter feeder communities likely possess less frequent and lower magnitude phytoplankton bloom conditions as a result of these ecological interactions. The net increase in production can be due to an increase in production rates due to increase temperature, light or limiting nutrient availability, or a reduction in the relative magnitude of loss terms such as reducing mixing or grazing (Cloern 1996).

Hydrologic residence time has a strong influence on the spatial and temporal variability of phytoplankton community community structure and biomass in estuarine systems. Several studies have found that elevated flows and reduced residence times directly reduce the ability of any phytoplankton groups to accumulate biomass and form blooms. Monbet (1992) found that the degree of tidal mixing had a profound impact on the average water column chlorophyll-a concentrations across coastal

systems for any given nitrate load. Cloern (2006) attributed the relatively low average concentrations of chlorophyll-a in North San Francisco Bay to the effects of tidal mixing, despite similar annual nitrate concentrations and loads there as in Chesapeake Bay and South San Francisco Bay where high chlorophyll concentrations are measured. Paerl et al (2003) documented shifts in dominant taxonomic groups in response to seasonally different estuarine flushing rates in the Neuse Estuary. Little data exists on the relationship between community composition and residence time in California estuaries.

Variability in Phytoplankton Blooms

The factors that control algal blooms have different time- and spatial scales of variability, so algal blooms can be recurrent seasonal phenomena, short-term episodic events, or very rare as when associated with exceptional climatic or hydrologic conditions. Generally, the temporal patterns in phytoplankton abundance generally vary by latitude (Day et al. 1989). In temperate latitudes, phytoplankton biomass often exhibits peak biomass in spring and fall, with the spring peak generally larger. Spring blooms are usually associated with river discharge and with increasing temperature, or from strong upwelling events. An example of this is the predictable spring phytoplankton bloom that occurs in the South San Francisco Bay each spring as a result of natural water column stratification from seasonal circulation changes that decouples of benthic grazers with the surface phytoplankton community (Cloern 2006). In relatively deep estuaries dominated by spring runoff and strong exchange with the coastal ocean, peak phytoplankton blooms can occur in relationship to coastal upwelling or spring river discharge, and summertime blooms associated with benthic recycling of nutrients is less important.

In subtropical or Mediterranean systems, seasonality is less pronounced and peak blooms can be associated with river nutrient discharge, which can be seasonally variable. High production rates occur in shallow or stratified systems during the summer. Extended periods of stratification from early spring through late summer allow for a long phytoplankton growing season (Sinclair et al. 1981). In many shallow systems, dominant summertime blooms may be controlled by temperature-regulated nutrient regeneration from sediments (Kemp and Boynton 1984). Blooms are more likely to develop during periods of weak tidal energy, and dissipate during periods of strong tidal energy. In intermittently or ephemerally tidal estuaries in California, which are typically closed during portions of the summertime, the summer low flow conditions during warmer climatic periods present conditions more favorable to induce phytoplankton community blooms.

Phytoplankton biomass and community composition can be highly variable and difficult to predict, particularly in response to extreme climatic events. Chlorophyll measurements on 15 minute time scales in estuarine systems illustrate extreme daily fluctuations in phytoplankton biomass due to different concentrations associated with ebb and flood tides, vertical migration or grazing. At the decadal timescale, episodic events such as hurricanes in Neuse Estuary in North Carolina have caused significant blooms by transiently increasing N loading from the watershed. Variability in the phytoplankton presents a challenge to its use as an indicator of eutrophication. In perennially tidal estuaries, where the signal-to-noise ratio is lower, the use of phytoplankton biomass or community composition would require long term monitoring to document the typical spatial and temporal patterns, sources of

variability and factors driving variability. In intermittently or ephemerally tidal estuaries in a "closed" condition, this signal-to-noise ratio is typically better as tidal forcing is greatly reduced.

4.3   Effects of Altered Phytoplankton Biomass and Community Structure on Ecosystem Services and Estuarine Beneficial Uses

Phytoplankton provide numerous ecosystem services and have a direct relationship to many estuarine beneficial uses designated by the State of California. The primary services to the ecosystem provided by phytoplankton can be classified as food-based, nutrient uptake and sequestration, and the oxygenation of the water column. Perturbation of the natural phytoplankton community can result in the impairment of one or more of these essential functions. Phytoplankton are a major primary producer in many enclosed bays in California and provide the ultimate source of carbon underpinning many coastal benthic and pelagic food webs. Because of estuarine dependency of many marine species, the nursery and trophic benefits of estuaries extend far out into the coastal zone and marine realm, supporting many commercially and recreationally important open ocean marine and estuarine fish and invertebrates. Valued species may feed either directly on the phytoplankton, on the consumers of phytoplankton, or on detrital carbon derived from phytoplankton production. For example, in San Francisco Bay, many zooplankton, mysid shrimp, and clam communities, are limited by carbon productivity of the primary producer community (Cloern et al., 2003) and it is calculated that the rate of carbon production there is sufficient to keep pace with consumption only during periodic phytoplankton blooms (Cloern 1996).

Many commercially and recreationally (COMM) important fisheries species have a life-history stage that is estuarine dependent; examples include, salmonids, smelt, herring, Dungeness crab, shellfish (Blackmon et al. 2006). Phytoplankton also support shellfisheries (SHELL) and aquaculture (AQUA), as a variety of bivalves used for bait and human consumption are based on healthy phytoplankton communities. The biomass and community composition of phytoplankton can influence the population structure and growth rates of invertebrates and fish (Peterson et al. 1984). Phytoplankton is also important in supporting migratory birds and spawning fish during critical life stages, thus linking to MIGR, SPWN, and RARE beneficial uses.

As the magnitude of nutrient loading to estuaries increase or the ratios of nutrients (C:N:P:Si) change, phytoplankton biomass increases, favoring phytoplankton species tolerant of or favored by high nutrient levels (Figure 4.1). Under conditions of sustained loading, four basic ecosystem effects may occur:

- Increased accumulation of organic matter in waters and sediments, resulting in higher biological oxygen demand, increased activity of heterotrophic bacteria, including pathogenic bacteria, and poor water quality and poor benthic and pelagic habitat quality
- Shifts in taxonomic composition of phytoplankton to poorer quality assemblages, altered trophic energy transfer and food web support, and
- Increased frequency and duration of harmful algal blooms
- Decreased light penetration and reduced light availability to benthic primary producers
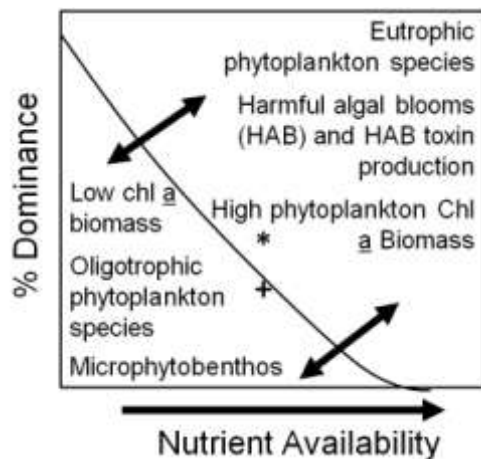
Figure 4.1 Conceptual model of relationship between nutrient availability and relative dominance of primary producers in California estuaries by major habitat type: (a) intertidal flats, (b) shallow subtidal and (c) deepwater or turbid subtidal.

4.3.1 Degradation of Pelagic and Benthic Habitat Quality

Under conditions of persistent algal blooms, one potential consequence is a higher concentration of organic matter in the water column and, ultimately, in benthic sediments (Brussard et al. 1996). The resulting increase in oxygen demand can result in hypoxic or anoxic conditions that cause stress to benthic and pelagic organisms. As heterotrophic microbes consume the organic matter from the primary producers, oxygen is removed by aerobic microbes and reduced compounds are created as metabolic byproducts. Even in natural, non-eutrophic conditions, these processes occur in both muddy and sandy sediment environments and the fauna are adapted to deal with low-oxygen, reducing environments. As the amounts of organic matter produced and accumulated in the system, the low oxygen and reduced conditions begin to either smother or poison the benthic fauna. Increased sediment organic matter accumulation can affect benthic habitat quality via microbially-mediated, indirect paths of water column hypoxia/anoxia or the accumulation of toxic reduced sulfides and ammonia in the sediment. These processes lead to changes in the abundance and composition of the macrobenthic community (see Chapter 7 for detailed information) and eventually lead to azoic conditions.

The effects can include decreased growth rates, poor reproduction, larval recruitment, impaired foraging behavior, migration of motile species out of the affected area and elevated mortality. Anoxia has been implicated as a cause of widespread water quality and ecological impacts throughout the world (Nixon 1995, Diaz 2001). Many invertebrate and fish species become stressed in low oxygen conditions (<3 mg L^{-1}), making them more susceptible to diseases and death (Theede 1973, Diaz 2001). Fish, shellfish and benthic organisms cannot survive in anoxic conditions (DO=0 mg L^{-1}) or even hypoxic (DO<=2 mg L^{-1}) for extended periods of time. Increased heterotrophic bacterial activity in the surface waters is associated with increased pathogenic bacteria concentration which can contribute to a decline in water quality and increased transmission of waterborne illness affecting human and animal health

through primary contact during recreation activities (REC-1). These changes also adversely impact aesthetics (REC-2) through nuisance buildup and smell during decay.

4.3.2   Changes in the Taxonomic Composition of Phytoplankton

Studies have shown that the taxonomic composition of phytoplankton communities change as a function of increased nutrient availability primarily related to differential nutrient kinetics, nutrient storage, and the growth and loss rates of the community. In low nutrient estuaries, phytoplankton is typically characterized as having low biomass with taxonomic composition dominated by species adapted to oligotrophic conditions. Primary productivity in these systems may be dominated by benthic microalgae rather than phytoplankton (Underwood and Kromkamp 1999). With moderate nutrient enrichment, biomass becomes higher and more seasonally variable, with often with moderate increases in diversity and a shift towards more nutrient tolerant species. As the estuary becomes more increasingly eutrophic or hypereutrophic, phytoplankton can sustain very high algal biomass, with dominance by very few nutrient tolerant taxa and larger individual cells (Figure 4.1). Shifts in the dominant size class of phytoplankton can also occur with increasing eutrophication.

In general, larger sized plankton, such as diatoms, are favored in higher concentration, variable nutrient environments where it is advantageous to sequester and store nutrients when available. However, the relationship between cell size and functional group is complex, as there are many factors involved. As nutrient concentrations increase, at a certain point, silica can become limiting, and diatoms are unable to utilize all of the excess N and P. Silica is provided at a relatively constant concentration as it is dependent on the upstream weathering of rock material in the watershed. The effect of further increases in N and P then are to favor phytoplankton forms that are able to grow without using silica. Often these species are less desirable as a food source and food web base than diatoms. Malone (1982) showed that small forms had 3-5 times higher alpha (efficiency) and maximum light-limited growth rate than large forms such as diatoms, making the smaller celled taxa more growth-efficient at lower nutrient concentrations.

The benefits of enhanced primary production during blooms are directly correlated with the species that dominate the bloom. Size of the phytoplankton determines largely whether the linear foodchain is dominant or the microbial foodweb is more important (Underwood and Kromkamp 1999). Larger, readily grazed phytoplankton (e.g. most diatoms and chlorophytes and some dinoflagellates) dominate the community the trophic transfer of carbon and energy will occur within the water column as a result of grazing (Paerl et al 2003). For example, in San Francisco Bay, large cell diatom production tends to fuel the pelagic food web supporting zooplankton including jellyfish, filter feeding shell fish and crustaceans, fishes, and mammals including humans. Blooms of smaller-celled flagellates can lead to suppression of herbivores (Cloern 1996, Ning et al. 2000, Cloern et al. 2005b).

The efficiency of energy transfer from phytoplankton to consumers and ultimate production at upper trophic levels varies with species composition of the algae. Brett and Müller-Navarra (1997) estimated that diatom-dominated marine upwelling systems sustain 50 times more fish biomass per unit of phytoplankton biomass than cyanobacteria-dominated lakes. If internal energy transfer is poor, the

production of phytoplankton tends to be diverted through the microbial foodweb, known as the microbial loop (Azam et al. 1983). This pathway short circuits the transfer of trophic energy to higher trophic levels and essentially wastes energy through inefficient diversion through the microbial community, producing less useable carbon biomass for desirable trophic groups such as invertebrates and fisheries. Further, with excessive microbial biomass production and higher rates of metabolism and water column respiration, this pathway tends to exacerbate the formation of hypoxic and anoxic conditions, creating deleterious conditions for potentially the entire ecosystem.

4.3.3. Effects Associated with Harmful Algal Blooms

Harmful algal blooms (HABs) are blooms of phytoplankton, macroalgae, or cyanobacteria that cause negative effects in human populations, beneficial uses of the estuary, or ecosystem processes. HABs can produce a variety of responses, such as the production of potent chemical compounds that are toxic to humans or other consumers, nuisance levels of biomass that reduce light, visual efficiency, consume oxygen. HABs also tend to be dominated by one or a small number of taxa, reducing trophic efficiencies, and increasing instability in the ecosystem.

In recent years, HABs have increased in frequency and distribution throughout the world; they have been reported in nearly every US coastal state (Anderson et al 2008, Glibert et al. 2008). The ecological impacts of HABs include impairment of fisheries, shellfish, and other related ecosystem Beneficial Uses. Human health is a significant concern with respect to ingestion of toxins accumulated in shellfish (SHELL and COMM) as well as recreational impacts (REC1 and REC2) when contact, respiration or direct ingestion may result in skin, eye, respiratory irritation and neurological damage (Burkholder et al. 1993, 2002; Glibert et al. 2008). HABs can result in hypoxia, shellfish disease, fish kills and the mortality of other aquatic species (Glibert et al. 2002), thus impacting beneficial uses associated with SHELL, AQUA, COMM, and MUN. *Alexandrium* spp., *Gymnodinium catenatum, Pyrodinium bahamense,* and *Karenia brevis* can create significant blooms or "red tides" which result in toxin accumulation in shellfish that can cause illness in humans if ingested. Blooms of the dinoflagellate species *Pfiesteria piscicida (P. piscicida)* have co-occurred with fish kills and human illness reports and in 1998, Congress appropriated funds to the Centers for Disease Control and Prevention (CDC) to address concerns about human health effects possibly associated with exposure to *P. piscicida. Lyngbya* is a toxic cyanobacteria (blue-green algae) that has been documented to cause eye, skin and respiratory irritation in exposed humans.

The increased frequency and duration of some harmful algal bloom species has been attributed to the cultural eutrophication of coastal waters as a result of human practices (Paerl 1988, Hallegraeff 1993, Paerl 1997, Richardson 1997). The frequency of blooms of red tide species (*K. mikimotoi, G. polygramma, N. scintillans* and *P. minimum=cordatum*) showed a direct correlation with increases in population in the watershed and the resultant increase in nutrient loading in Hong Kong (Lam and Ho 1989). In the European North Sea, alteration of nutrient loads and changes in nutrient stoichiometry were linked to shifts from the normal spring and fall siliceous diatom blooms to dominance by nuisance flagellate species (Officer and Ryther 1980, Ryther and Dunstan 1971, Symada 1990). For estuarine or marine HAB species typically found in California, there is a relative dearth of knowledge of controls on

abundance and of the production of toxins. Therefore, the ability to use HABs as NNE indicators is limited at this time.

Proliferation of cyanobacteria in freshwater and oligohaline portion of estuaries are often driven by decreases in the N:P ratio of available nutrients, as well as hydrological conditions favoring low-turbulence (e.g., Peel-Harvey Inlet, Hilman et al. 1990). Cyanobacterial blooms such as *Nodularia* or *Microsystis* can produce heptatoxic peptides, while species such as *Anabaena* or *Aphanizomenon* can produce neurotoxins that can kill domestic and wild animals. These toxins can accumulate shellfish or drinking water, with teratogens and tumor promoters as an important concern to humans (Hallegraeff et al. 2003.). Studies found that Microcystis thrives when the ratio of N:P falls below 15 (Havens et al. 2001). When the ratio of N:P is greater than 20:1, non-toxic algae predominate. Mixing of the water column and lower water temperature decrease the conditions that form toxic Microcystis blooms. In rivers, any actions that increase flow rate will minimize the conditions which foster blooms. These results imply that in fresh reaches of upper estuaries, implementation of nitrogen reduction measures without a simultaneous decrease of phosphorus can lead to unanticipated and negative shifts in community structure.

In general information on the prevalence of HABs in California estuaries is limited, with the exception of San Francisco Bay and the Delta and limited work in Elkhorn Slough. Microcystis aeruginosa blooms have occurred in the Delta and the North San Francisco Bay during July through November of each year since 1999. Several surveys of M. aeruginosa blooms conducted in the Northern San Francisco Bay and the Sacramento and San Joaquin Delta have documented that the blooms can be widespread, often with microcystin concentrations that exceed World Health Organization guidelines for risks to humans and wildlife (e.g., Lehman and Walker 2003; Lehman et al. 2005, 2008). For example, Lehman et al. (2005) documented that an extensive M. aeruginosa bloom was found to extend 180 km from Benicia to near Rio Vista on the Sacramento River to 20 km downstream from Tracy on the San Joaquin River side of the Delta, with toxicity exhibited at all stations. Concentrations of microcystins were measured in greater concentrations in zooplankton and clam tissue relative to algal tissue although concentrations were not greater than lethal limits known to cause acute death (Lehman et al. 2005, 2008). Concentrations found in lower Sacramento River may be chronically obstructive to food quality, feeding ability, growth, and fecundity in zooplankton (Lehman et al. 2008). Recently, Miller et al. (2010) found evidence of microcystin toxicity in Dead Sea otters in Monterey Marine Sanctuary, attributed to a land-sea trophic transfer through marine invertebrates as the most likely route of exposure. These studies appear to support the hypothesis that microcystins are transferred or perhaps biomagnified in the food web (Lehman et al. 2008). Change in flow regime may influence the prevalence of cyanobacteria. Given M. aeruginosa seems to prefer high light and warm shallow water eutrophic conditions, any change in the management of the flows from the Sacramento River that leads to increased or more persistent but steady flow rate and improved salinity stratification may expand the population in the late summer/autumn.

In seasonally tidal lagoons in the Central Coast California with higher nutrient availability, phytoplankton blooms were dominated by a few dinoflagellate species such as Gynmnodium fuscum, the species that has been associated with toxic red tides on the east coast, English Channel and Gulf of Mexico (Paerl

1988). In Southern California, a one-time survey of 12 intermittently tidal and ephemerally tidal coastal lakes and lagoons in 2009 indicated that cyanobacteria were the prevalent taxonomic group in these blooms, with the dominant species being Microsystis spp. The microcystin toxin was detected in the majority of sites (Magrun, unpublished data).

While increased anthropogenic nutrients increase the potential for development of some HAB species, the conversion of nutrients into biomass is also dependent on other factors including presence of required trace metals and chelating agents, water clarity, temperature, physical factors such as water column stability and stratification, and the presence of seed populations.(Cloern et al. 2005, Hallegraeff et al. 2003). In a well-documented case in New Jersey, a brown tide caused by the chrysophyte picoplankton *Aureococcus* has been related to discharge of chelators in detergents and lawn fertilizers, together with suppression of zooplankton grazing by pesticides. The bloom was responsible for reduction in both eelgrass bed habitat and the commercial scallop that utilize them (Cosper et al. 1993). Suppression of zooplankton grazing by overharvesting of piscivorous fish can release HAB species from grazing pressure. Thus an understanding of drivers for eutrophication is more complicated than can be captured by a monitoring program that revolves around phytoplankton biomass and nutrients.

4.3.4   Decreased Light Penetration to Benthic Primary Producers

However, under continued high nutrient loads, nutrient-tolerant species of phytoplankton are superior competitors to microphytobenthos or submerged aquatic vegetation such as seagrass, so increased phytoplankton abundance can be deleterious to these benthic primary producers. Initial indications of eutrophication issues include decreased SAV bed density and increased abundance of macroalgae. Under very high nutrient loading, the system can become dominated by algal competitors (phytoplankton, epiphytes or macroalgae) resulting in the degradation or loss of rooted vegetation and the seagrass community (for more detailed explanation, see Chapter 5 on Effect of Eutrophication on Seagrass and SAV). The primary mechanism of seagrass loss is through light reduction caused by shading or smothering from algal competitors Short et al. 1991, 1995; McGlathery 2001; Havens et al. 2001).

Seagrasses have a high light requirement relative to other aquatic autotrophs, principally because they have a high non-photosynthetic biomass in belowground biomass to support. Moreover, the direct rooting of SAV in benthic sediments exposes plants to a reduced environment; additional oxygen demand to maintain the rhizosphere in an oxidized state requires higher levels of photosynthetic production and energy expenditure than in non-vascular aquatic plants. Reduced light from excessive photosynthetic growth creates a light deficit that impairs and eventually kills rooted SAV while macroalgal competitors, generally considered a lower quality habitat, thrive. Coupled with increased delivery of labile organic detritus to sediments from senescent macroalgae and phytoplankton the enrichment of water column phytoplankton can amplify other stressors such as hypoxia/anoxia and sulfide toxicity that further exacerbate the hostile environmental conditions for SAV. Thus, a positive feedback loop develops between nutrient enrichment of the water column and eutrophic or dystrophic conditions. Degraded seagrass beds tend to be sparse or patchy, heavily epiphytized with macroalgae and experience large diurnal swings in dissolved oxygen concentrations. A hysteresis effect sometimes

occurs when the SAV are eliminated, the bottom becomes denuded and sediments are more easily suspended into the water column, further reducing light penetration.

Degradation of seagrass habitat by phytoplankton clearly affects estuarine beneficial uses. Seagrass and SAV are designated marine and/or estuarine habitat that have an obligate requirement for seawater (MAR and EST BUs). They are also wildlife habitat particularly waterfowl and shorebirds (WILD BUs). Seagrass and SAV beds function as habitat and nursery areas for commercially and recreationally important open ocean marine and estuarine fish and invertebrates, and provide critical structural environments for resident bay and estuarine species (COMM, MAR, EST). Seagrass also support shellfisheries (SHELL), as a variety of bivalves used for human consumption and bait occur in seagrass beds. Besides providing important habitat for fish, seagrass and SAV are considered to be an important resource supporting migratory birds and spawning fish during critical life stages (MIGR, SPWN and RARE beneficial uses). Seagrass and SAV meet the spawning BUs as they provide a refuge for anadromous fish (salmonids), particularly during the transition from freshwater to seawater (see reviews in Kennedy 1982 and Blackmon et al. 2006), thus linking to SPWN, and RARE BUs. Seagrass and SAV habitat provide a direct food source for migrating waterfowl such as Brant geese (Ward 1983, Derksen and Ward 1993, Moore et al. 2004). Healthy Seagrass and SAV support REC-2 BUs in a number of ways. These habitats are prime areas for recreational crabbing and fishing as well as kayaking and waterfowl hunting. Seagrasses are a source of primary production in nearshore marine systems, underpinning detrital-based food webs. Eelgrass beds are also a source of secondary production and can have up to 15% greater secondary production and greater species richness (Orth et al. 2006, Ferraro and Cole 2007) than mudflats and sand flats. Additionally, these communities stabilize sediments and improve water quality. It can readily be understood that the effect of excessive phytoplankton growth will have a variable effect on the benthic producer community depending on the species of SAV, the water depth and the sediment type, even in different areas within the same estuary.

4.3.5   Approaches to Setting Numeric Endpoints Based on Phytoplankton

Thresholds for Phytoplankton Biomass in Seagrass

Among examples found in the literature, the approaches used to set thresholds for phytoplankton biomass in seagrass have the greatest number of examples for how it is applied in a regulatory context. Light available to seagrass for photosynthesis has been determined to be the major criterion limiting their distribution because of their high light requirements (15-25% surface irradiance) compared to that of other aquatic primary producers such as algae and phytoplankton (<5% surface irradiance). Several examples of numeric criteria exist for seagrass habitat throughout the US, derived through the use of biooptical models to that link chlorophyll-a biomass, turbidity or total suspended sediment (TSS), and colored dissolved organic matter (CDOM) to light limitation for photosynthesis of seagrass beds at given depths (e.g., Janicki et al. 2000, Kemp et al. 2004, Brown et al. 2004, Kaldy et al. 2011). Figure 4.2 shows an example of the output of a biooptical model that shows the relationship between chlorophyll-a and TSS relative to acceptable light attenuation for North River North Carolina (Biber et al. 2008). Bio-optical models predicting light attenuation under various environmental conditions have been calibrated for the Chesapeake Bay (Gallegos 2001), Indian River Lagoon in Florida (Gallegos and Kenworthy 1996), and

North River in North Carolina (Biber et al. 2008), Yakina Estuary in Oregon (Brown et al. 2007), and Tampa Bay in Florida (Janicki et al, 2000). Table 4.1 summarizes the numeric thresholds for chlorophyll-a and light attenuation/water clarity derived using a biooptical models. Other use of biooptical models is to determine the suitability for SAV survival of water quality conditions not encountered in existing field conditions.
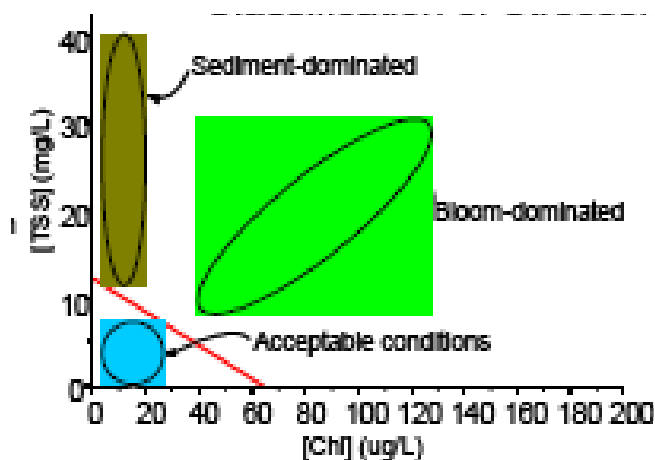
Figure 4.2. Output of a biooptical model showing relationship between chlorophyll-a turbidity and minimum light requirements for seagrass growth (Biber et al. 2008). Stressors related to water-quality conditions fall into turbidity-dominated (brown), or chlorophyll = phytoplankton-dominated (green) regions. Acceptable water-quality conditions (blue) for SAV occur below the red line of constant light attenuation (minimum light requirement).

Table 4.1. Examples of chlorophyll-a and light requirements for seagrass habitats in various estuaries.

Location	Chl a (ug L^{-1})	Light Requirement	Source
Chesapeake Bay[4]	< 15	9-15% attenuation of surface irradiance	Batiuk et al. 2001, 1992
Yaquina Bay	< 3-5^{-1}	0.8– 1.5 m^{-1}, expressed as water clarity	Brown et al. 2007
Tampa Bay	<3.8-9.8	0.65-1.04 m^{-1}, expressed as water clarity	Janicki et al. 2000
Sarasota Bay	<6.1-11.0	Not given	Janicki et al. 2009
Maryland Coastal Bays	<15 in lower bays <60 in upper tributaries	15% attenuation of surface irradiance	Wazniak and Hall 2005

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[4] Light attenuation is primary requirement, chlorophyll-a and turbidity is secondary

Derivation of seagrass chlorophyll-a numeric endpoints for applicable California estuaries is likely to be an estuary-specific exercise. Explicit studies are needed to understand the precise light requirements of seagrass in California estuaries, which may vary as a function of species, depth and other site-specific factors (Figure 4.3). This information would then be used to develop a biooptical model that could be used to establish a combination of chlorophyll-a thresholds and turbidity to establish levels of light attenuation that will be protective of seagrass habitats, given site-specific factors.
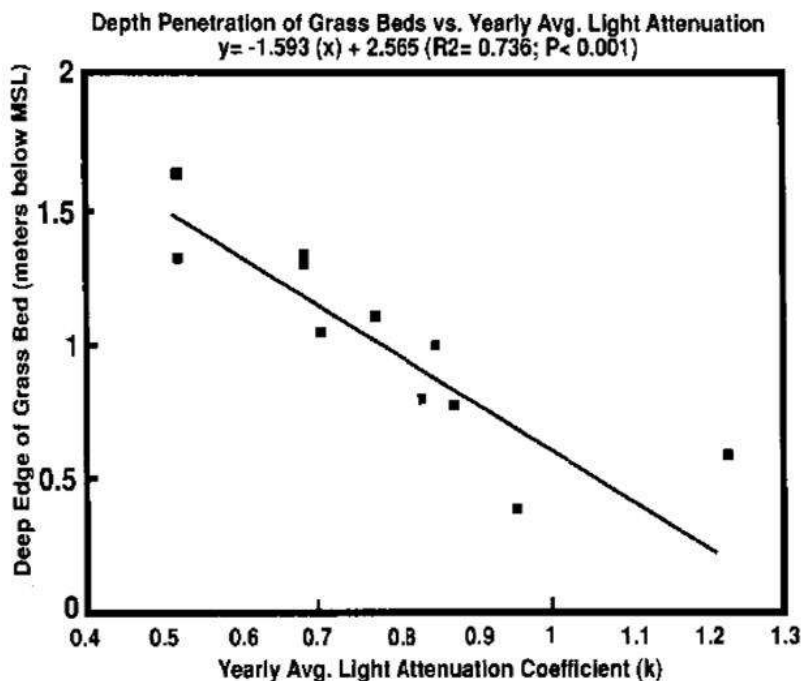
Figure 4.3. Relationship between light attenuation and maximum depth of seagrass beds in Sarasota Bay (Morris and Tomasko 1993).

Tampa Bay Estuary Program has utilized chlorophyll-a, TSS and light attenuation targets to determine nutrient loads that must be reduced in order to achieve the restoration of seagrass habitat in that estuary. Beginning in the 1960s, reductions in effluent nutrient concentrations succeeded in reducing phytoplankton biomass with resulting expansion of seagrass coverage. Steady improvements in treatment of waste water (accounting for about 40% of nitrogen loading) and storm water (accounting for 60%) have improved water quality and water clarity have resulted in SAV recovery to about 90% of the goal through 2010. These reductions have shown marked impact on the chlorophyll concentrations observed in four identified homogeneous regions in the bay, with each segment now meeting its chlorophyll-a numeric target.

For unvegetated subtidal habitats, there is precedent for chlorophyll-a endpoints in various assessment framework (e.g., Soucho et al. 2000, Bricker et al. 2003, Ferreira et al. 2006, Zalidvar et al. 2008) and on phytoplankton productivity (Devlin et al. 2007) to assess eutrophication. Several examples of these assessment frameworks are reviewed in this section.

The NOAA Assessment of Estuarine Trophic Status (ASSETS) is a protocol for evaluating eutrophication based on the National Estuarine Eutrophication Assessment (NEEA) database by Bricker et al. (2003). It is an integrated method for eutrophication assessment that combines an index of pressure from human influence, an assessment of eutrophication status, and an index of the level of management of the system. The OHI (Overall Human Influence) index uses a simple mass balance model based on land nutrient loading and system susceptibility. The OEC (Overall Eutrophication Conditions) index is a symptoms-based evaluation of state calculated by aggregating primary and secondary eutrophication symptoms using a combination matrix. Symptoms are evaluated using logic gates based on chlorophyll-a and macroalgae for the Primary Symptoms Method (PSM) and dissolved oxygen, submerged aquatic vegetation (SAV) loss and nuisance and toxic algal blooms for the Secondary Symptom Method (SSM).

The thresholds for chlorophyll-a employed by the ASSETS protocol were developed with a group of regional experts (Table 4.2; Bricker et al. 2003), though some studies were cited in justification for the break points including: 1) estuaries with highest annual chl $\underline{a}$ less than 5 µg L^{-1} appear unimpacted, with the exception of corrals (Nixon and Pilson 1983, Lapointe and Matzie 1996); 2) at 20 µg L^{-1} chlorophyll-a, SAV shows declines (Stevenson et al. 1993) and phytoplankton community shifts from diverse mixture to monoculture (Twilley et al. 1985); and 3) at 60 µg L^{-1} chlorophyll-a, high turbidity and low bottom water dissolved oxygen are observed (Jaworski 1981). The ASSETS protocol establishes a separate set of chlorophyll ranges for oligotrophic Florida and Biscayne Bays, special cases in the population of US estuaries; nutrient inputs are extremely low in these waters and chlorophyll levels commensurately low as well.

Table 4.2. Thresholds for phytoplankton biomass used in the ASSETS methodology (Bricker et al. 2003).

Eutrophication Category	Threshold for Chlorophyll-a (µg L^{-1})
Low	< 5
Moderate	5-20
High	20-60
Hypereutrophic	> 60

Within the European Union, the Water Directive Framework intends to use phytoplankton biomass, taxonomic composition, abundance, and frequency of plankton blooms as the "biological quality elements in a framework to categorize waterbodies by ecological condition (Zalidvar et al. 2008). Towards this end, an assessment framework has been proposed to use phytoplankton (Souchu et al.

2000; Table 4.3). The Souchu et al. (2000) scheme classifies lagoons into five eutrophication levels, formalized by five different colors from blue, signifying no eutrophication to red, signifying high eutrophication, the same color scheme as used in the Water Framework Directive (Table 4.3) Chlorophyll-a thresholds are based on total chlorophyll-a and phaeophytin, with ranges of total chlorophyll-a from <5 µg L^{-1} in the undisturbed or slightly disturbed categories to >30 µg L^{-1} for highly disturbed or hypereutrophic.

Table 4.3. Thresholds for phytoplankton chlorophyll-a and phaeophytin and other variables in used by Souchu et al. (2000) for the eutrophication assessment of French Mediterranean estuaries in Zalidvar et al. (2008).

YEAR			BLUE		GREEN		YELLOW		ORANGE		RED
Δ%O2 SAT		0		20		30		40		50	
TUR	NTU	0		10		20		30		40	
PO$_4$$^{3-}$	µM	0		0.3		1		1.5		4	
diN	µM	0		15		20		40		60	
NO$_2$ –N	µM	0		0.5		1		5		10	
NO$_3$ –N	µM	0		7		10		20		30	
NH$_4$ –N	µM	0		7		10		20		30	
Chl- a	µgl^{-1}	0		5		7		10		30	
Chl- a+ phaeo	µgl^{-1}	0		7		10		15		40	
TN	µM	0		50		75		10 0		12 0	
TP	µM	0		1		2		5		8	

For intermittently and ephemerally tidal lagoons, whose hydrology can often be described as lacustrine rather than tidally driven per se, no comparable set of chlorophyll-a endpoints were found in the literature. It is worth considering whether chlorophyll-a biomass thresholds developed for lakes could be applied, with appropriate caveats, to these systems. The California NNE framework for lakes consists of chlorophyll-a thresholds for warm and cold water, REC-1, REC-2 beneficial uses that range from <5-10 µg L^{-1} for the BURC I (presumptive unimpaired) to >10-25 µg L^{-1} for the BURC III category (presumptive impaired; TetraTech 2006).

Use of the concept of a "summer time mean" for intermittently and ephemerally tidal estuaries has similar applicability as that considered for lakes. The summer mean chlorophyll-a target is related to the frequency of severe bloom conditions (defined as concentrations greater than 30 µg L^{-1}). In work on USACE reservoirs, Walker (1985, 1987) determined an estimate of the frequency of time that concentrations are greater than 30 µg L^{-1} can then be made from the arithmetic mean target concentration and a coefficient of variation on the log-transformed values (CV; standard deviation divided by the mean), using the algorithm found in Walker (1985). Based on this analysis, setting a summer mean target of 5 µg L^{-1} means that blooms will almost never occur, while a target of 10 µg L^{-1} implies that such blooms will be rare (Table 4.5: TetraTech 2006). A target of 20 µg L^{-1} suggests blooms will occur about 15-20 percent of the time, which is suggested as the maximum allowable level consistent with full support of contact recreation use. A target mean concentration of 25 µg L^{-1} corresponds to blooms about one quarter of the time.

Table 4.4. Table of NNE thresholds for lakes and streams (TetraTech 2006).

Beneficial Use Risk-Category I. Presumptive unimpaired (use is supported).
Beneficial Use Risk Category II. Potentially impaired (may require an impairment assessment)
Beneficial Use Risk Category III. Presumptive impaired (use is not supported or highly threatened)

RESPONSE VARIABLE	RISK – CATEGORY BOUNDARY	BENEFICIAL USE						
		COLD	WARM	REC-1	REC-2	MUN[1]	SPWN	MIGR
Benthic Algal Density (mg chl-a/m^2)	I / II	100	150	C	C	100	100	B
Maximum	II / III	150	200	C	C	150	150	B
Chlorophyll-a (µg/l)	I / II	5	10	10	10	5	A	B
Lakes and Reservoirs[2] – summer mean	II / III	10	25	20	25	10	A	B
Clarity (Secchi depth, meters.)[3] – lakes	I / II	A	A	2	2	A	A	B
summer mean	II / III	A	A	1	1	A	A	B
Dissolved Oxygen (mg/l)	I / II	9.5	6.0	A	A	A	8.0	C
Streams – the mean of the 7 daily minimums	II / III	5.0	4.0	A	A	A	5.0	C
pH maximums –	I / II	9.0	9.0	A	A	A	C	C
photosynthesis driven	II / III	9.5	9.5	A	A	A	C	C
DOC (mg/l)	I / II	A	A	A	A	2	A	A
	II / III	A	A	A	A	5	A	A

A = No direct linkage
B = More research needed to quantify linkage
C = Addressed by Aquatic Life Criteria

[1] For application to zones within water bodies that include drinking water intakes.
[2] Reservoirs may be composed of zones or sections that will be assessed as individual water bodies
[3] Assumes that lake clarity is a function of algal concentrations, does not apply in waters of high non-algal turbidity

Table 4.5. Frequency of chlorophyll-a concentrations greater than 30 µg L^{-1} using the method of Walker (1985) in TetraTech (2006).

Summer Mean Chlorophyll a (µg/L)	CV = 0.62	CV = 0.26	CV = 0.17
5	0.4 %	0.0 %	0.0 %
10	5.9 %	1.2 %	0.1 %
20	16.7 %	17.7 %	14.1 %
25	20.4 %	26.8 %	27.1 %

Evaluation of a numeric endpoint for chlorophyll-a or productivity also needs to consider questions of temporal and spatial applicability consistent with the protection of specific beneficial uses. Temporally, a chlorophyll-a target can be defined as a point-in-time measurement (or frequency of such

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measurements) or as an average over a year, season, or other period. Spatially, the target could be applied as an estuary-wide average, a concentration at a specific point, or in relation to specific sub-habitat areas such as seagrass.

Ultimately, confidence in setting NNE endpoints based on biomass and/or community structure is more easily accomplished with long-term data sets that describe the range in variability in these indicators and relationship to consumer communities linked to beneficial uses. In San Francisco Bay, the only perennially tidal estuary which has a long-term chlorophyll-a data set (McKee et al. 2011), this would be done by convening a workshop of experts to synthesize data that could be used to establish thresholds based on biomass, productivity and community structure. For intermittently and ephemerally tidal estuaries, this work should begin by compiling historical chlorophyll-a, nutrient, and water clarity data to determine: 1) if information on reference condition exists; and 2) if it is consistent with ranges of concentrations associated with blooms, HAB species cell counts, and toxin concentrations.

Thresholds for Harmful Algal Bloom Species Abundance and Toxin Concentrations

In estuarine and nearshore marine environments, certain species of harmful algae can produce algal toxins to levels that can be lethal to humans or other consumers. For example, among marine HABs, phytoplankton blooms consisting of toxic species of the diatom genus Pseudo-nitzschia spp. produce the neurological toxin domoic acid (DA) which, when accumulated through trophic activities, has lead to sickness or mortality in sea mammals, seabirds and humans (Amnesic Shellfish Poisoning, ASP; (Bates et al. 1989, Scholin et al. 2000). At least 1,500 km^2 along the southern California coastline were affected by a toxic event in May/June of 2003 when some of the highest particulate DA concentrations reported for US coastal waters were measured inside the Los Angeles harbor DA-poisoning has been implicated in greater than 1,400 mammal stranding incidents within the SCB during 2003 and 2004. These events do not adequately document the scale of toxic HAB impacts, as adverse effects on viability, growth, fecundity, and recruitment can occur within different trophic levels, either through toxin transmitted directly from the algae to the affected organism or indirectly through food web transfer. In fresh and brackish water habitats, cyanobacteria blooms produce toxins that, in high concentrations, have caused deaths in South America and Asia. In the U.S. they have been associated with waterfowl kills and health problems in people and animals that have come in contact with them.

With respect to establishing action limits or thresholds for HAB species or toxin concentrations, experience is much more evident with fresh to brackish water cyanobacteria species rather than marine HABs. The World Health Organization (WHO) Guidelines for Drinking-water Quality (WHO, 1993, 1996) features guidelines for cyanobacteria cell counts, toxin concentrations and chlorophyll-a representative of safe levels for primary contact. These WHO guidelines represent a scientific consensus, based on very broad international participation, of the health risks to humans presented by cyanobacteria. It does not necessarily reflect an adequate protection level for aquatic organisms. WHO (1996) has a recommended limit of 1 µg L^{-1} microcystin-LR (the most common microcystin) for drinking water.

Table 4.6. Thresholds associated with risks from human exposure to cyanobacterial blooms in recreational or drinking waters. From WHO (1996).

Risk Level	Cyanobacterial Cell Counts	Expected Toxin Concentration	Chlorophyll-a ($\mu g\ L^{-1}$)[5]
Low probability of health effect	20,000 cells per ml	2-4 $\mu g\ L^{-1}$ with concentrations up to 10 in highly toxic blooms	< 10
Moderate [6] probability of health effect	100,000 cells per ml	50 $\mu g\ L^{-1}$	<50

The California Office of Environmental Health Hazard Assessment is currently conducting a toxicological summary of thresholds of adverse effects of anatoxin-a, cylindrospermospin, and four microcystins (LA, LR, RR, and YR) and will recommend specific action levels based on a variety of human and animal endpoints (Kim Ward, SWRCB personal communication). The OEHHA summary, currently undergoing peer review, may be an excellent starting point for consideration of NNE for some common cyanobacterial toxins.

While it is clear that these thresholds are most applicable for fresh to brackish water environments, where cyanobacteria are most prevalent, they should also be considered for polyhaline and euhaline portions of estuaries, because of the potential for risk via downstream transport from freshwater areas. Consumption of the *Microcystis* cells can amplify the toxin up the food chain and can be harmful or fatal to higher trophic levels. For example, sea otter populations are declining due to harmful effects of microcystin, the toxin produced by cyanobacteria *Microsystis* that grows mainly in low-salinity waters and is delivered to the upper estuary from the watershed (Miller et al. 2010). The toxins are accumulated in filter feeders such as clams and mussels which are preferred food items of the sea otter (Wilkerson et al. 2006).

For other estuarine or marine HAB species typically found in California, there is a lack of understanding on the controls of relative abundance and toxins production that limit our ability to establish NNE thresholds at this time. Additional research is needed to understand controls on marine harmful algal bloom frequency and occurrence and controls on toxin production. Additional work is required to understand chemical controls on community structure (ammonia, trace elements, and micronutrients). For example, not all Pseudo-nitzschia species are capable of producing domoic acid, and toxic species do not produce domoic acid constitutively. Laboratory studies have demonstrated that toxin production in some species of Pseudo-nitzschia may increase under silicate or phosphate limitation (Bates et al. 1991, Fehling et al. 2004). In addition, domoic acid can chelate iron and copper, and thus the molecule may affect trace metal acquisition or metal detoxification by phytoplankton (Rue and Bruland 2001, Wells et al. 2005). Thus, the scenario(s) under which Pseudo-nitzschia blooms and domoic acid is produced in nature may be varied and complicated, making it difficult to develop a strategy to mitigate the

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[5] Assumes dominance by cyanobacteria

[6] At these concentrations, risk level may be high if cyanobacteria are scum forming, which is often the case with *Microsystis spp*. and *Anabena spp.*

occurrence of these events. Thus, while action limits can be identified, it is not possible to link these HAB species which nutrients at this time.

4.4 Modeling the Relationships Between Nutrients, Change in Phytoplankton Biomass and Taxonomic Composition

The relationship between nutrient dose and biotic response is fundamentally one of nutrient supply and growth response. If the process were merely a transformation of one form of nutrient (dissolved, inorganic, bioavailable) into another form (particulate, organic) sequestered as phytoplankton, the prediction of the biomass response would require only a simple mass balance calculation. Duarte and Agusti (1998) suggested that 5 g N m^{-2} y^{-1} may be a "balance point" above which eutrophication responses become pronounced. This indicates that recipient waters have an upper limit to assimilative capacity and they begin to reflect increasing system responses once the limit is exceeded. However, the task of forecasting the effects of nutrient loading on phytoplankton communities is complicated because the numerous co-factors that control, either from top-down or bottom up, the biomass and productivity of phytoplankton (e.g. vertical and horizontal transport, grazing, nutrient transformations, turnover time, nutrient preference and uptake kinetics of the phytoplankton, incident light and water clarity, water depth, temperature, and many other factors).

Modeling of the relationships between phytoplankton and nutrient loads et al. cofactors come in two basic varieties: 1) empirical statistical load/concentrations vs. response and 2) dynamic simulation models that account for mechanistic controls on the load and biological response of nutrients.

4.4.1 Statistical Models Based on Empirical Observations

Chlorophyll-a and Productivity

The availability of a nutrient at a given point in time and space is not necessarily related to the biomass at that same point due to effects of these variables. In fact, a poor relationship can be expected for synoptically collected data on stress-response variables, and this is largely borne out in the data, worldwide. This is exemplified for example in Delaware Bay (Sharp 2010) in which synoptic data for ambient DIN and paired chlorophyll-a concentration at the same location show almost no functional relationship whatsoever (Figure 4.4). Nutrient concentrations in situ can sometimes provide information regarding the potential for nutrient impairment of an estuary when analyzed on the appropriate timescale. Nutrient concentrations are highly dynamic and are rapidly modified and transformed by biogeochemical processing. The concentration of a nutrient measurable in the water column represents the instantaneous net "remainder" after processing by all other factors. In the best case, a "steady state" concentration represents the balance of uptake of the nutrient and production of biomass, which can be used to infer something about the relationship between concentration of a limiting and phytoplankton growth. However, instantaneous snapshots of concentration levels produced by most monitoring programs gives no information about the time-varying component of a dynamic stressor and the response variable. Moreover, often growth can be co-limited or sequentially limited by two or more

nutrients. As one becomes sufficient, another nutrient becomes limiting, clouding the relationship between the stressor and growth response.

Figure 4.4. Relationship between dissolved inorganic nitrogen and chlorophyll-a in Delaware Bay. From Sharp 2010.

The relationship of phytoplankton to increased nutrient availability in estuaries has been best studied with respect to phytoplankton biomass and productivity; Table. 4.7). There has been some success in relating phytoplankton to both external nutrient loads and in-situ nutrient concentrations in estuaries, particularly when data are averaged over annual time periods. Table 4.7 shows relatively high correlation coefficients published by various authors for both phytoplankton biomass and production. In general, variations in N loading rates are reflected in concentrations of N in receiving water bodies, particularly when residence time of that water body is long (on the order of weeks). Although many processes act at various rates to modify nutrient concentrations, mean total nitrogen (TN) concentrations are significantly correlated to TN loading for 5 sub-systems of Chesapeake Bay averaged over a decadal period (Boynton and Kemp 2008). Conley et al. (2000) reported that on an annual basis about 70% on the variation in TN concentration could be explained by variation in TN loads in a large sample of Danish estuaries. Madden et al. (2010) found a strong correlation between SEAWIFS remotely sensed chlorophyll-a and TN loading for 108 estuaries in the United States (Figure 4.5).

A survey of the fundamental nutrient forms and processes in several major estuaries was performed by Smith (2006) using data from 92 estuarine and coastal sites worldwide (Figure 4.5). The analysis demonstrated a strong correspondence between log transformed annual mean concentrations of total P and standing stock of chlorophyll-a and a still stronger relationship between log transformed annual mean total N and standing stock of chlorophyll-a. Nitrogen accounted for a significant portion of the variability of phytoplankton production or algal biomass on an annual basis, but the form and relative proportion of each nutrient was also important in the response. Note that the stressor variable is in units of concentration and that the axes are log plots. Deeper analysis showed that the strength of the relationship depended on whether the nutrient data were reported as DIN concentration only (NO3 - +

NO2 - + NH4 +) or as TN (DIN + DON + PON) being generally stronger with TN than with DIN. Many states are considering TN as the N form to be regulated by establishing numeric criteria.

$$C = \sum_{K=1\,\text{TO}\,K} E_k \left[\beta_k + \beta_{kN} \ln \left\{ [(Nt/V)] \, [1/(1+\alpha\ t)] \right\} + \beta_{kT} T \right] + E_R \beta_R + \varepsilon$$

Where C is predicted chlorophyll-a concentration in mg m-3; N is TN loading in g d-1; t is freshwater residence time in days; α is an assumed loss rate from settling and denitrification (estimated through trial and error to be .001 per day); V is estuarine volume in m3; T is mean annual air temperature in degrees Celsius [°C]; Ek is an indicator variable for a specific class of estuaries; K is the number of estuarine classes; β k, βkN, and βkT are estimated regression coefficients for the effect of estuarine class, the class-specific effect of nutrient loading, and the class-specific effect of temperature, respectively (i.e., a total of 3K coefficients); ER and βR are the indicator variable and estimated regression coefficient for the effect of region; and ε is the model error.

Figure 4.5 Statistical regression model (left) and plot of predicted versus measured values for a regression of SeaWiFS maximum monthly chlorophyll-a for 108 estuaries,. From Madden et al. (2010).

Measurement of inorganic forms of N alone may underestimate the true influence of nitrogen inputs. Several recent studies (Antia et al. 1991, Seitzinger et al. 2002, Berman and Bronk 2003) show that significant fractions of the dissolved organic N pool, which is not measured in the DIN fraction and often not monitored at all, can be assimilated by estuarine plants, including microalgae. Dissolved organic nitrogen (DON) can be the dominant form in the N pool in estuarine systems, especially during warm periods of the year when system metabolism is high. This pool can be very dynamic and variable. Similarly, PO4 3- does not necessarily reflect the total availability of the total P pool to phytoplankton.

It is also important to note that these results reflect correlative relationships. However, viewing the correlations as potentially causal interactions, important points emerge from the analyses: 1) in the absence of scaling factors such as time averaging, time lagging, and spatial apportionment, the relationship of nutrients and chlorophyll is generally weak; 2) selecting the appropriate timescales over which to average the data is important to the outcome of the analysis; and 3) total nutrients are often better correlated with chlorophyll-a response than is dissolved inorganic nutrients as the stressor.

Table 4.7. Modeled relationships between nutrient loading and phytoplankton response in world estuaries. (From Boynton and Kemp 2008).

Location	Variable, X (units)	Variable, Y	r^2 / n	Reference
Multiple estuaries	TN-loading (g N m^{-2} y^{-1})	Phytoplankton Production	0.60 / 14	Boynton et al. 1982
SF Bay and other estuaries	Composite parameter X = f(B, Z_p, I_0)		0.82 / 211	Cole and Cloern 1987
Narragansett Bay	Composite parameter X = f(B, Z_p, I_0)		0.82 / 1010	Keller 1988
Multiple estuaries	DIN-loading (mol N m^{-2} y^{-1})		0.93 / 19	Nixon et al. 1996
Multiple estuaries	TN-loading (g N m^{-2} y^{-1})		0.36 / 51	Borum and Sand-Jensen 1996
Boston Harbor	Composite parameter X = f(B, Z_p, I_0)		0.66 / 12	Kelly and Doering 1997
Waquoit Bay system	Annual average DIN conc (μM)		0.61 / 12	Valiela et al. 2001
Chesapeake Bay	TN(x_1), TP(x_2) load (kg mo^{-1})		0.67 / 11	Harding et al. 2002
Multiple estuaries	DIN (mM m^{-3}); tidal range (m)	Phytoplankton Biomass	na / 163	Monbet 1992
Multiple systems / MERL	DIN input (mmol m^{-3} y^{-1})		na / 34	Nixon 1992
Ches Bay mesohaline	River flow (m^3 d^{-1}); proxy for N-load		0.70 / 34	Harding et al. 1992
Maryland lagoons	TN load (g N m^{-2} y^{-1})		0.96 / 9	Boynton et al. 1996
Danish coastal waters	TN concentration (ug l^{-1})		0.64 / 168	Borum 1996
Canadian estuaries	TN concentration (ug l^{-1})		0.72 / 15	Meeuwig 1999
Ches Bay and Tributaries	TN Load; (mg N m^{-2} yr^{-1}) (R_{time}, yrs)$^{-1}$		0.82 / 17	Boynton and Kemp 2000
Danish estuaries	TN concentration (ug N l^{-1})		0.30 / 1347	Nielsen et al. 2002
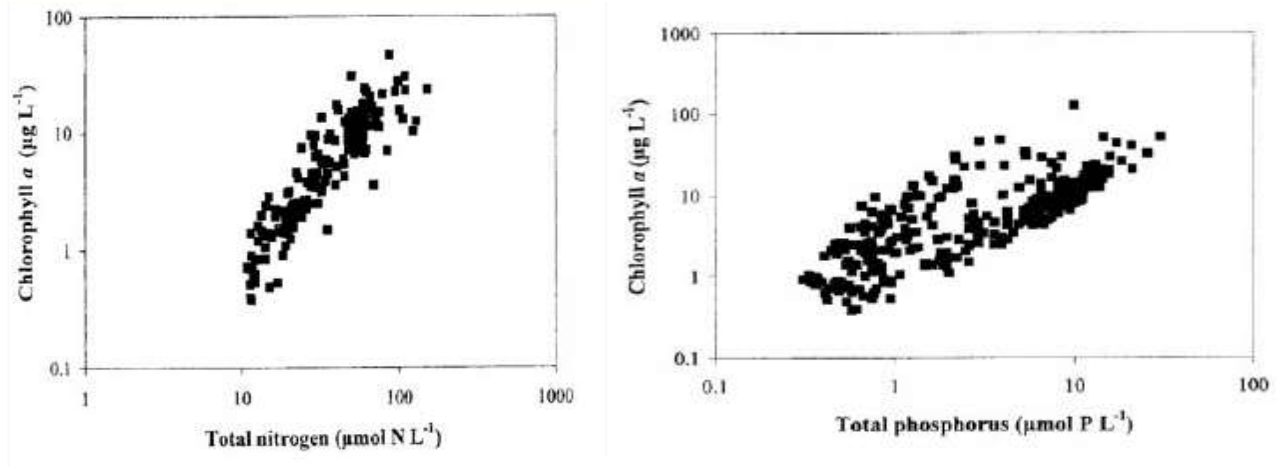
Figure 4.6. Relationship between total nitrogen (left panel), total phosphorus (right panel) and chlorophyll-a. From Smith (2006).

Taxonomic Composition

The dynamic life history of phytoplankton, their microscopic size and the numerous individual species that exist, has resulted in the pursuit of phytoplankton functional groups as the viable indicator of eutrophication when evaluations go beyond typical biomass observations. The portioning of phytoplankton species into functional taxonomic groups (chlorophytes, cryptophytes, cyanobacteria, diatoms, and dinoflagellates) and the relative dominance, distribution, and successional changes at the taxonomic group level can be indicative of the physiochemical conditions, nutrient availability, and food web dynamics within estuarine systems (Cottingham and Carpenter 1998; Pinckney et al. 2001; Paerl et al. 2003, 2006, 2007). The use of diagnostic photosynthetic pigments of various microalgal groups has been included in coastal monitoring programs to quantify indicators of phytoplankton taxonomic group biomass (Millie et al. 1993, Mackey et al. 1996, Pinckney et al. 2001).

There has been some success at modeling the relationship between phytoplankton taxonomic composition and environmental factors, particularly through the use of artificial neural networks (Paerl et al. 2005, Millie et al. 2004). Rothenburger et al (2009) analyzed 13 years of environmental data and phytoplankton species and assemblage structure in the Neuse River Estuary to identify potential environmental predictors of phytoplankton assemblage patterns in a hypereutrophic estuary. They found that seasonally, species composition was strongly related to temperature and total TN:TP phosphorus ratios. Inter-annual variability in river discharge influenced whether phytoplankton assemblages were dominated by diatoms and phototrophic flagellates or by mixotrophic and heterotrophic dinoflagellates. A significant increase in chlorophytes and bloom-forming dinoflagellates, some harmful, was correlated with high ammonium concentrations, suggesting a potentially important role of ammonium in controlling phytoplankton community structure.

4.4.2 Dynamic Simulation Modeling of Nutrient Load Phytoplankton Response

Where available, perhaps the best tool for developing load-response relationships is dynamic modeling. Dynamic simulation models are mathematical representations of the real world that estimate environmental events and conditions. Models can be used to predict pollutant delivery as well as simulate how various changes or pollution-reduction actions could affect a waterbody's beneficial uses, especially with respect to water quality, aquatic life, and wildlife. Because estuaries and their watersheds are typically complex, scientists and managers can rely on computer models to synthesize information about the ecosystem's characteristics and the effects of various environmental actions to reduce pollution. The Chesapeake Bay water quality requirements were developed using extensive modeling of light behavior in the water column (Batiuk et al. 2001). Dynamic modeling is being used to predict the transport and production of nutrients and chlorophyll within the bay (Cerco et al. 2010). To take this approach, two types of models would need to be developed:

1. Watershed model, which incorporates information about loadings or exchanges from land use, fertilizer applications, wastewater plant discharges, septic systems, wet and dry air deposition, exchange with the coastal ocean, weather and other variables to estimate the amount of nutrients and sediment reaching the estuary and where these pollutants originate.

2. Estuary water quality model, which simulates the ecosystem response to pollutant loads, which would consist of two sub-models: a) A hydrodynamic sub-model that will simulates the exchange with rivers, oceans, mixing of waters in the Estuary and its tidal tributaries and b) A water quality sub-model that simulates the Estuary's biological, chemical and physical dynamics in response to nutrient loads and other factors (light, temperature, grazing, etc.).

The models would be used to establish load allocations of nutrients that the estuary can sustainably assimilate. It would also be used to generate simulations of the past, present or future state of the Estuary, ocean, watershed, and airshed (e.g., population growth, climate change, etc.) to explore potential effects of management actions and evaluate alternatives. Thus these models would be a key component of a strategy to adaptively manage an estuary.

However, such models are very data and resource intensive to develop. For most California estuaries, only rudimentary data on the nutrient loads and rates of transformation are available, making it difficult to develop mechanistic or statistical predictive models. For systems where sufficient data are available, the complexities of nutrient processing and the translation of these processes into expressions of eutrophication can be parameterized into predictive models.

4.5 Review of Phytoplankton Indicators of Eutrophication

The purpose of this section is to review each of the available phytoplankton indicators with respect to the evaluation criteria, and summarize data gaps and make specific recommendations indicators to pursue for further development. Three types of phytoplankton indicators are reviewed:

1) Measures of chlorophyll-a biomass and productivity
2) Measures of taxonomic composition
3) Harmful algal bloom species abundance and toxin concentration

Measures of water clarity have also been used in tandem with phytoplankton indicators to assess eutrophication. Water clarity indicators are reviewed in Chapter 6 (sediment and water chemistry) and specifically for seagrass habitats in Chapter 5.

4.5.1   Sound and Practical Measurement

Chlorophyll-a

Water column chlorophyll-a (the green pigment found in most aquatic plants) is a proxy for total phytoplankton biomass. The relationship is derived from a general understanding of the ratio of organic matter to chlorophyll-a in algal cells (Carbon: chlorophyll-a), together with cell biovolume. Though species-specific differences can alter these relationships, the general approach is very well accepted among the scientific community. As an indicator, water column chlorophyll-a is one of the most common measures of eutrophication in aquatic ecosystems and has been extremely well studied and vetted through 40 years of research and use in long-term monitoring programs. Phytoplankton biomass responds rapidly to pulsed nutrient inputs that might otherwise go undetected by regular nutrient sampling and integrates available nutrient loads over time periods of days, making it a more stable indicator of eutrophication. That being said, phytoplankton biomass can be highly spatially and temporally variable, so use of this primary producer group as an indicator requires the ability to document trends over time and space in order to strengthen the ability to detect trends over time. Chlorophyll-a samples require minimal processing and storage in the field and are not easily contaminated and the cost of laboratory analysis is low in comparison with nutrient analyses. Monitoring of chlorophyll-a is conducted through three basic types of instrumentation (Table. 4.8): 1) discrete water samples, 2) continuous monitoring through in situ probes, or 3) through remote sensing. The advantages and disadvantage of each are summarized below.

Analysis of Chlorophyll-a in Water Samples. Chlorophyll-a is usually collected from water samples of a known volume that are filtered through fine mesh filter paper (0.45 or 0.7 µm) to concentrate the chlorophyll-containing organisms, mechanical rupturing of the collected cells, and extraction of the chlorophyll from the disrupted cells into the organic solvent acetone. The extract is then analyzed by either a spectrophotometric method (absorbance or fluorescence), using the known optical properties of chlorophyll, or by HPLC. Fluorometers are among the most common instruments used; they transmit an excitation beam of light in the blue range (440 nm - 460 nm) and detect the light fluoresced by chlorophyll in a sample in the red wavelength (685 nm). This fluorescence is directly proportional to the concentration of chlorophyll. HPLC is the most accurate, but costly and slow. It provide information about chlorophyll types (a, b, and c) as well as accessory pigments (see information on taxonomic analysis below) and thus can be extremely useful in certain applications. As mentioned above, water column chlorophyll-a analysis does not require expensive instrumentation and is considered to be fairly cost-effective. Frequent grab samples are required to establish sources of variability and detect long-term trends. For this reason, many established monitoring programs include analysis of chlorophyll-a on frequencies of weekly to monthly. Field based monitoring of chlorophyll-a has the greatest precision and accuracy, but spatial and temporal coverage is limited. Laboratory analyses are time-consuming and usually require an experienced, efficient analyst to generate consistently accurate and reproducible

results. In addition, they do not lend themselves readily to continuous monitoring of chlorophyll (and thus phytoplankton) since the collection of samples at reasonable time intervals, e.g., every hour, would be extremely time-consuming.

Table 4.8. Summary of methods for measuring phytoplankton biomass and community structure.

Group	Indicator	Methods	Information
Biomass	Discrete water column chlorophyll-a	Grab samples with laboratory analysis	Precise measure of water column chlorophyll-a
	Chlorophyll-a fluorescence	In situ probes and flow through instrumentation	Chlorophyll-a fluorescence, which must be calibrated to grab samples
	Remote sensing of color	Satellite (SeaWiFS, MERIS, MODIS) or wide variety of multispectral and hyperspectral airborne sensors	Water color as a proxy for chlorophyll-a
Productivity	Photosynthesis versus irradiance curves	Modeled production	Rate of carbon fixation per unit time
	Isotope	Direct measure of gross and net productivity	
Community Structure	Number of species and relative abundance	Taxonomy and cell counts	Dominant species and presence/absence of rare or pollutant tolerant taxa
	Chemotaxonomic phaeopigments	HPLC	Relative composition of broad taxonomic group composition by determining chlorophyll and carotenoid presence phaeopigments (e.g., Chlorophytes, Cryptomonads, diatoms, dinoflagellates and zeaxanthin)
HAB species and toxin concentrations	HAB species abundance	Taxonomy/cell counts or Q-PCR	Abundance of HAB species
	Toxin concentrations	HPLC or Elisa Assay	Concentration of toxins associated with water column or sediment

Chlorophyll-a Fluorescence. Sensors or probes measuring chlorophyll-a fluorescence for both in spot sampling and in continuous monitoring applications are now enjoying widespread use. Hand held meters, data sondes, and flow through systems can log measure chlorophyll-a fluorescence *in situ*, albeit

with the potential loss of accuracy because of potential interferences from CDOM and other optical properties of the waterbody. Thus the results of in-situ analysis will not be as accurate as results from the laboratory analysis procedure. Some sources of inaccuracy can be minimized by combining extractive analysis of a few samples during a sampling or monitoring study with the sensor data, though it is not recommended that the in-situ studies replace the standard procedure. The advantage of this methodology is observations can be relatively cost-effective using probes and calibration of readings with laboratory samples, thus net phytoplankton biomass can be simply mapped spatially and/or easily tracked over time. Many data sondes have the ability to add probes such as salinity, temperature, turbidity, and PAR along with chlorophyll-a fluorescence, thus allowing for collateral data which help to interpret controls on phytoplankton productivity.

Chlorophyll-a fluorescence have been deployed as probes on buoys or at piers or as flow through instruments on boats (Madden and Day 1992) and ferries (Buzzelli et al. 2003, Petersen et al. 2003). Flow through instruments, if deployed on passenger ships, ferries or other boats with regular routes, have the advantages of acquiring data with both spatial and temporal resolution with less change for biofouling of the instrumentation. Chlorophyll-a fluorescence probes deployed through data sondes are affected by biofouling and must be maintained regularly (weekly-monthly, depending on the environment).

Remote Sensing of Ocean Color (Remotely Sensed Chlorophyll-a). Remote sensing of ocean color is an established method of detecting phytoplankton blooms in the coastal ocean and in large estuaries (Stumpf and Tomlinson 2005), with sensors mounted on onboard an aircraft or satellite (e.g., Robinson 1985, Martin 2004). Remote sensing has the advantage of providing large spatial and temporal coverage. Ocean-observing satellite sensors like Advanced Very High Resolution Radiometer (AVHRR), Sea-viewing Wide-Field-of-view Sensor (SeaWiFS) and MODerate-resolution Imaging Spectroradiometer (MODIS) are regularly used to estimate chlorophyll-a concentration and track spatial and temporal variability in phytoplankton blooms. These platforms have a swath width of 1500-2000 km (close to the distance between successive orbit tracks) and, as such, cover the earth surface on almost a daily basis, i.e., revisit each area every 1-2 days; this high temporal sampling frequency lends itself to capturing spatial and temporal variability of algal blooms. At the same time, such a wide scan width limits the pixel size of multispectral sensors to ~1 km. Satellite sensors designed for land observations (e.g., Landsat) have narrow swath width (100–200 km), resulting in significantly longer revisit period (~16 days), but collect data at high spatial resolution (~30 m for Landsat TM, 20 m for Spot, etc.).

Modern satellite ocean color data has limited capability in distinguishing between HABs versus other blooms due to low sensitivity to the differences in phytoplankton taxonomic composition (Garver et al. 1994). The optical signatures of the many phytoplankton taxonomic groups (e.g., diatoms and dinoflagellates) are different only in short wavelengths, which are not measured by modern satellite sensors (Kahru and Mitchell 1998). Discrimination of phytoplankton taxonomic groups on the basis of remotely-sensed ocean color is possible for the taxons characterized by unique optical properties, e.g., coccolithophores (Balch et al. 1991), cyanobacteria (Westberry et al. 2005) or large dinoflagellates characterized by decreased backscattering (Cannizzaro et al. 2008).

Remote sensing of phytoplankton blooms in estuaries has some limitations. First, use of freely available imagery like SeaWiFS and MODIS is limited in California's predominantly small estuaries because of the large pixel size (~1 km). The exception to this is San Francisco Bay, where remote sensing of phytoplankton blooms has been applied in the past through the development of customized algorithms calibrated by empirical data (e.g. Catts et al. 1980). High resolution multi- and hyperspectral imagery can be deployed via airborne sensors. However, costs to use airborne sensors in a long-term monitoring program with high temporal frequency are high. Second, remotely sensed color in estuaries is cofounded by a number of factors, leading to the overestimation of chlorophyll-a by standard algorithms. Examples include dissolved and suspended matter [e.g., Muller-Karger et al. 2005], especially in the regions affected by freshwater discharge, bottom reflectance, which depends on bathymetric depth and water transparency (e.g., Maritorena et al. 1994), landmass reflection and terrigenous absorbing aerosols found in high concentrations in urban areas.

Phytoplankton Productivity

Phytoplankton productivity is the measure of the rate of biomass production and is in fact a more immediate measure of the influence of nutrients on autotrophic production and potential eutrophication than biomass concentration. However, there are numerous problems associated with the measurement and use of this parameter as an indicator. First, it is relatively difficult and time consuming to measure productivity, and gathering data over a large and representative spatial area is neither efficient nor widely conducted in monitoring programs for coastal waters. Secondly, though the rate of productivity may be a good indicator of nutrient concentration, the ultimate disposition of the production may vary across estuaries or even within an estuary based on several factors. High productivity in deep and well-mixed waters may not result in problematic levels of phytoplankton biomass as the biomass produced can be mixed throughout the water column, and the balance of productivity to respiration (P:R) within the entire water column constrains the production within acceptable limits. Moreover, even in shallow estuaries where biomass may accumulate in the euphotic zone, if grazer or filter feeding communities are present, the biomass may be efficiently removed, contributing to a healthy and productive estuary, without causing negative impacts. These factors point to the importance of site-specific assessment when using either biomass or productivity as an index of eutrophication.

Phytoplankton Community Composition

There are many different methods that will provide measurements of phytoplankton community composition (Table 4.8). Methods that provide a broad analysis of community composition are relatively cheap and fast whereas methods that provide more detail are labor intensive and can be expensive to employ. The determination of which method is best depends on the scientific question to be addressed and the level of effort supplied.

Chemotaxonomic Pigments. The use of chemotaxonomic photopigments to determine phytoplankton functional groups allows a cost-effective alternative to detailed methods to determine the phytoplankton community assemblage (Millie et al. 1993, Mackey et al. 1996, Pinckney et al. 2001). This method will provide information on broad taxonomic group composition by determining chlorophyll and carotenoid presence in environmental samples. While chlorophyll-a is present in all phytoplankton functional groups, chlorophyll-b is produced only by Chlorophytes, alloxanthin is produced only by Cryptomonads, fucoxanthin is produced by diatoms, peridinin is produced by dinoflagellates and zeaxanthin is produced by cyanobacteria. The presence of any of these pigments in a sample will determine taxonomic groups of phytoplankton that are present. The photopigment composition of each functional group is typically well correlated with species cell counts or biovolume estimates and can be quantified using high performance liquid chromatography (HPLC) coupled with diode array spectrophotmetrey (PDAS; Paerl et al. 2006).

A suite of easily deployed, broadly applicable phytoplankton-based water quality indicators has been intensively used in eastern coast estuaries. Analyses and interpretation of long term data from the Neuse River Estuary are being performed to develop qualitative and quantitative relationships between the abundance of specific phytoplankton functional groups and various estuarine chemical and physical variables. These analyses will yield information that will link the abundance of each phytoplankton functional group with a particular set of environmental conditions (Paerl et al. 2003). Thus, based on work from the east coast it appears that the use of phytoplankton community assemblage using diagnostic pigment indicators may hold promise as potential indicator of California estuarine eutrophication, yet a large amount of taxonomic data gaps currently exist.

Taxonomic Composition. Community composition can also be determined to the species level to provide information on species shifts and diversity. There are two main methods that provide information to the species level. The first is the gold standard of evaluating community composition, quantification of microalgae by estimating cell numbers. This method involves preserving the samples and using microscopy to determine cell numbers to the species level. While this method is commonly used it is labor intensive, costly and requires a high level of expertise.

Faster and less labor intensive molecular methods have been developed to identify and enumerate microalgae communities to the species level. These methods can be categorized as either "whole cell" or "cell homogenate" based methods. The whole cell methods include fluorescent in situ hybridization (FISH) where the whole cell remains intact and uses fluorescent probes that bind to the complementary target DNA sequence. Fluorescence microscopy can be used to enumerate the number of cells in a sample. Alternatively, cell homogenate based methods include sandwich hybridization (Scholin et al., 1997, 1999), microarray hybridization (Gescher et al. 2008) and quantitative polymerase chain reaction (qPCR; Ausubel et al. 1995). These methods also use molecular probes but typically disrupt the cells resulting in a cell homogenate. For a review of molecular methods used in oceanography see Kudela et al. (2009).

While phytoplankton community assemblage provides more insightful data to improve our understanding of primary producer ecological responses to increased nutrient availability, the cost and

complexity of obtaining, validating and understanding these dynamics in west coast estuaries to meet current regulator and management means are likely prohibitive. Similar to the wide-spread application of benthic invertebrate community composition for management and restoration of freshwater stream systems, there is little doubt that phytoplankton community composition doesn't hold similar promise for estuarine systems. Taxonomic information in addition to phytoplankton biomass provides much greater insight on the health of the primary producer community. A future understanding of the species natural distribution and seasonal dynamics is necessary for west coast estuaries. In addition, knowledge of the competitive interactions between tolerant and intolerant species, the conditions that select for tolerant species proliferation, the ecological and water quality implications of blooms of certain phytoplankton species, etc will greatly improve the application of phytoplankton taxonomy for estuarine management decisions. Unfortunately, the current greatest limitation for immediate application is the lack of detailed, spatially representative taxonomy and community dynamic data statewide.

One use of phytoplankton community structure data is to combine it into an index of biological integrity (IBI). IBIs are becoming more common for assessment of estuarine ecological condition and management focus in the face of physical and chemical transformation, habitat destruction, and changes in biodiversity (Borja et al. 2008). An IBI describes the biological condition of an assemblage of plants or animals, typically based on the diversity and relative abundance of species or the presence or absence of pollution tolerant species. A key element of developing an IBI is the ability to describe the community response of the assemblage (e.g., benthic invertebrates, phytoplankton, etc.) along gradient of physical or chemical stress from minimally disturbed or "reference state" to highly disturbed. IBIs developed and used in Chesapeake Bay present an example of how phytoplankton community structure data can be synthesized to provide information about the ecological health of the Estuary and about the ability to support specific beneficial uses. A Phytoplankton Index of Biotic Integrity (P-IBI) was developed in Chesapeake Bay using an 18 year data set (Lacouture et al. 2006). The P-IBI combined the scores of pollution-sensitive, biologically important metrics of the phytoplankton community into a single index. Like other multi-metric indexes, the P-IBI is more sensitive to habitat conditions than its component metrics, which include chlorophyll-a, the abundances of several potentially harmful species, and various indicators of cell function and species composition (Lacouture et al. 2006). Currently within California, the only estuary that potentially has sufficient data to develop a P-IBI is San Francisco Bay (see McKee et al. 2011).

HAB Toxins. The detection of HAB toxins can also be used to identify which phytoplankton groups are present although this method is less precise than the photopigment analysis. For example, domoic acid is produced by a diatom (Pseudo-nitzschia spp.), saxitoxin is produced by dinoflagellates and cyanobacteria, okadaic acid and yessotoxin are produced by dinoflagellates. Please see review of HAB toxins in California by Caron et al. (2010). Some of these species are considered indicators of nutrient enrichment and would thereby suggest impairment of a waterbody. There are numerous HAB toxin detection methodologies which include both rapid assay methods such as immunoassays, receptor assays and chemical methods such as high-pressure liquid chromatography and liquid-chromatography-mass spectrometry. Additionally, there are a number of field test kits being developed that are used to determine rapid (<20 minute) analysis of environmental samples.

4.5.2   Clear Linkage to Beneficial Uses

Indicators of phytoplankton (phytoplankton biomass, productivity, taxonomic composition, and HAB species toxin concentrations) have a well-documented linkage to beneficial uses of California estuaries (see Section 4.3), with a broad base of evidence that phytoplankton provide food web support for marine and estuarine aquatic organisms (EST, MAR) including the commercial and sport fisheries (COMM), shellfish such as clams, oysters and mussels (SHELL and AQUA), migratory (MIGR) birds and fish, support for fish nursery habitat (SPAWN). Harmful algal blooms can adversely affect the health of humans (REC-1) by irritation and injury to recreational swimmers, sailboarders, and boaters (Lehman et al., 2005). In addition, elevated phytoplankton biomass could impact estuarine and wildlife habitat by shading and degrading eelgrass habitat and impact aesthetics (REC-2) through nuisance buildup and smell during decay.

4.5.3   Acceptable Signal to Noise Ratio

Phytoplankton are subject to a high degree of spatial and temporal variability due to a number of physical, chemical and biological co-factors (see Section 4.2 summarizing this variability). However, it has been possible to determine statistically-significant trends with respect to phytoplankton biomass when long-term datasets are available (e.g., Cloern et al. 2006). For ephemeral system responses such as changes in species composition and occurrence of HABs, because phytoplankton species populations appear and disappear within weeks, assessing change on shorter timescales may require higher resolution monitoring of annual cycles over many years (Smetacek and Cloern 2008). It should be noted that while high spatial and temporal variability is characteristic of all biological indicators, these indicators tend to integrate better over time and space than stressors, such as nutrient concentrations. Ultimately, our understanding and the various hypotheses about controls on spatial and temporal variability in phytoplankton biomass and community structure and linkages to consumers can be tested and refined through predictive models.

4.5.4   Predictive Linkage with Nutrient Loads and Other Management Controls

Phytoplankton biomass has a well vetted relationship with nutrient loads in lakes and estuaries (see section 4.4 this chapter) through the use of statistical load-response models (Madden et al. 2010, Boyton and Kemp 1998, and others), simplified spreadsheet models (lakes biomass spreadsheet tool, TetraTech 2006) or dynamic simulation models (e.g. Cerco et al. 1996, Bowen and Hieronymous 2003). Models that predict taxonomic composition at the assemblage level also exist but have been most often used in an academic rather than regulatory setting.

4.6 Summary, Data Gaps and Recommendations

Table 4.9 summarizes data gaps and recommended next steps with respect to the use of phytoplankton as a NNE indicator.

Table 4.9. Summary of how phytoplankton indicators met review criteria, data gaps and recommended next steps.

Indicator	Met Review Criteria	Data Gaps	Recommended Next Steps
Phytoplankton biomass and productivity	Four of four	Wealth of data exist in selected estuaries. Need a review of science supporting selection of endpoints	Recommend development of a white paper and a series of expert workshops to develop NNE assessment framework for phytoplankton biomass, productivity, taxonomic composition/assemblages, abundance and/or harmful algal bloom toxin concentrations in "open" and "closed" estuaries.
Phytoplankton taxonomy, abundance, and/or harmful algal bloom toxin conc.	Cyanobacteria cell counts and toxin = four of four; 'taxonomic composition/assemblage and HAB cell counts and toxin = three of four	Data exist on taxonomic composition and HAB species and toxin concentration in California estuaries.	Recommend monitoring to include measurement of HAB toxin concentrations in water and faunal tissues.
Phytoplankton biomass, (with epiphyte load and light attenuation) in Seagrass Beds	Phytoplankton biomass = primary, epiphyte load and light attenuation = secondary	Lack of coordinated monitoring on seagrass areal extent and condition in California estuaries, with some notable exceptions Lack of data on relative importance of phytoplankton, epiphyte, and macroalgal blooms as stressors to seagrass beds in California.	Recommend 1) studies to establish light requirements for California seagrass species, 2) development of a statewide workgroup to develop an assessment framework for seagrass based on phytoplankton biomass, macroalgae, and epiphyte load, using biooptical models when appropriate and 4) collection of baseline data to characterize prevalence of algal blooms associated with on seagrass beds.

4.7 References

Anderson, D.A., P.M. Glibert, and J.M. Burkholder. 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25: 562-584.

Anderson, D.A., J.M. Burkholder, W. Cochlan, P. M. Glibert, C. Gobler, Heil, R. Kudela, T. Parsons, V. Trainer and G. Vargo. 2008. Harmful algal blooms in the United States: Linkages to eutrophication. Harmful Algae. 8: 39-53.

Antia N.J, P.J. Harrison, J. Oliveira. 1991. The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycology, 30: 1-89.

Ausubel, F., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., Struhl, K., 1995. Short Protocols in Molecular Biology, third ed. Wiley, New York.

Azam F., T. Fenchel, J.G. Field, J.S. Gray, L.A. Meyer-Reil , F. Thingstad. 1983. The ecological role of water column microbes in the sea. Mar Ecol Prog Ser 10:257-263

Balch, W.M., Holligan, P.M., Ackleson, S.G., Voss, K.J., 1991. Biological and optical properties of mesoscale Coccolithore bloom in the Gulf of Maine. Limnology and Oceanography, 36 (4), 629-643.

Bates, S., et, et al. (1989). "Pennate diatom Nitzschia pungem as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada." Canadian Journal of Fisheries and Aquatic Sciences 46: 1203-1215.

Batuik, R.A., P. Bergstrom, M. Kemp, E. Koch, L. Murray, J.C. Stevenson, R. Bartleson, V. Carter, N. Rybicki, J.M. Landwehr, C. Gallegos, L. Karrh, M. Naylor, D. Wilcox, K.A. Moore, S. Alistock, M.Teichberg. 2000. Chesapeake Bay Submerged Aquatic Vegetation Water Quality and Habitat-based Requirements and restoration targets: A second technical synthesis. US Environmental Protection Agency, Annapolis, MD. http://www.chesapeakebay.net/publication.aspx?publicationid=13051

Batiuk RA, Orth RJ, Moore KA, et al. 1992. Submerged Aquatic Vegetation Habitat Requirements and Restoration Targets: a Technical Synthesis. Annapolis, Maryland: USEPA, Chesapeake Bay Program.

Batiuk, R., P. Bergstrom, M. Kemp, E. Koch, L. Murray, C. Stevenson, R. Bartleson, V. Carter, N. Rybicki, J. Landwehr, C. Gallegos, L. Karrh, M. Naylor, D. Wilcox, K. Moore, S. Ailstock, M. Teichberg. 2001. Chesapeake Bay submerged aquatic vegetation water quality and habitat-based requirements and restoration targets: A second technical synthesis. CBP/TRS 245/00. EPA 903- R-00-014, U.S. EPA, Chesapeake Bay Program, Annapolis, MD.

Berman T, Bronk DA (2003) Dissolved organic nitrogen: a dynamic participant in aquatic ecosystems. Aquatic Microbial Ecology 31:279–305

Biber P., C. L. Gallegos, W.J. Kensworthy. 2008. Calibration of a Bio-optical Model in the North River,North Carolina (Albemarle–Pamlico Sound): A Tool to Evaluate Water Quality Impacts on Seagrasses. Estuaries and Coasts: J CERF (2008) 31:177–191

Blackmon, D., T. Wyllie-Echeverria, D.J. Shafer. 2006. The role of seagrasses and kelps in marine fish support. WRAP Technical Notes Collection (ERDC TN-WRARP-06-1). US Army Engineering Research and Development Center, Vicksburg, MS. 39180. . http://el.erdc.usace.army.mil/elpubs/pdf/tnwrap06-1.pdf

Borja, A., Bricker, S.B., Dauer, D.M., Demetriades, N.T., Ferreira, J.G., Forbes, A.T., Hutchings, P., Jia, X.P., Kenchington, R., Marques, J.C., Zhu, C.B., 2008. Overview of integrative tools and methods in assessing ecological integrity in estuarine and coastal systems worldwide. Marine Pollution Bulletin 56 (2008) 1519–1537.

Boynton, W.R. and Kemp, W.M., 2008. Estuaries, pp. 809-856. In: Capone, D.G., Bronk, D.A., Mulholland, M.R., and Carpenter, E.J. (Eds.), Nitrogen in the Marine Environment 2nd Edition. Elsevier Inc., Burlington, Massachusetts.

Bricker, S. B., J. G. Ferreira, and T. Simas. 2003. An integrated methodology for assessment of estuarine trophic status. Ecological Modelling 169:39-60.

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology, 85, 1771–1789.

Brown, C.A., W.G. Nelson, B.L. Boese, T.H. DeWitt, P.M. Eldridge, J.E. Kaldy, H. Lee II, J.H. Power, and D.R. Young. 2007. An approach to developing Nutrient Criteria for Pacific Northwest Estuaries: A Case Study of Yaquina Estuary, Oregon. USEPA Office of Research and Development, National Health and Environmental Effects Laboratory, Western Ecology Division. EPA/600/R-07/046. http://www.epa.gov/wed/pages/publications/authored.htm

Brussard, C. P. D., R. Riegman, A. A. M. Noordelos, G. C. Cade'e, H. Witte, A. J. Kop, G. Nieuland, F. C. Van Duyl, and R. P. M. Bak. 1995. Effects of grazing, sedimentation and phytoplankton cell lysis on the structure of a coastal pelagic food web. Mar. Ecol. Prog. Ser. 123: 259–271.

Burkholder J., Noga E., Hobbs C., Glasgow H. 1992. New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills. Nature 358, 407- 410.

Burkholder, J.M., D.A. Tomasko, B.W. Touchette. 2007. Seagrasses and eutrophication. Journal Experimental Marine Biology Ecology 350: 46-72.

Buzzelli, C.P., J. Ramus, and H.W. Paerl. 2003. Ferry-based monitoring of surface water quality in North Carolina estuaries. Estuaries 26: 975–984.

Cannizzaro, J.P., Carder, K.L., Chen, F.R., Heil, C.A., Vargo, G.A., 2008. A novel technique for detection of the toxic dinoflagellate Karenia brevis in the Gulf of Mexico from remotely sensed ocean color data. Continental Shelf Research.

Caron, D.A. et al. 2010. Harmful algae and their potential impacts on desalination operations off southern California. Water Research 44(2), SI, 385-416.

Catts, G., Khorram, S., Cloern, J.E., Knight, A.W., and DeGloria, S.D., 1985. Remote sensing of tidal chlorophyll a variations in estuaries: International Journal of Remote Sensing, v. 6, page 1685-1706.

Cerco, C. (1995) "Simulation of Long-Term Trends in Chesapeake Bay Eutrophication," Journal of Environmental Engineering 121: 298-310

Cerco, C. (2000) "Phytoplankton Kinetics in the Chesapeake Bay Eutrophication Model," Water Quality and Ecosystem Modeling 1: 5-49

Cerco, C. and Cole, T. (1993) "Three Dimensional Eutrophication Model of Chesapeake Bay," Journal of Environmental Engineering 119: 1006-1025

Cerco, C., and Moore, K. (2001) "System-wide Submerged Aquatic Vegetation Model for Chesapeake Bay," Estuaries 24(4): 522-534

Cerco, C., S.C. Kim, and M.R. Noel. 2010. The 2010 Chesapeake Bay Eutrophication Model. A Report to the US Environmental Protection Agency and to the US Army Corps of Engineer Baltimore District. US Army Engineer Research and Development Center, Vicksburg, MD.

CLEAP 2008. Central Coast Lagoon Ecological Assessment Project. Prepared for the California Coastal Conservancy by 2nd Nature LLC.

Cloern, J.E. 1982. Does the benthos control phytoplankton biomass in South San Francisco Bay (USA)? Marine Ecology Progress Series 9: 191–202.

Cloern, J.E. 1991. Tidal stirring and phytoplankton bloom dynamics in an estuary. Journal of Marine Research. 49: 203-221.

Cloern, J.E. 1996. Phytoplankton bloom dynamics in coastal ecosystems: a review with some general lessons from sustained investigation of San Francisco Bay, California. Reviews of Geophysics. 34 (2): 127-168.

Cloern, J.E. 1999. The Relative Importance of Light and Nutrient Limitation of Phytoplankton Growth: A Simple Index of Coastal Ecosystem Sensitivity to Nutrient Enrichment: Aquatic Ecology 33: 3-15.

Cloern, J.E. 2001. Our evolving conceptual model of the coastal eutrophication problem. Marine Ecology Progress Series. 210:223–253.

Cloern, J.E. (Lead Author) 2006. "Eutrophication." In: Encyclopedia of Earth. Eds. Cutler J. Cleveland (Washington, D.C.: Environmental Information Coalition, National Council for Science and the Environment) http://www.eoearth.org/article/Eutrophication

Cloern, J.E., 2007. Habitat connectivity and ecosystem productivity: Implications from a simple model. The American Naturalist 169, E21-E33.

Cloern J.E. and Dugdale R. 2010. San Francisco Bay. In Nutrient in Estuaries: A Summary Report of the

National Estuarine Experts Workgroup 2005–2007. P.M. Glibert, C.J.. Madden, W. Boynton, D. Flemer, C. Heil and J. Sharp(eds). US Environmental Protection Agency. Available online http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/upload/Nutrients-in-Estuaries-November-2010.pdf

Cloern, J.E., Cole, B.E. and Hager, S.W. 1994. Notes on a Mesodinium rubrum Red Tide in South San Francisco Bay (USA): Journal of Plankton Research, v. 16, p. 1269-1276.

Cloern, J.E., T.S. Schraga and C. Burns Lopez. 2005. Heat wave brings a red tide to San Francisco Bay. Eos Transactions of the American Geophysical Union 86(7):66

Cloern, J.E., T.S. Schraga, C.B. Lopez, N. Knowles, R.G. Labiosa, and R. Dugdale. 2005. Climate anomalies generate an exceptional dinoflagellate bloom in San Francisco Bay. Geophysical Research Letters 32, LI4608.

Cloern, J.E., A.D. Jassby, T.S. Schraga and K.L. Dallas. 2006. What is causing the phytoplankton increase in San Francisco Bay? 2006 Pulse of the Estuary. San Francisco Estuary Institute, Oakland. Available online: http://www.sfei.org/rmp/pulse/2006/index.html

Cloern, J. E. and F. H. Nichols. 1985. "Time scales and mechanisms of estuarine variability, a synthesis from studies of San Francisco Bay. Hydrobiologia 129(OCT): 229-237.

Cloern, J.E. and Jassby, A.D., 2010. Patterns and scales of phytoplankton variability in estuarine-coastal ecosystems. Estuaries and Coasts 33, 230-241.

Cloern, J.E. and R. Dufford. 2005. Phytoplankton community ecology: principles applied in San Francisco Bay. Marine Ecology Progress Series. 285: 11-28.

Cloern, J.E. et al., 2010. Biological communities in San Francisco Bay track large-scale climate forcing over the North Pacific. Geophysical Research Letters 37, L21602. 6pp.

Cloern, J.E., A.D. Jassby, J.K. Thompson, and K.A. Hieb. 2007. A cold phase of the East Pacific triggers new phytoplankton blooms in San Francisco Bay. Proceedings of the National Academy of Sciences. 104 (47): 18561-18565.

Cloern, J.E., and Jassby, A.D., 2008. Complex seasonal patterns of primary producers at the land-sea interface. Ecology Letters 11, 1294-1303.

Cloern, J.E., B.E. Cole, R.L.J. Wong, and A.E. Alpine. 1985. Temporal dynamics of estuarine phytoplankton: a case study of San Francisco Bay. Hydrobiologia. 129: 153-176.

Conley, D.J., H. Kaas, F. Mohlenberg, B. Rasmussen and J. Windolf. 2000. Characteristics of Danish estuaries. Estuaries. 23: 820-837.

Cosper, E. M. , R. T Garry, A. J. Milligan, and D. H. Doall. 1993. Iron, selenium, and citric acid are critical to the growth of the "brown tide" microalga, Aureococcus unophugeflerens, p, 667-673. In T. J. Smyda and Y. Shimizu [eds.], Toxic phytoplankton blooms in the sea. Elsevier.

Cottingham, K.L. and S.R. Carpenter. 1998. Population, community, and ecosystem variates as indicators: phytoplankton response to whole-lake enrichment. Ecological Applications 8(2):508-530.

Dagg, M. J. 1983. A method for the determination of copepod feeding rates during short time intervals.

Mar. Biol. 75: 63-67. -. 1985. The effects of food limitation on diel migratory behavior in marine zooplankton. Ergeb. Limnol. 21: 247-255.

Day J. et al. 1989. Estuarine Ecology. John Wiley & Sons Inc. 543 pp.

Dennison, W. C., R.J. Orth, K.A. Moore, J.C. Stevenson, V. Carter, S. Kollar, P.W. Bergstrom, and R.A. Batiuk. 1993. Assessing water quality with submersed aquatic vegetation. Bioscience 43(2): 86-94

Derksen, D. V., and D. H. Ward. 1993. Life history strategies and habitat needs of the black brant. Fish and Wildl. Leaflet 13.1.15.

Devlin, M., Best, M., Coates, D., Bresnan, E., O'Boyle, S., Park, R., Silke, J., Cusack, C., Skeats, J., 2007. Establishing boundary classes for the classification of UK marine waters using phytoplankton communities. Mar. Pollut. Bull. 55, 91–103.

Devlin, M., Barry, J., Painting, S., and Best, M. 2009. Extending the phytoplankton tool kit for the UK Water Framework Directive: indicators of phytoplankton community structure. Hydrobiologia 633: 151–168.

Diaz, R. J. 2001. Overview of Hypoxia around the World. Journal of Environmental Quality 30:275-281.

Duarte, C. M.,and S. Agusti´. 1998. The CO2 balance of unproductiveaquatic ecosystems. Science 281: 234–236.

Dugdale, R.C., F.P. Wilkerson, V.E. Hogue, and A. Marchi. 2007. The role of ammonium and nitrate in spring bloom development in San Francisco Bay. 2007. Estuarine, Coastal and Shelf Science. 73: 17-29.

Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. Fish. Bull. 70: 1063–1085.

Ferraro, S.P., and F.A. Cole. 2007. Benthic macrofauna--habitat associations in Willapa Bay, Washington, USA. Estuarine, Coastal and Shelf Science 71:491-507.

Ferreira J.G. A.M. Nobre, T.C. Simas, M.C. Silva, A. Newton, S.B. Bricker, W.J. Wolff, P.E. Stacey, A. Sequeira. 2006. A methodology for defining homogeneous water bodies in estuaries. Application to the transitional systems of the EU Water Framework Directive. Estuarine, Coastal and Shelf Science. 66: 468-482

Gallegos C.L.2001. Calculating optical water quality targets to restore submerged aquatic vegetation: Overcoming problems partitioning diffuse attenuation coefficient for photosynthetically active radiation.

Gallegos, C.L. and J.W. Kenworthy. 1996. Seagrass depth limits in the Indian River Lagoon (Florida): application of an optical water quality model. Est. Coast. Shelf Sci., 42:267-288.

Garver, S. A., D. A. Siegel, and B. G. Mitchell. 1994. "Variability in near-surface particulate absorption spectra: What can a satellite ocean color imager see?," Limnol. Oceanogr. 39, 1349-1367 (1994).

Gescher, C., Metfies, K., Medlin, L.K., 2008. TheALEX CHIP –development of aDNAchip for identification and monitoring of Alexandrium. Harmful Algae 7, 485–494.

Glibert P.M., J. Harrison, et al. (2006). "Escalating Worldwide Use of Urea: A Global Change Contributing to Coastal Eutrophication." Biogeochemistry 77(3):441-463.

Glibert, P. M., R. Magnien, et al. (2001). "Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events." Estuaries 24(6A): 875-883.

Glibert, P.M., 2010. Long-term changes in nutrient loading and stoichiometry and their relationships with changes in the food web and dominant pelagic fish species in San Francisco Estuary, California. Reviews in Fisheries Science 18, 211-232.

Gilbert, P., Anderson, D.A., Gentien, P., Granéli, E., Sellner, K.G.. 2005a. The global complex Phenomena of Harmful Algal Blooms. Oceanography, 18 (2), 136-147.

Gilbert, P., Seitzinger, S., Heil, C.A., Burkholder, J.M., Parrow, M.W., Codispoti, L.A., Kelly, V.. 2005b. The role of eutrophication in the global proliferation of Harmful Algal Blooms: New perspectives and new approaches. Oceanography, 18 (2), 198-209.

Glibert, P.M., J.M. Burkholder, E. Graneli and D.M. Anderson. 2008. Advances and insights in the complex relationships between eutrophication and HABs : Preface to the special issue. Harmful Algae 8: 1-2.

Glibert, P.M., J. Landsberg, J. Evans, M.A. Al-Sarawi, M. Faraj, M.A. Al-Jarallah, A. Haywood, S. Ibrahem, P. Klesius, C. Powell, and C. Shoemaker. 2002. A fish kill of massive proportion in Kuwait Bay, Arabian Gulf, 2001: The roles of infectious bacteria, harmful algae, and eutrophication. Harmful Algae. 1: 215-231

Hallegraeff, G.M. 1993. A review of harmful algae blooms and their apparent global increase. Phycologia 32: 79-99.

Hallegraeff, G.M. and Anderson, D.M. and Cembella, A.D., 2003. Manual on Harmful Marine Microalgae, UNESCO, Landais, pp. 792. ISBN 9231038710 (2003)

Havens, K. E., J. Hauxwell, A. C. Tyler, S. Thomas, K. J. McGlathery, J. Cebrian, I. Valiela, A. D. Steinman and S.-J. Hwang. 2001. Complex interactions between autotrophs in shallow marine and freshwater ecosystems: implications for community responses to nutrient stress. Environmental Pollution 113:95-107.

Heck K.L, Hays C., and Orth R.J.. 2003. A critical evaluation of the nursery role hypothesis for seagrass meadows. Marine Ecology Progress Series 253:123–136.

Heisler, J., P. Glibert, J. Burkholder, D. Anderson, W. Cochlan, W. Dennison, Q. Dortch, C. Gobler, C. Heil, E. Humphries, A. Lewitus, R. Magnien, H. Marshall, K. Sellner, D. Stockwell, D. Stoecker, and M. Suddleson. 2008. Eutrophication and harmful algal blooms: A scientific consensus. Harmful Algae. 8: 3-13.

Herndon, J., W.P. Cochlan, R.A. Horner. 2003. Heterosigma akashiwo blooms in San Francisco Bay. IEP Newsletter. 16 (2): 46-48.

Hillman, K., Lukatelich R. J. & McComb, A. J. 1990. The impact of nutrient enrichment on nearshore and estuarine ecosystems in Western Australia. Proc Ecol Soc Aust 16 , 39-53.

Howarth, R. W. 1988. Nutrient limitation of primary production in marine ecosystems. Annual Review of Ecology and Systematics 19:89-110.

Howarth, R. W., A. Sharpley, and D. Walker. 2002. Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals. Estuaries 25:656-676.

Howarth, R. W., A. Sharpley, et al. (2002). "Sources of nutrient pollution to coastal waters in the United States: Implications for achieving coastal water quality goals." Estuaries 25(4B): 656-676.

Janicki, A.J., D. Wade, and J.R. Pribble. 2000. Establishing a process for tracking chlorophyll-a concentrations and light attenuation in Tampa Bay. Prepared for: Tampa Bay Estuary Program. Prepared By: Janicki Environmental, Inc.

Janicki, A., M. Dema, R. Nijbroek. 2009. Seagrass Targets for the Sarasota Bay Estuary Program.Prepared for the Sarasota Bay Estuary Program. Prepared by Janicki Environmental, Inc.

Jaworski, N. A. (1981). Sources of nutrients and the scale of eutrophication problems in estuaries. In: Nielson, B. J., Cronin, L. E. (ed.) Estuaries and nutrients. Humana Press, Clifton

Kahru, M., Mitchell, G.. 2001. Seasonal and nonseasonal variability of satellite-derived chlorophyll and colored dissolved organic matter concentration in the California Current. Journal of Geophysical Research, 106 (C2), 2517-2529.

Kemp, W.M., R. Batiuk, R. Bartleson, P. Bergstrom, V. Carter, C.L. Gallegos, and W. Hunley. 2004. Habitat requirements for submerged aquatic vegetation in Chesapeake Bay: Water quality, light regime, and physical-chemical factors. Estuaries 27: 363– 377.Kennedy, V.S. (ed). 1982. Estuarine Comparisons. Academic Press, New York.

Kudela, R. M., J. Q. Lane, et al. (2008). "The potential role of anthropogenically derived nitrogen in the growth of harmful algae in California, USA." Harmful Algae 8(1): 103-110.

Lacouture, R.V., Johnson, J.M., Buchanan, C., Marshall, H.G., 2006. Phytoplankton index of biotic integrity for Chesapeake Bay and its tidal tributaries. Estuaries and Coasts 29, 598–616.

Lam C.W.Y., Ho K.C. 1989. Red Tides in Tolo Harbour, Hong Kong. In: Red Tides. Eds. Okaichi T., Anderson D.M. Nemoto T. Elsevier. 49-52.

LaPointe, B. E., and W. R. Matzie. 1996. Effects of stormwater nutrient discharges on eutrophication processes in nearshore waters of the Florida Keys. Estuaries 19: 422-43

Lehman, P.W., and Walker, S., 2003. Microcystis blooms in the Delta. IEP News Letter 16, 18-19.

Lehman, P.W., G. Boyer, C. Hall, S. Waller, K. Gehrts. 2005. Distribution and toxicity of a new colonial Microcystis aeruginosa bloom in San Francisco Bay Estuary, California. Hydrobiologia. 541: 87-99.

Lehman, P.W., G. Boyer, M. Satchwell, and S. Waller. 2008. The influence of environmental conditions on the seasonal variation of Microsystis cell density and microcystins concentration in San Francisco Estuary. Hydrobiologia. 600: 187-204.

Lewitus A.J., Koepfler E.T., Morris J.T. 1998. Seasonal variation in the regulation of phytoplankton by nitrogen and grazing in a saltmarsh estuary. Limnol Oceanogr 43:636–646

Li Y. and T.J. Smayda. 1998. Temporal variability of chlorophyll in Narragansett Bay, 1973-1990. ICES J Mar Sci 55:661–667

Macky, M. D., D. J. Mackey, H. W. Higgins, and S. W. Wright. 1996. CHEMTAX-A program for estimating class abundanc- es from chemical markers: Application to HPLC measure- ments of phytoplankton. Marine Ecology Progress Series 144:265- 283.

Madden C., R. Smith, E. Dettman, J. Kurtz, W. Nelson, N. Detenbeck, J. Latimer, and S. Bricker. 2010. Estuarine Typology Development and Application. In Nutrient in Estuaries: A Summary Report of the

National Estuarine Experts Workgroup 2005–2007. P.M. Glibert, C.J.. Madden, W. Boynton, D. Flemer, C. Heil and J. Sharp(eds). US Environmental Protection Agency. Available online http://water.epa.gov/scitech/swguidance/standards/criteria/nutrients/upload/Nutrients-in-Estuaries-November-2010.pdf

Madden, C. and J. Day. 1992. An instrument system for high-speed mapping of chlorophyll a and physico-chemical variables in surface waters. Estuaries. 15(3):421-427.

Madden, C.J., D.T. Rudnick, A.A. McDonald, K.M. Cunniff, J.W. Fourqurean. 2009. Ecological indicators for assessing and communication seagrass status and trends in Florida Bay. Ecological Indicators 9S: S68-S82. doi:10.1016/j.ecolind.2009.02.004

Malone, T C. (1982). Phytoplankton photosynthesis and carbon-specific growth light-saturated rates in a nutrient saturated Environment. Limnol. Oceanogr 27: 226-235

Maritorena S., A. Morel, and B. Gentili. 1994. Diffuse reflectance of oceanic shallow waters: Influence of water depth and bottom albedo. Limnol. Oceanogr., 39(7) 1689-1703.

Martin S. 1994. An Introduction to Ocean Remote Sensing, University of Washington Press.

McGlathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient enriched coastal waters. Journal of Phycology 37:453-456.

McKee, L.J., Sutula, Gilbreath, A.N., Gillett D., Beagle, J., Gluchowski, D., and Hunt, J., Numeric nutrient endpoint development for San Francisco Bay- Literature review and Data Gaps Analysis. Southern California Coastal Water Research Project Technical Report No. 644. April 2011.

Miller MA, Kudela RM, Mekebri A, Crane D, Oates SC, et al. 2010 Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. PLoS ONE 5(9): e12576.

Millie D., H. W. Paerl, and J. P Hurley 1993. Microalgal pigment assessments of high-performance liquid chromatography: Asynopsis of organismal and ecological applications. Can. J. Fish. Aquat. Sci. 50: 2513-2527.

Millie, D.F., G.R. Weckman, H.W. Paerl, J.L. Pinckney, B.J. Bendis, R.J. Pigg, G.L. Fahnenstiel. 2004. Neural net modeling of estuarine indicators: Hindcasting phytoplankton biomass and net ecosystem production in the Neuse (North Carolina) and Trout (Florida) Rivers, USA. Ecological Indicators

Monbet, Y. 1992. Control of phytoplankton biomass in estuaries: A comparative analysis of microtidal and macrotidal estuaries. Estuaries 15(4): 563–571.

Monod. J. 1942. Recherches sur la croissance des cultures bactriennes,2 11 pp.

Moore, J.E., M.A. Colwell, R.L. Mathis, J.M. Black. 2004. Staging of Pacific flyway brant in relation to eelgrass abundance and site isolation, with special consideration of Humboldt Bay, California. Biological Conservation 115: 475-486.

Morris, L.J., and D.A. Tomasko (eds.). 1993. Proceedings and Conclusions of Workshops on: Submerged Aquatic Vegetation and Photosynthetically Active Radiation. Special Publication SJ93-SP13. Palatka, Fla.: St. Johns River Water Management District.

Muller-Karger, F. E., C. Hu, S. Andréfouët, and R. Varela. 2005. The Color of the Coastal Ocean and applications in the solution of research and management problems. In: Remote Sensing of Coastal

Aquatic Environments: Technologies, Techniques and Application, R.L. Miller, C.E. Del Castillo and B.A. McKee [Eds.], Springer, 101-127.

Ning, X., J.E. Cloern, and B.E. Cole. 2000. Spatial and temporal variability of picocyanobacteria Synechococcus sp. in San Francisco Bay. Limnology and Oceanography. 45 (3): 695-702.

Nixon, S. W. and M. E . Q. Pilson. 1983. Nitrogen in estuaries and marine ecosystems. Pp. 565–648 In: Carpinter, E. J., Capone, D. G. (eds.), Nitrogen in the marine environment. Academic Press, New York.

Officer, C. B. and J. H. Ryther. 1980. The possible importance of silicon in marine eutrophication. Marine Ecology Progress Series 3:83-91.

Orth, R.J., T.J.B. Carruthers, W.C. Dennison, C.M. Duarte, J.W. Fourqurean, K.L. Heck Jr., A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott, S.L. Williams. 2006. A global crisis for seagrass ecosystems. BioScience 56:987-996.

Paerl, H.W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnology and Oceanography, 33(4) Part 2: Comparative Ecology of Freshwater and Marine Ecosystems, 823-847.

Coastal eutrophication and harmful algal blooms: Importance of atmospheric deposition and groundwater as "new" nitrogen and other nutrient sources. Limnology and Oceanography 42:1154-1165.

Paerl, H., et al., 2003. Phytoplankton photopigments as indicators of Estarine and Coastal Eutrophication. Bioscience 53: 1-12.

Paerl, H.W., Dyble, J., Pinckney, J.L., Valdes, L.M., Millie, D.F., Moisander, P.H., Morris, J.T., Bendis, B., and Piehler, M.F., 2005. Using Microalgal Indicators to Assess Human- and Climate-Induced Ecological Change in Estuaries. In S. Bertone (ed.) Estuarine Indicators. Boca Raton Florida: CRC Press, Inc. p 145-172.

Paerl, H.W., Valdes, L.M., Peierls, B.L., Adolf, J.E., Harding, L.W., 2006. Anthropogenic and climatic influences on the eutrophication of large estuarine ecosystems. Limnology and Oceanography Vol. 51, 448-462.

Paerl, H.W., L.M. Valdes, A.R. Joyner, and V. Winkelmann. 2007. Phytoplankton Indicators of Ecological Change in the Nutrient and Climatically-Impacted Neuse River-Pamlico Sound System, North Carolina . Ecological Applications 17(5): 88-101.

Paerl, H.W. 2007. Nutrient and other environmental controls of harmful cyanobacterial blooms along the freshwater-marine continuum. In, H. K. Hudnell (Ed.) Proceedings of the Interagency, International Symposium on Cyanobacterial Harmful Algal Blooms. Advances in Experimental Medicine and Biology, Ch. 10, 215-241.

Peterson, T. William, et al. (2003). A new climate regime in northeast pacific ecosystems. Washington, DC, ETATS-UNIS, American Geophysical Union.

Peterson, C.H., H.C. Summerson, P.B. Duncan. 1984. The influence of seagrass cover on population structure and individual growth rate of a suspension-feeding bivalve, Mercenaria mercenaria. Journal of Marine Research 42: 123-138.

Pinckney, J., T. Richardson, D. Millie & H. Paerl. 2001. Application of photopigment biomarkers for quantifying microalgal community composition and in situ growth rates. Organic Geochemistry 32:585-595.

Richardson, K., 1997. Harmful or exceptional phytoplankton blooms in the marine ecosystem. Adv. Mar. Biol. 31, 301–385.

Robinson I.S. 1985. Satellite Oceanography. Ellis Horwood, Chichester, England.

Rothenberger, M.B., Burkholder, J.M., and Wentworth T.R. 2009. Use of long-term data and multivariate ordination techniques to identify environmental factors governing estuarine phytoplankton species dynamics. Limnology and Oceanography 54: 2107–2127.

Rue, E. L. and K.W. Bruland. 2001. Domoic acid binds iron and copper: A possible role for the toxin produced by the marine diatom Pseudo-nitzchia. Marine Chemistry, 76, 127-134.,

Ryther, J. H. and Dunstan, W. M. 1971. Nitrogen, phosphorus and eutrophication in the coastal marineenvironment. Science 171: 1008–1013.

Scholin, C.A., Gulland, F., Doucette, G.J., Benson, S., Busman, M., Chavez, F.P., Cordarok, J., DeLong, R., De Vogelaere, A., Harvey, J., Haulena. M., LefebvreI, K., Lipscomb, T., Loscutoff, S., Lowenstine, L.J., Marin, R., Miller, P.E., McLellan, W.A., Moeller, P.D.R., Powell, C.L., Rowleskk, T., Silvagni, P., SilverI, M., Spraker, T., Trainer, V., Van Dolah, F.M.. 2000. Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature, 403, 80-84.

Scholin, C.A., Miller, P.E., Buck, K.R., Chavez, F.P., Harris, P., Haydock, P., Cangelosi, J., 1997. Detection and quantification of Pseudo-nitzschia australis in cultured and natural populations using LSU rRNA-targeted probes. Limnology and Oceanography 42, 1265–1272.

Scholin, C., Marin, R., Miller, P., Doucette, G., Powell, C., Howard, J., Haydock, P., Ray, J., 1999. Application of DNA probes and a receptor binding assay for detection of Pseudo-nitzschia Bacillariophyceae) species and domoic acid activity in cultured and natural samples. Journal of Phycology 35, 1356–1367.

Scholin, C.A., Vrieling, E., Peperzak, L., Rhodes, L., Rublee, P., 2003. Detection of HAB species using lectin, antibody and DNA probes. In: Hallegraeff, G., Anderson, D., Cembella, A. (Eds.), Manual on Harmful Marine Microalgae. Intergovernmental Oceanographic Commission, UNESCO, Paris, pp. 131–164.

Seitzinger, S. R.W. Sanders, and R. Styles, 2002. Bioavailability of DON from natural and anthropogenic sources to estuarine plankton. Limnol. Oceanogr. 47: 353–366.

Sharp, J.H., 2010. Estuarine oxygen dynamics: what can we learn about hypoxia from long-time records in the Delaware Estuary? Limnology and Oceanography 55, 535–548.

Short, F. T., D. M. Burdick and J. E. Kaldy. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, Zostera marina. Limnology and Oceanography 40:740-749

Short, F. T., G. E. Jones and D. M. Burdick. 1991. Seagrass decline: Problems and solutions. Coastal Zone '91 Conference - ASCE. Long Beach, CA/July 1991. Pp 439-453.

Sinclair, M., D. V.Subba Rao and R. Courture 1981. Phytoplankton temporal distributions in estuaries., Oceanol. Acta, 4, 239-246.

Smayda T. 1990. Novel and nuisance phytoplankton blooms in the sea: Evidence for a global epidemic, p. 29-40. In Toxic marine phytoplankton: Proc. 4th Int. Conf. on Toxic Marine Phytoplankton. Elsevier.

Smayda T. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnology and Oceanography. 42(5, part 2): 1137-1153

Smetacek, V. and J.E. Cloern. 2008. On phytoplankton trends: How are phytoplankton at coastal sites around the world responding to ongoing global change? Science. 319: 1346-1348.

Smith, V. H. 2006. Responses of estuarine and coastal marine phytoplankton to nitrogen and phosphorus enrichment. Limnology and Oceanography, 51: (1, part 2) 377-384.

Souchu, P., Ximenes, M.C., Lauret, M., Vaquer, A., Dutrieux, E., 2000. Mise à jour d'indicateurs du niveau d'eutrophisation des milieux lagunaires méditerranéens, août 2000, Ifremer-Créocean-Université Montpellier II, 412 p.

Stevenson, J. C., L.W. Staver, and K.W. Staver. 1993. Water quality associated with survival of submersed aquatic vegetation along an estuarine gradient. Estuaries 16(2): 346-361

TetraTech 2006. Technical approach to develop nutrients numeric endpoints for California. Prepared for: U.S. EPA Region IX (Contract No. 68-C-02-108-To-111)

Theede, H. 1973. Comparative studies on the influence of oxygen deficiency and hydrogen sulphide on marine bottom invertebrates. Netherlands Journal of Sea Research 7:244-252.,

Thurman, H. V. 1997. Introductory Oceanography. New Jersey, USA: Prentice Hall College.

Twilley, R. R. (1985). "The exchange of organic carbon in basin mangrove forests in a southwest Florida estuary." Estuarine Coastal and Shelf Science 20: 543-557.

Underwood, G. J. C. and J. Kromkamp. 1999. Primary production by phytoplankton and microphytobenthos in estuaries. Page 306 in D. B. Nedwell and D. G. Raffaell, editors. Advances in ecological research estuaries. Academic Press, London

Valiela, I. 1995. Marine Ecological Processes. 2nd edition. Springer-Verlag, New York.

Walker, W.W. 1985. Statistical bases for mean chlorophyll a criteria. Lake and Reservoir Management, 1:57-62. [alternate volume title: Lake and Reservoir Management – Practical Applications; North American Lake Management Society.]

Walker, W.W. 1987. Empirical Methods for Predicting Eutrophication in Impoundments. Report 4–Phase III: Applications Manual. Technical Report E-81-9. U.S. Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS.

Ward, D.H. 1983. The relationship of two seagrasses: Zostera marina and Ruppia maritima to the Black Brant, Branta bernicla nigricans, San Ignacio lagoon, Baja California, Mexico. MS Thesis. University of Oregon, Eugene OR. 60 p.

Wazniak, C.E. and M.R. Hall [Ed]. 2005. Maryland's Coastal Bays: Ecosystem Health Assessment 2004. DNR-12-1202-0009. Maryland Department of Natural Resources, Tidewater Ecosystem Assessment, Annapolis, MD

Wells, M.L., Trick, C.G., Cochlan, W.P., Hughes, M.P., Trainer, V.L., 2005. Domoic acid: the synergy of iron, copper, and the toxicity of diatoms. Limnology and Oceanography 50, 1908–1917.

Westberry T.J, D. Siegal , and A. Subramaniam. 2005. An improved bio-optical model for the remote sensing of Trichodesmium spp. blooms. Journal of Geophysical Research, VOL. 110, C06012

WHO 1993 Guidelines for Drinking-water Quality, Second edition. Volume 1, Recommendations. World Health Organization, Geneva.

WHO 1996 Guidelines for Drinking-water Quality, Second edition. Volume 2, Health Criteria and Other Supporting Information. World Health Organization, Geneva.

Wilkerson, F.P., R.C. Dugdale, V.E. Hogue, and A. Marchi. 2006. Phytoplankton blooms and nitrogen productivity in San Francisco Bay. Estuaries and Coasts. 29 (3): 401-416.

Zaldívar, J.M., Cardoso, A.C., Viaroli, P., Newton, A., de Wit, R., Ibañez, C., Reizopoulou, S., Somma, F., Razinkovas, A., Basset, A., Holmer, M. and Murray, N. 2008. Eutrophication in transitional waters: An overview. Transitional Waters Monographs : 1(2008), 1-78

5. Suitability of Seagrasses and Submerged Aquatic Vegetation as Indicators of Eutrophication

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5.1 Introduction

Rooted submerged aquatic vegetation (SAV) encompasses a large diversity of species that range from obligate halophytes (e.g., seagrasses, *Zostera marina, Z. japonica*) to euryhaline species (e.g., *Ruppia maritima, Vallisneria* spp., *Stuckenia pectinata*) to freshwater obligates (e.g., *Elodea canadensis, Nuphar* spp.). The primary features distinguishing between groups of SAV are salinity tolerance and pollination vectors. Throughout the course of this review, the term "seagrass" will be applied exclusively to genera that are obligate halophytes, exhibit hydrophilous (underwater) pollination and form meadows; this includes but is not limited to, *Zostera, Phyllospadix, Halodule, Thalassia, Halophila*, etc. "Brackish SAV" or "aquatic beds" will be applied to genera that are euryhaline species, exhibit aerial or surface pollination and tend to form canopies; this includes but is not limited to *Ruppia, Stuckenia, Zannichellia, Myriophyllum*, etc.

All seagrass and SAV provide key biological functions within the enclosed bays, estuaries, and lagoons where they are found. They provide three dimensional structure in the water column that functions as habitat and nursery areas for commercially and recreationally important marine and freshwater fish and invertebrates, including threatened and endangered species (Blackmon et al. 2006, Waycott et al. 2009, Hughes et al. 2009). In addition to providing important habitat for fish, seagrass and SAV are considered to be important resources supporting migratory birds (Derksen and Ward 1993); for example the non-indigenous seagrass *Zostera japonica* may support shorebirds (Lamberson et al. 2011). Seagrass and SAV can be significant sources of primary production in nearshore marine systems, supplying the base of detrital food webs (Fenchel 1977). In addition, several organisms directly graze upon the plants or consume epiphytes and epifauna supported by plant structures, thus these plants contribute to the system at multiple trophic levels (Kikuchi and Pérès 1977, Phillips 1984, Thayer et al. 1984 Heck and Valentine 2006). Finally, seagrass and SAV can improve water quality by facilitating nutrient cycling, oxygenating the water column, trapping suspended particulates, and reducing erosion by stabilizing the sediment (Phillips 1984, Thayer et al. 1984).

These macrophytes have a variety of characteristics that make them good candidates to be "end-points of concern" for numeric nutrient criteria or "bio-indicators". First, many of these species, especially the seagrasses, are perennial and form persistent rhizomes; consequently they act as "long term integrators" responding to environmental change (Burkholder et al. 2007). Second, as rooted organisms, they are not mobile and cannot move in response to changing environmental drivers. Third, for a number of key seagrass and SAV species (including *Zostera marina* and *R. maritima*) the biological and physiological requirements are known well enough to develop models of how the plants respond to stressors. Finally, a number of very well designed monitoring programs currently use seagrasses as bio-indicators (Foden and Brazier 2007, Madden et al. 2009) including government organizations such as

Washington State Department of Natural Resources[7] and non-governmental organizations (http://seagrassnet.org).

The purpose of this review is to summarize existing information on seagrass and brackish SAV with regard to their suitability as indicators to diagnose eutrophication in California estuaries, utilizing the criteria specified in Chapter 1. Although many estuarine systems do support SAV it is important to recognize that not all systems would be expected to support these plant communities based on the morphology and hydrology of the system.

5.2 General Ecology of Seagrass and Brackish Water SAV Common to California Estuaries

Seagrasses and brackish SAV generally occur as beds in shallow, soft-sediment environments of bays and estuaries. Their distribution is naturally limited to high light environments where photosynthesis can support the roots buried in soft sediments. Along the West coast of the US including California, the upper limit of vertical distribution is generally controlled by desiccation and the lower boundary by light limitation (Boese et al. 2005). In some areas of higher energy enclosed bays (see definition, Appendix B), the upper limit of SAV can be controlled by wave energy and sand scour. Within California estuaries (see definition, Appendix B), seagrass is typically found in perennially tidal, enclosed bays and coastal lagoons. A handful of enclosed bays represent the majority of seagrass habitat found in California, including: San Diego Bay, Mission Bay, Newport Bay, Morro Bay, San Francisco Bay, Tomales Bay, and Humboldt Bay (Merkel et al. 2009). Brackish SAV species are commonly found near sources of freshwater input in enclosed bays, perennially tidal lagoons, and some river mouth estuaries, particularly during low flow periods. They are also found in intermittently, ephemerally tidal lagoons in the closed condition (Grewell et al. 2007).

In California enclosed bays and estuaries, *Zostera marina* (commonly known as eelgrass) is the dominant seagrass species. Taxonomically, the genus Zostera consists of about 9 species worldwide; *Zostera marina* has a circum-global distribution in the north Pacific and Atlantic Oceans and is the most widely distributed of all seagrass species (Green and Short 2003). The biology and ecology of *Z. marina* and other species are well characterized from North America and Europe and have been summarized in a number of volumes (Burkholder and Doheny 1968, Thayer et al. 1984, Phillips 1984, Hemminga and Duarte 2000, Bortone 2000, Larkum et al. 2006). Two seagrasses, *Phyllospadix scouleri* and *P. torreyi*, occur on the rocky intertidal open coast and are outside the consideration of this document.

Two recognized species of *Zostera* are documented from California systems. The native eelgrass *Zostera marina* is known from the entire Pacific Coast of North America, from Alaska into Mexico (Green and Short 2003). The second species is the non-native "dwarf eelgrass" *Zostera japonica* known from Oregon and Washington (Kaldy 2006 and others) that has recently been documented in Humboldt Bay, CA. California Fish and Game has attempted to eradicate the known Humboldt populations (Eicher 2006).

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[7] www.dnr.wa.gov/ResearchScience/Topics/AquaticHabitats/Pages/aqr_nrsh_eelgrass_monitoring.aspx

Both *Zostera marina* and *Z. japonica* are obligate halophytes that require marine salinity regimes (Tyerman 1982, Touchette 2007); although *Z. japonica* does appear to be more euryhaline and has been observed growing in marsh channels with low salinity (Shafer et al. 2011). Many aspects of the physiology of *Z. marina* have been well documented especially with regard to the light and nutrient requirements of this species for growth. There are a variety of models and indicators that have been developed to evaluate *Z. marina* response to stressors. In contrast the physiology of *Z. japonica* is not as well documented; although this is an active area of research and there is little information on the ecological consequences of *Z. japonica* colonization.

Some information is available on the taxonomy of SAV species found in brackish to euryhaline portions of California estuaries (Grewell et al. 2007). In predominantly brackish estuaries of the North Coast *Stuckenia pectinata* (sago pondweed), *Ruppia maritima* (widgeon grass), *Zannichellia palustris* (horned pondweed) and *Myriophyllum verticillatum* (whorl-leaf water milfoil) are characteristic (Grewell et al. 2007). In the muted brackish tidal ponds of San Francisco Bay *Stuckenia pectinata*[8] and *Ruppia maritima* were noted SAV species (Grewell et al. 2007), and *S. pectinata* is abundant in the brackish northern San Francisco Estuary (K. Boyer, San Francisco State University, pers. comm.). Along the central coast brackish SAV species include *S. pectinata* and *R. cirrhosa* (Grewell et al. 2007). The California South Coast estuaries with strong freshwater influence are characterized by R. cirrhosa, and those with hypersalinity are dominated by *Ruppia maritima* (Grewell et al. 2007). *Ruppia* spp. dominated SAV species found in the closed, brackish lagoons and river mouth estuaries during a regional survey of eutrophication in Southern California estuaries (K. McLaughlin, SCCWRP, pers. comm.). *Ruppia* spp. was the only SAV species observed in a survey conducted in Klamath River estuary (H. Lee and C. Brown, PCEB US EPA, unpublished data).

Although seagrass and SAV do support a wide variety of beneficial uses and ecosystem services it is important to recognize that not all estuarine systems would be expected to support seagrass or SAV. Haines et al. (2006) found a relationship between water level variability and seagrass coverage for "intermittently closed and open lake or lagoon" (ICOLL). Systems with highly variable water levels did not generally contain seagrass or SAV. These systems generally did not contain seagrass or SAV because they tend to "break-out" frequently which "re-sets" the system when the accumulated substrate is washed out. These "break-out" events result in the removal of pelagic organisms and dessication of epiphytes, macrophytes and benthos. The period of fluctuating water level cycles associated with these break outs is likely to exceed the critical rate of seagrass and SAV recruitment via seed when the water level is highly variable (see Haines et al. 2006 for additional details).

Further details on the ecology of *Ruppia* spp. and Zostera spp., the dominant seagrass and brackish SAV species in California enclosed bays and estuaries, are given below. Other SAV species, particularly *Stuckenia pectinata* are locally important; however, comprehensive review of all SAV is beyond the scope of this document. The interested reader is directed to the following documents as an

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[8] *Stuckenia pectinata* is the currently accepted nomenclature for *Potamogeton pectinatus*

introduction to the biology and ecology of *Stuckenia* (Kantrud 1990, Orth et al. 2010) and its use as assessment end-point (Hoven and Miller 2009, Hoven et al. 2011).

5.2.1   Ruppia maritima L.

Taxonomically, the genus *Ruppia* consists of at least 4 to 6 species and a number of varieties depending on the classification scheme (Kantrud 1991). Recent molecular phylogeny recognizes 5 species and a *R. maritima* complex consisting of diploid and triploid hybrids (Yu Ito, Univ. of Tokyo, Japan, pers. comm.). *R. maritima* is nearly cosmopolitan in distribution between about 69 °N and 55 °S latitude and is reported from salinities ranging between freshwater and hypersaline (Kantrud 1991). Kantrud (1991) provides an excellent literature review of the autecological and physiological characteristics of *R. maritima*. The extreme tolerance of *Ruppia* spp. to salinity has been an intense area of study, especially with respect to reproductive allocation, seed germination and seedling survival. *Ruppia* spp. appear to osmoregulate by accumulating the amino acid proline and soluble carbohydrates (Brock 1981, Murphy et al. 2003); additionally, low salinity appears to stimulate seed germination (Koch and Dawes 1991, Kahn and Durako 2005)

The life cycle of *Ruppia maritima* is population specific; some populations exhibit a perennial life-cycle while other populations appear to be annual. In some areas, *Ruppia* dominated beds are known for their seasonal and annual fluctuations, lower peak biomass and production (relative to *Zostera marina* or *Thalassia testudinum*) which may affect food webs, increase exposure to predation and negatively affect survival of fishery species (Cho et al. 2009). There also appears to be a correlation between the annual form and water permanence, where ephemeral water bodies generally contain annual populations. Warm temperatures associated with the evaporation of ephemeral waters also appear to contribute to expression of an annual life cycle (Malea et al. 2004). To date, few experiments have been conducted to investigate if these are "truly" obligate annual populations or if they are stressed perennial plants that have gone reproductive. Work conducted by Setchell (1924) found that "annual" plants cultured in the lab flourished and flowered throughout the year. Biomass and productivity patterns have been examined from a variety of systems ranging from tropical to temperate. In tropical and sub-tropical systems, both biomass and productivity generally exhibit a bi-modal distribution with a mid-summer depression due primarily to high temperatures. Alternatively, pulsed salinity changes caused reduced growth of *R. maritima* from the Gulf of Mexico (La Peyre and Rowe 2003). In temperate systems, biomass and productivity appear to be unimodal with a summer maximum. Reproductive effort appears to be strongly correlated with spring biomass (Bonis et al. 1993).

The nutrient requirements for laboratory culture of *R. maritima* were described by Thursby (1984); maximum growth occurred at 110 μM NO_3 and 2.3 μM PO_4 and had a critical N content of 2.5-3.0%. The critical nitrogen level is the internal nitrogen concentration that is just limiting growth. Furthermore, field populations in Rhode Island appeared to be N limited during summer; however, this conclusion may be confounded by translocation to support floral development (Thursby 1984). Nitrogen uptake rates for both leaves (24 to 46 nM mg dw^{-1} h^{-1}) and roots (0.1 to 8.5 nM mg dw^{-1} h^{-1}) of *R. maritima* were measured by Thursby and Harlin (1984). Minimum light requirements for *R. maritima* have not been established in the literature; although, growth was not light saturated at 2000 foot-candles (Thursby

1984). It should be noted that there is not direct conversion for light measured in foot-candles to equivalent photosynthetically active radiation. Much of what is known about brackish SAV light requirements is summarized by Batuik et al. (2000); with saturating irradiance (Ik) ranging between 40 and 700 µmol photons $m^{-2} s^{-1}$ and compensating irradiances (Ic) ranging between 2 and 80 µmol photons $m^{-2} s^{-1}$. Based on the paucity of information, Batuik et al. (2000) suggest that substantial new research is required to better define the light requirements for many SAV species. Leaf nutrient content and exogenous nitrogen and phosphorus additions appear to increase the rate of *Ruppia cirrhosa* decomposition from the Mediterranean (Menéndez et al. 2003, Menéndez 2009). *Ruppia* below-ground biomass appears to be closely related to sediment biogeochemical cycling, anaerobic decomposition of sediment organic matter can lead to the release of free-sulfides which can cause rhizome/root mortality (Azzoni et al. 2001). The linkage between below-ground biomass and sediment biogeochemistry (sulfide production from sulfate reducing bacteria) may have contributed to the loss of *Ruppia* from Mediterranean estuaries (Heijs et al. 2000).

5.2.2   Zostera marina L.

Zostera marina is generally considered a perennial species; however, "annual" populations have been identified in the literature at the southern end of the distribution on the West Coast (Meling-Lopez and Ibarra-Obando 1999). Although genetic differentiation has been detected between populations (Muñiz-Salazar et al. 2005), it is not clear if these are truly annual plants or if they exhibit a compressed life-cycle due to environmental (temperature) forcing. A better description of this life-history is "semelparous", organisms which reproduce once and then die (Silvertown 1987); since the term "annual" implies an inherent genetically controlled trait. In addition, there is at least one semeparous population in San Francisco Bay (near Alameda) that relies on seed for recruitment each spring at least partly due to Canada geese herbivory during the fall months (Boyer and Wyllie-Echeverria 2010,). Most populations, including those in Humboldt Bay, have a vertical distribution from about -2 m to about +0.3 m relative to mean lower low water (Keller and Harris 1966, Boese et al. 2005). Underwater irradiance has been identified as a major factor controlling the survival of transplants at different depths in San Francisco Bay (Zimmerman et al. 1995). Recent studies suggest that water temperature warming associated with El Nino events may lead to declines in eelgrass (Thom et al. 2003) and in some cases replacement with *Ruppia maritima* (Johnson et al. 2003). Little is known about the factors controlling the spread of *Z. japonica* and its interactions with *Z. marina* and the response of these congeners to sea-level rise and global climate change. The minimum light requirements for eelgrass from the Pacific Northwest have been determined for field populations to be about 3 moles photons $m^{-2} d^{-1}$ (Kaldy and Lee 2007, Thom et al. 2008) and are unlikely to be very different for California populations. The nitrogen requirements for *Z. marina* have been well studied (see Kaldy 2009 and Lee et al. 2007 for reviews) as have the effects of nutrient over enrichment reviewed by Burkholder et al. (2007). Nitrogen uptake rates are typically on the order of 0.01 to 4 µmoles N $gdw^{-1} h^{-1}$ and C:N:P ratios range between 255:17:1 to 576:24:1 depending on tissue type and location (Kaldy 2009). However, much higher rates have also been reported. For example, Thursby and Harlin (1982) determined that *Z. marina* uptake rates ranged between 20 and 210 µmoles N $gdw^{-1} h^{-1}$ for leaf and root tissue, respectively. Other work has shown that *Z. marina* can acquire up to 70% of its nitrogen from the water column (Hemminga et al. 1994); but that

the amount of N acquired from sediment versus water column is dependent on actual concentrations (Zimmerman et al. 1987). More recent work has described the electrochemical mechanisms of N uptake by *Z. marina* and suggests that the plant has an even higher affinity for N than previously believed (Rubio et al. 2007 and references therein). Larned (2003) suggests that *Z. japonica* may be a factor influencing water column N concentrations in Oregon estuaries. However, other calculations suggest that *Z. japonica* only incorporates about 13.5 g N m^{-2} y^{-1} (Kaldy 2006) which, given the limited distribution, would be unlikely to have a measureable drawdown of nitrogen except in some locations with very expansive populations (e.g., Padilla and Willapa Bays, WA).

5.3 Seagrasses and Brackish Water SAV: Ecosystem Services and Relationship with Beneficial Uses

Seagrasses and other SAV are considered to be plant communities that form physical structures like expansive meadows or smaller beds. As a result, they are considered to be "habitat forming" species that create unique biological, physical, and chemical environments when they occur in the form of submerged or intertidal aquatic beds or larger meadows. Eelgrass beds are important ecological communities of shallow bays and estuaries because of the multiple ecological services they sustain (Orth et al. 2006; Figure 5.1). Seagrass and SAV are directly related to a variety of beneficial uses (BU) applied to many of estuaries by the State of California (Table 5.2). Table A.1 in Appendix 1 provides the definitions of the applicable BUs.

Seagrass and SAV are designated marine and/or estuarine habitat that have an obligate requirement for seawater (MAR and EST BUs). They are also wildlife habitat, particularly for waterfowl and shorebirds (WILD BUs). Seagrass and SAV beds function as habitat and nursery areas for commercially and recreationally important open ocean marine and estuarine fish and invertebrates, and provide critical structural environments for resident bay and estuarine species. Many commercially and recreationally (COMM BU) important fisheries species have a life-history stage that is estuarine dependent and many of them utilize seagrass beds; examples include, salmonids, herring, Dungeness crab, and shellfish (Hoffman 1986, Blackmon et al. 2006, Carr et al. 2011). Seagrass also support shellfisheries (SHELL), as a variety of bivalves used for human consumption and bait occur in seagrass beds. Presence of seagrass can influence the population structure and growth rates of clams (Peterson et al. 1984); additionally seagrass patch size and structural characteristics affect bivalve survivorship (Irlandi 1997). Peterson and Heck (2001) suggest that bivalves and seagrass have positive interactions resulting in a facultative mutualism.

Besides providing important habitat for fish and invertebrates, seagrass and SAV are considered to be an important resource supporting migratory birds and spawning fish during critical life stages. Bortolus et al. (1998) found that *Ruppia maritima* was an important food sources for a variety of waterfowl species in Argentina; including swans and ducks. Along the Pacific flyway, both *Ruppia maritima* and *Z. marina* are food resources for Black Brant geese (Ward 1983, Derksen and Ward 1993, Moore et al. 2004). Seagrass and SAV meet the spawning BUs as they provide a refuge for anadromous fish (salmonids)

particularly during the transition from freshwater to seawater (see reviews in Kennedy 1982 and Blackmon et al. 2006). Seagrass and SAV habitat provide a direct food source for migrating waterfowl (Moore et al. 2004) as well as an acclimation refuge for anadromous fish species (Blackmon et al. 2006), thus linking to MIGR, SPWN, and RARE BUs. Healthy seagrass and SAV support REC-2 BUs in a number of ways. These habitats are prime areas for recreational crabbing and fishing as well as kayaking and waterfowl hunting. Additionally, seagrass beds are a major focus of marine life studies on every coast of the United States, including California.

Seagrasses are a source of primary production in nearshore marine systems, underpinning detrital-based food webs. Carbon budgets on seagrass communities in the Gulf of Mexico indicate that seagrass generally contribute about 30% of total ecosystem net primary production, with the other 66% distributed among the various algal groups e.g., epiphytes, macroalgae and phytoplankton (Heffernan and Gibson 1983, Moncreiff et al. 1992, Kaldy et al. 2002). In addition, several organisms directly graze upon eelgrass or consume epiphytes and epifauna supported by eelgrass plant structures, thus these plants contribute to the system at multiple trophic levels (Fenchel 1977, Phillips 1984, Thayer et al. 1984). Eelgrass beds are also a source of secondary production and can have up to 15% greater secondary production (Heck et al. 1995) and greater species richness (Ferraro and Cole 2007) than mudflats and sandflats.
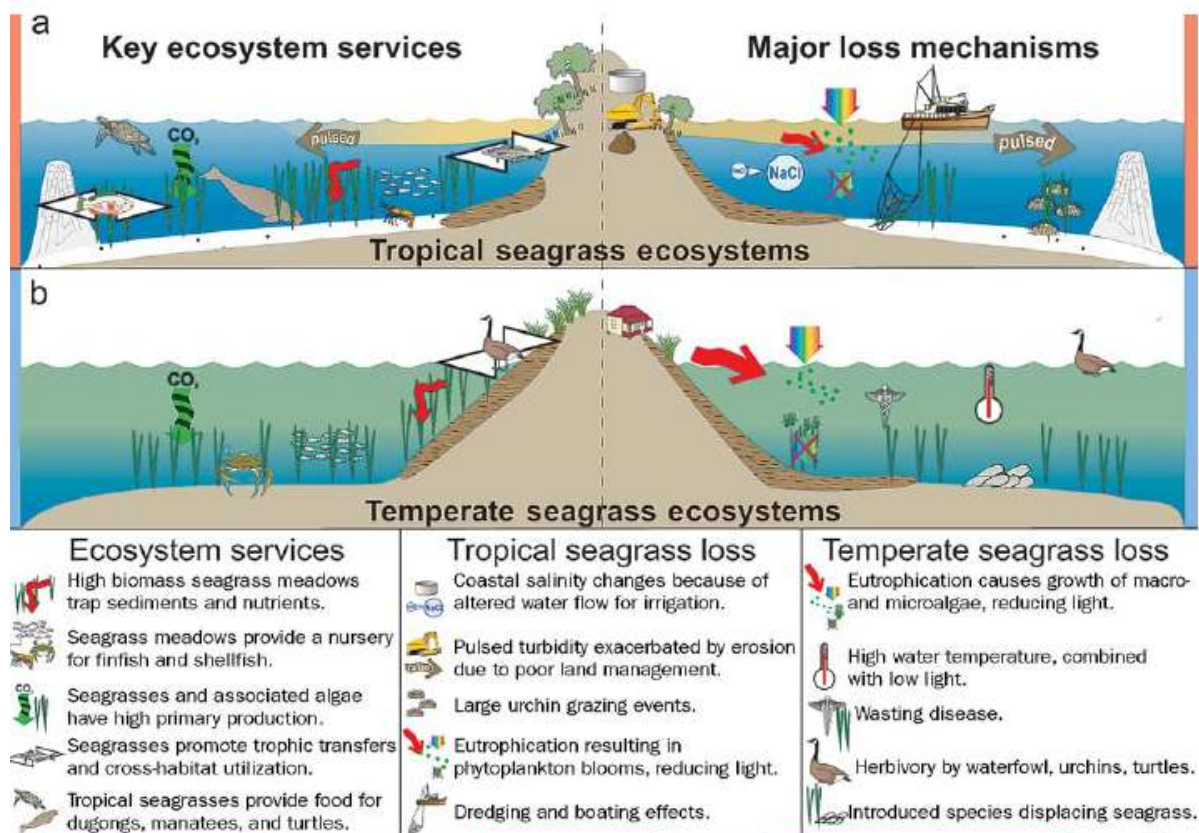
Figure 5.1. Conceptual diagram of ecosystem services and major mechanisms of loss for tropical (a) and temperate seagrass ecosystems (b; modified from Orth et al. 2006).

5.4 Effects of Eutrophication on Seagrass and Other SAV

5.4.1 General Conceptual Model of Effects of Eutrophication and Linkage to Management Controls

Under oligotrophic conditions, increased nutrient loads may initially be beneficial to seagrass communities by stimulating primary production, leading to greater secondary production by consumers. However, under continued high nutrient loads, algae are superior competitors and their increased abundance can be deleterious to seagrass. Initial indications of eutrophication issues include decreased bed density and increased abundance of the algal flora. Under very high nutrient loading, the system can become dominated by algal competitors (phytoplankton, epiphytes or macroalgae) resulting in the degradation or loss of the seagrass community (Figure 5.2). The primary mechanism of seagrass loss is through light reduction caused by shading or smothering from algal competitors. Reduced light coupled with increased delivery of labile organic detritus (senescent algae and seagrass) to the sediments can lead to additional biogeochemical stressors (hypoxia/anoxia, sulfide toxicity, etc.) that further exacerbate the problem. Consequently, there is a positive feedback loop between nutrient enrichment and expression of eutrophic or dystrophic conditions. Degraded seagrass beds tend to be sparse or patchy, heavily epiphytized with accumulations of macroalgae and experience large diurnal swings in dissolved oxygen concentrations as a result of the large respiration rates from the autotrophic and heterotrophic communities.
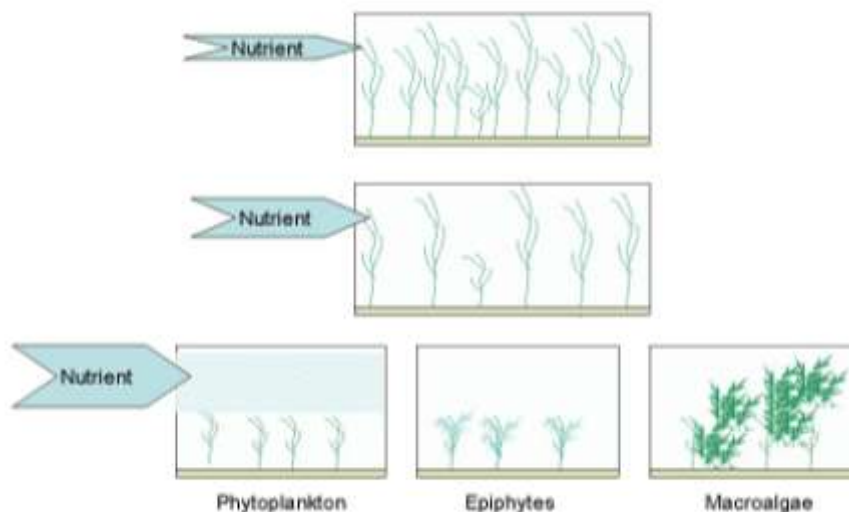
Figure 5.2. Conceptual model of how seagrass and some SAV communities respond to increased nutrient loading and resulting eutrophication. Adapted from Short et al. 1991.

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Dynamic simulation models (as ecosystem or stress-response) have been used in many instances to develop and evaluate nutrient and other water quality criteria and restoration potential. The Chesapeake Bay approach has been to define the water quality parameters (e.g., light, temperature, salinity, nutrients) associated with SAV declines and to develop a suite of environmental characteristics that are protective of the resource and allow restoration of SAV habitat (Batuik et al. 1992, 2000). As part of this approach, light attenuation by epiphyte load, in addition to light attenuation by the water column (k_d) from suspended sediment and phytoplankton, is considered in efforts to evaluate SAV light requirements. These relationships have been based on extensive published and unpublished data sets developed over more than 30 years of research on a single, albeit large estuary. A dynamic simulation model is then used to model the relationship between the seagrass habitat and factors that control light availability, and other stressors that require management (nutrient loads, sediment inputs, etc.). Similarly, the US EPA Western Ecology Division has developed and used a seagrass stress response model to evaluate the impact of proposed nutrient criteria on eelgrass in Yaquina Bay, Oregon, a Pacific Northwest estuary (Brown et al. 2007). Potential nutrient criteria were developed using the in situ observations as a basis for the "Estuarine Reference Condition" using cumulative distribution functions. Proposed criteria were then incorporated into the *Zostera* stress response model to determine whether particular percentile values would adequately protect *Zostera* resources within the estuary (Brown et al. 2007). Based on this data analysis and modeling exercise, median values of most water quality parameters were protective of seagrass habitat.

5.4.2 Effects of Eutrophication on Zostera spp.

Eelgrass response to nutrient loading and eutrophication has been a major research focus over the last couple of decades (Nixon et al. 2001, Nielsen et al. 2004a, Burkholder et al. 2007, Leoni et al. 2008 and references therein). Most seagrass eutrophication studies have examined the community level response in experimental systems ranging from aquaria to mesocosms to the natural environment (Table 5.1). For *Z. marina* much of this work has been conducted along the East Coast of North America and has resulted in a general theory of seagrass response. Specifically, that enhanced nutrient loading leads to a degradation of *Z. marina* habitat (Figure 5-2) by stimulating algal production (micro- and macroalgae) and shading out seagrass (Short et al. 1991, 1995, McGlathery 2001, Havens et al. 2001). A number of dose response experiments have been undertaken with *Z. marina*; however, most studies have been monocultures in experimental mesocosm experiments and this work has been primarily conducted with plants from the North Atlantic populations (Burkholder et al. 2007 and references therein). The experimental mesocosm and load-response experiments clearly show that the taxonomy of the community shifts from seagrass dominance to either macroalgae or phytoplankton dominance (Burkholder et al. 2007). Field based sampling along eutrophication gradients (e.g., Waquoit Bay, MA) and field manipulations of water column nutrients exhibit similar patterns (Burkholder et al. 2007). Thus, the seagrass declines through indirect effects (e.g., shading, increased hypoxia, increased respiration, etc.). However, there does not appear to be a predictable trajectory of development between nutrient input and the algal type (epiphyte vs. macroalgae vs. phytoplankton) that can adversely affect seagrass (Nixon et al. 2001). That is, for any given load of nutrients there is no capacity to predict if the system

will become dominated by macroalgae, epiphytes (e.g., microphytobenthos) or phytoplankton. The ultimate primary producer dominance is determined by initial starting condition of the system (spore or propagules availability) interacting with various biotic and abiotic cofactors (see Chapter 1). Additionally, because seagrass occurs in shallow subtidal habitats, characterization of external loads versus internal recycling of nutrients through the sediments is important for achieving a predictable load-response. These data gaps would affect the accuracy and precision of dynamic simulation models that attempt to capture the relationship between seagrass community structure and nutrient loads and other co-factors. In general, an understanding of the relationship between nutrient loading and algal primary producers within seagrass habitats is a data gap that requires additional research.

Table 5.1. Selected list of literature examining the effect of eutrophication on *Z. marina* communities. Abbreviations are as follows: Zm = *Zostera marina,* SAV = Submerged Aquatic Vegetation, Epi = Epiphytes, Phyto = Phytoplankton, Macro= Macroalgae, Algae = epiphytes + phytoplankton + macroalgae, nd= no data. Positive (+) or negative (-) response in biomass is denoted (Kaldy 2009).

Organizational Level	Experimental System	Response	Location	Reference
Community	aquaria	+Epi, -*Zm*	Virginia	Neckles et al. 1993
	aquaria	+*Zm*, -*Zm*[3]	Netherlands	van Katwijk et al. 1999
	aquaria	+ Epi	Washington	Williams & Ruckleshaus 1993
	aquaria	+Epi, -*Zm*[2]	Virginia	Moore & Wetzel 2000
	field	+*Zm*, +Macro	Rhode Island	Harlin & Thorne-Miller 1981
	field	+Zm	Netherlands	van Lent et al. 1995
	field	nd	Maryland	Stevenson et al. 1993
	field	+Phyto, +Macro, -Zm	Mass.	Valiela et al. 1992
	field	- Epi, -*Zm**	Washington	Williams & Ruckleshaus 1993
	field	+Macro, -*Zm*[4]	Finland	Bostrom et al. 2002
	field	+Macro, -Zm	Mass.	Hauxwell et al. 2003
	mesocosm	+Epi, -SAV	Maryland	Twilley et al. 1985
	mesocosm	-*Zm*[1]	North Carolina	Burkholder et al. 1992, 1994
	mesocosm	+algae, -*Zm*	New Hampshire	Short et al. 1995
	mesocosm	+Phyto, -*Zm*, -Macro, -Epi	Rhode Island	Taylor et al. 1995
	Lit. review	nd		Worm et al. 2000
	Lit. review	nd		Nixon et al. 2001

*Suggest nutrient limitation of Z. marina
[1] Suggest nitrate toxicity of Z. marina
[2] Conclude light dominant factor, only +Epi and -Zm at highest light level.
[3] Positive and negative effects were dependent on source of seagrass and salinity.
[4] Conclusion based on inference.

Some work has been done on the direct effects of nutrients on seagrasses. Tenant (2006) conducted an in situ fertilization experiment in Humboldt Bay and concluded that phosphate toxicity explained field observations. However, the study suffers from a poor design and lack of data to evaluate the sediment nutrient pools as well as the tissue nutrient pools. Claims of phosphate toxicity are premature and not supported by the primary literature. Work from North Carolina and Europe suggests that some populations of *Z. marina* may exhibit declines in response to low level concentrations of NO_3 or NH_4 (Burkholder et al. 1992, 1994, van Katwijk et al. 1997). However, these conclusions may be confounded by other factors (e.g., high temperatures and reduced light). Oregon populations of *Z. marina* are regularly exposed to >30 µM NO_3 from coastal upwelling (Kaldy and Lee 2007, Brown and Ozretich 2009) and have shown no declines associated with nitrate toxicity. Furthermore, exposure to 1000 µM NO_3 for two weeks in a laboratory experiment (temp 8°C, 12:12 L:D with saturating irradiance) did not produce mortality or evidence of stress (J. Kaldy, US EPA, unpubl. data). Additionally, this seagrass-nutrient loading paradigm from east coast systems may not be directly transferable to California or the West Coast in general. Since there are a variety of local and regional processes that affect nutrient dynamics

on the West Coast that are not as prevalent on the east coast. For example, seasonally closed estuaries and large scale upwelling are not primary features in East Coast systems and as a result are not considered in many of the conceptual models that develop from research in that region. Other factors include land use patterns and the fact that, in some portions of *Zostera*'s range "natural" nutrient loads far exceed those calculated for even the most eutrophic systems on the East Coast (e.g., Waquoit Bay, MA).

There have been relatively few studies of *Zostera marina* nutrient interactions on the West Coast or in California. In a field study from Padilla Bay, WA, Williams and Ruckelshaus (1993) demonstrated that eelgrass growth was influenced by both sediment nutrient availability and higher order effects of epiphytes and isopod grazer interactions. They conclude that "Consideration of sediment nitrogen, epiphytes or herbivores alone is unlikely to yield a predictable understanding of the control of eelgrass productivity in nature, particularly given the complexity of the eelgrass habitat with respect to its dual nutrient sources" (Williams and Ruckelshaus 1993). This conclusion suggests that external nutrient loads and internal recycling alone do not control eelgrass growth and production, but that integrated water quality, biological and environmental factors play a considerable role (Koch, 2001). Therefore, the use of seagrass health as a primary indicator of eutrophication is problematic in that other stressors (temperature, excessive sedimentation, climate change) may be adversely impacting health.

Estuarine macrophyte community response to nutrient loading occurs primarily as a shift in dominant primary producers (see Figure 5.2). Macroalgae interact with *Zostera* spp. in a variety of ways. An overabundance of macroalgae can cause degradation of *Zostera* habitat through two related mechanisms. First, mats or rafts of algae can develop over or among seagrass shoots, limiting the amount of light available to seagrass. Two California studies found high abundances in experimental enclosures led to reductions in eelgrass growth or density, perhaps due to light limitation (Huntington and Boyer 2008a using *Gracilariopsis* sp. in Tomales Bay, and Olyarnik 2008 using *Ulva* sp. in Bodega Bay). Secondly, mats or rafts of algae that settle on top of the seagrass effectively smother the plants, cutting off light and oxygen leading to anaerobic conditions with a build of toxic metabolites (e.g., sulfides). However, some systems can exhibit large accumulations of macroalgae growing among *Z. marina* shoots with no apparent decline in seagrass condition driven by seasonal upwelling of nutrients (J. Kaldy, US EPA, pers. observation). The interactions between *Zostera* and macroalgae are explored in more detail in Chapter 3.

A variety of physiological measures (tissue nutrient content, amino acid content, enzyme activities, δ^{15}N values) and morphological measures have been used to assess seagrass response to nutrient enrichment; however, "at present there are no available, reliable tools for early assessment of nutrient enrichment in seagrasses" (Burkholder et al. 2007). The closest early assessment tool was developed by Lee et al. (2004) and combines morphology and leaf tissue N content. Unfortunately, leaf tissue N is highly variable (within and between plants) and is of limited use to detect early stages of nutrient enrichment leading to eutrophication (Burkholder et al. 2007). However, the ratio of leaf N:leaf mass may be useful as a "nutrient pollution indicator" (Lee et al. 2004). This ratio in combination with other biomarkers (Ferrat et al. 2003) or information may be a useful indicator of early eutrophication; however, additional research will be required to verify that the indicator works along the Pacific Coast.

5.4.3   Effects of Eutrophication on Brackish - Euryhaline SAV

Work conducted in European estuaries indicates that the brackishwater SAV species *Ruppia* spp. acts as a seagrass analog and is susceptible to degradation based on the same types of interactions (shading, smothering, biogeochemical stressors, etc.). There are several examples from Europe that examine how the systems respond to losses of *Ruppia* associated with eutrophication or other anthropogenic activities (Bachelet et al. 2000, Lenzi et al. 2003, Pergent et al. 2006, Shili et al. 2007). Bachelet et al. (2000) investigated a eutrophication gradient along the coast of France; the intermediate site was characterized by *Ruppia* with a constant biomass with sporadic spring blooms of macroalgae. In contrast, the eutrophic site was dominated by macroalgae and had low biomass and abundance of macrozoobenthos (Bachelet et al. 2000). In the Orbetello lagoon (Italy), eutrophication abatement measures (macroalgal harvesting, increased circulation and waste water phytotreatment) resulted in reductions of algal biomass and increased SAV (Giusti and Marsili-Libelli 2005). More recent macroalgal blooms appear to be a "legacy effect" of sediment nutrient release (Lenzi et al. 2003). At Biguglia lagoon in Corsica, a healthy bed of R. cirrhosa was replaced by Ulvoid algae between 1997 and 1998 with re-appearance of R. cirrhosa in 1999. Pergent et al. (2006) attribute these shifts to nutrient availability related to agricultural runoff and wastewater discharge. Several studies have investigated the response of *Ruppia* spp. nutrient loading; however, these studies use a gradient approach where there is little control over or quantification of the loading to the system. Thus, for European *Ruppia*, well defined load -response experiments do not appear to exist. The US EPA Chesapeake Bay Program explicitly assumes that all SAV species follow the same conceptual model where nutrients increase light attenuation by phytoplankton and epiphytes leading to declines of SAV (Batuik et al. 2000). Manipulative experiments in Maryland concluded that epiphytes, stimulated by nutrient additions, caused declines in Potamogeton perfoliatus (Staver 1984).

Numerous California lagoon systems are known to support very dense and apparently healthy *Ruppia* populations under very eutrophic conditions (high nutrient loading, high organic loading to the sediments, fish kills, large diurnal dissolved oxygen swings, etc.). The presence of dense *Ruppia* populations has been observed primarily in Southern California intermittently closed estuaries (Sutula & McLaughlin, SCCWRP, unpubl. data), but also occurs in the Klamath River in Northern CA (Lee and Brown, US EPA, unpubl. data). It is not clear if these *Ruppia* beds are adapted to and thrive under high nutrient conditions or if these populations are an expression of eutrophication symptoms. The beds tend to be seasonal and it is unknown what triggers the reduction of biomass and subsequent decline of these apparently annual populations.

Alternatively, the presence of these dense, ephemeral California populations may be an expression of the natural life cycle of this species. In Chesapeake Bay, the growth form of seagrass and SAV are classified as "meadow forming" and "canopy forming", respectively (Batuik et al. 2000). Brackish SAV species tend to be "canopy formers" with biomass concentrated in the top half of the water column and exhibit rapid growth toward the surface early in the growing season. Canopy formation results in shading of older portions and the sloughing of lower leaves. Epiphytes accumulate on the older portions of the leaves and continued growth results in epiphyte free apical leaves near the surface of the water that actively photosynthesize. In contrast, "meadow forming" species concentrate biomass in the lower

portion of the water column and new leaf production occurs near the base of the plant. Older leaf tissue near the surface may be heavily epiphytized but rapid leaf turn-over rates allow the plants to maintain positive carbon balance. Additionally, it should be noted, that changes in the distribution of *Ruppia*, and probably other brackish SAV as well, can be related to factors other than nutrients and eutrophication. In the Ichkeul lagoon (Tunisia) rapid changes (1993-1998) in the species composition and distribution of SAV, including *Ruppia cirrhosa* were linked primarily to water management activities (e.g., dams) coupled with drought and not eutrophication (Shili et al. 2007). In San Diego, California, a shift in community dominance from *Z. marina* to *R. maritima* in San Diego Bay were likely related to increased water temperature associated with the 1997-1998 El Niño event (Johnson et al. 2003).

In general, a better understanding of the response of *Ruppia* spp. to alterations in nutrient loading requires substantial research before it could be used as an indicator. Key research questions that need to be addressed before brackish SAV will be useful indicators of eutrophication include: First, the basic physiological requirements (salinity tolerances, temperature tolerances, nutrient requirements, minimum light requirements, etc.) of brackish SAV species need to be defined for California. Second, the environmental triggers to seasonal cycles of biomass (temp, salinity, day length, etc.) of both meadow and canopy forming SAV need to be elucidated. Third, nutrient dose-response relationships need to be determined with emphasis on how the response is manifested (e.g., epiphyte loads, light reduction, self-shading from canopy development, etc.).

5.5   Indicators of Seagrass Health and Effects from Eutrophication

A suite of indicators are generally used to assess seagrass health and effects from stressors. For the purposes of this review, these indicators can be grouped into three categories:

- Indicators of seagrass and SAV community structure (taxonomy, biomass, aerial distribution, density)

- Factors that affect seagrass health through reduced light availability to the plant (e.g., water column light attenuation, total suspended solids, phytoplankton biomass, epiphyte load, macroalgal biomass or cover)

- Other indicators (environmental or water quality)

5.5.1   Indicators of Seagrass and SAV Community Structure

Overview

Indicators of seagrass community structure have been identified and used by various organizations to monitor the health of the habitat. The European Union, Water Framework Directive, specifically identifies taxonomic composition, abundance determined by shoot density and areal distribution to be the "biological quality elements" for defining ecological status (Foden and Brazier 2007). In Florida Bay,

indicators have been targeted to reflect how salinity regime controls seagrass distribution, cover and species composition (Madden et al. 2009). Specifically, they evaluate spatial extent, abundance, species dominance and presence of desired target species which is combined into an index of community status (Madden et al. 2009). In Puget Sound, Washington State, video transect surveys are used to evaluate spatial extent, depth distribution and patchiness (Gaeckle et al. 2007). The Texas Seagrass Conservation Plan (TSCP) outlines a broad spectrum of objectives and actions for evaluating seagrass and estuary health (Texas Parks and Wildlife 1999). Specifically, the TSCP lists seagrass distribution, abundance, species composition and interactions with biogeochemical and environmental stressors (Texas Parks and Wildlife 1999). Additionally, all of the programs described above are long-term, iterative programs that monitor over decadal scales. Duarte and Kirkman (2001) suggest that the time frame "to determine real changes brought about by human disturbance may take 5-10 years".

Seagrass physical structure (e.g. biomass, shoot density, leaf length, etc.) responds to a variety of stressors, including temperature, light availability, sedimentation, eutrophication, and hydromodification (Dennison 1987, Dennison and Alberte 1985, Fonseca and Kenworthy 1987, Dawes and Tomasko 1988, Masini et al. 1995, Moore et al. 1993, Fonseca et al. 1983, Williams and McRoy 1982, Backman and Barilotti 1976, Thom and Albright 1990, Zimmerman et al. 1990, 1994). Thus, community structure indicators would lend themselves to bioassessment of seagrass health on a habitat scale, but not specifically to diagnose eutrophication, for the reasons explored in detail below. Community structure data would be important collateral information to provide context to a more specific diagnostic assessment of eutrophication in seagrass communities. In contrast, community species diversity indicators for brackish SAV may be useful to diagnose eutrophication; however, there are major research gaps that need to be addressed and tested prior to incorporation into an assessment framework.

Established seagrass monitoring programs exist in many of the estuaries where extensive *Zostera marina* beds are found (e.g., Humboldt Bay, Morro Bay, San Francisco Bay, San Diego Bay); however, no standardized monitoring program has been established and these programs tend to use a mix of methods and time scales to assess the areal distribution of seagrass beds (Merkel et al. 2009). Southern California recently created a workgroup of stakeholders to develop recommendations for an eelgrass regional monitoring program. These efforts could serve as a template for attempting to develop a minimum standard for monitoring of the major *Zostera* beds in California estuaries (M. Sutula, SCCWRP, personal communication). Limited data are available on the areal distribution and density/biomass of brackish SAV species in California estuaries. Currently, the only known comprehensive survey is the Bight '08 Regional Survey, which characterized the biomass and % cover of brackish SAV species where they were found in 25 estuaries in Southern California (M. Sutula, SCCWRP personal communication). Results of this assessment are still pending. In addition, a survey of brackish SAV is underway (summer 2011) to map distribution and cover in San Francisco Bay (K. Boyer, San Francisco State University, pers. comm.). Currently, it is not clear if structural indicators (taxonomy, density, and biomass) of canopy forming brackish SAV will be useful indicators to diagnose anthropogenic eutrophication for reasons detailed below. There are a number of key research questions (outlined in section 5.4) that need to be addressed in order to better evaluate the application of these potential indicators.

Taxonomy

The species composition of seagrass habitats (hereto referred to as "taxonomy," has been used as an indicator for assessing eutrophication, especially in areas that are taxonomically diverse such as Florida Bay which has 6 species of seagrass. However, the diversity of the seagrass/SAV in California is limited and therefore not likely to be useful as an indicator. There are several species of *Zostera* that are documented from California, although, *Z. marina* is the dominant. The non-indigenous *Z. japonica* is known only from the Humboldt Bay system; where the California Department Fish and Game is actively trying to eradicate it (Eicher 2006). There has been no research conducted to evaluate the beneficial uses associated with the presence of *Z. japonica* (Williams 2007). Earlier reports of Z. asiatica from California have been evaluated using molecular techniques and appear to be morphological variants of *Z. marina* (Talbot et al. 2006). Recently, there has been molecular work identifying 2 populations of Z. pacifica based on nuclear and mitochondrial DNA (Coyer et al. 2008). However, Z. pacifica is not currently recognized as a valid taxon according to most authorities and further research is required to clarify the taxonomy. Furthermore, the populations identified as Z. pacifica are only known from the California Channel Islands and as such are outside the scope of this document. *Z. marina* is native and occurs along the entire CA coast extending into Mexico. The *Zostera* spp. are restricted to the most saline portions of the estuary where salinity is generally greater than 15 ppt.

Within the brackish to euryhaline estuaries or zones of estuaries, there are two dominant species of *Ruppia*, *Ruppia maritima* and *Ruppia cirrhosa*, but they tend to occupy different habitats (Ferren et al. 1995). Again the limited species diversity of brackish *Ruppia* spp. may limit the utility of this metric for diagnosing eutrophication. Similarly, extensive *Stuckenia pectinata* beds in northern San Francisco Bay (Suisun Bay) generally occur as monocultures (K. Boyer, pers. comm.). Additionally, it will be important to quantify the response of the SAV community to N loading and to quantify thresholds for community structure shifts (SAV to algae). A wide variety of algal taxa from all divisions coexist with the macrophyte species, primarily as epiphytes although some species can occur as an understory (e.g., Gracilaria spp., Ulva spp.). Algal-seagrass interactions are described in more detail in Chapter 3.

Areal Distribution

Areal distribution is the cover of seagrass habitat within an estuary. It has been a backbone of modern seagrass monitoring programs in both tropical and temperate systems and has been used to evaluate water quality. The accepted paradigm is that good water quality (e.g., low nutrient loads and high light penetration) leads to a larger areal distribution of SAV and seagrass. Poor water quality (high nutrient loads, high suspended solids, high chlorophyll-a, and low light) has been associated with decreased areal distribution of seagrass (Batuik et al. 1992, Dennison et al. 1993, Short and Wyllie-Echeverria 1996, Short et al. 1996, Short and Burdick 1996, Batuik et al. 2000, Orth et al. 2006, Wazniak et al. 2007, Beem and Short 2009). In contrast, improved water quality, has in a few cases, lead to increased areal distribution of seagrass (Orth et al. 2006). However, it should be noted that changes are expressed over long time periods and generally require 5 to 10 years of data to quantify. For example, changes in the distribution of seagrass in Laguna Madre, Texas in response to salinity reduction are occurring decades after dredging the Gulf Intracoastal Waterway (Quammen & Onuf 1993). As described above, it is very

difficult to separate the effects of individual stressors (e.g., response to nutrient load versus reduced light penetration from increased suspended sediment) without additional monitoring of water quality parameters. Thus, as an indicator of eutrophication, areal distribution may not have enough specificity to identify when a problem with eutrophication may be occurring. To assess problems with respect to eutrophication, areal distribution would be used to provide context for trends, rather than diagnose eutrophication. Within well-established seagrass habitats, it should be part of a larger program of bioassessment and monitoring that can be used to follow long-term trends over time.

Methods to map the areal distribution of seagrass and other SAV include diver surveys, singlebeam fathometer surveys, towed video and ROV surveys, color and multispectral aerial photographic surveys, and sidescan sonar surveys (Sargent et al. 1995, Pulich et al. 1997, Fourqurean et al. 2001. Clinton et al. 2007 and others, Virnstein and Morris 1996, Norris et al. 1997, Berry et al. 2003, Steward et al. 2005, Fourqurean et al. 2001a, 2001). No single methodology has fully dominated the techniques employed to map seagrass habitat. However, for systemwide surveys with repeatable results, mapping methodologies have gravitated towards the application of two technologies, aerial imagery (color, color infrared and multispectral) and video surveys with data being managed in geographic information systems (GIS) software. The determination of the type of technology to use is driven by habitat type (deep or shallow beds) as well as trade-offs in cost versus precision of mapping required. These mapping programs are usually conducted over the long-term and require significant personnel and infrastructure investments. Infrastructure investments include extensive GIS mapping capacity (servers, back up systems, data storage, specialized software), specialized GIS expertise and access or ability to create appropriate data streams (aerial imagery, video or acoustic surveys, etc.).

With respect to brackish SAV, a large number of mapping programs have been developed to determine the areal extent of freshwater and brackish SAV, especially with regard to monitoring the spread of invasive species. For example, Underwood et al. (2006) evaluate the use of remote sensing imagery to target two invasive species in the Sacramento-San Joaquin Delta. Many large-scale freshwater and brackish SAV mapping programs use hyperspectral remote sensing imagery (Williams et al. 2003, Alberotanza et al. 2006, Underwood et al. 2006, Yuan & Zhang 2008). More traditional aerial photography or transect mapping methods could probably also be adapted to SAV mapping. Areal distribution and presence/absence data may be useful indicators of SAV response to eutrophication and have been used in other assessment programs within California.

Density

Seagrass density, or the number of shoots per unit area, is a commonly used metric of seagrass abundance. Measures of seagrass density have been part of the backbone of most seagrass monitoring plans in both temperate and tropical systems. In some systems, the density of seagrass has been used as a response variable to evaluate changes in water quality; for example in Chesapeake and Tampa Bays (Orth et al. 2006). As with areal distribution, seagrass density may not have enough specificity to identify when a problem with eutrophication may be occurring. To assess problems with respect to eutrophication, seagrass density and areal distribution would be used to provide context for trends, rather than diagnose eutrophication per se.

Density estimates are typically made by counting the number of shoots in a pre-defined area (e.g., quadrat) of known area using divers or coring methods. Field counts are generally scaled and reported per unit area. Alternatively, large scale seagrass monitoring programs (e.g., Florida Bay) utilize Braun-Blanquet methods to assess seagrass density (Fourqurean et al. 2001b), which is a rapid visualization method that classifies shoot density into categories. Both methods require reasonable visibility to make estimates and putting divers or snorkelers into the water in order to make measurements. The use of the Braun-Blanquet method also requires some training and quality assurance validation between individual surveyors. However, both methods are robust and repeatable. These methods are directly applicable to both seagrasses and brackish SAV.

Measures of density (and areal distribution) need to be interpreted in context of the expected variability known to occur. Throughout its range, West Coast beds of *Zostera* spp. exhibit seasonality in areal distribution and abundance. In the most northerly portions of its range (e.g., portions of Alaska), eelgrass production becomes very slow due to cold temperatures and low light and can survive under 1 meter of ice in the Bering Sea (McRoy 1969). At the southernmost extreme (e.g., the Sea of Cortez) and in at least one bed in San Francisco Bay, eelgrass populations exhibit an apparent annual life cycle perhaps due to stresses of high temperatures or herbivory, respectively, that lead to mortality and replacement through seedling recruitment in the fall as water temperatures cool. However, this semelparous life cycle may be a stress response and not a true annual life history. Between these extremes, eelgrass response is variable with seasonal declines and expansions being reflective of the range of environmental conditions experienced during a given year or within the particular waterbody in which eelgrass occurs (Thom 1990). In southern California, seagrass grows year-round and flowering may occur during any month, although it is most pronounced in the late spring (Ewanchuck 1995, Ruckelshaus 1996). While seagrass may be present at any period of the year, the site specific environmental conditions may cause fluctuations between years and seasons (Johnson et al. 2003, Kaldy and Lee 2006).

There is also evidence for estuarine specific gradients in seagrass distribution and density. Generally, seagrass density decreases with decreased salinity, thus abundance is typically higher near the ocean end and decreases up estuary (Young et al. 2009). The actual patterns of abundance are likely to be estuary specific depending on the strength of the salinity gradient. Despite these observations, field work has shown the capacity of density metrics to detect change is weak and requires large sample sizes to detect modest 10% changes (Heidelbaugh and Nelson 1996). Fewer samples are required to detect larger 50% reductions but by that point the loss of the bed is imminent (Orth et al. 2006).

A number of mesocosm experiments and field surveys designed to evaluate seagrass (*Zostera*) response to nutrient loading have been conducted (Short et al. 1995, Burkholder et al. 1992, 1994, 2007, Hauxwell et al. 2003, Hauxwell and Valiela 2004). One consistent finding is that *Zostera* density decreases with increased N loading. The mechanism for reduced abundance is decreased light availability from algal over-growth (both phytoplankton and macroalgae). Thus for seagrass, the linkage between density and N load appears to be through indirect effects.

The available information suggests that brackish SAV respond to nutrient enrichment in a similar manner as seagrasses (as described above). However, additional regional research is required to quantify the linkages between SAV density and N loading. Additionally, although the same methods for quantifying seagrass shoot density could be applied to brackish canopy forming SAV, other methods may need to be developed due to health risks associated with diver surveys in heavily eutrophic systems. SAV density is also likely to suffer from high variability resulting in a weak capacity to detect change. Furthermore, this variability may be exacerbated for canopy forming species by temporal dynamics (e.g. seasonal biomass patterns) and differences in water depth.

Biomass

Biomass is a measure of the standing stock or weight of a tissue per unit area and is often used as a metric of seagrass and SAV. Typically high biomass of seagrass or SAV is considered an indication of favorable water quality characteristics. Biomass estimates are made by harvesting all plant tissue within a pre-defined area, cleaning to remove epiphytes and then obtaining a dry weight. Often this is done as either core samples or a combination of clipping/coring although "tongs" and custom designed "post-hole" diggers have been used to sample seagrass biomass. Coring methods provide a complete picture of total biomass since it includes the below ground tissues which in some species can account for 80% of the total. The "rake" or "tong" method was recently used in the California Bight '08 Regional Monitoring study to sample brackish SAV (K. McLaughlin, SCCWRP, pers. comm.). Additionally, methods for visual estimates of biomass have also been described (Duarte and Kirkman 2001), although they are not used as commonly as harvest methods. Visual estimates quantify only the above-ground portion of the plants which typically account for 20 to 50% of the total biomass. Previous work has suggested that the ratio of below to above ground biomass may be a crude indicator of sediment nutrient availability (Short 1983).

Within the realm of seagrass monitoring at the large regional scale, biomass is typically not preferred because it is very time and labor intensive, requiring trained technical support. Data interpretation is not clear-cut with large expected variability and slow response time, thus yielding a poor signal-noise ratio. In general, sampling this parameter is labor intensive, expensive and destructive. Additionally, by the time detectable trends in biomass are evident, it is often too late to respond with management actions that will remediate the situation. Furthermore, changes in biomass should also be expressed in alterations of abundance (density) and distribution such that it becomes a redundant metric.

The methods used for seagrass biomass estimates can be directly applied to estimate brackish SAV biomass. However, there currently is not sufficient regional data to evaluate if brackish SAV biomass can be used as an indicator to diagnose eutrophication. Additional research will be required to determine if SAV biomass will be a good candidate indicator.

5.5.2   Factors Resulting in Reduced Light Availability

The primary mechanism of seagrass loss from eutrophication is through the reduction in available light to plant leaves caused by shading or smothering from algal competitors. Reduced light coupled with increased delivery of labile organic detritus (senescent algae and seagrass) to the sediments can lead to additional biogeochemical stressors (hypoxia/anoxia, sulfide toxicity, etc.) that further exacerbate the

problem. Seagrass and SAV beds adversely affected by eutrophication tend to be sparse or patchy, heavily epiphytized with microalgae, and/or shaded with phytoplankton or macroalgal blooms. Thus, epiphyte load, water column light attenuation (from attendant phytoplankton biomass and turbidity), and macroalgal biomass are indicators of eutrophication that directly affect light availability to seagrass. Canopy forming SAV are even more complicated because, in addition to all of the other factors that attenuates irradiance, the canopy formers also self-shade. That is, by having most of their biomass at the surface of the water, the plant absorbs and attenuates light before it can reach the deeper leaves.

Water column light penetration is a dominant factor controlling the growth and distribution of seagrass and SAV. Although, water column light attenuation cannot be directly related to nutrient loading, monitoring of underwater light is likely to be a critical component of evaluating eutrophication because all of the algal groups that respond to nutrients influence the underwater light field. Water column turbidity, is generally not related to nutrient loading except in some circumstances and is not likely to be a useful indicator of eutrophication, although it does contribute to water column light attenuation. Water column chlorophyll-a *(*chl a*)*, which is a surrogate measure for phytoplankton, responds to nutrient loading and influences underwater light availability for seagrasses and SAV. Consequently, monitoring of chl *a* may be a strong indicator of eutrophication under some conditions.

Water Column Light Penetration

The single most important variable for aquatic plants is adequate irradiance (light availability). Without adequate light, regardless of the cause, aquatic macrophytes will not survive. Underwater irradiance is indirectly linked to nutrient loading, since the water column phytoplankton and algae stimulated by nutrients rapidly reduce light availability to aquatic plants. The minimum light requirements for eelgrass in the Pacific Northwest have been determined experimentally to be about 3 moles photons m^{-2} d^{-1} (Kaldy and Lee 2007, Thom et al. 2008). The minimum light requirements for most brackish SAV species have not yet been determined (Batuik et al. 2000).

Photosynthesis is a quantum process, requiring absorption of about 8 photons to initiate electron transport in the chloroplast. As light penetrates through water, it is subjected to scattering and absorption, resulting in a diffuse (i.e., non-parallel) light field that decreases exponentially with depth. Plants have adapted to this diffuse light field by distributing leaves vertically in the water column providing maximum surface area for light absorption. Consequently, it is important to measure light that impinges upon the leaf surface from all angles. Underwater irradiance or photon flux density (PFD) is commonly measured using spherical (4 π) quantum sensors attached to data loggers. These spherical sensors which integrate light from all angles, are a reasonable analog for aquatic plants and are used for direct measurement of minimum light requirements. Cosine corrected sensors (2π or flat) can be used to measure diffuse light attenuation coefficients (k_d) but are inappropriate for developing minimum light requirements because they only accurately measure downwelling or upwelling (based on orientation) irradiance. Data from either type of sensor can be used to calculate k_d using the Beer-Lambert equation which is a measure of how much light is removed from the water column per meter of depth.

$$k_d = \frac{\ln I_0 / I_z}{z}$$

Where I_o = the surface PFD, I_z is the PFD at depth z (m), the final units are m^{-1}. This formula can also be re-arranged to predict the PFD at a given depth for a given k_d and surface irradiance. This is useful for setting restoration targets (e.g., Chesapeake Bay). Irradiance or photon flux density (moles photons m^{-2} s^{-1}) is the appropriate SI unit for measuring photosynthetic quanta. Additionally, because only a portion of the light spectrum is used in photosynthesis, commonly referred to as photosynthetically active radiation or PAR (400-700 nm λ), it is critical that instrumentation only measure in the PAR region of the spectrum. The standard instrumentation for measurement of underwater PAR is the LI-COR spherical quantum sensor attached to a datalogger contained in watertight housing (Dunton 1994, Onuf 2006, Kaldy and Lee 2007). The biggest drawback to the continuous measurement of underwater PFD is biofouling, which requires frequent cleaning of the sensors (Onuf 2006). Seagrass monitoring programs often use a combination of light measurements, with some long-term in situ continuous measurement stations as well as instantaneous profiles for calculating k_d at other stations. The methods used for development of seagrass light criteria could also be applied to meadow-forming brackish SAV, although there appears to be very little information on SAV light requirements (Batuik et al. 2000). Since much of the brackish SAV observed in California estuaries may be canopy forming (K. McLaughlin, SCCWRP, personal observation), this approach may not be applicable or would require extensive new research.

Currently, there are no empirical light attenuation-nutrient load-response relationships that can be directly applied to seagrasses or SAV. Numerous empirical studies have shown that under nutrient loading, seagrass/SAV systems shift to either a phytoplankton, epiphyte, or macroalgal dominated system (Figure 5.2, section 5.4). However, since there is no way to predict which algal type will become dominant, there is also no way to predict how much light will be attenuated by that particular component. Consequently, the best we can do is monitor the existing conditions and use a dynamic simulation model to explore the impacts to seagrass and SAV, given the dominant algal type. Monitoring data taken in conjunction with other information may be useful as an indicator of eutrophication. Empirical field work has shown that light attenuation coefficients can be highly variable both spatially and temporally. A long term data set in Yaquina Bay (Oregon), shows a strong increase in k_d with increasing distance from the mouth; this pattern was also prevalent in individual cruises and multiple estuaries (Boese et al. 2009).

Historically, Secchi depth (depth at which a black and white colored disk disappears from view) has been used to evaluate light penetration; however, this metric although simple and inexpensive does not provide a quantum estimate of light availability. Most seagrass monitoring programs use quantum measurements of underwater light. Relationships between Secchi depth and percent surface irradiance have been developed (see Batuik et al. 2000 Chapter 3). However, these are still very site specific because of variations in dissolved constituents that absorb light and the instantaneous nature of the measurement. These relationships have been used to mine existing historical data; although most current monitoring programs either call for or have updated their methods to measure PFD (Batuik et al. 2000). Some citizen based groups collect Secchi depth but the utility of this data is limited. HOBO$^{©}$ light loggers have also been used in the literature; however, these measure in units of lumens ft^{-2} which is not

directly related to quanta, and they measure outside the range of PAR and as a result dramatically overestimate PAR levels. Relationships between Hobo loggers and LI-COR spherical quantum sensors have been developed but these are instrument specific, utilize differently configured sensors and still often overestimate available light (Kaldy and Lee 2007, J. Kaldy, US EPA, unpubl. data). Finally, a new generation of low cost, cosine corrected quantum sensors are available. However, the efficacy of these systems has not been determined by the scientific community (Data Flow Systems, New Zealand).

Although long term continuous records are desirable for determination of base-line, changes in trends and the development of minimum light requirements, short-term continuous (2 weeks) or even instantaneous measurements of underwater light would be useful in the context of monitoring. Instantaneous measures are least preferred because of the highly dynamic nature of underwater light and the fact that seagrass and SAV are long term integrators responding to trends over weeks to months rather than single events. Light penetration integrates measures of turbidity and chlorophyll-a and can be more easily linked with dose –response (i.e. low light = adverse effects). But within a diagnostic framework for eutrophication, could produce a false-positive if the reduced light is from high suspended sediment loads. It should be noted that neither light penetration nor chlorophyll-a capture effects of reduced light availability from epiphyte loads.

Turbidity

Another water column parameter that can be used in conjunction with seagrass and SAV monitoring is turbidity. Turbidity is an optical measure of light attenuation (nephelometric turbidity units, NTU's) which can be used as a proxy for the total suspended solids (TSS) in the water column. Using long term data, site specific empirical relationships can be derived for NTU's and TSS; however, these relationships are often weak and noisy. TSS can be measured directly from grab samples as mg L^{-1}; although TSS affects light penetration, high TSS may or may not be related to nutrient loading. Both chl a and turbidity affect light transmission through the water column, higher concentrations of either constituent lead to less light available at depth. Several states have adopted the TSS concentration of 15 mg L^{-1} as protective of seagrass resources (Batuik et al. 2000, Brown et al. 2007, Wazniak et al. 2007). In Chesapeake Bay, TSS concentration standards (<15 mg L^{-1}) are the same for both eelgrass and brackish SAV (Batuik et al. 2000); substantial new research would need to be conducted in order to evaluate the efficacy of this standard for California systems.

Water Column Chlorophyll-a

Water column chlorophyll-a (chl $\underline{a}$) concentrations (μg L^{-1}) can be used as a proxy for phytoplankton biomass. Both chl $\underline{a}$ and turbidity affect light transmission through the water column, and higher concentrations lead to less light available at depth. Several states have adopted the chl a concentration of 15 μg L^{-1} as protective of seagrass resources (Batuik et al. 2000, Brown et al. 2007, Wazniak et al. 2007). In Chesapeake Bay, chl $\underline{a}$ concentration standards are the same for both eelgrass and brackish SAV (Batuik et al. 2000). Use of chl $\underline{a}$ and other indicators related to light availability in a rapid or intensive assessment framework would require additional research in California. In particular, additional research would need to be conducted in order to evaluate the efficacy of the chl $\underline{a}$ standard developed

for the East Coast (e.g. Chesapeake Bay) or Europe (e.g. European Water Framework Directive) for California estuaries.

Chl a can be measured via discrete water samples or via in situ data loggers. A wide variety of in situ instruments are available to measure both of these parameters and several others (e.g., salinity, temperature, turbidity, dissolved oxygen, pH); these generally fall under the category of water quality meters (e.g., YSI sondes and Hydrolab). Instrumentation is frequently used for developing base line, continuous data sets to evaluate long term trends in water quality. Many monitoring programs also utilize grab samples analyzed using bench top instrumentation or chemistry in the laboratory. The relationships between water column chl a and nutrient loading are explored in more detail in Chapter 4.

Epiphyte Load

The epiphyte[9] community is actually a complex mixture consisting of living and dead algal cells, secreted extracellular polysaccharide material from algal cells, microfauna (bacteria, rotifers, ciliates, etc.) and mineral particles such as silt and clay. Epiphyte load is the amount of biomass of epiphyte material accumulated per unit area of seagrass or SAV leaf commonly expressed as a dry weight per unit leaf area e.g., gdw cm^{-2} leaf (Frankovich and Fourqurean 1997). In some cases, epiphyte load is expressed as epiphyte chl a content per unit leaf area (µg chl a cm^{-2} leaf). Measurements of epiphyte load are generally made by scraping and collecting the epiphyte matrix from a known leaf surface area. Alternatively, epiphyte load can be estimated using artificial substrate incubated in the field, with the assumption that the substrate mimics the plants (Staver 1984). Epiphyte load and subsequent light reduction are highly variable both spatially and temporally, even at the scale of individual plants. Boese et al. (2009) found that epiphyte load on *Z. marina* in Oregon varied by almost a factor of 10 (<1 to >8 mg cm^{-2}) on an annual basis. There were differences in epiphyte load between wet and dry seasons, location in the estuary and between younger inner leaves and older outer leaves. Additionally, they found a strong relationship between epiphyte load and light reduction (Boese et al. 2009) in Yaquina Bay, Oregon. Epiphyte load is generally not quantified in most seagrass or SAV monitoring programs or is quantified using relative abundance.

Epiphyte load on seagrass or SAV is likely to be directly related to nutrient loading and as such has been considered as an indicator of eutrophication. The Chesapeake Bay Program utilizes light attenuation by epiphyte load, in addition to light attenuation by the water column (k_d) from water column suspended sediment and phytoplankton, in efforts to evaluate SAV light requirements. An epiphyte attenuation coefficient is also calculated (k_e) and used with epiphyte biomass (B_e) to predict the percent light reaching the leaf surface (PLL) as described by Batuik et al. (2000).

$$PLL = e^{(-k_d)(z)} \, e^{(-k_e)(b_e)} * 00$$

This is based on empirical measurements made over 30 years throughout Chesapeake Bay. Exporting this concept to other estuaries may be problematic. For example, researchers in Oregon attempted to

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[9] "Periphyton" is another term that is often used synonymously with epiphytes.

make these calculations for a local *Z. marina* population; however, the calculations suggested that with the measured epiphyte loads, PLL was insufficient to support eelgrass. This directly contradicted our observation of a permanent seagrass bed located in the area for more than 30 years (Eldridge and Kaldy, US EPA, unpublished data). Developing the relationships between epiphyte biomass and light attenuation were difficult and could not be directly applied in California. A complete exploration of the light epiphyte modeling conducted in Chesapeake Bay is beyond the scope of this document, the interested reader is directed to Chapter V in Batuik et al. (2000). The Chesapeake Bay epiphyte- light work described above has also been conducted in brackish SAV communities. Minimum light requirements and photosynthetic data for meadow and canopy-forming SAV species was identified as an important data gap by Batuik et al. (2000).

Empirical relationships between nutrient and epiphyte loads are hard to quantify because a number of other factors (e.g., herbivore pressure, light availability, etc.) can be confounding (Neckles et al. 1993, 1994, Williams and Ruckelshaus 1993, Balata et al. 2008, McCall et al. 2009). Work conducted in Florida Bay concluded that epiphyte response to nutrient enrichment is pronounced but very localized and that epiphyte response to moderate enrichment may not be a sensitive metric (Frankovich and Fourqurean 1997). More recent work has concluded that epiphytes and microphytobenthos are ambiguous indicators of nutrient availability in pristine oligotrophic environments (Fourqurean et al. 2010). In contrast, other researchers have concluded that both microalgal and macroalgal epiphytes may be useful indicators of eutrophication (Cambridge et al. 2007, Balata et al. 2008). Epiphytic opportunistic green macroalgae were consistently associated with seagrass decline in Cockburn Sound, Australia (Cambridge et al. 2007). Differences in species composition and abundance patterns appeared to be related to nutrient availability in the Mediterranean Sea (Balata et al. 2008).

Mesocosm studies have shown that seagrass beds are impaired by nutrient loading favoring algal competitors; however, currently there is no way to predict how a given system will respond to nutrient loading with regard to which primary producer group will dominate (e.g., phytoplankton, macroalgae, or epiphytes; Short et al. 1995). Some systems will become macroalgal dominated and others will become phytoplankton or epiphyte dominated. The resulting dominance of primary producer groups in eutrophic systems may be related to water residence time, because low residence time favors macroalgae while longer residence time favors phytoplankton (Nielsen et al. 2004b). The lack of clear predictable relationships between nutrient load and algal type or epiphyte biomass is an impediment to the use of epiphyte load on seagrass as a stand-alone indicator of eutrophication (Batuik et al. 2000). However, used in combination with other metrics it could be a diagnostic tool for eutrophication. One possible approach would be to develop a rapid assessment method with categories that tie directly to the impact on light reduction. Since epiphyte load is directly related to light reduction experienced by the seagrass or SAV leaf (Batuik et al. 2000, Drake et al. 2003, Boese et al. 2009) relative categories of epiphyte load could be related to quantifiable light reductions that may impact macrophyte photosynthesis. Results of a rapid assessment could trigger additional in-depth study to better quantify the degree to which the habitat is impacted by eutrophication. Use of epiphytes and other indicators related to light availability in a rapid or intensive assessment framework would require additional research in California.

Macroalgal Mat Cover or Biomass

Accumulations of drift macroalgae or the development of macroalgal mats may also lead to degraded seagrass or brackish SAV habitat (Figure 5.2). These macroalgal mats can develop intertwined among seagrass shoots or can be deposited on top of existing beds. These interactions frequently, but not always, lead to light limitation of the seagrass and a series of secondary impacts (e.g., low dissolved oxygen, sulfide toxicity, etc.). The interactions between seagrass, brackish SAV and macroalgae are examined in detail in Chapter 3. Use of macroalgal biomass/cover and other indicators related to light availability in a rapid or intensive assessment framework would require additional research in California.

5.5.3   Other Indicators

Physiological parameters

Physiological parameters consist of measurements made at the organism level that provide a snapshot of the biochemistry of that organism. For aquatic plants, physiological parameters include, but are not limited to, measures of photo pigment concentrations (Chl a, Chl b and accessory pigments), carbohydrate content, enzyme activity, tissue C:N:P, photosynthetic rate measurements (e.g., maximum rate of photosynthesis, dark respiration, photosynthetic efficiency, quantum yield, electron transport rate, etc.). Some of these measurements can be made in situ using sophisticated instrumentation (e.g., diving Pulse Amplitude Modulated Fluorometry) while other measurements require destructive sampling followed by bench top analytical chemistry (e.g., pigment concentrations, carbohydrate content, etc.). Many of these parameters have been suggested as indicators of stressors such as high temperature, low light and nutrient over-enrichment. However, changes in these physiological parameters in field populations are almost never a response to a single stressor. That is, changes in a wide variety of environmental factors can lead to physiological changes, thus direct cause-effect type relationships are often difficult to verify or validate. Additionally, measurement of these parameters requires a highly trained technical staff and often produces data that are difficult to interpret; for example, interpretation of the causes for shifts in fluorescence data, ratio of F_v/F_m, are not always obvious.

There are several seagrass physiological parameters that show promise as indicators of nutrient loading; however, physiological parameters are especially difficult to use in a predictive capacity. The use of most physiological parameters to assess eutrophication will require substantial new research to validate the findings of others. As mentioned earlier, Lee et al. (2004) have proposed using the ratio of tissue N content to leaf mass as a nutrient pollution index. Additional field and laboratory manipulative work would need to be conducted in order to evaluate the efficacy of this method to detect changes in nutrient loading. The use of stable nitrogen isotopes also holds promise for detecting nutrient enrichment as long as there is sufficient isotopic separation of sources. Several recent studies suggest that the $\delta^{15}N$ of eelgrass tissue may be useful to determine sources of nitrogen entering estuaries (Fourqurean et al. 1997, Huntington and Boyer 2008). The development of eelgrass $\delta^{15}N$ as an indicator of anthropogenic nutrient loading will require substantial new research to evaluate the many caveats associated with this technique including differentiating between sediment and water column N sources

as well as any fractionation. It should also be noted that evidence of anthropogenic nutrient loads do not imply that an adverse effect of eutrophication has occurred. These metrics may also be applicable to brackish SAV species; however, this will require substantial new research to evaluate these potential metrics since there appears to be little or no published information available.

Dissolved Oxygen and pH

Seasonal or stochastic variation of dissolved oxygen (DO) and even pH can also have an impact upon seagrass and SAV indicators. Seagrasses have also been found to be sensitive to water column hypoxia and anoxia which can cause lethal concentrations of sulfide at the plant meristem (Greve et al. 2003, Borum et al. 2005). Many species of seagrass, including eelgrass, can withstand going into anaerobic respiration for limited durations (Smith et al. 1984, Pregnall et al. 1984, Alcoverra et al. 1999); however, prolonged anoxia can be lethal (Alcoverra et al. 1999). Seagrasses production is CO_2 limited in estuarine and marine waters (Zimmerman et al. 1997, Invers et al. 2001, Palacios and Zimmerman 2007). The availability of CO_2 in aqueous media is controlled primarily by pH. Increasing CO_2 and associated decreased pH of the surface waters could lead to increases in the area specific productivity of seagrass meadows (Palacios and Zimmerman 2007). pH and CO_2 are also critical for carbon availability in brackish and freshwater systems. Although changes in pH and CO_2 availability undoubtedly affect brackish SAV there does not appear to be many publications on the topic. Understanding the impacts of pH on brackish SAV will likely require substantial new research. Measurement of pH is easy to accomplish using a variety of water quality sondes or multiprobes. The linkages between these secondary physiochemical responses and eutrophication are reviewed in Chapter 7.

5.6   Summary and Recommendations

5.6.1   Seagrass

Within California, seagrass indicators are only applicable to perennially tidal enclosed bays and estuaries in which seagrasses are known to occur and in which the beds are relatively persistent. Seagrass habitat is known to be affected by a variety of natural and anthropogenic stressors (heat, desiccation, chemical, eutrophication, grazing, etc.). As such, changes in the physical structure of seagrass beds (areal distribution, biomass, density) are not specific to eutrophication and are not recommended for use as an NNE indicator (Table 5.2). Additional indicators such as pH and DO are considered separately in Chapter 7. Due to the extensive research still required physiological parameters are not recommended for further consideration. Indicators whose mode of action results in the reduced light availability to the plant and therefore reduced photosynthesis include: epiphyte load, macroalgal biomass/cover, and water column light attenuation (which integrates water column chlorophyll-a biomass and turbidity). **It is recommended that indicators associated with reduced light availability be pursued for use in the NNE framework for seagrass habitat as they have more specificity to the diagnosis of eutrophication.**

Key data gaps would need to be addressed in order to pursue the use of these indicators for diagnosing and managing eutrophication. These include:

- Development of light requirements for seagrass and SAV beds in different regions of the state and for different species.
- Assessment of duration of reduced light/photosynthesis that results in adverse effects to the seagrass bed.
- Development and validation of site-specific dynamic simulation models that simulate reduced light availability to seagrass beds from nutrient loads and other co-factors.

One possible approach is to develop a field-based rapid assessment method that combines the assessment of each of the indicators associated with reduced light availability into an index of risk of reduced photosynthesis within the seagrass bed. Repeated field-based rapid assessments of bed condition indicating low light stress could trigger additional in-depth study to better quantify the degree to which the habitat is impacted by eutrophication versus other stressors. Long-term trends in light, temperature and nutrient loading would be important ancillary data to collect for data interpretation. Clearly, use of these indicators to assess eutrophication will require additional research in California and it is recommended that these methods be developed in conjunction with the appropriate user communities.

Many of the estuaries with persistent seagrass beds have established long-term monitoring programs that facilitate detection of trends within appropriate time scales. Field-based rapid or intensive assessments of seagrass bed condition and stressors could be nested within these programs. Several states and agencies use a tiered monitoring and assessment approach that include indicators such as canopy height, leaf morphology, Hydrographic data, invasive species, wasting index, etc. depending on the assessment level. Model programs for this kind of monitoring have been developed and used in Florida (Fourqurean et al. 2001b), the National Estuarine Research Reserve System (Moore et al. 2009), Washington State [http://www.dnr.wa.gov/ResearchScience/Topics/AquaticHabitats/Pages/aqr_nrsh_eelgrass_stressor_response.aspx)](http://www.dnr.wa.gov/ResearchScience/Topics/AquaticHabitats/Pages/aqr_nrsh_eelgrass_stressor_response.aspx) and Texas (Dunton et al. 2010). Data on persistent seagrass from Southern California systems have been summarized by Bernstein et al. (2010). Compilation of existing data for known beds in other California estuaries of the state should occur and agreement among stakeholders on minimum standards for monitoring physical structure (areal extent and density) should be made in order to introduce consistency. This type of study would be similar to the "Status and Trends" reports commonly used in the National Estuary Program (e.g., Corpus Christi Bay NEP). In estuaries where seagrass beds are a minor portion of the habitat (because of habitat suitability or niche constraints), other indicators such as phytoplankton (chlorophyll-a) or macroalgal biomass may be more useful in diagnosing eutrophication.

5.6.2   Brackish SAV

Though brackish water SAV is an important component of intermittent and ephemerally tidal estuaries in California as well as brackish open waters of northern San Francisco Bay (i.e., Suisun Bay), little documentation exists on the extent and ecology of these primary producer communities. Literature from intermittently tidal Mediterranean estuaries and the Chesapeake Bay suggests that brackish SAV species decline in response to eutrophication. The mechanism of decline is presumably mediated

through light limitation caused by epiphytes, phytoplankton or macroalgal blooms, though there is relatively poor documentation of response of SAV with nutrient loads and other co-factors (temperature, salinity, etc.). This literature contradicts anecdotal observations of brackish water SAV in intermittently or ephemerally tidal estuaries, where very dense and apparently healthy *Ruppia* populations exist under very eutrophic conditions (high nutrient loading, high organic loading to the sediments, fish kills, large diurnal dissolved oxygen swings, etc.). It is not clear if these *Ruppia* beds are adapted to and thrive under high nutrient conditions or if these populations are an expression of eutrophication symptoms. The beds tend to be seasonal and it is unknown what triggers the reduction of biomass and subsequent decline of these apparently annual populations. No clear documentation exists of dose-response relationship between elevated biomass of SAV and secondary consumers, such as water column macroinvertebrates, nor documentation of changes in bed extent, biomass or density as a function of nutrient loading. **Because brackish water SAV can be a large component of the primary producer community when these estuaries are closed and the closed condition is a critical time period in which eutrophication symptoms worsen, this constitutes an important data gap for these types of estuaries within California.**

The following key data gaps should be addressed in order to pursue the use of brackish water SAV for diagnosis of eutrophication:

- Document nutrient load- SAV community response through long-term monitoring at individual estuaries (preferred) or through synoptic studies of load-response in multiple estuaries that span a disturbance gradient. Data sets should capture co-factor influence on load-response (e.g., timing and duration of inlet opening-closure, salinity regime, etc.).
- Document the relationship between SAV community structure, indicators of light availability (epiphyte load, chlorophyll-a biomass, macroalgal cover/biomass, etc.), dissolved oxygen, pH, and indicators of aquatic life use (macroinverbrates, fish, etc.).
- Document the growth habits of these plants and elucidate mechanisms of water column versus sediment response to nutrient loads. Detailed physiological and autecological studies of brackish SAV species need to be undertaken in order to better understand the habitat requirements of these communities.
- Data sets exist to begin to explore these data gaps. These include the Bight 08 Eutrophication Assessment Project (SCCWRP 2009) and the Central Lagoon Ecosystem Assessment Project (CLEAP 2009), data sets that should be used to explore the co-occurrence of increased SAV biomass with other symptoms of eutrophication (DO, pH, macroalgal biomass and cover, etc.). These existing data sets should be explored to elucidate whether any apparent load-response exists.

Table 5.2. Summary of literature reviews for candidate SAV and seagrass related indicators for E-NNE.

Group	Indicator	Methods	Information	Summary of Review
Community and physical Structure	Taxonomic composition	Field/lab identification	Changes in species diversity	The science to support its use is available; however, the indicator will not be useful in California because of limited species diversity and overlap in environmental tolerances. **Not recommended for further evaluation as an NNE indicator.**
	Density	Shoot counts in situ Braun-Blanquet % density estimates from In situ photos or side scan sonar	Changes in density of seagrass at a particular site	Changes in abundance often related to changes in light availability, but density can also change in response to other natural and anthropogenic factors. Density is a core component of seagrass monitoring programs, but not specific enough to diagnose eutrophication. Methods are well developed and currently used in a number of monitoring programs within the USA. **Not recommended for further evaluation as an NNE indicator.**
	Areal Distribution	Aerial photographs Video transects Transect mapping	Evaluate trends at the basin scale	Areal distribution is a core component of seagrass monitoring programs, but not specific enough to diagnose eutrophication. Methods are well developed and currently used in a number of monitoring programs within the USA, but not likely to be a powerful, early detection tool due to coarse resolution. **Not recommended for further evaluation as an NNE indicator.**
	Biomass	Cores Regression analysis	Diver-based clip plots or SAV rakes	Sampling is destructive and the data are difficult to interpret with limited power to detect change without very large sample size. Changes in biomass may be linked to factors other than nutrients, and therefore **not recommended as an NNE indicator for seagrass habitats.** Possible metric for canopy-forming brackish SAV, though no linkage with nutrient loads has been documented and will require additional research and development of long-term data sets.
Light Attenuation	Epiphyte Cover or Load	Visual rapid assessment, empirical sampling	Relative abundance of competing primary producers	There is a relationship between nutrient loading and epiphyte biomass but epiphyte load is confounded with a variety of other parameters and is unlikely to have a good "signal to noise ratio". As a result it is not likely to be a good stand-alone tool for detecting eutrophication. A "Rapid Assessment" type approach may work in conjunction with additional metrics, but will require additional research to develop rapid assessment method. **Recommend to pursue in conjunction with other metrics associated with light attenuation.**
	Light Attenuation	Grab samples or Continuously deployed Instrumentation (e.g., data sondes)	Attenuation of light reaching seagrass or SAV bed	Science exists, but assessment framework needs to be refined for California use. Due to species specific requirements and location specific characteristics application of this metric will require additional research and validation. **Recommend to pursue in conjunction with other metrics associated with light attenuation (epiphyte load, chlorophyll-a, turbidity), possibly as a rapid assessment to determine whether additional intensive diagnosis is warranted.**
	Chlorophyll-a, Turbidity or TSS		Surface water Chl a biomass and turbidity or TSS	Science exists, but assessment framework needs to be refined for use in California. Most useful as ancillary information to aid with the interpretation of other metrics. **Recommend to pursue in conjunction with other metrics associated with light attenuation (epiphyte load, chlorophyll-a, turbidity, macroalgal cover/biomass), possibly as a rapid assessment to determine whether additional intensive diagnosis is warranted.**
	Macroalgae	Field survey	Biomass or cover	See Chapter 4 for review

*parameters may include enzyme assays, photosynthetic characteristics, carbohydrate content, etc.

Table 5.2. Continued

Group	Indicator	Methods	Information	Summary of Review
Miscellaneous	Dissolved Oxygen and pH		Surface water DO and pH	See Chapter 7 for review.
	Physiological parameters*	Various methods	Site specific changes in physiology	Science to support their use exists and metrics are commonly used in seagrass monitoring programs. Sampling is expensive, destructive and has long time lags between sample collection and processing. Sample size limits the detection limits with most of these methods. Data are highly variable (temporally and spatially) and respond to a variety of natural cycles as well as anthropogenic stressors other than nutrients. Data interpretation can be difficult. Generally, poor signal to noise ratio. Some physiological measures (stable isotopes and ratio of N content to leaf mass) show promise but will require extensive new research for both seagrass and brackish SAV (7-10 y). **Not recommend to pursue as part of diagnostic assessment of eutrophication because, while it provides information about anthropogenic enrichment of seagrass, it does not indicate whether an adverse effect has occurred.**

*parameters may include enzyme assays, photosynthetic characteristics, carbohydrate content, etc.

5.7 References

Alberotanza, L., R.M. Cavalli, S. Pignatti, A. Zandonella. 2006. Classification of submersed aquatic vegetation in the Venice Lagoon using MIVIS airborne data. Annals of Geophysics 49: 271-276.

Alcoverra, T., R.C. Zimmerman, D.G. Kohrs, R.S. Alberte. 1999. Resource allocation and sucrose mobilization in light-limited eelgrass *Zostera marina*. Marine Ecology Progress Series 187: 121-131.

Azzoni, R., G. Giordani, M. Bartoli, D. T. Welsh, P. Viaroli. 2001. Iron, sulphur, and phosphorus cycling in the rhizosphere sediments of a eutrophic *Ruppia cirrhosa* meadow (Valle Smarlacca, Italy). Journal Sea Research 45: 15-26.

Bachelet, G., X. de Montaudouin, I. Auby, P-J Labourg. 2000. Seasonal changes in macrophyte and macrozoobenthos assemblages in three coastal lagoons under varying degrees of eutrophication. ICES Journal Marine Science 57: 1495-1506.

Balata, D., I. Bertocci, L. Piazzi, U. Nesti. 2008. Comparison between epiphyte assemblages of leaves and rhizomes of the seagrass *Posidonia oceanica* subjected to different levels of anthropogenic eutrophication. Estuarine Coastal Shelf Science 79: 533-540.

Batuik, R.A., R.J. Orth, K.A. Moore, W.C. Dennison, J.C. Stevenson, L.W. Staver, V. Carter, N. Rybicki, R.E. Hickman, S. Kollar, S. Bieber, P. Heasly. 1992. Chesapeake Bay Submerged Aquatic Vegetation Habitat Requirements and restoration targets: A technical synthesis. U.S. Environmental Protection Agency, Annapolis, MD. CBP/TRS 83/92

Batuik, R.A., P. Bergstrom, M. Kemp, E. Koch, L. Murray, J.C. Stevenson, R. Bartleson, V. Carter, N.

Rybicki, J.M. Landwehr, C. Gallegos, L. Karrh, M. Naylor, D. Wilcox, K.A. Moore, S. Alistock, M. Teichberg. 2000. Chesapeake Bay Submerged Aquatic Vegetation Water Quality and Habitat-based Requirements and restoration targets: A second technical synthesis. US Environmental Protection Agency, Annapolis, MD. http://www.chesapeakebay.net/publication.aspx?publicationid=13051

Beem, N.T., F.T. Short. 2009. Subtidal eelgrass declines in the Great Bay Estuary, New Hampshire and Maine, USA. Estuaries and Coasts 32: 202-205.

Berry, H.D., A.T. Sewll, S. Wyllie-Echeverria, B.R. Reeves, T,F, Mumford, J.R. Skalski, R.C. Zimmerman, J. Archer. 2003. Puget Sound Submerged Vegetation Monitoring Project: 2000-2002 monitoring report. Washington State Dept. of Natural Resources, Olympia WA. 60 pp. plus appendicies. http://www.dnr.wa.gov/Publications/aqr_nrsh_00_02svmp_rpt.pdf

Blackmon, D., T. Wyllie-Echeverria, D.J. Shafer. 2006. The role of seagrasses and kelps in marine fish support. WRAP Technical Notes Collection (ERDC TN-WRARP-06-1). US Army Engineering Research and Development Center, Vicksburg, MS. 39180. . http://el.erdc.usace.army.mil/elpubs/pdf/tnwrap06-1.pdf

Boese, B.L., B.D. Robbins, G. Thursby. 2005. Dessication is a limiting factor for eelgrass (*Zostera marina* L.) distribution in the intertidal portion of a northeastern Pacific (USA) estuary. Botanica Marina 48:274-283.

Boese, B.L., W.G. Nelson, C.A. Brown, R.J. Ozretich, H. Lee II, P.J. Clinton, C.L. Folger, T.C. Mochon-Collura, T.H. DeWitt. 2009. Chapter 8: Lower depth limit of *Zostera marina* in seven target estuaries. IN: Lee II, H. and C.A. Brown (eds). Classification of Regional Patterns of Environmental Drivers and Benthic Habitats in Pacific Northwest Estuaries. US EPA, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Western Ecology Division. EPA 600/R-09/140. http://www.epa.gov/wed/pages/publications/authored.htm

Bonis, A., P. Grillas, C. van Wijck, J. Lepart. 1993. The effect of salinity on the reproduction of coastal submerged macrophytes in experimental communities. Journal Vegetation Science 4: 461-468.

Bortolus, A., O. O. Iribarne, M.M. Martínez. 1998. Relationship between waterfowl and the seagrass *Ruppia maritima* in a Southwestern Atlantic coastal lagoon. Estuaries 21: 710-717.

Bortone, S.A. (ed). 2000. <u>Seagrasses Monitoring, Ecology, Physiology and Management</u>. CRC Press, Boca Raton, Florida.

Borum, J., O. Pedersen, T.M. Greve, T.A. Frankovich, J.C. Zieman, J.W. Fourqurean, C.J. Madden. 2005. The potential roles of plant oxygen and sulphide dynamics in die-off events of the tropical seagrass, *Thalassia testudinum*. Journal of Ecology 93: 148-158.

Bostrom, C., E. Bonsdorff, P. Kangas and A. Norkko. 2002. Long-term changes of a brackish water eelgrass (*Zostera marina* L.) community indicate effects of coastal eutrophication. Estuarine, Coastal Shelf Science 55:795-804

Boyer, K. E. and S. Wyllie-Echeverria. 2010. Eelgrass Conservation and Restoration in San Francisco Bay: Opportunities and Constraints. Report for the San Francisco Bay Subtidal Habitat Goals Project. Included as Appendix 8-1. http://www.sfbaysubtidal.org/report.html

Brock, M.A. 1981. Accumulation of proline in a submerged aquatic halophyte, *Ruppia* L. Oecologia 51: 217-219

Brown, C.A., W.G. Nelson, B.L. Boese, T.H. DeWitt, P.M. Eldridge, J.E. Kaldy, H. Lee II, J.H. Power, and D.R. Young. 2007. An approach to developing Nutrient Criteria for Pacific Northwest Estuaries: A Case Study of Yaquina Estuary, Oregon. USEPA Office of Research and Development, National Health and Environmental Effects Laboratory, Western Ecology Division. EPA/600/R-07/046. http://www.epa.gov/wed/pages/publications/authored.htm

Brown, C.A., R.J. Ozretich. 2009. Coupling between the coastal ocean and Yaquina Bay, Oregon: Importance of oceanic inputs relative to other nitrogen sources. Estuaries and Coasts 32:219-237

Burkholder, P.R., T.E. Doheny. 1968. The Biology of Eelgrass. Town of Hempstead, Long Island, N.Y. Dept. of Conservation and Waterways, Point Lookout, N.Y. PP. 120.

Burkholder, J. M., H. B. Glasgow and J. E. Cooke. 1994. Comparative effects of water-column nitrate enrichment on eelgrass *Zostera marina*, shoal grass *Halodule wrightii* and widgeongrass *Ruppia maritima*. Marine Ecology Progress Series 105:121-138.

Burkholder, J. M., K. M. Mason and H. B. Glasgow. 1992. Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments. Marine Ecology Progress Series 81:163-178.

Burkholder, J.M., D.A. Tomasko, B.W. Touchette. 2007. Seagrasses and eutrophication. Journal Experimental Marine Biology Ecology 350: 46-72.

Cambridge, M.L., J.R. How, P.S. Lavery, M.A. Vanderklift. 2007. Retrospective analysis of epiphyte assemblages in relation to seagrass loss in a eutrophic coastal embayment. Marine Ecology Progress Series 346: 97-107.

Carr, L. A., K. E. Boyer, and A. Brooks. 2011. Spatial patterns in epifaunal community structure in San Francisco Bay eelgrass (*Zostera marina*) beds. *Marine Ecology* 32:88-103.

Cho, H.J., P. Biber, C. Nica. 2009. The Rise of *Ruppia* in Seagrass beds: Changes in coastal environment and research needs. P. 333-347. In: E.K. Druruy, T.S. Pridgen (eds.) Handbook on Environmental Quality. Nova Science Publishers, Inc. N.Y.

Cho, H.J., M.A Poirrer. 2005. Seasonal growth and reproduction of *Ruppia maritima* L. s.l. in Lake Ponchartrain, Louisiana, USA. Aquatic Botany 81: 37-49.

Clinton, P.J., D.R. Young, D.T. Specht, H. Lee II. 2007. A guide to mapping intertidal eelgrass and non-vegetated habitats in estuaries of the Pacific Northwest USA. US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, EPA 600/R-07/062.

Dennison, W.C. R.J. Orth, K.A. Moore, J.C. Stevenson, V. Carter, S. Kollar, P.W. Bergstrom, R.A. Batuik. 1993. Assessing water quality with submersed aquatic vegetation. BioScience 43: 86-94.

Derksen, D.V., D.H. Ward. 1993. Life History and Habitat needs of the Black Brant. In: Waterfowl Management Handbook. US. Fish and Wildlife Service. Fish and Wildlife Leaflet 13. University of Nebraska- Lincoln. http://digitalcommons.unl.edu/icwdmwfm/41.

Drake, L.A., F.C. Dobbs, R.C. Zimmerman. 2003. Effects of epiphyte load on optical properties and photosynthetic potential of the seagrasses *Thalassia testudinum* Banks ex König and *Zostera marina* L. Limnology and Oceanography 48:465-463

Duarte, C.M., H. Kirkman. 2001. Methods for the measurement of seagrass abundance and depth distribution. In: F.T. Short, R.G. Coles (eds.). Global Seagrass Research Methods. Elsevier Science B.V., Amsterdam.

Dunton, K.H. 1994. Seasonal growth and biomass of the subtropical seagrass Halodule wrightii in relation to continuous measurements of underwater irradiance. Marine Biology 120: 479-489.

Dunton, K.H., W. Pulich, T. Mutchler. 2010. A seagrass monitoring program for Texas Coastal Waters: multiscale integration of landscape features with plant and water quality indicators. Coastal Bend Bays & Estuaries Program, Corpus Christi, TX. USA. http://texasseagrass.org/documents/A%20Seagrass%20Monitoring%20Program%20for%20Texas%201-10-11.pdf

Eicher, A. 2006. Humboldt Bay Harbor, Recreation and Conservation District Permit No. 03-03 Annual Report: 2006. UC Sea Grant Extension, Humboldt Bay Cooperative Eelgrass Project, 2 Commercial Street, Suite 4, Eureka, CA. 95501. (http://coastalwatersheds.ca.gov/portals/1/humboldtbay/monitor/docs/InvSp_SeaGr_Zostjap_2.pdf)

Fenchel, T. 1977. Aspects of the decomposition of seagrasses. PP 123-145. IN: C.P. McRoy and C. Helfferich (eds.). Seagrass Ecosystems: A scientific perspective. Marcel Dekker, Inc., N.Y.

Ferrat, L., C. Pergent-Martini, M. Roméo. 2003. Assessment of the use of biomarkers in aquatic plants for the evaluation of environmental quality: application to seagrasses. Aquatic Toxicology 65: 187-204.

Ferren, W.R., P.L. Fiedler, R. Leidy (eds.). 1995. Wetlands of the Central and Southern California Coast and Coastal Watersheds: A methodology for their classification and description. http://ucjeps.berkeley.edu/wetlands/

Ferraro, S.P., F.A. Cole. 2007. Benthic macrofauna-habitat associations in Willapa Bay, Washington, USA. Estuarine Coastal and Shelf Science 71: 491-507.

Foden, J., D.P. Brazier. 2007. Angiosperms (seagrass) within the EU water framework directive: a UK perspective. Marine Pollution Bulletin 55: 181-195.

Fonseca, M., P.E. Whitfield, N.M. Kelly, S.S. Bell. 2002. Modeling seagrass landscape pattern and associated ecological attributes. Ecological Applications 12: 218-237.

Fourqurean, J.W., M.F. Muth, J.N. Boyer. 2010. Epiphyte loads on seagrasses and microphytobenthos abundance are not reliable indicators of nutrient availability in oligotrophic coastal ecosystems. Marine Pollution Bulletin. In Press. Doi:10.1016/j.marpolbul.2010.03.003

Fourqurean, J.W., T.O. Moore, B. Fry, J.T. Hollibaugh. 1997. Spatial and temporal variation in C:N:P ratios, δ^{15}N, and δ^{13}C of eelgrass *Zostera marina* as indicators of ecosystem processes, Tomales Bay, California, USA. Marine Ecology Progress Series 157: 147-157.

Fourqurean, J.W., M.J. Durako, M.O. Hall, L.N. Hefty. 2001a. Seagrass distribution in south Florida: a multiagency perspective. In: Porter, J.W., K.G. Porter (eds). The connections between Ecosystems in the South Florida Hydroscape: The River of Grass Continues. Taylor & Francis, Inc., London. http://www.fiu.edu/~seagrass/papers/seagrass_distribution/distribu.pdf

Fourqurean, J.W., A. Willsie, C.D. Rose, L.M. Rutten. 2001b. Spatial and temporal pattern in seagrass community composition and productivity in South Florida. Marine Biology 138: 341-354.

Frankovich, T.A., J.W. Fourqurean. 1997. Seagrass epiphyte loads along a nutrient availability gradient, Florida Bay, USA. Marine Ecology Progress Series 159: 37-50

Gaeckle, J., P. Dowty, B. Reeves, H. Berry, S. Wyllie-Echeverria, T. Mumford. 2007. Puget Sound Vegetation Monitoring Program 2005 Monitoring Report. Washington State Dept. of Natural Resources. 111 Washington St., SE., Olympia, WA. http://www.dnr.wa.gov/htdocs/aqr/nshr/index.html

Giusti, E., S. Marsili-Libelli. 2005. Modelling the interactions between nutrients and the submersed vegetation in the Orbetello Lagoon. Ecological Modelling 184: 141-161.

Green, E.P. and F.T. Short. 2003. World Atlas of Seagrasses. University of California Press, Berkeley, California, USA.

Greve, T.M., J. Borum, O. Pedersen. 2003. Meristematic oxygen variability in eelgrass (*Zostera marina*). Limnology & Oceanography 48:210-216.

Grewell, B.J., J.C. Callaway, W.R. Ferren, Jr. 2007. Estuarine Wetlands. P. 124-154. In: M.G. Barbour, T. Keeler-Wolf, A.A. Schoenherr (eds.). Terrestrial vegetation of California. University of California Press, Berkeley. http://ddr.nal.usda.gov/dspace/bitstream/10113/7132/1/IND43980831.pdf

Haines, P.E., R.B. Tomlinson, B.G. Thom. 2006. Morphometeric assessment of intermittently open/closed coastal lagoons in New South Wales, Australia. Estuarine, Coastal and Shelf Science 67: 321-332.

Harlin, M. M., and B. Thorne-Miller. 1981. Nutrient enrichment of seagrass beds in a Rhode Island coastal lagoon. Marine Biology 65:221-229.

Hauxwell, J., I. Valiela. 2004. Effects of nutrient loading on shallow seagrass-dominated coastal systems: patterns and processes. In: Nielsen, S., G. Banta, M. Pedersen. (eds.). Estuarine Nutrient Cycling: The influence of Primary Producers. Kluwer Academic Publishers, the Netherlands, pp. 59-92.

Hauxwell, J., J. Cebrian and I. Valiela. 2003. Eelgrass *Zostera marina* loss in temperate estuaries: relationship to land-derived nitrogen loads and effect of light limitation imposed by algae. Marine Ecology Progress Series 247:59-73

Havens, K. E., J. Hauxwell, A. C. Tyler, S. Thomas, K. J. McGlathery, J. Cebrian, I. Valiela, A. D. Steinman and S.-J. Hwang. 2001. Complex interactions between autotrophs in shallow marine and freshwater ecosystems: implications for community responses to nutrient stress. Environmental Pollution 113:95-107.

Heck., K.L., J.F. Valentine. 2006. Plant-herbivore interactions in seagrass meadows. Journal of Experimental Marine Biology Ecology 330: 420-436.

Heffernan, J.J., R.A. Gibson. 1983. A comparison of primary production rates in Indian River, Florida seagrass systems. Florida Scientist 46:295-306.

Heidelbaugh, W.S., W.G. Nelson. 1996. A power analysis of methods for assessment of change in seagrass cover. Aquatic Botany 53: 227-233.

Hemminga, M.A., B.P. Koutstaal, J. van Soelen, A.G.A. Merks. 1994. The nitrogen supply to intertidal eelgrass (*Zostera marina*). Marine Biology 118:141-158

Hemminga, M.A., C.M. Duarte. 2000. Seagrass Ecology. Cambridge University Press, Cambridge, UK.

Heijs, S.K., R. Azzoni, G. Giordani, H.M. Jonkers, D. Nizzoli, P. Viaroli, H. van Gemerden. 2000. Sulfide-induced release of phosphate from sediments of coastal lagoons and the possible relation to the disappearance of *Ruppia* sp. Aquatic Microbial Ecology 23: 85-95.

Hoven, H.M., T.G. Miller. 2009. Developing vegetation metrics for the assessment of beneficial uses of impounded wetland surrounding Great Salt Lake, Utah, USA. Natural Resources and Environmental Issues: 15: 63-72. http://digitalcommons.usu.edu/nrei/vol15/iss1/11

Hoven, H.M., D. Richards, W.P. Johnson, G.T. Carling. 2011. Plant metric refinement for condition assessment of Great Salt Lake impounded wetlands. Report to South Davis Sewer District, Box 140111, Salt Lake City, UT 84114-0111, USA

Hughes, A.R., S.L. Williams, C.M. Duarte, K.L. Heck Jr., M. Waycott. 2009. Associations of concern: declining seagrasses and threatened dependent species. Frontiers Ecology Environment; 7, doi:10.1890/080041

Huntington, B. E. and K. E. Boyer. 2008a. Impacts of red macroalgal abundance (*Gracilariopsis* sp.) on eelgrass (*Zostera marina*) in Tomales Bay, California. Marine Ecology Progress Series 367:133-142.

Huntington, B.E., K. E. Boyer. 2008. Evaluating patterns of nitrogen supply using macroalgal tissue content and stable isotopic signatures in Tomales Bay, CA. Environmental Bioindicators 3: 180-192.

Invers, O., R.C. Zimmerman, R.S. Alberte, M. Pérez, J. Romero. 2001. Inorganic carbon sources for seagrass photosynthesis: an experimental evaluation of bicarbonate use in species inhabiting temperate waters. Journal of Experimental Marine Biology and Ecology 265: 203-217

Irlandi, E.A. 1997. Seagrass patch size and survivorship of an infaunal bivalve. Oikos 78: 511-518.

Johnson, M.R., S.L. Williams, C.H. Lieberman, A. Solbak. 2003. Changes in the abundance of the seagrasses *Zostera marina* L. (eelgrass) and *Ruppia maritima* L. (widgeongrass) in San Diego, California, following an El Niño event. Estuaries 26: 106-115.

Kahn, A.E., M.J. Durako. 2005. The effect of salinity and ammonium on seed germination in *Ruppia maritima* from Florida Bay. Bulletin of Marine Science 77: 453-458.

Kaldy, J.E. 2009. Water column and sediment nutrients as limits to growth of *Zostera marina* and *Thalassia testudinum*. In: Nelson, W.G. (ed). Seagrasses and Protective Criteria: A Review and Assessment of Research Status. Office of Research and Development, National Health and Environmental Effects Research Laboratory, EPA/600/R-09/50. http://www.epa.gov/wed/pages/publications/authored/Seagrasses%20and%20Protective%20Criteria%20EPA600R-09050.pdf

Kaldy, J.E. 2006. Production ecology of the non-indigenous seagrass, dwarf eelgrass (*Zostera japonica* Ascher. & Graeb.), in a Pacific Northwest Estuary, USA. Hydrobiologia 553: 201-217.

Kaldy, J.E., K.S. Lee. 2007. Factors controlling *Zostera marina* L. growth in the eastern and western Pacific Ocean: Comparisons between Korea and Oregon, USA. Aquatic Botany 87: 116-126.

Kaldy, J.E., C.P. Onuf, P.M. Eldridge, L.A. Cifuentes. 2002. Carbon budget for a subtropical seagrass dominated coastal lagoon: how important are seagrasses to total ecosystem net primary production? Estuaries 25: 528-539.

Kantrud, H.A. 1990. Sago Pondweed (Potamogeton pectinatus L.): A literature review. U.S. Fish Wildl. Serv., Fish Wildl. Res. 176. 92 pp. http://www.dtic.mil/dtic/tr/fulltext/u2/a322631.pdf

Kantrud, H.A. 1991. Widgeongrass (*Ruppia maritima*): A literature review. U.S. Fish Wildl. Serv., Fish Wildl. Res. 10. 58 pp. http://www.dtic.mil/cgi-bin/GetTRDoc?AD=ADA322676&Location=U2&doc=GetTRDoc.pdf

Kennedy, V.S. (ed). 1982. Estuarine Comparisons. Academic Press, New York.

Kikuchi, T., J.M. Pérès. 1977. Consumer ecology of seagrass beds. PP 147-193. : C.P. McRoy and C. Helfferich (eds.). Seagrass Ecosystems: A scientific perspective. Marcel Dekker, Inc., N.Y.

Koch, E.M. 2001. Beyond Light: physical, geological and geochemical parameters as possible submersed aquatic vegetation habitat requirements. Estuaries 24:1-17.

Koch, E.W., C.J. Dawes. 1991. Influence of salinity and temperature on the germination of *Ruppia maritima* L. from the North Atlantic and Gulf of Mexico. Aquatic Botany 40: 387-391.

La Peyre, M.K., S. Rowe. 2003. Effects of salinity changes on growth of *Ruppia maritima* L. Aquatic Botany 77: 235-241.

Lamberson, J.O., M.R. Frazier, W.G. Nelson, P.J. Clinton. 2011. Utilization patters of intertidal habitats by birds in Yaquina Estuary, Oregon. US Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Western Ecology Division, Newport, OR. EPA/600/R-11/118

Larkum, A.W.D., R.J. Orth, C.M. Duarte. (eds.). 2006. Seagrasses: Biology, Ecology and Conservation. Springer, The Netherlands.

Larned, S.T. 2003. Effects of the invasive, nonindigenous seagrass *Zostera japonica* on nutrient fluxes between the water column and benthos in a NE Pacific estuary. Marine Ecology Progress Series 254: 69-80.

Lee, K.S., F.T. Short, D.M.Burdick. 2004. Development of a nutrient pollution indicator using the seagrass *Zostera marina* along nutrient gradients in three New England estuaries. Aquatic Botany 78: 197-216.

Lee, K.S., S.R. Park, Y.K. Kim. 2007. Effects of irradiance, temperature, and nutrients on growth dynamics of seagrasses: A Review. Journal of Experimental Marine Biology and Ecology 350: 144-175.

Lenzi, M., R. Palmieri, S. Porrello. 2003. Restoration of the eutrophic Orbetello lagoon (Tyrrhenian Sea, Italy): water quality management. Marine Pollution Bulletin 46: 1540-1548.

Leoni, V., A. Vela, V. Pasqualini, C. Pergent-Martini, G. Pergent. 2008. Effects of experimental reduction of light and nutrient enrichments (N and P) on seagrasses: a review. Aquatic Conservation: Marine and Freshwater Ecosystems 18: 202-220. DOI: 10.1002/aqc.842

Lepoint, G., J., Jacquemart, J.M. Bouquegneau, V. Demoulin, S. Gobert. 2007. Field measurements of inorganic nitrogen uptake by epiflora components of the seagrass *Posidonia oceanica* (Monocotyledons, Posidoniaceae). Journal of Phycology 43: 208-218.

Madden, C.J., D.T. Rudnick, A.A. McDonald, K.M. Cunniff, J.W. Fourqurean. 2009. Ecological indicators for assessing and communication seagrass status and trends in Florida Bay. Ecological Indicators 9S: S68-S82. doi:10.1016/j.ecolind.2009.02.004

Malea, P., T. Kevrekidis, A. Mogias. 2004. Annual versus perennial growth cycle in *Ruppia maritima* L.: temporal variation in population characteristics in Mediterranean lagoons (Monolimni and Drana Lagoons, Nothern Aegean Sea). Botanica Marina 47: 357-366.

McCall, D.D., C.F. Rakocinski, J.L. Pinckney. 2009. Effects of grass shrimp versus nutrient addition on epiphytic algae associated with the ephemeral widgeongrass *Ruppia maritima*. Marine Ecology Progress Series 379: 151-162.

McGlathery, K. J. 2001. Macroalgal blooms contribute to the decline of seagrass in nutrient enriched coastal waters. Journal of Phycology 37:453-456.

McRoy, C.P. 1969. Eelgrass under arctic winter ice. Nature 224: 818-819.

Meling-López, A.E., S.E. Ibarra-Obando. 1999. Annual life cycles of two *Zostera marina* L. populations in the Gulf of California: contrasts in seasonality and reproductive effort. Aquatic Botany 65: 59-69.

Menéndez, M., D. Carlucci, M. Pinna, F.A. Comin, A. Basset. 2003. Effect of nutrients on the decomposition of *Ruppia cirrhosa* in a shallow coastal lagoon. Hydrobiologia 506-509: 729-735.

Menéndez, M. 2009 Response of early *Ruppia cirrhosa* litter breakdown to nutrient addition in a coastal lagoon affected by agricultural runoff. Estuarine Coastal Shelf Science 82: 608-614.

Merkel, K., M. Sutula, B. Bernstein, B. Chesney. 2009. Southern California Regional Eelgrass Monitoring Program. Unpublished Report.

Moncreiff, C.A., M.J. Sullivan, A.E. Daehnick. 1992. Primary production dynamics in seagrass beds of Mississippi Sound: The contributions of seagrass, epiphytic algae, sand microflora and phytoplankton. Marine Ecology Progress Series 74:161-171.

Moore, K. A. and R. L. Wetzel. 2000. Seasonal variations in eelgrass (*Zostera marina* L.) responses to nutrient enrichment and reduced light availability in experimental ecosystems. Journal Experimental Marine Biology Ecology 244:1-28.

Moore, et al. 2009. Long-term monitoring for estuarine submersed and emergent vegetation communities. NERR SWMP Biomonitoring protocol. National Estuarine Research Reserve System. NOAA Ocean Service, Silver Spring, MD. http://nerrs.noaa.gov/Doc/PDF/Research/TechReportSWMPBio-MonitoringProtocol.pdf

Moore, J.E., M.A. Colwell, R.L. Mathis, J.M. Black. 2004. Staging of Pacific flyway brant in relation to eelgrass abundance and site isolation, with special consideration of Humboldt Bay, California. Biological Conservation 115: 475-486.

Muñiz-Salazar, R., S.L. Talbot, G.K. Sage, D.H. Ward, A. Cabello-Pasini. 2005. Population genetic structure of annual and perennial populations of *Zostera marina* L. along the Pacific Coast of Baja California and the Gulf of California. Molecular Ecology 14: 711-722.

Murphy, L.R., S.T. Kinsey, M.J. Durako. 2003. Physiological effects of short-term salinity changes on *Ruppia maritima*. Aquatic Botany 75: 293-309.

Neckles, H.A., E.T. Koepfler, L.W. Haas, R.L. Wetzel, R.J. Orth. 1994. Dynamics of epiphytic photoautotrophs and heterotrophs in *Zostera marina* (eelgrass) microcosms: response to nutrient enrichment and grazing. Estuaries 17: 597-605.

Neckles, H. A., R. L. Wetzel and R. J. Orth. 1993. Relative effects of nutrient enrichment and grazing on epiphyte-macrophyte (*Zostera marina* L.) dynamics. Oecologia 93:285-295

Nielsen, S.L., G.T. Banta, M.F. Pedersen (eds). 2004a. Estuarine Nutrient Cycling : The Influence of Primary Producers. Kluwer Academic Publishers, Dordrecht, Netherlands.

Nielsen, S.L., M.F. Pedersen, G.T. Banta. 2004b. Attempting a Synthesis-Plant/nutrient interactions. Pp281-292. IN: Nielsen, S.L., G.T. Banta, M.F. Pedersen (eds). 2004. Estuarine Nutrient Cycling : The Influence of Primary Producers. Kluwer Academic Publishers, Dordrecht, Netherlands.

Nixon, S., B. Buckley, S. Granger, J. Bintz. 2001. Responses of very shallow marine ecosystems to nutrient enrichment. Human and Ecological Risk Assessment 7: 1457-1481.

Norris, J.G., S. Wyllie-Echeverria, T. Mumford, A. Bailey, T. Turner. 1997. Estimating basal area coverage of subtidal seagrass beds using underwater videography. Aquatic Botany 58: 269-287.

Olyarnik, S. 2008. The causes and consequences of macroalgal blooms on an eelgrass (*Zostera marina*) community in Bodega Harbor, CA. Dissertation, University of California, Davis.

Onuf, C.P. 2006. Biofouling and the continuous monitoring of underwater light from a seagrass perspective. Estuaries and Coasts 29: 511-518.

Orth, R.J., T.J.B. Carruthers, W.C. Dennison, C.M. Duarte, J.W. Fourqurean, K.L. Heck Jr., A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, S. Olyarnik, F.T. Short, M. Waycott, S.L. Williams. 2006. A global crisis for seagrass ecosystems. BioScience 56:987-996.

Orth, R.J., M.R. Williams, S.R. Marion, D.J. Wilcox, T.J.B. Carruthers, K.A. Moore, W.M. Kemp, W.C. Dennison, N. Rybicki, P. Bergstrom, R.A. Batuik. 2010. Long-term trends in submersed aquatic vegetation (SAV) in Chesapeake Bay, USA, related to water quality. Estuaries and Coasts 33: 1144-1163

Palacios, S.L., R.C. Zimmerman. 2007. Response of eelgrass *Zostera marina* to CO2 enrichment: possible impacts of climate change and potential for remediation of coastal habitats. Marine Ecology Progress Series 344: 1-13

Peterson, B.J., K.L. Heck Jr. 2001. Positive interactions between suspension-feeding bivalves and seagrass – a facultative mutualism. Marine Ecology Progress Series 213: 143-155.

Peterson, C.H., H.C. Summerson, P.B. Duncan. 1984. The influence of seagrass cover on population structure and individual growth rate of a suspension-feeding bivalve, *Mercenaria mercenaria*. Journal of Marine Research 42: 123-138.

Pergent, G., V. Pasqualini, C. Pergent-Martini, L. Ferrat, C. Fernandez. 2006. Variability of *Ruppia cirrhosa* in two coastal lagoons with differing anthropogenic stresses. Botanica marina 49: 103-110.

Phillips, R.C. 1984. The ecology of eelgrass meadows in the Pacific Northwest: A community profile. U.S. Fish Wildl. Serv. FWS/OBS-84/24. 85 pp.

Pregnall, A.M., R.D. Smith, T.A. Kursar, R.S. Alberte. 1984. Metabolic adaptation of *Zostera marina* (eelgrass) to diurnal periods of root anoxia. Marine Biology 83: 141-147.

Pulich, W., C. Blair, W.A. White. 1997. Current status and historical trends of seagrasses in the Corpus Christi Bay National Estuary Program Study Area. Texas Natural Resource Conservation Commission, Austin, TX. http://www.cbbep.org/publications/virtuallibrary/ccbnep20.pdf

Quammen, M.L., C.P. Onuf. 1993. Laguna Madre: seagrass changes continue decades after salinity reduction. Estuaries 16: 302-310.

Rubio, L., A. Linares-Rueda, M.J. García-Sánchez, J.A. Fernández. 2007. Ammonium uptake kinetics in root and leaf cells of *Zostera marina* L. Journal Experimental Marine Biology and Ecology 352: 271-279.

Sargent, F.J., T.J. Leary, D.W. Crewz, C.R. Kruer. 1995. Scarring of Florida's seagrasses: assessment and management options. FMRI Technical Report TR-1. Florida Marine Research Institute, St. Petersburg, Florida. 37 p. plus appendicies.

Setchell, W.A. 1924. *Ruppia* and its environmental factors. Proceedings National Academy Sciences 10: 286-288.

Shafer, D.J. J.E. Kaldy, T.D. Sherman, K.M. Marko. 2011. Effects of salinity on photosynthesis and respiration of the seagrass *Zostera japonica*: A comparison of two established populations in North America. Aquatic Botany 95: 214-220.

Shili, A., N.B. Maïz, C.F. Boudouresque, E.B. Trabelsi. 2007. Abrupt changes in *Potamogeton* and *Ruppia* beds in a Mediterranean lagoon. Aquatic Botany 87: 181-188.

Short, F.T. 1983. The seagrass *Zostera marina* L.: plant morphology and bed structure in relation to sediment ammonium in Izembek lagoon, Alaska. Aquatic Botany 16: 149-161.

Short, F. T., D. M. Burdick and J. E. Kaldy. 1995. Mesocosm experiments quantify the effects of eutrophication on eelgrass, *Zostera marina*. Limnology and Oceanography 40:740-749

Short, F.T., D.M. Burdick. 1996. Quantifying eelgrass habitat loss in relation to housing development and nitrogen loading in Waquoit Bay, Massachusetts. Estuaries 19: 730-739.

Short, F.T., S. Wyllie-Echeverria. 1996. Natural and human-induced disturbance of seagrasses. Environmental conservation 23: 17-27.

Short, F.T., D.M. Burdick, S. Granger, S.W. Nixon. 1996. Long-term decline in eelgrass, *Zostera marina* L., linked to increased housing development. IN: Kuo,J., R.C. Phillips, D.I. Walker, H. Kirkman (eds). Seagrass Biology: Proceedings of an International Workshop. Rottnest Island, Western Australia, 25-29 January 1996. pp. 291-298.

Short, F. T., G. E. Jones and D. M. Burdick. 1991. Seagrass decline: Problems and solutions. Coastal Zone '91 Conference - ASCE. Long Beach, CA/July 1991. Pp 439-453.

Silvertown, J.W. 1987. Introduction of plant population ecology. Second edition. Longman Scientific & Technical. Essex, England.

Smith, R.D., W.C. Dennison, R.S. Alberte. 1984. Role of seagrass photosynthesis in root aerobic processes. Plant Physiology 74: 1055-1058.

Staver, K.W. 1984. Responses of epiphytic algae to nitrogen and phosphorus enrichment and effects on productivity of the host plant, *Potamogeton perfoliatus* L., in estuarine waters. MSc thesis. University of Maryland, College Park, Md.

Stevenson, J. C., L. W. Staver and K. W. Staver. 1993. Water quality associated with survival of submersed aquatic vegetation along an estuarine gradient. Estuaries 16:346-361

Steward, J.S., R.W. Virnstein, L.J. Morris, E.F. Lowe. 2005. Setting seagrass depth, coverage, and light targets for the Indian River Lagoon System, Florida. Estuaries 28: 923-935

Talbot, S.L., W. Wyllie-Echeverria, D.H. Ward, J.R. Rearick, G.K. Sage, B. Chesney, R.C. Phillips. 2006. Genetic characterization of *Zostera asiatica* on the Pacific Coast of North America. Aquatic Botany 85: 169-176.

Taylor, D. I., S. W. Nixon, S. L. Granger, B. A. Buckley, J. P. McMahon and H.-J. Lin. 1995. Responses of coastal lagoon plant communities to different forms of nutrient enrichment- a mesocosm experiment. Aquatic Botany 52:19-34

Tennant, G.A. 2006. Experimental effects of ammonium on eelgrass (*Zostera marina* L.) shoot density in Humboldt Bay, California. MA Thesis. Humboldt State University, Humboldt, CA. Pp. 86.

Texas Parks and Wildlife. 1999. Seagrass Conservation Plan for Texas. Texas Parks and Wildlife, Resource Protection Division, 4200 Smith School Rd., Austin, Texas. 79 pp.
http://www.tpwd.state.tx.us/publications/pwdpubs/media/pwd_bk_r0400_0041.pdf

Thayer, G.W., W.J. Kenworthy, M.S. Fonseca. 1984. The ecology of eelgrass meadows of the Atlantic Coast: A community profile. U.S. Fish Wildl. Serv. FWS/OBS-84/02. 147pp.

Thom, R.M., S.L. Southard, A.B. Borde, P. Stoltz. 2008. Light requirements for growth and survival of eelgrass (*Zostera marina* L.) in Pacific Northwest (USA) estuaries. Estuaries and Coasts 31: 969-980.

Thom, R.M., A.B. Borde, S. Rumrill, D.L. Woodruff, G.D. Williams, J.A. Southard, S.L. Sargeant. 2003. Factors influencing spatial and annual variability in eelgrass (*Zostera marina* L.) meadows in Willapa Bay, Washington and Coos Bay, Oregon, estuaries. Estuaries 26: 1117-1129.

Thursby, G.B. 1984. Nutritional requirements of the submerged angiosperm *Ruppia maritima* in algae-free culture. Marine Ecology Progress Series 16:45-50.

Thursby, G.B., M.M. Harlin. 1982. Leaf-root interaction in the uptake of ammonium by *Zostera marina*. Marine Biology 72:109-112

Thursby, G.B., M.M. Harlin. 1984. Interaction of leaves and roots of *Ruppia maritima* in the uptake of phosphate, ammonia and nitrate. Marine Biology 83: 61-67.

Touchette, B.W. 2007. Seagrass-salinity interactions: physiological mechanisms used by submersed marine angiosperms for a life at sea. Journal Experimental Marine Biology Ecology 350: 194-215

Twilley, R. R., W. M. Kemp, K. W. Staver, J. C. Stevenson and W. R. Boynton. 1985. Nutrient enrichment of estuarine submersed vascular plant communities. 1. Algal growth and effects of production of plants and associated communities. Marine Ecology Progress Series 23:179-191.

Tyerman, S.D. 1982. Water relations of seagrasses. Plant Physiology 69: 957-965

Underwood, E.C., M. J. Mulitsch, J.A. Greenberg, M.L. Whiting, S.L. Ustin, S.C. Kefauver. 2006. Mapping invasive aquatic vegetation in the Sacramento-San Joaquin Delta using hyperspectral imagery. Environmental Monitoring and Assessment 121: 47-64.

Valiela, I., K. Foreman, M LaMontagne, D. Hersh, J. Costa, P. Peckol, B. DeMeo-Anderson, C. D'Avanzo, M. Babione, C.-H. Sham, J. Brawley and K. Lajtha. 1992. Couplings of watersheds and coastal waters: sources and consequences of nutrient enrichments in Waquoit Bay, Massachusetts. Estuaries 15:443-457

van Lent, F., J. M. Vershuure and M. L. J. van Veghel. 1995. Comparative study on populations of *Zostera marina* L. (eelgrass): in situ nitrogen enrichment and light manipulation. Journal Experimental Marine Biology Ecology 185:55-76

van Katwijk, M. M., L.H.T. Vergeer, ., G. H. W. Schmitz, J.G.M. Roelofs. 1997. Ammonium toxicity in eelgrass *Zostera marina*. Marine Ecology Progress Series 157: 159-173.

van Katwijk, M. M., G. H. W. Schmitz, A. P. Gasseling and P. H. van Avesaath. 1999. Effects of salinity and nutrient load and their interaction on *Zostera marina*. Marine Ecology Progress Series 190:155-165

Virnstein, R.W., L.J. Morris. 1996. Seagrass preservation and restoration: a diagnostic plan for the Indian River Lagoon. Technical Memorandum #14. St. Johns River Water Management District, Palatka, FL. 43 pp.

Ward, D.H. 1983. The relationship of two seagrasses: *Zostera marina* and *Ruppia maritima* to the Black Brant, *Branta bernicla nigricans*, San Ignacio lagoon, Baja California, Mexico. MS Thesis. University of Oregon, Eugene OR. 60 p.

Waycott, M., C.M. Duarte, T.J.B. Carruthers, R.J. Orth, W.C. Dennison, S. Olyarnik, A. Calladine, J.W. Fourqurean, K.L. Heck, Jr., A.R. Hughes, G.A. Kendrick, W.J. Kenworthy, F.T. Short, S.L. Williams. 2009. Accelerating loss of seagrasses across the globe threatens coastal ecosystems. Proceedings National Academies of Science 106:12377-12381.

Wazniak, C.E., M.R. Hall, T.J.B. Carruthers, B. Sturgis, W.C. Dennison, R.J. Orth. 2007. Linking water quality to living resources in a Mid-Atlantic lagoon system, USA. Ecological applications 17 (supplement):s64-78.

Williams, S. L. and M. H. Ruckelshaus. 1993. Effects of nitrogen availability and herbivory on eelgrass (*Zostera marina*) and epiphytes. Ecology 74:904-918

Williams, S.L. 2007. Introduced species in seagrass ecosystems: Status and Concerns. Journal Experimental Marine Biology and Ecology 350:89-110.

Williams, D.J., N.B. Rybicki, A. V. Lombana, T.M. O'Brien, R.B. Gomez. 2003. Preliminary investigation of submerged aquatic vegetation mapping using hyperspectral remote sensing. Environmental Monitoring and Assessment 81: 383-392.

Worm, B., T. B. H. Reusch and H. K. Lotze. 2000. In situ nutrient enrichment: methods for marine benthic ecology. International Review of Hydrobiologia 85:359-375

Young, D.R., P.J. Clinton, H. Lee II, D.T. Specht, T.C. Mochon-Collura. 2009. Aerial measures of estuarine intertidal and shallow subtidal *Zostera marina* coverage. PP 158-172. IN: Lee II, H. and C.A. Brown (eds.) Classification of regional patterns of environmental drivers and benthic habitats in Pacific Northwest estuaries. US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Western Ecology Division. EPA/600/R-09/140

Yuan, L., L. Q. Zhang. 2008. Mapping large-scale distribution of submerged aquatic vegetation coverage using remote sensing. Ecological Informatics 3:245-251

Zimmerman, R.C., J.L. Regguzoni, R.S. Alberte. 1995. Eelgrass (*Zostera marina* L.) transplants in San Francisco Bay: Role of light availability on metabolism, growth and survival. Aquatic Botany 51:67-86

Zimmerman, R.C., R.D. Smith, R.S. Alberte. 1987. Is growth of eelgrass nitrogen limited? A numerical simulation of the effects of light and nitrogen on the growth dynamics of *Zostera marina*. Marine Ecology Progress Series 41: 167-176.

Zimmerman, R.C., D.G. Kohrs, D.L. Steller, R.S. Alberte. 1997. Impacts of CO2 enrichment on productivity and light requirements of eelgrass. Plant Physiology 115: 599-607.

6. Altered Water and Sediment Chemistry: A Review of Existing Science

Karen McLaughlin and Martha Sutula

6.1 Introduction

Most proposed conceptual models of development of eutrophication in estuaries show that the primary response to increased nutrient loads is a change in the biomass and relative distribution of species of aquatic plants and algae expression as discussed in earlier chapters (Chapters 3, 4, 5, and 6) (Neinhuis 1992; Raffaelli, Balls et al. 1999; McGlathery, Sundbäck et al. 2004; Viaroli, Bartoli et al. 2004; Viaroli, Bartoli et al. 2008; Zaldivar, Cardoso et al. 2008). These changes are accompanied and enhanced by basic changes in chemistry of the water column and sediments including increased water column and sediment oxygen demand, leading to hypoxia (Nixon 1995; Zaldivar, Cardoso et al. 2008), decreased water clarity (Kemp, Boynton et al. 2005; Zaldivar, Cardoso et al. 2008), increased production of ammonium and sulfide at concentrations toxic to fauna (Azzoni, Giordani et al. 2001; Middelburg and Levin 2009), and changes in the rates of fundamental biogeochemical cycles such as denitrification (Viaroli, Bartoli et al. 2008; Middelburg and Levin 2009). Alteration in the ambient concentrations and predominant forms of nitrogen (N), phosphorus (P), silica (Si), as well as micronutrients such as iron can also result in direct and indirect effects on the ecosystem (Azzoni, Giordani et al. 2001; Viaroli, Bartoli et al. 2008). Information on these changes is important for understanding how estuaries respond to nutrient enrichment and could potentially be used as indicators of the extent of eutrophication within these systems (Zaldivar, Cardoso et al. 2008).

The purpose of this review is to summarize existing information on metrics describing changes in sediment and water column biogeochemistry and explore their suitability as indicators of eutrophication in California estuaries, utilizing the criteria specified in Chapter 2.

6.2. Direct Effects of Nutrients on Estuarine Biogeochemistry

While increased nutrient availability can result in eutrophication, nutrients themselves are generally not considered to directly impair beneficial uses, with several well noted exceptions: 1) ammonia, 2) nitrate and 3) urea. Information on the effects of these particular nutrient forms are given below.

6.2.1 Nutrient Forms and Effects on Aquatic Habitat

The nutrients nitrogen (N) and phosphorus (P) are elements that can be found in the natural environment in a number of different forms (Table 6.1). Total nitrogen (TN) is a measure of all forms of dissolved and particulate N present in a water sample. Nitrogen exists in water both as inorganic and organic species, and in dissolved and particulate forms. Inorganic nitrogen is found both as oxidized species (*e.g.* nitrate (NO_3^-) and nitrite (NO_2^-)) and reduced species (*e.g.* ammonia ($NH_4^+ + NH_3$) and nitrogen gas (N_2)). DIN comprises $NO_2^- + NO_3^- + NH_4^+$. These species of DIN can be found in both surface waters as well as sediment pore waters. Total dissolved nitrogen (TDN) consists of dissolved inorganic

nitrogen (DIN) and dissolved organic nitrogen (DON). Dissolved organic nitrogen is found in a wide range of complex chemical forms such as amino acids, proteins, urea and humic acids, found in both surface waters and sediment pore waters. The particulate nitrogen pool consists of plants and animals, and their remains, as well as ammonium adsorbed onto mineral particles. Nitrogen is an integral component of organic compounds such as amino acids, proteins, DNA and RNA. Particulate N can be found in suspended in the water column (e.g. as phytoplankton or detrital organic matter) or in the sediment. Some portion of the particulate N pool is subject to rapid mineralization, and is biologically available.

Table 6.1. Nutrient species relevant to estimating nutrient loads in relation to standing nutrient concentrations and impacts to beneficial uses.

Form	Nitrogen	Phosphorus
Dissolved Inorganic	Nitrate (NO_3^-) + nitrite (NO_2^{2-})	Ortho-phosphate (PO_4^{-2}) is considered freely dissolved. Measurements of phosphate are "soluble reactive phosphorus (SRP)," which includes o-phosphate plus P that is loosely adsorbed to particles.
	Ammonium ($NH4^+$; in dynamic equilibrium in natural waters with unionized or free ammonia)	
Dissolved Organic	Dissolved organic nitrogen (often a large portion of total nitrogen in natural waters especially those less impacted by human activities)	Dissolved organic phosphorus (can be a large portion of total phosphorus in natural waters unless impacted by human activities or during periods of active decomposition of organic matter (e.g. algal bloom die-off).
Particulate	Particulate organic nitrogen (detritus left from pieces of undecayed or partially decayed organic matter)	Particulate organic phosphorus (associated with live or dead organic matter)
	Particulate inorganic nitrogen (insignificant in natural waters and usually not considered)	Particulate inorganic phosphorus (typically associated with minerals)

Total phosphorous (TP) is a measure of all the various forms of P (dissolved and particulate) found in water. Dissolved P is available for APP uptake, and consists of inorganic orthophosphate (*e.g.* $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) and organic phosphorus-containing compounds (DOP), found in both surface waters and sediment pore waters. The particulate P pool consists of plants and animals, and their remains, P in minerals and phosphate adsorbed onto iron oxyhydroxides on mineral surfaces. Particulate P can be found in suspension or in the sediment. The adsorption and desorption of phosphate from mineral surfaces forms a buffering mechanism that regulates dissolved phosphate concentrations in rivers and estuaries.

6.2.2   Direct Effects of Ammonia on Estuarine Ecosystem Health

Toxicity of Unionized Ammonia to Aquatic Organisms

Ammonia nitrogen is a common byproduct of industrial and municipal wastewater streams, fertilizers, and natural processes. It includes both the ionized form (ammonium, NH_4^+) and the unionized form (ammonia, NH_3). Elevated unionized ammonia (NH_3) can be acutely toxic to aquatic animals and is a

common cause of fish kills, because it can readily diffuse across gill membranes (Sampaio et al. 2002). Invertebrates, particularly arthropods, are generally less susceptible than fish. However, recent information suggests that some types of mussels (Unionidae)—particularly the glochidia and juvenile stages—are very sensitive to ammonia (Newton and Bartsch 2007). Environmental conditions can greatly alter the toxicity of unionized ammonia to organisms. An increase in pH favors formation of the more toxic unionized form (NH_3), while a decrease favors the ionized (NH_4^+) form. Temperature also affects the toxicity of ammonia to aquatic life.

In most fish, ammonia is excreted by passive diffusion of ammonia across the gills according to its partial pressure gradient (Wilson et al. 1998). Disruption of this gradient causes internal ammonia concentrations to increase, affecting internal organs, nervous system function, respiration, fish growth, gill condition, organ weights, and hematocrit (Milne et al. 2000). A list of specific types of effects is given in Table 6.2. Ionized ammonia (NH_4^+) does not pass as easily through gill membranes, so it is appreciably less toxic than the unionized form (Camargo and Alonso 2006). Sensitivities to chronic exposures are less taxonomically consistent. Exposure duration and frequency strongly influence the severity of effects (Milne et al. 2000). Early life stages of fish are more sensitive than juveniles or adults. Hence, effects are more likely to occur during seasons when early life stages are present.

Table 6.2. Effects of elevated ammonia in fish (from US EPA 2010).

Effects	Reference
Decreased respiratory function causing hyperventilation	Lease et al. 2003,; Twitchen and Eddy 1994; IPCS 1986
Impairment of nerve function; peripheral and central nervous system effects causing hyperexcitability	Sampaio et al. 2002, Twitchen and Eddy 1994, IPCS 1986
Convulsions and coma	Twitchen and Eddy 1994,; IPCS 1986
Damage to gill epithelia causing asphyxiation, proliferation of gill tissue	Lang et al. 1987
Stimulation of glycolysis and suppression of Krebs cycle, causing progressive acidosis and reduction in blood oxygen-carrying capacity	Camargo and Alonso 2007
Uncoupling of oxidative phosphorylation, causing inhibition of ATP production and depletion of ATP in the basilar region of the brain	Camargo and Alonso 2007, Sampaio et al. 2002
Disruption of blood vessels and osmoregulatory activity, causing stress to the liver and kidneys	Camargo and Alonso 2007, Sampaio et al. 2002, Bosakowski and Wagner 1994
Repression of immune system, causing increased susceptibility to bacteria and parasitic diseases	Camargo and Alonso 2007, Sampaio et al. 2002
Reduction of Na^+ to potentially fatally low levels	Twitchen and Eddy 1994

In addition, unionized ammonia can cause toxicity to *Nitrosomona*s and *Nitrobacter* bacteria, inhibiting the nitrification process, the conversion of ammonium to nitrate. This inhibition can result in increased ammonium accumulation in the aquatic environment, intensifying the level of toxicity to bacteria and aquatic animals (Carmargo and Alonso 2006).

Typically, the concentrations at which toxicity of unionized ammonia occur are typically an order of magnitude above those concentrations considered to be elevated in natural waters. All Regional Water Quality Control Boards have basin plan objectives for unionized ammonia in surface waters, with guidance from USEPA on saltwater (US EPA 1989) and freshwater (US EPA 2011). Therefore, a direct toxicity endpoint is not currently included among the candidate indicators for the estuarine NNE framework.

Ammonium Inhibition of Nitrate Assimilation by Phytoplankton

Nitrate (NO_3^-) and ammonium (NH_4^+) are the two primary dissolved inorganic nitrogen forms in aquatic systems that are assimilated by primary producers (Peterson, 1985). Ammonium is the preferred nitrogen source for assimilation by phytoplankton and can become limiting in the environment (Peterson, Smith et al. 1985; Hogue, Wilkerson et al. 2001; Hogue, Wilkerson et al. 2005; Wankel, Kendall et al. 2006). However, studies have shown that in some estuaries when nitrate is generally in high supply, phytoplankton can switch to nitrate assimilation when ammonium is depleted (Hogue, Wilkerson et al. 2001).

There is evidence in North San Francisco Bay (Suisun Bay and lower Sacramento River Delta) that ammonium has an inhibitory effect on phytoplankton nitrate uptake when ammonium concentrations exceed 4 µmol/L (Wilkerson, Dugdale et al. 2006; Dugdale, Wilkerson et al. 2007). During springtime blooms in the North Bay there is an initial ammonium uptake by phytoplankton, subsequent ammonium depletion below 4 µM, followed by high rates of nitrate uptake resulting in a bloom period (Wilkerson, Dugdale et al. 2006). This inhibitory effect may prevent phytoplankton blooms from occurring since phytoplankton uptake of ammonium occurs at slower rates than nitrate uptake thus limiting rates of primary productivity (Dugdale, Wilkerson et al. 2007). Thus elevated ammonium concentrations have been suggested as a major mechanism by which spring diatom blooms appear to be suppressed in the North Bay and lower Sacramento River (Wilkerson, Dugdale et al. 2006; Dugdale, Wilkerson et al. 2007). As nitrate became less available relative to ammonium in North Bay, it has been suggested that the competitive advantage has shifted to phytoplankton taxa that can more efficiently use reduced forms of N (e.g. cyanobacteria and many flagellates; Berg et al., 2001; Glibert 2010). Despite this evidence, the ecological importance of ammonium inhibition of spring diatom blooms is not well understood relative to factors known to control primary productivity. Thus the linkage between ammonium concentrations and adverse effects on phytoplankton primary productivity is not at this time universally accepted.

Ammonium and Nitrate Toxicity of Seagrasses

Ammonium and nitrate toxicity has been documented for both *Zostera marina* (Burkholder, Mason et al. 1992; vanKatwijk, Vergeer et al. 1997; Touchette and Burkholder 2007) as well as Zostera noltii (Brun, Hernandez et al. 2002; Cabaco, Machas et al. 2008). Ammonium toxicity is thought to be a consequence of the uncoupling of ATP synthesis during photosynthesis, whereas nitrate toxicity is thought to be due to a diversion of energy and carbon toward nitrate reduction and ammonium assimilation, and amino acid synthesis which causes a `drain' of internal carbon that otherwise would have been allocated as food reserves in the plant (Burkholder, Mason et al. 1992; Touchette and Burkholder 2000). Toxicity

effects have been shown to be mitigated by phosphate additions due to an acceleration of carbon production by photosynthesis under addition of both nitrogen and phosphorus which helped to mitigate the effects of toxicity(Brun, Hernandez et al. 2002). Toxicity was affected by the frequency and duration of exposure as well as season. A single high pulse of ammonium or continuous exposure have the greatest inhibitory effects on seagrasses compared to lower concentrations pulsed at larger intervals (Brun, Hernandez et al. 2002). Continuous exposure negatively affected growth through a continuous supply of nitrogen that increased carbon demands and thus drained the carbon pool (Touchette and Burkholder 2000). At the other extreme, an initial high dose of ammonium can exceed the capacity of the seagrass to assimilate nitrogen into carbon skeletons, causing a high transitory drop in carbon (Turpin 1991; Touchette and Burkholder 2000), which may enhance the negative effect of ammonium on photosynthesis. Increased temperature accelerates the effects of both nitrate and ammonium toxicity (vanKatwijk, Vergeer et al. 1997; Touchette and Burkholder 2000). Studies characterizing thresholds for ammonium and nitrate toxicity on seagrasses are limited. A few macrocosm experiments have shown evidence of ammonium toxicity at concentrations as low as 25 μM and nitrate toxicity as low as 3.5-7 μM after approximately five weeks of elevated exposure (vanKatwijk, Vergeer et al. 1997; Touchette and Burkholder 2000). However, how ammonium and nitrate toxicity compare to other factors known to affect seagrass communities (e.g. shading by epiphyte and macroalgae communities and competition) is not well understood.

6.2.3   Role of Urea in Promoting Nuisance Blooms

Urea has been identified as a nutrient source that can be taken up by phytoplankton (Gilbert, Magnien et al. 2001; Anderson, Gilbert et al. 2002; Kudela, Lane et al. 2008). Loadings of urea have increased in certain areas, primarily due to the increased use of urea-based fertilizers (Anderson, Gilbert et al. 2002; Gilbert, Harrison et al. 2006). There is some evidence that certain phytoplankton species, mostly flagellates, prefer uptake of urea over other nitrogen forms and that urea can increase the toxicity of a bloom (as reviewed in (Anderson, Gilbert et al. 2002; Kudela, Lane et al. 2008). Some of these flagellates have been identified as harmful or nuisance species (Gilbert, Magnien et al. 2001; Anderson, Gilbert et al. 2002; Gilbert, Harrison et al. 2006; Kudela, Lane et al. 2008). Experiments using local harmful algal species (coastal California and Bay species) showed some preferential uptake of urea when ambient nutrient concentrations were low (Kudela, Lane et al. 2008). It has also been suggested that urea may sustain harmful blooms (Gilbert, Magnien et al. 2001; Kudela, Lane et al. 2008). In Chesapeake Bay, high urea concentrations were measured prior to a spring HAB bloom (Gilbert, Magnien et al. 2001). The unusually high urea levels were correlated with high springtime precipitation that may have increased urea loading prior to the bloom. However, there is some evidence that urea may have an inhibitory effect on nitrate uptake by phytoplankton (Kudela, Lane et al. 2008). Given urea use as fertilizer continues to rise, urea will likely to continue to form a greater portion of the dissolved organic nitrogen (DON) pool available for primary production in coastal ecosystems (Gilbert, Harrison et al. 2006), potentially driving future blooms.

6.2.4   Effects of Alteration in N:P Ratios

Aquatic algae and plants produce organic matter in a predictable stoichiometry or ratio of N:P (Table 6.3). With diatomaceous primary producers, this ratio also includes silica. The ratios of available nutrients in surface waters can convey an understanding of what nutrients or other factors (e.g. light) may be "limiting" or controlling the magnitude of primary production. Thus a nutrient may be "limiting" if its addition results in increased primary production (Howarth 1988). In general, there is scientific consensus around the idea that phosphorus limits primary production in freshwater aquatic habitats (Vollenweider 1976, Schindler 1977), while many, though not all, estuarine habitats are limited by nitrogen (Howarth 1988; Nixon 1995; Paerl, Pinckney et al. 1998), particularly those found in temperate climates with ample terrigenous sediment inputs.

Table 6.3. Literature values for Chla:C and C:N:P ratios of primary producer communities and assumptions to convert biomass to areal estimates of N and P associated with biomass.

Community	Stoichiometry (C:N:P)	Reference
Phytoplankton, assumed 1.5 m water depth	Chl a: C Ratio of 30:1 C:N:P = 106:16:1	(Cloern, Grenz et al. 1995), Redfield Ratio (Redfield 1958; Anderson and Sarmiento 1994)
Cyanobacteria mats	50% C by dry wt C:N:P = 550:30:1	(McLaughlin, Sutula et al. 2011) (Atkinson and Smith 1983)
Macroalgae	22% C by dry wt C:N:P = 80:5:1	(McLaughlin, Sutula et al. 2011), (Eyre and McKee 2002)
Benthic microalgae	Chl a: C ratio of 30:1 C:N:P = 90:15:1	(Sundbäck and McGlathery 2005) (Eyre and McKee 2002)

While the loads of N and P have increased over time, the rate of N loading has increased faster than P in many areas of the world (Glibert et al. 2006, Seitzinger et al., 2002), in some cases leading to expressions of eutrophication that differ from those classically considered, including inhibition of primary production by high N (Yoshiyama and Sharp, 2006, Dugdale et al. 2007). This has resulted in a increase in the N:P ratio of nutrient loads delivered from anthropogenic sources. This shift in N: P ratio, as well as the dominant form of nutrient (e.g. ammonium, urea) can alter the dominance of primary producer species composition (Gilbert, Harrison et al. 2006). For example, in freshwater and brackish water areas, a lowering of the N:P ratio can cause a shift phytoplankton taxonomic composition towards cyanobacteria. In lakes, thresholds of TN:TP lower than 22:1 differentiate lakes that will be controlled by cyanobacteria, versus those without such dominance (Smith et al. 1995, Smith 1983).

It has been suggested that the competitive advantage has shifted to phytoplankton taxa that can more efficiently use reduced forms of N (e.g. cyanobacteria and many flagellates; Berg et al., 2001, Glibert et al 2006). Furthermore, some cyanobacteria can proliferate in low P environments when other algal classes are P limited (Bertilsson et al., 2003; Van Mooey et al., 2009). Glibert (2010) suggests that the

proliferation of cyanobacteria during the most recent decade illustrates that nutrient stoichiometry may indirectly, as well as directly, affect phytoplankton assemblages: while cyanobacteria can tolerate elevated N:P levels, its dominance may also reflect the decline in other species without such tolerances.

In shallow estuaries, the shifting N:P ratios can result in shifts from seagrass communities to macroalgae dominance. As noted in the section above on ammonium and nitrate toxicity to seagrasses, an increase in the N:P ratio can result in a decline in seagrass populations as the plants cannot adequately process excess ammonium or nitrate without comparable phosphorus additions resulting in physiological stress and eventually senescence (Brun, Hernandez et al. 2002). Conversely macroalgae can increase their N-uptake rates and the N content of their tissues and tend not to be inhibited by low levels of irradiance as compared to seagrasses (Valiela, McClelland et al. 1997), all of which shifts primary producer dominance away from seagrasses in favor of macroalgae.

6.3   Effects of Organic Matter Accumulation (Eutrophication) on Estuarine Biogeochemistry

As the process of eutrophication progresses, the excessive production of aquatic plants and algal biomass result in an over-accumulation of labile organic matter in surface waters and sediments, altering the balance of basic biogeochemical cycles in the sediments and surface waters and leading to a cascade of adverse effects. These changes are described in detail below.

6.3.1   Increased Water Column and Sediment Hypoxia and Anoxia

Dissolved oxygen (DO) is necessary to sustain the life of all aquatic organisms that depend on aerobic respiration. DO concentrations reflect an equilibrium between oxygen-producing processes (*e.g.* photosynthesis) and oxygen-consuming processes (*e.g.* respiration), and the rates at which DO is added and removed from the system by atmospheric exchange (aeration and degassing) and hydrodynamic processes (*e.g.* accrual/addition from rivers and tides vs. export to ocean). Once dissolved, oxygen can be mixed into the bottom waters where it can support the life of epibenthic organisms. Oxygen diffuses into sediments or is advected in through the actions of benthic infauna (bioirrigation or bioturbation) and tidal pumping. Eutrophication produces excess organic matter that fuels the development of surface water hypoxia (i.e. surface water dissolved oxygen concentration $<$ 2 mg DO L^{-1}) and , in some cases, anoxia ($<$0.5 mg DO L^{-1}) as that organic matter is respired (Diaz 2001).

Consumption of oxygen (respiration) and production of carbon dioxide (CO_2) can occur through a variety of mechanisms (Figure 6.1). High rates of respiration from elevated biomass of live primary producers may reduce DO content of estuarine waters at night (e.g., Peckol and Rivers (1995), while decomposition of accumulated organic matter in both sediments and surface waters may cause a large microbial oxygen demand both day and night (Sfriso, Marcomini et al. 1987). Reduced compounds such ammonium also exerts a biochemical oxygen demand on surface waters (referred to as nitrogenous biological oxygen demand or NBOD) because dissolved oxygen is consumed as bacteria and other microbes oxidize ammonium into nitrite and nitrate (nitrification). When the supply of oxygen from the surface waters is cut off (via stratification), or the consumption of oxygen exceeds the resupply (via decomposition of excessive amounts of organic matter), oxygen concentrations can decline below the

limit for survival and reproduction of benthic (bottom-dwelling) or pelagic (water column dwelling) organisms (Stanley and Nixon 1992; Borsuk, Stow et al. 2001; Diaz 2001).
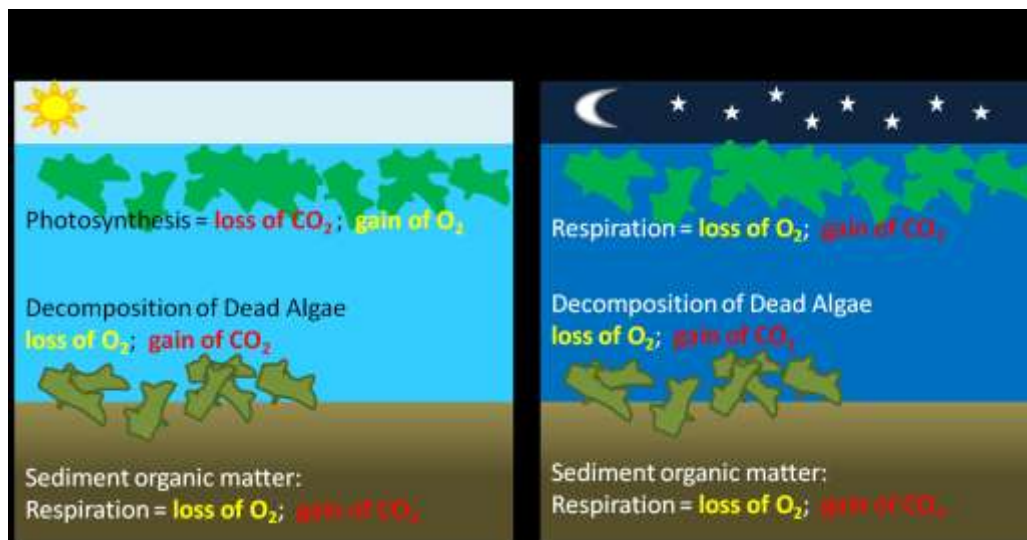
Figure 6.1. Day time primary production and respiration of decaying organic matter can result in large diurnal shifts in bottom water oxygen concentrations in eutrophic systems.

Hypoxia exhibits temporal variability, on diurnal, tidal, lunar, and seasonal timescales. Seasonal hypoxia often develops in association with stratification. Hypoxic water can occur as stratified water prevents the oxygenated surface water from mixing downward or when upwelled hypoxic water is advected into an estuary from offshore (Gilbert, Sundby et al. 2005; Howarth, Chan et al. 2011). Hypoxia appears in the bottom waters when respiration in the water and sediment depletes oxygen faster than it can be replenished. Breakdown of the stratification allows the surface and bottom waters to mix. Stratification can occur in both deepwater habitat of perennially tidal enclosed bays, such as San Francisco Bay, or in lagoonal or river mouth estuaries that are intermittently closed to tidal exchange and that are known to "trap salt" (Largier, Slinger et al. 1991). Diel cycles of hypoxia often appear in stratified or unstratified shallow habitats where nighttime respiration, in combination with water column and sediment dissolved oxygen demand, can deplete DO. Tidal and lunar frequencies can become apparent, particularly in poorly flushed areas where greater exchange occurs on flood or ebb tides or during a spring tide.

The response of aquatic organisms to low DO will depend on the intensity of hypoxia, duration of exposure, and the periodicity and frequency of exposure (Rabalais, Turner et al. 2002; Zaldivar, Cardoso et al. 2008). Organisms have developed several physiological and behavioral adaptations to deal with temporary periods of low oxygen availability. Organisms can: 1) temporarily utilize anaerobic pathways to produce energy (ATP); 2) scavenge oxygen from hypoxic waters and increase the efficiency of oxygen transport to cells; 3) emigrate from hypoxic zones; or 4) reduce demand for oxygen by reducing activity. However, these are all short-term strategies and will not enable the animal to survive long hypoxic periods. Adaptations are well developed in animals such as intertidal and burrowing animals that commonly experience hypoxia but poorly developed in animals that inhabit well-oxygenated

environments such as the upper water column. If oxygen deficiency persists, death will ensue. Sublethal effects also occur. For example, reduced motor activity from mild hypoxia may make the animal more vulnerable to predators or decrease its growth or reproduction. Changes in the survival and reproduction of benthic and pelagic organisms can result in a cascade of effects including loss of habitat and biological diversity, development of foul odors and taste, and altered food webs (NRC 2000). Sutula et al. (2011) provides a complete review of effects of dissolved oxygen on invertebrate and fish species of California estuaries.

6.3.2   Other Effects on Pelagic Habitat Quality

The consequences of organic matter accumulation in surface waters go beyond considerations of hypoxia. Overall, the water quality of pelagic habitat can be expected to decline, for several reasons. First, increased organic matter accumulation (as live or dead biomass) in surface waters reduces water clarity, which can cause self-shading and die-off phytoplankton blooms. Water column light penetration is a dominant factor controlling the growth and distribution of seagrass and benthic microalgae (microphytobenthos). Reduced light coupled with increased delivery of labile organic detritus (senescent algae and seagrass) to the sediments can lead to additional biogeochemical stressors (hypoxia/anoxia, sulfide toxicity, etc.) that further limit the productivity of these benthic primary producers (Short, Jones et al. 1991).

Increased organic matter accumulation, coupled with low dissolved oxygen concentration can cause a proliferation of heterotrophic bacteria, some of which may be pathogenic to aquatic organisms and humans (NRC 2000). In estuaries, the occurrence of microbial pathogens of concern to human health is generally associated with environmental contamination by human sewage or nonpoint source runoff and not with nutrients per se (NRC 1993). The exception is the group of pathogens *Vibrios*, a natural member of the microbial community in brackish estuarine and coastal waters (Colwell 1983). In laboratory studies, the growth rate of *Vibrio cholerae* has been positively correlated with organic enrichment (Singleton et al. 1982). Another species *V. vulnificus* has been identified as a dominant member of the heterotrophic bacterial community of the Chesapeake Bay (Wright et al. 1996). It is possible, therefore, that eutrophication promotes the growth of these pathogens under field conditions. Even if most pathogenic bacteria have anthropogenic sources, high concentrations of labile organic matter can provide substrates for attachment and regrowth. Thus, increased heterotrophic bacteria populations in estuarine surface waters can result in clogging of gills, increased frequency of disease, poor feeding behavior, etc.

6.3.3   Effects on Benthic Habitat Quality

Many of California's shallow estuaries and coastal lagoons that are always connected to the open ocean are not always prone to the formation of chronic hypoxic bottom waters. However, increased organic matter accumulation in sediment can still have direct effects on the habitat quality of macrobenthos that live upon (epifauna) and within sediments (infauna), otherwise known has benthic habitat quality. Gillett et al. (Chapter 7) provide a detailed explanation of effects of eutrophication on benthic habitat

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quality. This information is summarized here to explain the consequences of eutrophication that result in biogeochemical changes in the sediments and the link to benthic habitat quality.

There are relatively consistent and predictable changes in macrobenthic community structure with increasing accumulation of organic matter in marine sediments (Pearson and Rosenberg 1978), see Fig. 7.2 in Chapter 7). Under minimally disturbed conditions, a benthic community should be composed of a trophically and functionally diverse array of species that span different body sizes and lifespans, as well as live at various depths through the sediment, often extending 10's of cm below the sediment-water interface. As organic matter begins to accumulate in the sediment and there will be changes in the community, shifting towards a less diverse community composed of smaller fauna with relatively short lifespans living near the sediment surface. Eventually the sediments are devoid of macrofauna and are covered in mats of sulfur-oxidizing bacteria (i.e., *Beggiatoa*).

Changes in the macrobenthic community structure as a function of increased organic matter accumulation are driven by the complex suite of biogeochemical processes in occurring in the sediments (Pearson and Rosenberg 1978). All estuarine sediments receive oxygen via diffusion from the overlying water column (or from benthic autotrophs in shallow waters), so oxygen and oxidative processes will only penetrate relatively short distances, depending upon the grain size and porosity of the sediment, sediment mixing, as well as other hydrologic factors. The presence of benthic infauna will typically enhance the depth of oxygen penetration due to tube building/ventilating (bioirrigation) and bioturbation.

As sediment organic matter accumulates in the sediments, anoxic and reducing processes such as sulfate reduction and dissimilatory nitrate reduction dominate (see section 6.3.4 below), which leads to a variety of bacterial metabolic pathways that produce byproducts (primarily sulfide and ammonium) that are toxic to most metazoans (Figure 6.2, (Pearson and Rosenberg 1978; Jorgensen 1996; Gray, Wu et al. 2002; Hargrave, Holmer et al. 2008). Many of the species that are community dominants in disturbed habitats are always present at low densities and presumably at a competitive disadvantage to non-disturbed community dominants. Only when the non-disturbed dominants die off, are there available resources that allow tolerant fauna to flourish (e.g., (Gillet, Holland et al. 2007). The deposition and decomposition of this labile carbon may lead to the temporary disappearance of dissolved oxygen from the water column (hypoxia), and consequently the sediments become perched at a higher redox level and sulfide reduction occurs closer to the sediment surface (Figure 6.2). This can result in benthic effluxes of free sulfide and ammonium, which are toxic to estuarine fauna, contributing to a state known as 'dystrophic crises' which includes effects such as fish kills (Pugnetti et al. 1992, Viaroli et al. 1996). These compounds and the reducing environment of the sediments are thought to be the mechanism behind the mortality leading to changes in community structure.
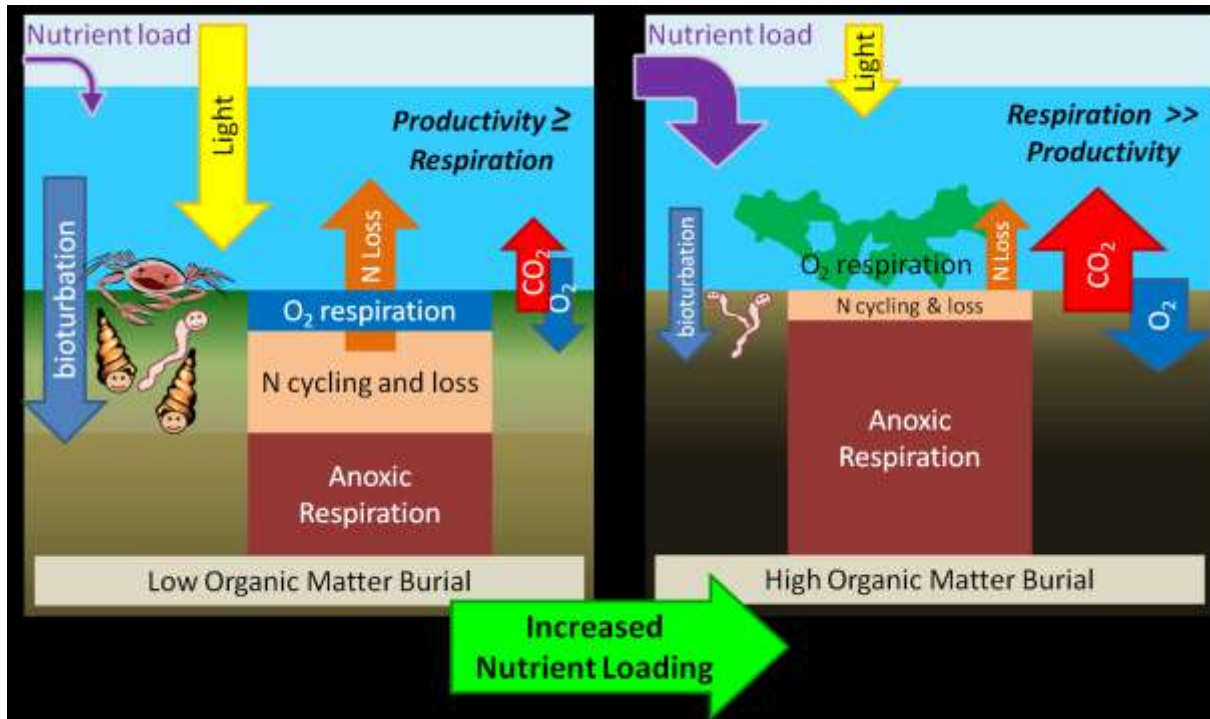
Figure 6.2. Conceptual model of estuarine response to increased nutrient loads in estuarine systems.

6.3.4   Effects on Nutrient Cycling

Primary production in most estuaries is primarily limited by N rather than P. Several factors make N more limiting in estuaries and coastal waters compared to freshwaters: 1) desorption (release) of P bound to fine grained particles as salinity increases, 2) lack of planktonic N fixation in most coastal ecosystems, and 3) flux of relatively P-rich, N-poor waters from coastal oceans into estuaries. However, given sufficient terrestrial N inputs, estuaries and coastal marine ecosystems can be driven to P limitation (Howarth, Chan et al. 2011).

During eutrophication, biogeochemical feedbacks within the sediments can further increase the supply of N and P, conditions that can favor the formation and persistence of harmful or nuisance algal blooms. Algal blooms (macro- or microalgal) in estuaries generally obtain nutrients directly from the water column, though studies have shown that algae may intercept nutrients fluxing out of sediments (Lavery and McComb 1991; McGlathery, Sundback et al. 2007). In California, wet-season particulate-nutrient loads deposited in estuaries can be a significant source of nutrients that can fuel excessive growth of submerged aquatic vegetation and macroalgae during the dry season (Boyle, Kamer et al. 2004; Sutula, Kamer et al. 2004; Sutula, Kamer et al. 2006). Thus, sediment-derived nutrients may cause algal blooms to persist even when nutrient loading from the watershed is reduced to levels calculated to limit biomass (Sutula, Kamer et al. 2004; Neto, Flindt et al. 2008). Sediment derived nutrients (i.e. benthic nutrient fluxes) can change in direction and magnitude according to changes in nutrient cycling within the sediments (Middelburg and Levin 2009).

Nutrient cycling within the sediments is comprised of several key processes. First is the decomposition of organic matter (respiration) which converts organic matter into inorganic carbon and dissolved inorganic nutrients (Figure 6.3). If oxygen is present in the surface sediments, respiration occurs as the reverse process of photosynthesis whereby heterotrophs use oxygen to decompose organic matter into inorganic nitrogen, phosphorus and carbon dioxide. As organic matter accumulates in the sediments, and bacterial production is further stimulated, the demand for oxygen outstrips the rates of diffusion. When dissolved oxygen is depleted, organic matter respiration proceeds in a series of reactions which represent successively lower energy levels (or redox states; Figure 6.3) (Froelich, Klinkhammer et al. 1979; Middelburg and Levin 2009). This redox series typically progresses from denitrification to manganese reduction to iron reduction, to sulfate reduction to finally methanogenesis. Denitrification, as well as iron and manganese reduction, occur in sub-oxic conditions. While sulfate reduction, dissimilatory nitrate reduction and methanogenesis occur largely in anoxic conditions. As these reactions progress, metabolites are released into sediment pore waters and may be advected or diffused into bottom waters. These metabolites include dissolved inorganic nutrients, carbon dioxide and, depending on the reaction, nitrate, manganese, iron, or sulfide.
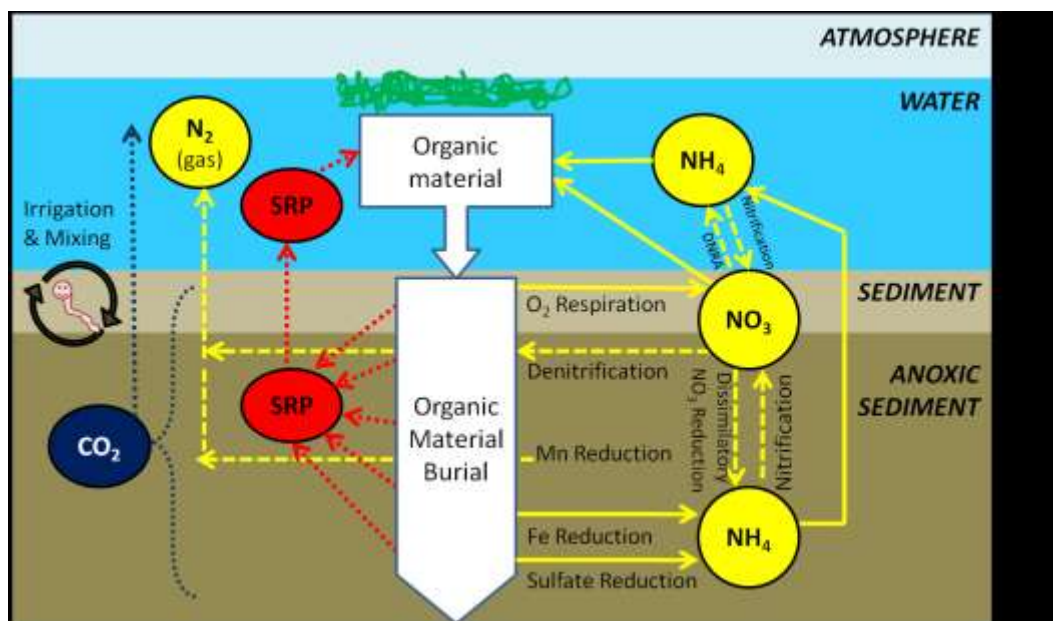
Figure 6.3. Typical zones for oxidation reduction reactions in estuarine sediments.

In minimally disturbed estuarine systems, seagrasses and other rooted plants and/ or benthic microalgae dominate the primary producer community and primary production is roughly equivalent to respiration (net autotrophic) (Figure 6.2) (Eyre and Ferguson 2002). The oxygenated bottom water and low organic matter content of the sediments allows a healthy benthic infaunal community to flourish. The combination of rooted plants and microalgae with bioturbation by benthic infauna oxygenate the upper sediment layers, which may enhance coupled nitrification-denitrification within the sediments

supporting a permanent loss of nitrogen from the system (Caffrey and Kemp 1990; Caffrey and Kemp 1992; Risgaard-Petersen and Jensen 1997). Furthermore, oxygenated sediments may contain high levels of oxidized iron compounds that can trap phosphorus thereby immobilizing this nutrient from use by primary producers (Azzoni, Giordani et al. 2001; DeWit, Stal et al. 2001). Low accumulation of organic matter minimizes remineralization of nutrients in the sediments resulting in low benthic nutrient fluxes, due to the low nitrogen and phosphorus content of the decomposing organic matter and efficient recycling within the plant/sediment system (Eyre and Ferguson 2002; Viaroli, Bartoli et al. 2008).

As the system progresses along the eutrophication gradient, low oxygen and toxicity will shift the benthic infauna community to smaller, more stress tolerant species that are not as efficient at bioturbation, limiting oxygen penetration into the sediments and effectively minimizing the zone of coupled nitrification/ denitrification in the sediments (Figure 6.2). Coupled nitrification/denitrification is a key pathway in the N budgets of coastal ecosystems affected by chemistry changes related to eutrophication. Because these processes permanently removes N from the ecosystem to the atmosphere it may help alleviate nutrient over-enrichment; however, nitrification is an aerobic process and therefore this process is shut off under hypoxic/anoxic conditions and thus, denitrification can also be inhibited (Middelburg, Soetaert et al. 1996). In hypoxic systems where bottom-water nitrate concentrations are very low, nitrate influxes cannot compensate for decreases in aerobic nitrification and denitrification may become very low (Kemp, Sampou et al. 1990; Kemp, Boynton et al. 2005). Moreover, the denitrification process is in competition with another dissimilatory reduction pathway, i.e. dissimilative reduction of nitrate into ammonium (DRNA). DRNA conserves N for the ecosystem and this process is stimulated with respect to denitrification when organic matter loadings are high (An and Gardner 2002). Therefore a highly eutrophic environment is often less favorable for denitrification. Furthermore, macroalgae can reduce denitrification by physically separating the denitrifiers from water-column nitrate, by efficient uptake of water-column nitrate, and by suppression of nitrification through anoxia below the algal mats (Krause-Jensen, Christensen et al. 1999; McGlathery, Sundback et al. 2007; Viaroli, Bartoli et al. 2008). Deposition of large quantities of organic matter may also result in sediment anoxia through microbial respiration. Reduced denitrification and increased organic matter decomposition enhances benthic ammonium fluxes from the sediment. Furthermore, sediment anoxia reduces iron compounds which decreases their affinity for phosphate; this, combined with the decomposition of organic matter in the sediments can result in large P fluxes from the sediments (Sfriso, Marcomini et al. 1987; Lavery, Lukatelich et al. 1991; Azzoni, Giordani et al. 2001; Eyre and Ferguson 2002). The remineralization of N and P in the sediments, increases benthic N and P fluxes which in turn stimulate further primary production in the estuary (Sfriso, Marcomini et al. 1987; Viaroli, Bartoli et al. 2008; Zaldivar, Cardoso et al. 2008).

6.3.5   Effects on Net Ecosystem Metabolism, Benthic Metabolism, and Carbon Sequestration

Metabolism is defined by the relative balance of production (photosynthesis) and respiration that can occur through a variety of biogeochemical processes. The balance of production versus respiration (P:R) is used to define the "trophic state" of the estuary, from oligotrophic (production> respiration) to eutrophic or hypereutrophic (production << respiration).

Whole ecosystem metabolism (or net ecosystem metabolism, NEM) has been used as a measure of the balance between primary production and respiration in estuarine systems (Caffrey 2003; Caffrey 2004; Russell and Montagna 2007). When NEM is positive the system is considered autotrophic and primary production exceeds respiration. When NEM is negative the system is considered heterotrophic and respiration exceeds production. Net ecosystem metabolism is problematic as in indicator of eutrophication because Interpretation of the data is not straightforward (Caffrey 2003). Recent studies have shown that most estuarine ecosystems tend to be net heterotrophic, especially systems that receive significant inputs of organic carbon from their respective watersheds (Duarte and Prairie 2005). Consequently NEM reveals more about the carbon budgets of these systems rather than the extent of eutrophication (Caffrey 2003; Caffrey 2004; Duarte and Prairie 2005). Furthermore, NEM can vary widely both spatially and temporally within a system (Caffrey 2004). It has been shown to be strongly dependent on physical and hydrodynamic factors such as temperature, salinity, habitat adjacent to the monitoring site, estuarine area, and water depth, in addition to nutrient loading (Caffrey 2004). Thus, NEM has been most effective at addressing questions such as how an individual systems vary spatially (i.e. which locations tend towards greater heterotrophy compared to other locations within an estuary) and how these systems are changing over time (Caffrey 2003), as well as to address questions of the relative importance of carbon loading from the watershed versus internal production (Duarte and Prairie 2005).

The balance between productivity and respiration within the sediments (benthic metabolism) can be used to determine the relative trophic status of estuarine ecosystems. The metabolism of benthic communities is a central component of the nutrient cycling and overall productivity of shallow estuarine ecosystems such as those prevalent in California (Eyre and Ferguson 2005). Benthic metabolism, while similar in concept to NEM, may be a more robust measure of estuarine eutrophication because it is a more direct measure and there are fewer confounding factors. As organic matter accumulation within estuaries increases, the benthic respiration rates, as measured by benthic carbon dioxide fluxes, increase and the ratio of P:R decreases. Thus, characterization of the benthic metabolism, either as benthic CO_2 fluxes or P:R ratios provides an index of eutrophication.

As estuaries becomes increasingly eutrophic and aquatic primary producer expression shifts to dominance by phytoplankton and/or macroalgae, the large amount of labile organic matter switches the system from being net autotrophic to one where respiration largely dominates primary production (net heterotrophic) and sediments tend to accumulate organic matter (Figure 6.3) (Eyre and Ferguson 2002; Viaroli, Bartoli et al. 2008; Zaldivar, Cardoso et al. 2008). Consequently, bottom waters often show large diurnal changes in oxygen concentrations associated with these high respiration rates and large sediment oxygen demand (Figure 6.4, (Sand-Jensen and Borum 1991; Viaroli, Bartoli et al. 1996; Viaroli, Bartoli et al. 2008). These rapid changes in benthic metabolism are enhanced by the composition of the primary producers.

Decomposition processes are regulated not only by organic matter quantity but depend also on its quality, which may control critical steps in the seasonal changes in oxygen availability. In turn, organic matter quality and its recalcitrance (resistance to decay) depends on types, growth rates, life cycles and elemental and macromolecular composition of the primary producer community (Enriquez, Duarte et al.

1993; Viaroli, Bartoli et al. 2008). Fast-growing plants such as macroalgae and phytoplankton decompose more quickly due to their high nutrient content and low N:C and P:C ratios (Enriquez, Duarte et al. 1993). Comparative studies demonstrate that under summer conditions the macroalgae *Ulva* sp. decomposes at an almost constant rate of 3% per day, which is three times greater than that of seagrasses like *Zostera marina* (Buchsbaum, Valiela et al. 1991). Similar differences have been found when comparing the degradation of the macroalga *Monostroma obscurum* and the seagrass *Zostera noltii* (Bourgues, Auby et al. 1996). Decomposition pathways and rates not only influence the extent of the oxygen deficit and sulfide release, but also strongly modify N and P pathways and fate (AmtoftNeubauer, UnderlienPedersen et al. 2004; Lomstein, BonneGuldberg et al. 2006). Furthermore, there is convincing evidence that recalcitrant compounds require oxygen to be degraded (Kristensen, Ahmed et al. 1995). More refractory, older material may degrade faster when exposed to oxygen, in particular at low overall mineralization rates (Hulthe, Hulth et al. 1998; Dauwe, Middelburg et al. 2001).

Bottom sediments can also be a net sink for carbon (i.e. sequester carbon) and nutrients through the burial of C, N, and P and the loss of N through denitrification. Conversely sediments can be a net source of C through CO_2 efflux. Organic matter produced in the water column or delivered from the watershed provides energy and nutrients for heterotrophic consumers inhabiting marine sediments (Middelburg and Levin 2009). The majority of the organic matter deposited is processed by a diverse community of microbes, protozoans and metazoans and only a small part is eventually buried (Hedges and Keil 1995; Middelburg and Meysman 2007). The net balance of sediment productivity and respiration (P:R ratio) appears to be an important control on the overall role of sediments as a source or sink of C and N (Eyre and Ferguson 2002; Eyre and Ferguson 2002; Eyre and Ferguson 2005).

6.3.6   Effects on Increasing Water Column Acidity

Increased anthropogenic CO_2 emissions have caused measureable declines in pH throughout the world's oceans, as the average concentration of CO_2 has increased (Doney, Fabry et al. 2009; Feely, Doney et al. 2009). Continued ocean acidification will affect the solubility of carbonate minerals, which comprise the shells and exoskeletons of benthic invertebrates, making these minerals less likely to precipitate and more likely to dissolve (Feely, Doney et al. 2009; Howarth, Chan et al. 2011). For estuaries and coastal marine ecosystems, eutrophication and its accompanying hypoxia, can exacerbate this acidification (Howarth, Chan et al. 2011). Hypoxic and anoxic waters are more acidic than open ocean waters, because respiration is coupled to the production of dissolved inorganic carbon including carbon dioxide, which is an acid (Borges and Gypens 2010). For a model saline estuary, the release of CO_2 associated with the development of hypoxia is sufficient to reduce pH levels by more than 0.5 units and to decrease aragonite solubility to levels where dissolution would be favored (Howarth, Chan et al. 2011). However, the relationship between ocean acidification and eutrophication is poorly understood, though the effects of pH declines are probably already occurring and are likely pervasive (Feely, Doney et al. 2009; Howarth, Chan et al. 2011). Whether shell-forming carbonate minerals dissolve or precipitate depends on a number of chemical conditions, including the concentrations of dissolved inorganic carbon and CO_2 as well as the alkalinity of the water and the calcium ion concentration (Salisbury, Green et al. 2008). These factors can vary widely along estuarine salinity gradients, but the strong tendency for alkalinity and calcium ion availability to decline toward freshwater end-members makes dissolution of carbonate

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minerals more likely (Howarth, Chan et al. 2011). This tendency is furthered by the very high levels of CO_2 in rivers entering estuaries. As a whole, the potential of acidification driven by eutrophication suggests that nutrient-enriched estuaries will be among the ecosystems most vulnerable to ecological and biogeochemical perturbations from ocean acidification (Howarth, Chan et al. 2011).
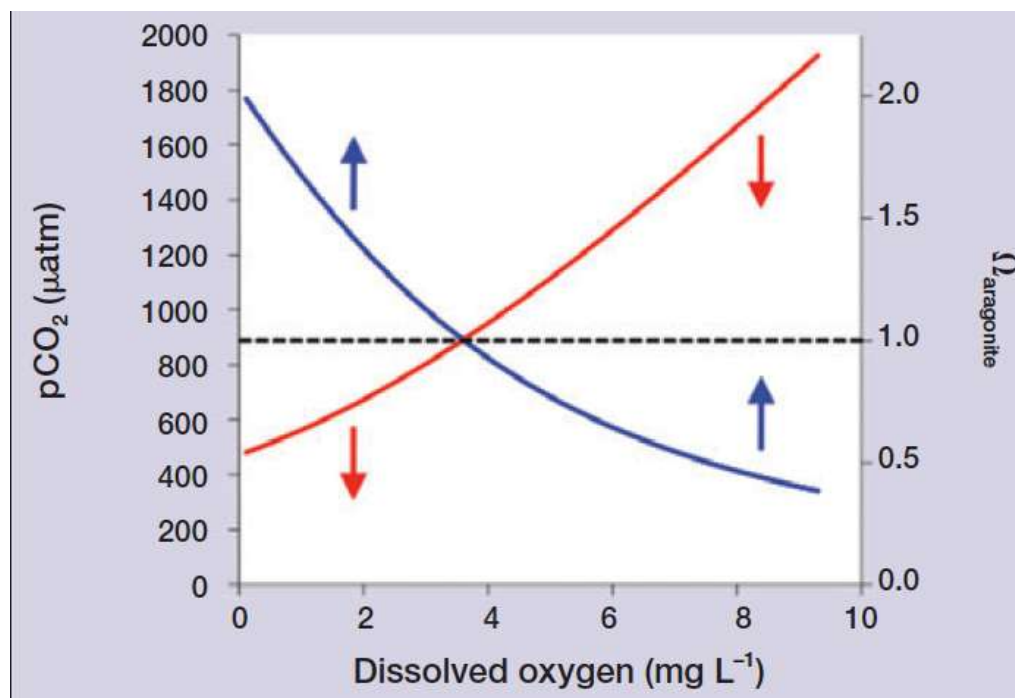
Figure 6.4. Illustration of the increases in partial pressure of CO_2 (pCO₂; blue line) and declines in the saturation state of aragonite($\Omega_{aragonite}$ red line) that stoichiometrically accompanies the onset of hypoxia. Above the horizontal dashed line, aragonite shell formation is favored, whereas dissolution is favored at levels below the line. Continued increases in CO_2 emissions from human activities will cause the system to reach dissolution conditions at increasingly higher threshold oxygen levels (arrows)(From: Howarth et al. 2011).

6.4 Estuarine Susceptibility to Altered Sediment and Water Column Biogeochemical Cycling

The physical characteristics of estuarine systems have a key role in its susceptibility to the effects of nutrient and organic matter loading that lead to eutrophication. In particular, morphology, hydrology and hydrodynamics, and regional climate conditions will affect the magnitude and expression of altered water column and sediment biogeochemistry (Zaldivar, Cardoso et al. 2008). Estuary morphology (surface area, volume, depth, etc.) controls internal hydrodynamics and exchange at the ocean inlet, which in turn affects water retention and flushing (Zaldivar, Cardoso et al. 2008). Therefore, systems with a higher flushing rate should experience less organic matter accumulation and consequently, sediment biogeochemical alterations would be less than in systems not as well flushed.

Estuarine susceptibility to eutrophication is also driven by hydrological factors that operate at several temporal scales (Paerl 2006; Arhonditsis, Stow et al. 2007; Zaldivar, Cardoso et al. 2008). Mixing and transport processes in estuaries are driven by tide, river flow and wind (Zaldivar, Cardoso et al. 2008). Overall, freshwater inputs contribute to eutrophication, while exchanges with adjacent coastal waters can prevent eutrophication through tidal flushing and dilution. The combination of freshwater and marine water inputs affects water column stratification, which in turn enhances the susceptibility to eutrophication, favoring APP growth in the photic zone and anoxia in the bottom waters (Largier, Hearn et al. 1996; Howarth, Swaney et al. 2000; Zaldivar, Cardoso et al. 2008). The relative contribution of hydrological factors to hydrodynamics depends on topographical characteristics of estuarine ecosystems. In macrotidal systems, tides control surface water retention time. In microtidal systems, tides also play an important role, mainly affecting the dynamics of salinity, suspended particulate matter and nutrients (Zaldivar, Cardoso et al. 2008). Such factors control the structure and composition of sediments and thus the biogeochemical cycles therein.

In terms of climatic context, solar radiation and temperature are also related to eutrophication susceptibility (Zaldivar, Cardoso et al. 2008). Therefore, the geographic location of estuarine ecosystems becomes a driving factor for eutrophication processes. California has a north-south gradient in temperature, solar irradiance, and precipitation, and therefore these factors will play a role in how the systems respond to nutrient and organic matter enrichment. Solar radiation and temperature are usually greater at lower latitudes, and influence the rate of nutrient cycling and the length of the growing season. Temperature affects biological process rates, and is a driver for evaporation. Evaporation intensity can be significant at lower latitudes, causing increases in salinity and nutrient concentrations especially in water basins with high residence times. Precipitation should also be considered as a factor increasing the susceptibility to eutrophication though several mechanisms, runoff from the land leading in turn to decreased salinity and increased N and P (the latter mostly from sediment transport) loadings from the watershed. In case of N limited systems the atmospheric deposition of N could also cause shifts in nutrient limitation (Zaldivar, Cardoso et al. 2008).

Sediment grain size, bulk density, water content and porosity are important determinants of diffusive properties and permeability and, therefore, of exchanges of oxygen and nutrients with the water column and the susceptibility of the system to changes in sediment biogeochemical cycling related to eutrophication. As mentioned above, these physical characteristics are largely driven by the system's

morphology, hydrodynamics and climatic context. Sediment structure and composition control the capacity to retain inorganic nutrients and toxic compounds, e.g. sulfide and ammonium. In terms of sediment granularity, two major sediment types can be distinguished, cohesive and non-cohesive. Muddy and silty sediments are cohesive and sandy sediments are non-cohesive and permeable. Under natural conditions, there is virtually no lateral movement or percolation in cohesive sediments. On intertidal flats when cohesive sediments emerge during ebb tide, they typically remain fully saturated with water and do not dry out. In contrast, in non-cohesive, sandy sediments the water is attracted much more loosely. Under quiet hydrodynamic conditions, this force is sufficient to keep the interstitial water in place, while under many natural conditions the hydrodynamic forcing induces water movement through the sediment comprising percolation, lateral flow and upward welling. Intertidal non-cohesive sediments lose a large part of their interstitial water during ebb tides (Zaldivar, Cardoso et al. 2008). In cohesive sediments, the transport of solutes is determined by molecular diffusion, which is an efficient process at the micrometer scale, but very inefficient for longer distances. Thus, fluxes through these sediments tend to be less than in larger grain sized sediments and these sediments tend to have typical redox profiles (Figure 6.3). The mineralization of organic matter in the sediment creates a high oxygen demand and because oxygen delivery is limited by molecular diffusion, cohesive sediments can become anoxic at very shallow depth. In contrast, sandy, non-cohesive are permeable and the interstitial water movements increase the transport rates of oxygen and other solutes including dissolved organic matter by orders of magnitude. This results in increased microbial process rates and such sediments are therefore very efficient bioreactors for the degradation of organic matter, by mixing reactants and diluting toxic end-products. As a result, these sediments show much more heterogeneity and it is often difficult to recognize the classical redox sequence. In addition, the exchange between water column and sediment is also strongly influenced by benthic fauna that contributes to bioturbation and bio-irrigation of the sediment (Zaldivar, Cardoso et al. 2008).

The nature of the sediment has a strong impact on the microbial process rates in conjunction with the hydrodynamic conditions. The sulfide produced in the anoxic sediment zones by sulfate reducing bacteria can partly be oxidized by aerobic processes and the intensity of these processes are determined by the physical characteristics and hydrodynamics of the system (Azzoni, Giordani et al. 2001; Zaldivar, Cardoso et al. 2008). In coastal areas, the biogeochemical interactions between iron and sulfide play a major role in regulating sulfur speciation and retention of phosphate in the Fe-bound pool (Howarth and Stewart 1992; Fenchel, King et al. 1998). Via a suite of redox reactions, sedimentary reactive iron immobilizes sulfides and phosphates as insoluble compounds. Therefore, the extent of toxic sulfide release and retention of phosphate in the sediments is regulated by the iron cycle (DeWit, Stal et al. 2001; Rickard and Morse 2005). Therefore factors influencing iron availability (e.g. oxygen and carbonate content), have a great influence on iron-based buffers within the sediment (Chambers, Fourqurean et al. 2001; Rozan, Tallillefert et al. 2002).

6.5 Review of Water and Sediment Chemistry Candidate Indicators

The purpose of this section is to review the utility of water and sediment chemistry indicators for inclusion in the NNE framework for estuaries. Indicators reviewed can be grouped into five main areas: 1) nutrients, 2) water clarity, 3) dissolved oxygen concentration and oxygen demand, 4) benthic metabolism, 5) organic matter accumulation, and 6) nitrogen cycling. Table 6.4 provides a list of the individual indicators evaluated under each of the six groups. Each indicator was reviewed, utilizing the evaluation criteria presented in Chapter 2.

Table 6.4. List of water and sediment chemistry indicators evaluated in this section.

Indicator Group	Indicator or Metric
Nutrients	Ammonia
	Urea
	N:P Ratio
Water Clarity	Secchi Depth
	Kd (Light extinction)
	Turbidity
Dissolved Oxygen	Dissolved Oxygen Conc.
	Biological or Chemical Oxygen Demand
	Sediment oxygen demand
Benthic Metabolism	Benthic production: respiration ratio
	Benthic TCO2 flux
Organic Matter Accumulation and Sediment Redox Status	Sediment %OC, %N, and %P
	Sediment C:N: P ratio
	Sediment TOC:TS and degree of pyritization
Nitrogen Cycling	Denitrification Efficiency

6.5.1 Utility of Ammonium, Urea and Nutrient Stoichiometry for NNE Framework in Estuaries

Within the realm of indicators of nutrient availability, three indicators were evaluated for application in the estuarine NNE framework: 1) ammonium, 2) urea and 3) nutrient stoichiometry. Note that toxicity of nitrate and ammonium on fish are not considered here, as objectives already exist and thus are beyond the scope of this document.

Several studies provide a broad base of evidence that phytoplankton and seagrasses have a direct linkage to important estuarine beneficial uses, including food web support for marine and estuarine aquatic organisms (EST, MAR) including the commercial and sport fisheries (COMM), shellfish such as clams, oysters and mussels (SHELL and AQUA), migratory (MIGR) birds and fish, support for fish nursery habitat (SPAWN). Increased incidence of harmful algal blooms can adversely affect the health of humans (REC-1) by irritation and injury to recreational swimmers, sailboarders, and boaters (Lehman et al., 2005). Thus adverse effects to phytoplankton and seagrass primary production and the occurrence of dominant assemblages known to support estuarine ecosystem services (e.g. fisheries) would be considered to adversely effecting beneficial uses. Ammonia, nitrate, urea and N:P:Si ratios in ambient surface water are all documented to have effects phytoplankton and seagrass productivity and taxonomic composition. In this section, the strength of the linkage to beneficial uses is reviewed for each.

Ammonium- Ammonium inhibition of nitrate uptake by diatoms has been documented in several regions of San Francisco Bay and ammonium concentrations above 4 μM have been suggested as a major mechanism by which spring diatom blooms appear to be suppressed in the North Bay and lower Sacramento River (Wilkerson, Dugdale et al. 2006; Dugdale, Wilkerson et al. 2007). Despite this evidence, the ecological importance of ammonium inhibition of spring diatoms blooms is not well understood relative to factors known to control primary productivity, particularly in other regions of the Bay where water column chlorophyll-a appears to be increasing.

It has been suggested that HABs in San Francisco Bay may be supported by regenerated ammonium in the summer and autumn (North Bay: (Dugdale, Wilkerson et al. 2007); South Bay: (Thompson, Koseff et al. 2008)) and that HAB species that utilize ammonium as a nitrogen source can produce greater toxicity (see review by (Gilbert, Harrison et al. 2006) and in (Kudela, Lane et al. 2008)). Likewise, some HAB organisms that can preferentially uptake urea may have a competitive advantage (Gilbert, Harrison et al. 2006). However, as with ammonium inhibition of diatom nitrate assimilation, the influence of ammonium on HABs in SF Bay has not been sufficiently investigated.

Ammonium toxicity has been documented for several estuarine seagrasses as well at concentrations as low as 25 μM (vanKatwijk, Vergeer et al. 1997; Brun, Hernandez et al. 2002; Touchette and Burkholder 2007; Cabaco, Machas et al. 2008); however, at what concentration and exposure frequency and duration these impacts will be felt is poorly understood. Furthermore, toxicity has been shown to be affected by other factors such as phosphorus loading, light availability and temperature in microcosms (vanKatwijk, Vergeer et al. 1997; Brun, Hernandez et al. 2002; Touchette and Burkholder 2007; Cabaco, Machas et al. 2008); but how these effects will interact within estuaries is unknown. Furthermore, how ammonium toxicity compares to other factors known to affect seagrass communities (e.g. shading by epiphyte and macroalgae communities and competition) are also not well understood.

In light of these studies, the linkage between ammonium concentrations beneficial uses is not at this time universally accepted. Additional review is required to better understand the role of ammonium in California estuaries.

Urea- Kudela et al. (2008) noted that some HAB species can show a preference for urea versus other inorganic nitrogen constituents (e.g. *A. sanguinea*) or similar affinities for urea versus other nitrogen substrates (*L. polyedrum*), which under conditions of high urea concentrations could favor these HAB species. However, the importance of urea in promoting HABs in SF Bay is not understood, in part because measurement of urea is not part of a routine research programs conducted in the Bay and the focus of HAB research has been largely restricted to cyanobacteria. Thus the linkage between urea and adverse effects on the phytoplankton community and Bay beneficial uses are not well documented.

N:P:Si Ratio-The ratio of nitrogen to phosphorus to silica has been found to be an important determinant of primary producer taxonomic composition. In freshwater and brackish water areas, a lowering of the N:P ratio can cause a shift phytoplankton taxonomic composition towards cyanobacteria, with thresholds of lower than 22:1 TN:TP showing a dominance by cyanobacteria in freshwater lakes (Smith et al. 1995, Smith 1983). In California, cyanobacteria dominance in brackish water coastal lakes, which in this document are considered estuaries, has been observed (Tracy Magrunn, personal communication November 2010). However, it is not clear that established thresholds for freshwater lakes can be translated to these brackish water habitats. In tidally well-flushed estuaries, however, thresholds of N:P ratios in surface waters are not so easily utilized. First, ambient water column nutrient concentrations can be highly variable due to tidal hydrology. In addition, it has been suggested that some cyanobacteria can proliferate in low P environments (high N:P ratio) when other algal classes either P limited or have a lack of tolerance for high nitrogen environments (Bertilsson et al., 2003; Van Mooey et al., 2009; Glibert 2010). Thus, for tidally well-flushed estuaries, the use of N:P:Si ratios is likely to be estuary specific and dependent on the typical phytoplankton taxonomic composition in "minimally disturbed" conditions. The linkage between nutrient stoichiometry and estuarine beneficial uses may be stronger in brackish, lentic environments, but this has not yet been shown for California's estuaries.

Shifting N:P ratios can result in shifts from seagrass communities to macroalgae dominance (Touchette and Burkholder 2000; Brun, Hernandez et al. 2002; Touchette and Burkholder 2007). An increase in the N:P ratio can result in a decline in seagrass populations as the plants cannot process excess nitrogen without comparable phosphorus additions resulting in physiological stress and eventual senescence (Brun, Hernandez et al. 2002). Conversely macroalgae can increase their N-uptake rates and the N content of their tissues (Valiela, McClelland et al. 1997), thus shifting primary producer dominance away from seagrasses in favor of macroalgae. However, thresholds for where these shifts occur are likely to be estuary specific and will depend upon a variety of other physical and biogeochemical factors, none of which are well characterized.

Predictive Relationships to Causal Factors

Conceptual models have shown a complex relationship between nutrients and primary productivity in estuaries. There are multiple factors that can limit primary productivity including light limitation, strong tidal/wind forcing that prevents long periods of stratification. An added challenge in developing linkages between nutrient loads, standing concentrations of nutrients and nutrient stoichiometry, and productivity response is that these are often out of phase in our dry summer Mediterranean climate (Kudela, Lane et al. 2008). There is growing evidence that DON, and in particular urea, is able to support greater population of HAB species. However at this time a causative linkage has not been established (Gilbert, Harrison et al. 2006); evidence suggests that HABs can utilize multiple sources of nitrogen; however this is not a unique characteristic for toxin producing species and should not imply that DON specifically selects for HABs (Kudela, Lane et al. 2008). With respect to ammonium and urea, dynamic simulation models have not yet been developed to understand the effects of these indicators vis-à-vis other controlling factors on phytoplankton and seagrass communities. Given these data gaps, the logical next step is to the develop models that synthesize understanding of the relative importance of ammonium, urea and nutrient stoichiometry versus other factors in controlling primary producer assemblages. For California, this is most appropriately done in an estuary such as San Francisco Bay, where long term water quality data sets exist and may provide the best understanding of how these indicators could be utilized.

Sound and Practical Measurement

The laboratory methods for analysis of total and dissolved nutrient forms exist and are low cost. Standard methods are available from EPA, USGS and the SWRCB Surface Water Ambient Monitoring Program (SWAMP) has a list of approved methods for the State.

Acceptable Signal to Noise Ratio

Nutrient concentrations vary over multiple temporal and spatial scales and tend to be less integrative of overall nutrient loads, thus having a tendency in general towards lower signal to noise ratios. Temporal scales of variability include daily (flux of nutrients with daily photosynthetic activity and tidal exchange), seasonal (influx in winter freshwater flows and flux from phytoplankton blooms/decay), and interannual (long-term variability due to changes in watershed management/use). Spatial variability includes vertical (vertical flux in the water column) and horizontal (freshwater/wastewater effluent inflow, tidal exchange) scales. The determination of whether ammonium, urea or N:P:Si ratios have acceptable signal:noise ratios should take into consideration: 1) to what extent ambient concentrations are anthropogenically influenced or have a natural source and 2) the degree of spatial and temporal variability that controls whether change can be detected.

Ammonium- Ammonium concentrations are typically low in natural waters but can be elevated when there is a distinct source such as wastewater treatment effluent, agricultural runoff, or benthic nutrient fluxes when sediments are suboxic, favoring production of ammonium via dissimilatory nitrate reduction (An and Gardner 2002). Thus, while variable, ambient ammonium concentrations are likely to be useful in determining nutrient overenrichment. Ultimately, the question of whether ammonium satisfies this

evaluation criterion is to determine how variable are the concentration-based effects of ammonium limitation of nitrate assimilation in diatoms or seagrass toxicity, and whether a concentration-based threshold effect would be highly influenced by other factors.   Currently, insufficient information is available on this topic, because the ecosystem-level effect of ammonium inhibition is not well understood. Development of models to conceptualized and synthesize the importance of ammonium inhibition of nitrate assimilation in phytoplankton and seagrass toxicity vis-à-vis other factors in utilizing ammonium as an NNE indicator.

*Urea-*Urea concentrations are typically low in minimally-disturbed, natural waters, with higher concentrations being associated with urea-based fertilizers and some wastewater treatment processes (Kudela, Lane et al. 2008). However, the influence of urea on the phytoplankton taxonomic composition is not very well understood and, as with ammonium, limited understanding exists on concentration-based effects of urea, and whether a concentration-based threshold effect would be highly influenced by other factors.

*N:P:Si Ratio-*N:P:Si ratio is influenced by anthropogenic loading to the estuary as well as the *in situ* sources and sinks for nutrients, such as primary producer uptake and release, benthic nutrient exchange, nitrification and denitrification, particulate deposition, etc. Therefore, use of the ambient water column N:P:Si ratio is not a precise indicator of eutrophication, and is much more likely to be of use as a supporting indicator of assessments based on primary producer community response.

Summary: Use of Ammonium, Urea, and N:P:Si as an NNE Indicator

Elevated ammonium concentrations have been suggested as a major mechanism by which spring diatom blooms appear to be suppressed in the North San Francisco Bay and lower Sacramento River (Wilkerson, Dugdale et al. 2006; Dugdale, Wilkerson et al. 2007). Furthermore, excess ammonium can result in toxicity for seagrass communities (vanKatwijk, Vergeer et al. 1997; Brun, Hernandez et al. 2002). Despite this evidence, the ecological importance of ammonium inhibition of spring diatom blooms and seagrass loss is not well understood relative to factors known to control primary productivity. Thus the linkage between ammonium concentrations and beneficial uses is not at this time universally accepted. Studies are ongoing and additional review of this topic is required to determine whether ammonium thresholds should be developed.

While there is growing evidence that urea has a role in support of an increased frequency of certain HAB species, a causative linkage has not been established (Gilbert, Harrison et al. 2006) and it is not possible to state that urea selects for HAB species (Kudela, Lane et al. 2008). Moreover little data are available on urea concentrations in California estuaries. Therefore, recommended next steps are to begin collecting urea data as a part of routine monitoring and to undertake a broader review of the effects of urea on phytoplankton community composition, with the intent of identifying critical data gaps for its use in the NNE.

Finally, water column N:P:Si ratios appears to be a useful indicator of shifts toward cyanobacteria in brackish water lentic environments (e.g. SF Bay Delta or ephemerally tidal coastal lagoons) and a shift from seagrasses to macroalgae in shallow estuaries. However, as with ammonium and urea, little data

are available specifically for California estuaries and additional experience is needed to understand the relationships between biomass, taxonomic composition and nutrient stoichiometry over the range of salinity gradients found in California estuaries.

For the above reasons, we recommend that ammonium, urea and nutrient stoichiometry be used as supporting indicators. With more available data and improved understanding of their utility to diagnose eutrophication, over time one of more of these indicators may prove to be useful for diagnosing eutrophication as a primary indicator.

6.5.2   Utility of Indicators of Water Clarity for NNE Framework in Estuaries

Several indicators for water clarity (underwater irradiance, Secchi depth, turbidity, TSS) have been reviewed by Kaldy and Sutula in Chapter 5, so this section will only briefly describe those indicators and summarize the conclusions. Water clarity can be expressed as the available light for aquatic plants and algae. Without adequate light, regardless of the cause, algae and aquatic macrophytes will not survive. Underwater irradiance is indirectly linked to nutrient loading, since the water column phytoplankton and algae stimulated by nutrients rapidly reduce light availability to aquatic plants.

Underwater Irradiance

Of methods available to measure the available light to aquatic primary producers, the most sophisticated is underwater irradiance, measured as irradiance or photon flux density (moles photons m^{-2} s^{-1}). Underwater irradiance measures the amount of light available at wavelengths required for photosynthesis (photosynthetically available radiation or PAR). Underwater irradiance is used calculate light attenuation coefficients (KD) using the Beer-Lambert equation, which is a measure of how much light is removed from the water column per m of depth. In seagrass habitats, underwater irradiance or photon flux density (PFD) is commonly measured using spherical (4 π) quantum sensors attached to data loggers, which integrate light from all angles and thus are a reasonable analog for aquatic plants. Cosine corrected sensors (2π or flat) can be used to measure diffuse light attenuation coefficients (k_d) tend to be used to determine light attenuation for phytoplankton rather than seagrass, since they only accurately measure downwelling or upwelling (based on orientation) irradiance. The standard instrumentation for measurement of underwater PAR is the LI-COR spherical quantum sensors attached to dataloggers contained in watertight housings (Dunton 1994; Onuf 2006; Kaldy and Lee 2007).

Secchi Depth

Historically, secchi depth (depth at which a black and white colored disk disappears from view) has been used to evaluate light penetration; however, this metric although simple and inexpensive does not provide a quantum estimate of light availability. Relationships between secchi depth and percent surface irradiance have been developed (see (Batuik, Bergstrom et al. 2000) Chapter 3); however, these are still very site specific because of variations in dissolved constituents that absorb light and the instantaneous nature of the measurement mostly used to mine existing historical data; although most current monitoring programs either call for or have updated their methods to measure PFD (Batuik, Bergstrom et al. 2000). Some citizen based groups do collect secchi depth but the utility of this data is

limited. HOBO[©] light loggers have also been used in the literature; however, these measure in units of lumens ft[-2] which is not directly related to quanta, they measure outside the range of PAR and as a result dramatically overestimate PAR levels.

Turbidity and TSS

Turbidity is an optical measure of light attenuation (nephelometric turbidity units, NTU) which can be used as a proxy for the total suspended solids (TSS) in the water column. Using long term data, site specific empirical relationships can be derived for NTU and TSS; however, these relationships are often rather weak and noisy. TSS can be measured directly from grab samples as mg l^{-1}; although TSS effects light penetration, it may or may not be related to nutrient loading. Both chl *a* and turbidity effect light transmission through the water column, higher concentrations lead to less light available at depth. Several states have adopted the TSS concentration of 15 μg l^{-1} as protective of seagrass resources (Batuik, Bergstrom et al. 2000; Brown, Nelson et al. 2007; Wazniak, Hall et al. 2007). In Chesapeake Bay, TSS concentration standards (<15 mg l^{-1}) are the same for both eelgrass and brackish SAV (Batuik, Bergstrom et al. 2000).

Summary: Use of Indicators of Water Clarity for the Estuarine NNE Framework

Light penetration integrates measures of turbidity and chlorophyll-a. Light penetration can be more easily linked with dose –response (i.e. low light = adverse effects), but within a diagnostic framework for eutrophication, could produce a false-positive if the reduced light is from high suspended sediment loads. Therefore, measures of water clarity are appropriate as supporting indicators for the NNE, particularly in seagrass habitats, but should not be used solely to diagnose eutrophication.

6.5.3 Utility of Dissolved Oxygen Concentration, Water Column or Sediment Oxygen Demand for the NNE Framework in Estuaries

Measures of dissolved oxygen (DO) refer to the amount of oxygen contained in water, and define the living conditions for oxygen-requiring (aerobic) aquatic organisms. DO concentrations reflect an equilibrium between oxygen-producing processes (*e.g.* photosynthesis) and oxygen-consuming processes (*e.g.* respiration), and the rates at which DO is added and removed from the system by atmospheric exchange (aeration and degassing) and hydrodynamic processes (*e.g.* accrual/addition from rivers and tides vs. export to ocean). Once dissolved, oxygen can be mixed into the bottom waters where it can support the life of benthic organisms. Dissolved oxygen can be expressed as a concentration (mg L^{-1}) or as percent saturation, relative to the concentration in air.

Biochemical oxygen demand (BOD) and sediment oxygen demand (SOD) are similar measures of rate of oxygen consumption, with BOD conducted on water samples, and SOD conducted on sediments. BOD is a chemical procedure for determining the rate of consumption of dissolved oxygen by aerobic biological organisms in a body of water to break down organic material present in a given water sample at certain temperature over a specific time period (APHA 1998). It is a measure that has been used commonly in wastewater monitoring and it is widely used as an indicator of the organic pollution from effluent. BOD is listed as a conventional pollutant in the U.S. Clean Water Act, so BOD limitation in NPDES permits are

a common practice (Claire et al 2003). It is most commonly expressed in milligrams of oxygen consumed per liter of sample during 5 days of incubation at 20 °C and is often used as a surrogate of the degree of organic matter loading in water. Carbonaceous BOD (or CBOD) is a measure of BOD, with inhibition of nitrification. BOD is similar in function to water column chemical oxygen demand (COD), in that both measure the amount of organic compounds in water. However, COD is less specific, since it measures everything that can be chemically oxidized, rather than just levels of biologically active organic matter.

Similar to water column BOD, SOD is a measure of the rate of dissolved oxygen consumed by biological and chemical processes in the sediment (APHA 1998). It can be measured in a variety of aquatic environments either through the use of benthic chambers or by incubation of sediment cores (Murray and Hicks 1986). In either case, the sediments are incubated with overlying water column and the rate of change of dissolved oxygen concentration recorded. SOD is expressed as mmol O_2 per unit area and per unit time.

Clear Linkage to Beneficial Uses

Dissolved oxygen has a clear and well established linkage to beneficial uses in estuaries. The response of aquatic organisms to low DO will depend on the intensity of hypoxia, duration of exposure, and the periodicity and frequency of exposure (Rabalais, Turner et al. 2002), and US EPA has an extensive database documenting adverse effects of low DO on a variety of fish and invertebrates with respect to juvenile and adult survival, reproduction and recruitment (EPA 2003). Impacts of hypoxia on estuarine pelagic and benthic organisms would have a direct impact on important beneficial uses, including food web support for marine and estuarine aquatic organisms (EST, MAR) including the commercial and sport fisheries (COMM), shellfish such as clams, oysters and mussels (SHELL and AQUA), migratory (MIGR) birds and fish, support for fish nursery habitat (SPAWN). Poor water quality and increased heterotrophic bacterial production would adversely affect the health of recreational swimmers, sailboarders, and boaters (REC-1) and decrease aesthetic enjoyment (REC-2) through nuisance buildup and smell during decay.

Dissolved oxygen concentration can be thought of as the integration of the sum of physical (stratification, aeration, mixing, etc.), chemical (e.g. nitrification, oxidation of reduced compounds) and biological processes (photosynthesis and respiration) that produce and consume oxygen. CBOD and SOD are two of the components that can be attributed as sinks for oxygen, measured as rates. However, high rates of CBOD and SOD do not necessarily imply hypoxia, because other factors may prevent the buildup of hypoxic conditions in the water column (e.g. aeration). Therefore, direct measures of dissolved oxygen concentrations have a clear linkage to beneficial uses, while CBOD and SOD are linked, but only indirectly.

Predictive Relationships to Causal Factors

Reduced dissolved oxygen is a measurable indirect impact of high nutrients and high primary productivity under certain conditions. Reduced dissolved oxygen can occur under conditions of water column stratification, high nutrients, ample sunlight, and high primary production and subsequent

decomposition by heterotrophs. Modeling these conditions will typically involve dynamic simulation models rather than empirical load-response models. Open-source dynamic simulation models exist to predict dissolved oxygen concentrations from nutrient and organic matter loading and other co-factors, with several excellent examples of application in estuaries such as Chesapeake Bay (Cerco and Cole 1994; Lung and Bai 2003). Though successful modeling of dissolved oxygen requires estimates of CBOD and SOD (DiTorro and Fitzpatrick 1993), the management endpoint of these models is typically dissolved oxygen concentration.

Sound and Practical Measurement

Dissolved oxygen has a well-established and practical means of measurement. There are standard methods for measuring dissolved oxygen including discrete measurements (Winkler Titration) and continuous measurements recorded at regular intervals for days to years (membrane or optical electrodes) deployed on data sondes.   Diurnal DO changes can be tracked over time using moored, continuously recording DO sensors. Measurements of DO taken from vertical profiles also help to indicate the extent of mixing or stratification at a monitoring site and are typically used in monitoring of deepwater subtidal areas.

BOD also has well-established, standard and practical means of measurement (APHA 1998). There are two commonly recognized methods for the measurement of BOD—the dilution and the manumetric method (Claire 2003). The dilution method is carried out by diluting the sample with oxygen saturated de-ionized water, inoculating it with a fixed aliquot of microbial "seed", measuring the change in dissolved oxygen due to respiration at 20 °C for five days. The difference between the final DO and initial DO is the BOD, corrected for the degree of dilution. For measurement of carbonaceous BOD (cBOD), a nitrification inhibitor is added after the dilution water has been added to the sample. The inhibitor hinders the oxidation of nitrogen. The manumetric method is similar to the dilution method, with the exception that it measures change in pressure of carbon dioxide and thus measures CBOD.

SOD has well-accepted methods for measurement, albeit not standard. Benthic flux chambers have been used in a wide variety of environments to assess rates of solute exchange (e.g. rivers (Callender and Hammond 1982), estuaries and embayments (Hammond, Fuller et al. 1985; McNichol, Lee et al. 1988; Berelson, Heggie et al. 1998), and open ocean environments (Jahnke, Nelson et al. 2000; Berelson, McManus et al. 2003). A benthic flux chamber approach to estimating SOD has certain advantages over incubation of sediment cores: 1) a direct determination is available from assessing the change in chamber concentration versus time, 2) chambers integrate the net reactions occurring within the sediments enclosed by the chamber, and 3) sediments are relatively undisturbed, relative to removal and incubation of sediment cores. However, field conditions do not always permit the use of benthic chambers, and it is possible to get greater spatial coverage with incubation of sediment cores.

Acceptable Signal to Noise Ratio

Dissolved oxygen concentrations in estuaries have a high degree of spatial and temporal variability. However, DO is a well-vetted indicator of eutrophication and there is considerable experience with its

use in a regulatory context to manage eutrophication, particularly with the use of data sondes to capture a continuous record in bottom and surface water dissolved oxygen. As stated earlier, both BOD and SOD are rates, and thus by themselves are not reliable indicators of eutrophication. The presence of high BOD or SOD does not necessarily imply that the estuary is suffering from eutrophication, nor does a low BOD or SOD imply that the estuary is minimally disturbed. Therefore BOD and SOD would not be considered to have an acceptable signal to noise ratio.

Summary: Use of Dissolved Oxygen, BOD, and SOD as NNE Indicator for Estuaries

Dissolved oxygen satisfies all four evaluation criteria and is recommended as an NNE indicator for the subtidal habitats of California estuaries. Existing basin plan objectives for dissolved oxygen exist for California estuaries. Because the lack of consistency in approach among these objectives, the SWRCB is undertaking a review of science supporting DO objectives (Sutula et al., 2011), using the US EPA Virginia Province Salt Water Dissolved Oxygen Criteria approach (USEPA 2003). The approach was developed for the region of the east coast of the US from Cape Cod, MA, to Cape Hatteras, NC. This approach has been adapted for use in setting DO criteria for Chesapeake Bay (Batiuk, Breitburg et al. 2009), and has been applied, with appropriate modification, the other coastal regions of the US including Maine and Alabama. BOD and SOD are can be helpful supporting indicators, but are not recommended for development of NNE endpoints at this time.

6.5.4   Utility of Indicators of Net Ecosystem Metabolism and Benthic Metabolism for the NNE Framework in Estuaries

While NEM has been used as an indicator of ecosystem condition, it is difficult to ascribe a threshold of NEM where a system becomes eutrophic (Caffrey 2003; Duarte and Prairie 2005). Because most aquatic ecosystems are net heterotrophic, especially systems that receive significant inputs of organic carbon from their respective watersheds and consequently NEM reveals more about the carbon budgets of these systems rather than the extent of eutrophication (Caffrey 2003; Caffrey 2004; Duarte and Prairie 2005). Furthermore, NEM is strongly dependent on physical and hydrodynamic factors such as temperature, salinity, habitat adjacent to the monitoring site, estuarine area, and water depth in addition to nutrient loading (Caffrey 2004), and tends to be a better indicator of the relative degree of carbon loading from watershed versus estuarine sources (Duarte and Prairie 2005). Thus, NEM is not expected to be a useful indicator of eutrophication in estuarine systems.

In Australia, sediment indicators of eutrophication have been developed to target a sustainable carbon loading for shallow coastal estuaries (Table 6.5) (Eyre and Ferguson 2002). These indicators are derived from measurements of carbon dioxide flux and oxygen flux from the sediments using benthic flux chambers or via incubation of sediment cores in the laboratory. Benthic carbon dioxide flux is the amount of CO_2 released from the sediment during the decomposition of organic matter. The balance between benthic production (P) and respiration (R) of carbon (sediment P:R ratio) is also an important component of coastal ecosystems (Eyre and Ferguson 2002). A low P:R ratio indicates a system that is decomposing excess organic matter and consuming oxygen which may lead to anoxia. Productivity: respiration (P:R) ratio was expressed as the hourly O_2 productivity times the daylight period divided by

the hourly O_2 respiration times 24 hr at the sediment/water interface (Eyre and Ferguson 2002). Productivity: respiration ratios decrease with increasing carbon loading due to a combination of factors. Much of the carbon loading comes from increased pelagic productivity which shades the water column and decreases the amount of light reaching the sediments; decreased light results in a decrease in benthic productivity. Increased carbon loading also increases sediment respiration which combined with lowered productivity decreases the P:R ratio (Eyre and Ferguson 2002; Eyre and Ferguson 2002). Systems are typically classified into four trophic states: oligotrophic (low organic matter supply), mesotrophic (moderate organic matter supply), eutrophic (high organic matter supply) and hypereutrophic (very high organic matter supply). Eutrophication represents a change in trophic state and it is this change that results in dramatic shifts in characteristic abundance, relative biomass and community structure of flora and fauna.

Table 6.5. Ranges of sediment indicators for each trophic state.

Indicator	Oligotrophic	Mesotrophic	Eutrophic	Hyper Eutrophic
Net Benthic CO_2 flux	< 48 mmol m^{-2} d^{-1}	49-96 mmol m^{-2} d^{-1}	97-144 mmol m^{-2} d^{-1}	> 144 mmol m^{-2} d^{-1}
P:R Ratio	> 0.48	0.34-0.48	0.25-0.34	< 0.25

Use of benthic TCO2 flux or benthic P:R ratios to assess eutrophication has two difficulties. First, systems may be naturally eutrophic or hypereutrophic based on geology and natural loadings from undeveloped land uses. Recent studies on estuaries in southern California have found that bar-built river mouth estuaries tend to regularly flush themselves of sediment organic matter, have a lower benthic CO_2 fluxes, and thus have a lower susceptibility to eutrophication than lagoonal environments (McLaughlin, Sutula et al. 2011). One river mouth estuary with extremely high algal biomass and chronic hypoxia showed low benthic TCO2 fluxes and high P:R ratios. Thus, organic matter loading or sediment organic matter accumulation is dependent on estuarine class. Indicators of benthic metabolism would be more appropriate for depositional habitats within lagoonal or enclosed bays and expectations for classification of estuaries from "oligotrophic" to "hypereutrophic" would require ample data on "minimally disturbed" estuaries.

The second difficulty of using benthic TCO2 fluxes or P:R ratios is the confounding effect that bioirrigation or bioturbation by benthic infauna can have on these rates. Sutula et al. (2006) found that sites with a higher abundance of benthic infauna greatly increased the magnitude of benthic TCO2 fluxes. At one site where sediment organic matter loading was the greatest, sediments were extremely anoxic and azoic (absent of benthic infauna), and benthic TCO2 fluxes were low. Thus, exclusive use of benthic TCO2 or P:R ratios would have resulted in a false negative at this site.

Clear Linkage to Beneficial Uses

Indicators of benthic metabolism can be considered to have an indirect linkage to estuarine beneficial uses in estuaries. Benthic TCO2 has linkages to the organic matter content and redox status of sediments, which have a direct linkage on benthic invertebrates (See Chapter 8 on Macrobenthos for

more detailed discussion). From the estuarine beneficial use perspective, macrobenthos are part of diversity of aquatic life and as such a direct measure of EST beneficial uses. In terms of commercial value, many species of macrobenthos are directly harvested (e.g., oysters, mussels, clams, shrimp, and lobsters) by humans, which would be classified as COMM and SHELL uses. Within California, commercial shellfish harvest represented approximately $100 million in fisheries landings in 2008 (NMFS pers. comm.), in addition to the creation of jobs and revenue related to harbor infrastructure, seafood processing and distribution, and tourism. Beyond their direct commercial value, the macrobenthos provide an important source of food for estuarine and marine fish, birds and marine mammals (EST), including migratory fish and marine mammals (MIGR), spawning fish (SPAWN), and threatened /endangered species of fish and birds (RARE). Numerous commercially important nekton (e.g. *Embiotaca jacksoni*, *Umbrina rancador*, or *Hypsopetta guttulata*) from California's estuaries are dependent upon the macrobenthos as a food source and thus provide indirect support for COMM beneficial uses (Allen 2006). Benthic metabolism can help to indicate the balance of autotrophy versus heterotrophy in sediments and plays a key role in sediment nutrient and contaminant cycling by maintaining bioturbation and bioirrigation (the mixing of sediment and advective exchange of sediment pore waters with surface waters), which is a key component in the maintenance of good estuarine and marine habitat and water quality (EST and MAR). Finally, from the biotic, food web perspective, balanced benthic metabolism can help to indicate an appropriate transfer of carbon from bacterial and primary production to higher trophic levels in estuaries, most of which cannot directly consume all of these types of organic matter (Gillet 2010). Therefore, indicators of benthic metabolism have an indirect connection on estuarine beneficial uses.

Predictive Relationships to Causal Factors

Benthic metabolism is a measurable indirect impact of high nutrients and high primary productivity (Boudreau 2005). Modeling these conditions will typically involve dynamic simulation models rather than empirical load-response models. Open-source dynamic simulation models exist to predict sediment diagensis and benthic metabolism (e.g. (DiTorro and Fitzpatrick 1993)), with several examples of application in estuaries such as Chesapeake Bay (Cerco and Cole 1994; Lung and Bai 2003). It is anticipated that sediment TCO2 fluxes and P:R ratios would be predicted with poor precision by dynamic simulation models, because of the number of co-factors, including benthic infauna, which control these indicators.

Sound and Practical Measurement

Benthic TCO2 fluxes and benthic P:R ratios have well-accepted methods for measurement, albeit not standard. As with SOD, typical means of estimating these parameters involved use of either benthic flux chambers or laboratory incubation of sediment cores (Hammond, Fuller et al. 1985; McNichol, Lee et al. 1988; Berelson, Heggie et al. 1998). A benthic flux chamber approach to estimating benthic metabolism has certain advantages over incubation of sediment cores: 1) a direct determination is available from assessing the change in chamber concentration versus time, 2) chambers integrate the net reactions occurring within the sediments enclosed by the chamber, and 3) sediments are relatively undisturbed, relative to removal and incubation of sediment cores. However, field conditions do not always permit

the use of benthic chambers, and it is possible to get greater spatial coverage with incubation of sediment cores.

Acceptable Signal to Noise Ratio

As stated earlier, use of benthic TCO2 and benthic P:R ratio has two difficulties. First, organic matter loading or sediment organic matter accumulation is dependent on estuarine class. Indicators of benthic metabolism would be more appropriate for depositional habitats within lagoonal or enclosed bays and expectations for classification of estuaries from "oligotrophic" to "hypereutrophic" would require ample data on "minimally disturbed" estuaries, which are largely now absent in California. Second, bioirrigation or bioturbation by benthic infauna can have a confounding effect on these rates, either a false negative or false positive diagnosis of adverse effect by eutrophication. Interpretation of these indicators is typically done with confidence when used as supporting indicators in a multiple lines of evidence. Therefore indicators of benthic metabolism would not be considered to have an acceptable signal to noise ratio.

Summary: Use of Indicators of Benthic Metabolism as NNE Indicator for Estuaries

Indicators of benthic metabolism can provide valuable information on effects of sediment organic matter loading on benthic habitat quality. However, because of poor signal:noise ratios and poor indirect linkages to beneficial uses, we recommend that indicators of benthic metabolism be used as supporting indicators, rather than primary indicators, of eutrophication in the NNE assessment framework.   These indicators are not likely to be of use in river mouth estuaries, but can be a helpful in diagnosis when used with multiple lines of evidence.

6.5.5   Utility of Indicators of Organic Matter Accumulation for NNE Framework in Estuaries

In shallow coastal systems pelagic and benthic compartments are tightly coupled and much of the carbon production and metabolism occurs in the sediments. As such, sediments and benthic communities are probably the most sensitive part of the coastal ecosystem to nutrient enrichment (Jorgensen and Richardson 1996), making them ideally suited as an indicator of change, and for defining management criteria such as sustainable carbon loading rates (Eyre and Ferguson 2002). Indicators of sediment organic matter accumulation can provide helpful information on effects of sediment organic matter loading on benthic habitat quality and potential for regeneration of nutrients to surface waters. Indicators of sediment organic matter accumulation generally fall into three categories: 1) sediment organic matter and nutrient content, 2) sediment organic matter lability, and 3) sediment iron and sulfide content.

The organic matter content of the sediment results from the difference between inputs (benthic primary production and sedimentation) and community respiration. It is typically expressed as the percent of organic carbon per unit dry weight of sediment. Since sediment organic matter is the fuel for both aerobic and anaerobic metabolism, total organic matter can be thought of as representing potential metabolism. However, the degree to which this potential is realized depends upon the composition of the organic matter pool, as this determines both the nutritive quality of organic matter for the benthic

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fauna and the degradability of the organic matter for microorganisms (Mann 1988). Organic matter content of marine sediments depends strongly on sediment texture, as fine-grained sediments have higher organic matter content. Comparison of sedimentary organic matter across gradients and systems (e.g. along the continental margin and river-ocean continuum) therefore requires normalization (Mayer 1994). The most common method is to express the organic content per unit specific surface area (OC/SS; (Mayer 1994; Hedges and Keil 1995; Hedges and Keil 1999)). The rationale is that organic carbon content correlates with specific surface area in sediments underlying oxic bottom waters and that additional organic carbon reflects enhanced preservation, which may be due to eutrophication (Mayer 1994). In addition to sediment organic matter, information on nutrient sediment nitrogen (N) and phosphorus (P) content and the form of N and P. Like organic carbon, N and P content also vary as a function of grain size, so normalization of N and P with specific surface area is required to utilize this information as an indicator.

Sediment organic matter lability refers to the extent to which sediment organic matter can be utilized by heterotrophic bacteria and benthic detritivores; "labile" carbon is that which is readily utilized. The overall degradability of sedimentary organic matter can be assessed directly by respirometric techniques, by determining benthic dark oxygen or CO_2 fluxes (see previous section on SOD or benthic TCO2 fluxes), or indirectly by molecular analyses of the organic matter pools (Fabiano, Danovaro et al. 1995) or its elemental (C:N:P) ratio (Enriquez, Duarte et al. 1993). Similarly, modeling studies have emphasized the role of organic matter quality and its depth distribution in the sediment as major factors regulating overall benthic metabolism and nutrient cycling (Fenchel, King et al. 1998). Sediments with low C:N or C:P ratios would be considered to have labile carbon sources, such as algal-derived carbon. High C:N:P ratios can signal terrestrial source of carbon, which can be high in refractory lignin. As a general pattern, organic matter at low concentrations can be a limiting resource for benthic animal communities. Increasing sedimentary organic matter content supplies more energy to benthic consumers, but also stimulates sediment oxygen demand. Above certain organic matter thresholds, the oxygen demand for bacterial respiration and the reoxidation of the products of anaerobic respiration become critical. These induce hypoxia or anoxia at the water–sediment interface and/or the accumulation of toxic reduced compounds in the surficial sediments, which negatively affect benthic animal communities (Viaroli, Bartoli et al. 2004). Thus, the lability of the organic matter is of crucial importance: it is the degree to which organic matter stimulates microbial metabolism, rather than the quantity of organic matter per se, that induces hypoxia or the accumulation of reduced compounds and, thereby, impacts on the macrofaunal community (Gray, Wu et al. 2002).

In estuaries, biological sulfate reduction and the biogeochemical interactions between iron and sulfide play a major role in regulating sulfur speciation and retention of phosphate in the iron-bound pool (Howarth and Stewart 1992; Fenchel, King et al. 1998; Viaroli, Bartoli et al. 2004). Sedimentary reactive iron can immobilize toxic sulfides as highly insoluble compounds and can buffer excess sulfide production. Under anoxic conditions dissolved sulfate is reduced to hydrogen sulfide gas (H_2S), which reacts with iron minerals to form iron sulfides. Iron monosulfides (FeS) form first, but are typically unstable, and are usually converted to pyrite (FeS_2). Sediment total sulfur will be comprised of iron monsulfides and potentially a very small amount of elemental sulfur (S^o). Thus the ratio of total organic

carbon: total sulfur (TOC:TS) ratios and degree of pyritization (DP- defined as the ratio of %pyrite iron/(%pyrite iron + %reactive iron) in sediment (Raiswell, Buckley et al. 1987). Furthermore, the potential iron buffering capacity against excessive sulfide production can be assessed by determining the content of reactive iron in the sediments as acid-volatile sulfide (AVS) which approximates the pool of iron already precipitated as iron sulfide, primarily iron monosulfides (Viaroli, Bartoli et al. 2004; Rickard and Morse 2005). The ratio of AVS to reactive iron represents the saturation state of the buffer. TOC:TS ratios, AVS, and DP reflect the importance of sulfide reduction in the decomposition of organic matter, and thus give a qualitative indication of the redox status of the environment of deposition. TOC:TS, AVS, and DP are also linked to the concentrations of pore water sulfide and ammonium, which are known to be toxic to benthic infauna at high concentrations.

Clear Linkage to Beneficial Uses

Indicators of sediment organic matter accumulation can be considered to have an indirect linkage to estuarine beneficial uses in estuaries. Sediment organic matter content and lability, TOC:TS, AVS, and DP can be related directly to the tolerances of macrobenthos to elevated levels of sulfide and ammonium associated with anoxic sediments. Macrobenthos are part of diversity of aquatic life and as such a direct measure of EST beneficial uses. As noted above, many species of macrobenthos are directly harvested by humans and other commercially important consumers, which would be classified as COMM and SHELL uses and provide an important source of food for estuarine and marine fish, birds and marine mammals (EST), including migratory fish and marine mammals (MIGR), spawning fish (SPAWN), and threatened /endangered species of fish and birds (RARE). Furthermore, factors that control benthic habitat quality play a key role in sediment nutrient and contaminant cycling through bioturbation and bioirrigation and thus are a key component of maintenance of good estuarine and marine habitat and water quality (EST and MAR). Therefore, indicators of sediment organic matter accumulation have an indirect connection to estuarine beneficial uses. Of the indicators considered, TOC:TS and DP have the strongest linkage, because they are proxies for direct measures of pore water ammonium and sulfide (Berner 1984).

Predictive Relationships to Causal Factors

As with benthic metabolism, indicators of sediment organic matter accumulation are an indirect indicator of high nutrients and high primary productivity (Boudreau 2005)and modeling these conditions will typically involve dynamic simulation models rather than empirical load-response models. Open-source dynamic simulation models exist to predict sediment diagensis and benthic metabolism (e.g. (DiTorro and Fitzpatrick 1993)), with several examples of application in estuaries such as Chesapeake Bay (Cerco and Cole 1994; Lung and Bai 2003). As mentioned with benthic metabolism, it is anticipated that sediment C:N:P and TOC:TS ratios as well as AVS and DP would be predicted with poor precision by dynamic simulation models, because of the number of co-factors, including benthic infauna, which control these indicators.

Sound and Practical Measurement

Measures of sediment organic matter accumulation have been scientifically well-vetted and, in some cases, standardized methods of measurement are available. Analytical methods for determining sediment total organic carbon and nitrogen concentration are standardized, using combustion techniques via a CHN analyzer (see Froelich (1980) and Viaroli et al. (2004)). Sediment P concentration is determined via digestion at high temperature combustion or by persulfate digestion and extraction followed by conventional spectrophotometry for ortho-P (Froelich 1980). C:N:P ratios are simply a calculation of the atomic ratio of %TOC, %TON and %TP content in sediments. Sediment sulfur (TS) can be estimated by X-ray fluorescence spectrometry. Some different methods are available to determine DP (Raiswell, Buckley et al. 1987). Reactive iron is best measured by the 1 N HCl, 24-hour procedure of Leventhal and Taylor (1990). Acid volatile sulfide (AVS) is measured as the H_2S released by addition of 6N HCl to 1 g of fixed sediment (Fossing and Jorgensen 1989). All of these measures can be considered low cost relative to measures of TCO2 flux or SOD.

Acceptable Signal to Noise Ratio

Use of indicators of sediment organic matter content, nutrient content, or C:N:P ratio do not have an acceptable signal to noise ratio to diagnose eutrophication. High %OC or low C:N:P ratios can reflect recent die-off of algae that, in some cases, can stimulate the production of benthic infauna. Rather than the quantity of organic carbon or nutrients per se, it is the degree to which labile organic matter stimulates microbial metabolism that induces sediment anoxia or the accumulation of reduced compounds and, thereby, impacts on the macrofaunal community (Gray, Wu et al. 2002).

TOC:TS ratio, AVS, and DP has the potential for better signal to noise ratio in diagnosing eutrophication, because it is has the capacity to integrate the net effect of labile organic matter on sediment redox status and benthic habitat quality because anoxic conditions are required for biological sulfate reduction (and pyrite formation) to occur. Theoretically, TOC:TS, AVS, and DP values are controlled by a number of factors: 1) sediment redox state (requiring sufficient time of sediment anoxia for pyrite to form), 2) Availability of dissolved sulfate, which is not limited in estuaries because sulfate concentrations are usually very high, even in brackish water, 3) loading of reactive iron minerals per unit surface area of sediment. This factor can be limiting in systems in which the underlying geology is iron poor (particularly in sand dominated environments) and 4) amount of reactive organic matter.

TOC:TS, AVS, and DP are further controlled by a number of co-factors that can either increase or decrease the susceptibility of an estuary to eutrophication. These include bioturbation, where the mixing of sediments by benthic invertebrates continually adds dissolved oxygen, causing the oxidation of pyrite and sedimentation rates. In addition, when sedimentation rates are low, more pyrite can form because organic matter is exposed to dissolved oxygen in the water column for longer periods of time. However, if the water column is anoxic and contains H_2S, pyrite formation is maximized because H_2S production is not hindered by reoxidation and because more time is available for iron minerals to react with H_2S (Berner 1983).

In Australia, TOC:TS ratios and DP values have been used to classify estuarine sediments with respect to adverse effects of eutrophication. Aerobic marine sediments typically have DP values < 0.42 (Berner 1983) and TOC:TS ratios greater than 5.0. Marine sediments undergoing sulfate reduction below an oxygenated water column typically have TOC:TS ratios in the range from 1.5 to 5.0 (Berner 1983; Hedges and Keil 1995), while marine sediments undergoing sulfate reduction under poorly laminated sediment with sparse bioturbation (e.g. anoxic bottom waters with high H_2S concentrations) typically have TOC:TS ratios lower than 1.5 (Berner 1983) and DP in the range from 0.46 - 0.80 (Raiswell, Buckley et al. 1987).

One criticism of the use of TOC:TS ratios, AVS, and/or DP is that these values represent the loss of capacity. For example, TOC:TS ratio do not apply when iron limitation is suspected or when TS concentrations are low (Raiswell, Buckley et al. 1987). Likewise, with DP and AVS, the ratio of pyrite or iron monosulfides to reactive iron (II) represents the saturation of buffering capacity. Finally, several studies indicate that the acid extraction used to determine reactive iron overestimates the true buffering capacity against sulfide (Azzoni, Giordani et al. 2001). Recently, a biological oxygen and sulfide monitor (BOSM) has been developed for monitoring the sediment capacity to trap sulfide (Heijs, Jonkers et al. 1999). BOSM experiments have confirmed previous observations showing that the sedimentary buffering capacity against sulfides is not a simple function of the reactive iron concentration, since not all of the iron is available to react with sulfide (Viaroli, Bartoli et al. 2004). Thus, use of TOC:TS, AVS and DP is dependent on the iron content and surface area (particle size), and these indicators are likely to be more appropriate of depositional habitats. Use of these indicators requires additional experience in order to determine whether they are adequately precise to use assess eutrophication in California estuaries.

Summary: Use of Indicators of Sediment Organic Matter Accumulation as an NNE Indicator for Estuaries

Indicators of organic matter accumulation such as sediment organic matter, nutrient content and C:N:P are helpful indicators of organic matter loading, but do not have a sufficiently strong linkage with beneficial uses and signal to noise ratio to be used to assess eutrophication. TOC:TS, AVS, and DP have greater promise; however, additional research is required to determine in what habitat types or estuarine classes these indicators can be employed and what are the appropriate thresholds. Therefore we recommend that TOC:TS, AVS, and DP be considered as supporting indicators for the NNE framework.

6.5.6   Utility of Denitrification Efficiency for NNE Framework in Estuaries

Denitrification, the microbially-mediated conversion of NO_3 to N gas, is typically a major pathway through which anthropogenic nitrogen is lost from the system (Seitzinger 1988). At low inputs of nitrate, the rate of denitrification is low. With increasing nitrate, the rate of denitrification increases to an optimum level, then declines as eutrophication causes increases in sediment anoxia, favoring dissimilatory nitrate reduction, the conversion of nitrate to ammonium (An and Gardner 2002; Brock 2006; Porubsky, Weston et al. 2009). When coupled to denitrification, nitrification, a process that occurs in relatively aerobic environments and converts NH_4 to NO_3, enhances the efficiency of nitrogen removal. When denitrification efficiencies are lowered, more nitrogen is recycled to the water column

as ammonium. Nitrogen that is recycled to the water column (and not denitrified) stimulates further cycles of primary production, and therefore can continue the supply of organic matter to the sediment, leading to more decomposition and more dissolved oxygen consumption, and potentially to anoxic and hypoxic events (Herbert 1999; Nedwell, Jickells et al. 1999; Welsh 2000; Viaroli, Bartoli et al. 2004). Thus denitrification efficiency (DE%), expressed as the percentage of the total inorganic nitrogen released from the sediments as nitrogen gas (N_2) can be used as a measure of eutrophication (Berelson, Heggie et al. 1998; Eyre and Ferguson 2002; Eyre and Ferguson 2009):

DE% = ((N_2- TN / (DIN + N_2-TN) * 100%)

In the absence of nitrogen (N_2) measurements, denitrification rates (DR) and efficiencies (%DE) can be estimated from the following equations:

DR = TDINp - DINm

DE% = [$TDIN_p$ - DIN_m] * 100 / $TDIN_p$

where $[DIN]_m$ is the measured dissolved inorganic nitrogen liberated from the sediment during the degradation of organic matter and $[TDIN]_p$ is the total dissolved inorganic nitrogen flux predicted on the basis of stoichiometric relationship with the benthic carbon dioxide flux (Heap et al. 2001). However, the stoichiometric approach requires that the molar proportions of carbon and nitrogen in the organic matter being degraded are known. Problems with the stoichiometric approach can occur if one assumes that nitrogen missing from the DIN flux has been denitrified, when it may have been utilized by bacteria in sediment. This is most likely to occur in sediments with high TOC:TN ratios (>20) because microbial decomposition can be nitrogen limited under such conditions (Twilley, Cowan et al. 1999).

Because of the link among carbon loading and decomposition, oxygen consumption, and coupled nitrification–denitrification, the DE of shallow estuaries should decrease as the carbon loading increases, making DE useful for defining critical loads (Eyre and Ferguson 2002; Eyre and Ferguson 2009). The rate of carbon decomposition (which can be considered a proxy for carbon loading) is an important control on the efficiency with which sediments in depositional mud basins with low water column nitrate concentrations recycle nitrogen as N_2. The relationship between DE and carbon loading is due to changes in carbon and nitrate supply associated with sediment biocomplexity. At the DE optimum (500–1,000 mol m^{-2} h^{-1}), there is an overlap of aerobic and anaerobic respiration zones (caused primarily by the existence of anaerobic micro-niches within the oxic zone, and oxidized burrow structures penetrating into the anaerobic zone), which enhances denitrification by improving both the organic carbon and nitrate supply to denitrifiers. On either side of the DE optimum zone, there is a reduction in denitrification sites as the sediment loses its three-dimensional complexity. At low organic carbon loadings, a thick oxic zone with low macrofauna biomass exists; resulting in limited anoxic sites for denitrification, and at high carbon loadings, there is a thick anoxic zone and a resultant lack of oxygen for nitrification and associated NO_3 production. Eyre and Ferguson (2009) proposed a trophic scheme for defining critical (sustainable) carbon loading rates and possible thresholds for shallow estuaries based on the relationship between denitrification efficiency and carbon loading. There is evidence that denitrification efficiencies of >~68%, ~40%, 18% and 8% are indicative of oligotrophic, mesotrophic,

eutrophic and hypertrophic conditions respectively, in shallow coastal waterways in southeastern Australia (Eyre and Ferguson 2009). The denitrification efficiency ''optimum'' occurs between carbon loadings of about 50 and 100 g C m^{-2} year^{-1} (Eyre and Ferguson 2002).

Clear Linkage to Beneficial Uses

Indicators of denitrification efficiency can be considered to have a direct linkage estuarine beneficial uses in estuaries. Denitrification is a key ecosystem service that is important in the maintenance of good water quality and healthy benthic and pelagic habitat. Thus, efficient denitrification is a measure of EST beneficial uses and can be considered to support all other beneficial uses in which maintenance of water quality is important to the use (e.g. COMM, SHELL, MIGR, SPAWN, and RARE).

Predictive Relationships to Causal Factors

Similar to benthic metabolism and indicators of sediment organic matter accumulation, denitrification efficiency (DE) is an indirect measure of high nutrients and high primary productivity (Boudreau 2005); however, DE in estuarine sediments has a bell shaped curve rather than a linear relationship with nutrient loading which complicates its predictive capacity. Thus, modeling DE will typically involve dynamic simulation models rather than empirical load-response models. Open-source dynamic simulation models exist to predict sediment diagensis and benthic metabolism (e.g. (DiTorro and Fitzpatrick 1993)); however, it is anticipated that DE would be predicted with poor precision by dynamic simulation models, because of the number of co-factors, including benthic infauna, which control these indicators.

Sound and Practical Measurement

The denitrification efficiency of sediments in estuaries is determined using benthic chambers. Benthic flux chambers have been used in a wide variety of environments to assess rates of solute exchange (e.g. rivers (Callender and Hammond 1982), estuaries and embayments (Hammond, Fuller et al. 1985; McNichol, Lee et al. 1988; Berelson, Heggie et al. 1998), and open ocean environments (Jahnke, Nelson et al. 2000; Berelson, McManus et al. 2003). The denitrification efficiency of estuarine sediments is determined by comparing the amount of nitrogen released as a gas to the total amount of nitrogen released. The released nitrogen gas is quantified using a Membrane Inlet Mass Spectrometry (MIMS) technique (Kana, Darkangelo et al. 1994). This involves stripping the gases from a water sample using a membrane under high vacuum. Carbon dioxide and water are removed from the gas-stream via a cold trap. The dry gas is then introduced to the mass spectrometer to measure N_2/Ar ratios. The N_2 concentrations are then calculated from the measured ratios using the N_2/Ar calibration curves and theoretically determined argon concentrations for the sampled waters. The N_2/Ar technique has several assumptions that must be met to achieve accurate rate calculations (Eyre, Rysgaard et al. 2002). Different tests can be applied to ensure these assumptions are being met (Eyre, Rysgaard et al. 2002). Overall, though scientifically well-accepted methods exist to measure denitrification efficiency, measurement requires a high level of expertise, sophisticated equipment, and is costly to perform. Thus, denitrification efficiency does not meet this evaluation criterion.

In benthically-driven habitats of estuaries, denitrification has a reasonably good signal to noise ratio. Carbon loading is the main control on the denitrification efficiencies of sediment (Eyre and Ferguson 2009). The lowering of denitrification efficiencies with increased carbon loadings results from the inhibition of nitrification caused by a lack of dissolved oxygen or exposure to hydrogen sulfide gas or from an increase in the quantitative importance of dissimilatory nitrate reduction to ammonium (An and Gardner 2002; Ferguson, Eyre et al. 2004; Brock 2006).

Denitrification efficiency is a good indicator of sustainable carbon loading rates in shallow water habitats of enclosed bays (Berelson, Heggie et al. 1998; Heggie, Skyring et al. 1999), shallow coastal lagoons ((Eyre and Ferguson 2002; Eyre and Ferguson 2009), where denitrification is linked to nitrification occurring in the sediment. The relationship between denitrification efficiency and carbon loading may not hold in coastal waterways where denitrification is linked to water column nitrate and limitation of benthic primary producers (Eyre and Ferguson 2002). Salinity (Rysgaard, Thastum et al. 1999) and water temperature (Carpenter and Dunham 1985) can also have physiological impacts on denitrifying bacteria, and may be additional controls on denitrification efficiencies. Finally, as with indicators of benthic metabolism, denitrification efficiency would be more appropriate for depositional habitats within lagoonal or enclosed bays and expectations for classification of estuaries from "oligotrophic" to "hypereutrophic" would require ample data on "minimally disturbed" estuaries, which are largely now absent in California.

Summary: Use of Denitrification Efficiency as an NNE Indicator for Estuaries

Denitrification efficiency can provide valuable information on effects of sediment organic matter loading on benthic habitat quality and has a direct linkage to estuarine beneficial uses. However, denitrification efficiency is complicated to measure and requires substantial expertise. Denitrification efficiency is likely to be better suited as a supporting indicator, particularly within the context of a special study. These indicators are not likely to be of use in river mouth estuaries, but can be helpful in diagnosing eutrophication when used with multiple lines of evidence.

6.6   Summary and Recommendations

Of the indicators evaluated, dissolved oxygen was the only indicator that met all four evaluation criteria and, as such, is recommended for inclusion as a primary indicator in the NNE framework (Table 6.4). Many of the other indicators evaluated would function well in the category of supporting indicator, providing additional lines of evidence, in addition to the primary NNE indicators, of eutrophication. However, because of issues identified they could not be used in isolation to diagnose eutrophication. These indicators include: 1) ammonia, urea (in phytoplankton-dominated enclosed bays), 2) N:P ratio in brackish water habitats, 3) light extinction (seagrass habitat only), and 4) Sediment C:N:P and TOC:TS ratios, AVS, and degree of pyritization (shallow water habitats). Indicators such as benthic TCO2 flux, benthic P:R ratios, and denitrification efficiency are good indicators of eutrophication in shallow water deposition habitats, but are require extensive expertise and therefore are not recommended to include as a routine part of an assessment framework.

Table 6.4. Summary of evaluation of sediment and water chemistry indicators of eutrophication.

Group	Indicator or Metric	Criteria Met	Recommended Action
Nutrients	Ammonia	Three of four	Evidence of ammonium inhibition of diatom nitrate assimilation, but ecological importance of process not well understood for entire Bay. **Recommend inclusion as supporting indicator for phytoplankton-dominated enclosed bays**
	Urea	Three of four	Causative link between urea and HABs has not been established and little data are available on urea concentrations in California estuaries. **Recommend inclusion as supporting indicator for phytoplankton-dominated enclosed bays**
	N:P Ratio	Three of four in brackish habitat	Linkage to beneficial uses and signal:noise ratio only strong in brackish water estuaries (e.g. coastal lakes). **Recommend inclusion as a supporting indicator for "closed" estuaries or brackish water regions of "open" estuaries**
Water Clarity	Secchi Depth	Two of Four	Phytoplankton biomass a component of light attenuation, but other factors unassociated with eutrophication can contribute (e.g. suspended sediment), so poor linkage to BUs and signal:noise. **Include as co-factor or supporting indicator for seagrass habitat. Otherwise not recommended.**
	Kd (Light extinction)		
	Turbidity		
Dissolved Oxygen	Dissolved Oxygen Conc.	Four of Four	**Recommend as primary indicator in NNE framework**
	Biological or Chemical Oxygen Demand	Two of four	Indirect linkage to beneficial uses and poor signal: noise ratio. **Can include as a co-factor in monitoring, but not recommended for inclusion in NNE framework.**
	Sediment oxygen demand	Two of four	
Benthic Metabolism	Benthic production: respiration ratio	Two of four	Indirect linkage to beneficial uses and poor signal: noise ratio. **Provides useful information as part of a special study, but not recommended for inclusion in NNE framework**
	Benthic TCO2 flux	Two of four	
Organic Matter Accum. & Sediment Redox	Sediment %OC, N, P	Two of four	Indirect linkage to beneficial uses and poor signal: noise ratio. **Recommended as a supporting indicator.**
	Sediment C:N:P ratio	Two of four	
	Sediment TOC:TS & DOP	Three of four	Moderate signal:noise ratio, that may be improved with additional work on expectations given geology and habitat type. **Recommend inclusion as supporting indicator to further evaluate performance.**
Nitrogen Cycling	Denitrification Efficiency	Two of four	Technically difficult to measure and poor-moderate signal:noise. **Not recommended for inclusion as supporting indicator**

6.7 Literature Citations

Allen, M. J. (2006). Contiental Shelf and Upper Slope. <u>The Ecology of Marine Fishes: California and Adjacent Waters</u>. L. G. Allen, D. J. Pondella and M. H. Horn. Berkeley, CA, University of California Press**:** 167-202.

AmtoftNeubauer, A.-T., A.-G. UnderlienPedersen, et al. (2004). "Benthic decomposition of *Zostera marina* roots: a controlled laboratory experiment." <u>Journal of Experimental Marine Biology and Ecology</u> **313**: 105-124.

An, S. and W. S. Gardner (2002). Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas). **237:** 41-50.

Anderson, D. M., P. M. Gilbert, et al. (2002). "Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences." <u>Estuaries</u> **25**(4B): 704-726.

Anderson, L. A. and J. L. Sarmiento (1994). "Redfield ratios and remineralization determined by nutrient data analysis." <u>Global Biogeochemical Cycles</u> **8**(1): 65-80.

Arhonditsis, G. B., C. A. Stow, et al. (2007). "Delineation of the role of nutrient dynamics and hydrologic forcing on phytoplankton patterns along a freshwater-marine continuum." <u>Ecological Modelling</u> **208**: 230-246.

Arroyo, N., K. Aarino, et al. (2006). "Drifting algae as a means of re-colonizing defaunated sediments in the Baltic Sea. A short-term microcosm study." <u>Hydrobiologia</u> **554**: 83-95.

Astill, H. and P. S. Lavery (2001). "The dynamics of unattached benthic macroalgal accumulations in the Swan-Canning Estuary." <u>Hydrological Processes</u> **15**: 2387-2399.

Atkinson, M. J. and S. V. Smith (1983). "C:N:P ratios of benthic marine plants." <u>Limnology and Oceanography</u> **28**(3): 568-574.

Azzoni, R., G. Giordani, et al. (2001). "Iron, sulfur and phosphorus cycling in the rhyzosphere sediments of a eutrophic Ruppia cirrhosa meadow of the Valle Smarlacca (Italy)." <u>Journal of Sea Research</u> **45**: 15-26.

Balducci, C., A. Sfriso, et al. (2001). "Macrofauna impact on *Ulva rigida* C. Ag. production and relationship with environmental variables in the lagoon of Venice." <u>Marine Environmental Research</u> **52** 27-49.

Bates, S., et, et al. (1989). "Pennate diatom Nitzschia pungem as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada." <u>Canadian Journal of Fisheries and Aquatic Sciences</u> **46**: 1203-1215.

Bates, S. S., A. S. W. DeFreitas, et al. (1991). "Controls on domoic acid production by the diatom Nitzschia pungens f. multiseries in culture: nutrients and irradiance." Canadian Journal of Fisheries and Aquatic Sciences **48**: 1136-1144.

Batiuk, R. A., D. L. Breitburg, et al. (2009). "Derivation of habitat-specific dissolved oxygen criteria for Chesapeake Bay and its tidal tributaries." Journal of Experimental Marine Biology and Ecology **381**: S204-S215.

Batiuk, R. A., P. Bergstrom, et al. (2000). Chesapeake Bay Submerged Aquatic Vegetation Water Quality and Habitat-Based Requirements and Restoration Targets: A Second Technical Synthesis. Annapolis, MD, U.S. Environmental Protection Agency.

Berelson, W., J. McManus, et al. (2003). "A time series of benthic flux measurements from Monterey Bay, CA." Continental Shelf Research **23**: 457-481.

Berelson, W. M., D. Heggie, et al. (1998). Benthic nutrient recycling in Port Phillip Bay, Australia, Elsevier. **46:** 917-934.

Berner, R. A. (1983). "Sedimentary pyrite formation- an update." Geochimica et Cosmochimica Acta **48**: 605-615.

Berner, R. A. (1984). "Sedimentary pyrite formation- an update." Geochimica et Cosmochimica Acta **48**: 605-615.

Bolam, S., T. Fernades, et al. (2000). "Effects of macroalgal mats on intertidal sandflats: an experimental study." Journal of Experimental Marine Biology & Ecology **249**: 123-137.

Bolam, S. G. and T. F. Fernandes (2002). "The effects of macroalgal cover on the spatial distribution of macrobenthic invertebrates: the effect of macroalgal morphology." Hydrobiologia **475/476**: 437-448.

Borges, A. C. and N. Gypens (2010). "Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification." Limnology and Oceanography **55**: 346-353.

Borsuk, M. E., C. A. Stow, et al. (2001). "Modelling Oxygen Dynamics in an Intermittently Stratified Estuary: Estimation of Process Rates Using Field Data." Esturine, Coastal and Shelf Science **52**: 33-49.

Boudreau, B. P. (2005). Modelling mixing and diagenesis. Macro-and microorganisms in marine sediment. E. Kristingsen, J. Kostaka and R. H. Haese. Washington, D.C., American Geophysical Union**:** 323-340.

Bourgues, S., I. Auby, et al. (1996). "Differential anaerobic decomposition of seagrass (*Zostera noltii*) and macroalgae (*Monostroma obscurum*) biomass from Arcachon Bay (France)." Hydrobiologia **329**: 121-131.

Boyle, K. A., K. Kamer, et al. (2004). "Spatial and temporal patterns in sediment and water column nutrients in a eutrophic Southern California estuary." Estuaries and Coasta **27**(3): 378-388.

Brock, D. (2006). Nitrogen fixation and dissimilatory nitrate reduction to ammonium (DNRA) support nitrogen dynamics in Texas estuaries, The American Society of Limnology and Oceanography: 558-568.

Brooks, K. and C. Mahnken (2003). "Interactions of Atlantic salmon in the Pacific northwest environment II. Organic wastes." Fisheries Research 62: 255-293.

Brown, C. A., W. G. Nelson, et al. (2007). An approach to developing Nutrient Criteria for Pacific Northwest Estuaries: A Case Study of Yaquina Estuary, Oregon, USEPA Office of Research and Development, National Health and Environmental Effects Laboratory, Western Ecology Division.

Brun, F. G., I. Hernandez, et al. (2002). "Assessing the toxicology of ammonium pulses to the survival and growth of *Zostera noltii*." Marine Ecology Progress Series 225: 177-187.

Buchsbaum, R., I. Valiela, et al. (1991). "Available and refractory nitrogen of coastal vascular plants and macroalgae." Marine Ecology Progress Series 72: 131-143.

Burkholder, J., E. Noga, et al. (1992). "New 'phantom' dinoflagellate is the causative agent of major estuarine fish kills." Nature 358: 407-410.

Burkholder, J. M., K. M. Mason, et al. (1992). "Water-column nitrate enrichment promotes decline of eelgrass *Zostera marina*: evidence from seasonal mesocosm experiments." Marine Ecology Progress Series 81: 163-178.

Cabaco, S., R. Machas, et al. (2008). "Imacts of urban wastewater discharge on seagrass meadows (Zostera Noltii)." Estuarine Coastal and Shelf Science 78(1): 1-13.

Cabral, J., M. Pardal, et al. (1999). "The impact of macroalgal blooms on the use of the intertidal area and feeding behavior of waders (Charadrii) in the Mondego estuary (west Portugal)." Acta Oecologica 20(4): 417-427.

Caffrey, J. M. (2003). "Production, respiration and net ecosystem metabolism in U.S. estuaries." Environmental Monitoring and Assessment 81: 207-219.

Caffrey, J. M. (2004). "Factors controlling net ecosystem metabolism in US estuaries." Estuaries 27(1): 90-101.

Caffrey, J. M. and W. M. Kemp (1990). "Nitrogen cycling in sediments with submersed macrophytes: Microbial transformation and inorganic pools associated with estuarine populations of *Potamogeton perfoliatus* and *Zostera marina*." Marine Ecology Progress Series 66: 147-160.

Caffrey, J. M. and W. M. Kemp (1992). "Influence of the submersed plant, *Potamogeton perfoliatus*, on nitrogen cycling in estuarine sediments." Limnology and Oceanography 37(7): 1483-1495.

Caldwell, R. (1975). Hydrogen sulfide effects on selected larval and adult marine invertebrates. U. D. o. Interior, Water Resources Research Institute.

Callender, E. and D. Hammond (1982). "Nutrient exchange across the sediment-water interface in the Potamac River Estuary." Estuarine Coastal and Shelf Science 15: 395-413.

Cardoso, P. G., M. A. Pardal, et al. (2004). "Macroinvertebrate response to different species of macroalgal mats and the role of disturbance history." Journal of Experimental Marine Biology and Ecology **308**: 207-220.

Carpenter, E. J. and S. Dunham (1985). "Nitrogenous nutrient uptake, primary production, and species composition of phytoplankton in the Carmans River estuary, Long Island, New York." Limnology and Oceanography **30**(3): 513-526.

Cerco, C. F. and T. M. Cole (1994). Three-dimensional eutrophication model of Chesapeake Bay volume I. Vicksburg, MS, U.S. Army Corps of Engienners Waterways Experiment Station.

Chambers, R. M., J. W. Fourqurean, et al. (2001). "Biogeochemical effects of iron availability on primary producers in a shallow marine carbonate environment." Limnology and Oceanography **46**: 1278-1286.

Chapman, P. (2001). "Utility and relevance of aquatic oligochaetes in Ecological Risk Assessment." Hydrobiologia **463**: 149-169.

Cloern, J. E., C. Grenz, et al. (1995). "An empirical model of the phytoplankton chlorophyll:carbon ratio - The conversion factor between productivity and growth rate." Limnology and Oceanography **40**(7): 1313-1321.

Cohen, R. and J. Pechenik (1999). "Relationship between sediment organic content, metamorphosis, and post larval performance in the deposit-feeding polychaete *Capitella* sp. I." Journal of Experimental Marine Biology & Ecology **240**: 1-18.

Cummins, S. P., D. E. Roberts, et al. (2004). "Effects of the green macroalga *Enteromorpha intestinalis* on macrobenthic and seagrass assemblages in a shallow coastal estuary." Marine Ecology Progress Series **266**: 77-87.

Cuomo, M. (1985). "Sulphide as a larval settlement cue for *Capitella sp I* " Biogeochemistry **1**: 169-181.

D'Avanzo, C. and J. Kremer (1994). "Diel oxygen dynamics and anoxic events in an eutrophic estuary." Estuaries **17**(1B): 131-139.

Dauer, D. M., C. A. Maybury, et al. (1981). "Feeding behavior and general ecology of several spionid polychaetes from the Chesapeake Bay." Journal of Experimental Marine Biology & Ecology **54**: 21-38.

Dauwe, B., J. J. Middelburg, et al. (2001). "Effect of oxygen on the degradability of organic matter in subtidal and intertidal sediments of the North Sea area." Marine Ecology Progress Series **215**: 13-22.

DeWit, R., L. J. Stal, et al. (2001). "ROBUST: The ROle of BUffering capacities in STabilising coastal lagoon ecosystems." Continental Shelf Research **21**: 2021-2041.

Diaz, R. J. (2001). "Overview of Hypoxia around the World." Journal of Environmental Quality **30**(2): 275-281.

DiTorro, D. and J. Fitzpatrick (1993). Chesapeake Bay sediment flux model. Vicksburg, MS, U.S. Army Corps of Engineers Waterways Experimental Station.

Doney, S. C., V. J. Fabry, et al. (2009). "Ocean acidification: the other CO2 problem." Annual Review of Marine Science **1**: 169-192.

Duarte, C. M. and Y. T. Prairie (2005). "Prevalence of heterotrophy and atmopsheric CO2 emissions from aquatic ecosystems." Ecosystems **8**: 862-870.

Dugdale, R. C., F. P. Wilkerson, et al. (2007). "The role of ammonium and nitrate in spring bloom development in San Francisco Bay." Estuarine Coastal and Shelf Science **73**: 17-29.

Dunton, K. H. (1994). "Seasonal growth and biomass of the subtropical seagrass Halodule wrightii in relation to continuous measurements of underwater irradiance." Marine Biology **120**: 479-489.

Enriquez, S., C. M. Duarte, et al. (1993). "Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content." Oecologia **94**(457-471).

Escartin, J. and D. G. Aubrey (1995). "Flow structure and dispersion within algal mats." Estuarine Coastal & Shelf Science **40**(4): 451-472.

Eyre, B. D. and A. J. P. Ferguson (2002). "Comparison of carbon production and decompostion, benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic microalgae- and macroalgae-dominated warm-temperature Australian lagoons." Marine Ecology Progress Series **229**: 43-59.

Eyre, B. D. and A. J. P. Ferguson (2002). Sediment biogeochemical indicators for defining sustainable nutrient loads to coastal embayments. Proceedings of Coast to Coast - "Source to Sea", Tweed Heads.

Eyre, B. D. and A. J. P. Ferguson (2005). "Benthic metabolism and nitrogen cycling in subtropical east Australian estuary (Brunswick): Temporal variability and controlling factors." Limnology and Oceanography **50**(1): 81-96.

Eyre, B. D. and A. J. P. Ferguson (2009). "Denitrification efficiency for defining critical loads of carbon in shallow coastal ecosystems." Developments in Hydrobiology **207**: 137-146.

Eyre, B. D. and L. J. McKee (2002). "Carbon, nitrogen, and phosphorus budgets for a shallow subtropical coastal embayment (Moreton Bay, Australia)." Limnology and Oceanography **47**(4): 1043-1055.

Eyre, B. D., S. Rysgaard, et al. (2002). "Comparison of isotope pairing and N2/Ar methods for measuring sediment denitrification rates- assumptions, modifications and implications." Estuaries **25**: 1077-1087.

Fabiano, M., R. Danovaro, et al. (1995). "A three-year time series of elemental and biochemical composition of organic matter in subidal sandy sediments of the Ligurian Sea (north western Mediterranean)." Continental Shelf Research **15**: 1453-1470.

Feely, R. A., S. C. Doney, et al. (2009). "Ocean acidification: present conditions and future changes in a high CO2 world." Oceanography **22**: 36-47.

Fenchel, T., G. M. King, et al. (1998). Bacterial Biogeocemistry. The Ecophysiology of Mineral Cycling. San Diego, CA, Academic Press.

Ferguson, A. J. P., B. D. Eyre, et al. (2004). "Benthic nutrient fluxes in euphotic sediments along shallow sub-tropical estuaries, northern New South Wales, Australia." Aquatic Microbial Ecology **37**(3): 219-235.

Fossing, H. and B. B. Jorgensen (1989). "Measurement of bacterial sulphate reduction in sediment: evaluation of a singel-step chromium reduction method." Biogeochemistry **8**: 205-222.

Froelich, P. N. (1980). "Analysis of organic carbon in marine sediments." Limnology and Oceanography **25**: 564-572.

Froelich, P. N., G. P. Klinkhammer, et al. (1979). "Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis." Geochimica et Cosmochimica Acta **43**: 1075-1090.

Gamenick, I., A. Jahn, et al. (1996). "Hypoxia and sulfide as structuring factors in a macrozoobenthic community on the Baltic Sea shore: colonization studies and tolerance experiments." Marine Ecology Progress Series **144**: 73-85.

Gianmarco, G., R. Azzoni, et al. (1997). "Seasonal variations of sulfate reduction rates, sulphur pools and iron availability in the sediment of a dystrophic lagoon (Sacca Di Goro, Italy)." Water, Air, & Soil Pollution **99**: 363-371.

Giere, O., J. Preusse, et al. (1999). "*Tubificoides benedii* (Tubificidae, Oligochaeta)- a pioneer in hypoxic and sulfidic environments. An overview of adaptive pathways." Hydrobiologia **406**: 235-241.

Gilbert, D., B. Sundby, et al. (2005). "A seventy-two year record of diminishing deep-water oxygen in the St. Lawrence estuary: the northwest Atlantic connection." Limnology and Oceanography **50**: 1654-66.

Gilbert, P. M. (2010). "Long-term changes in nutrient loading and stoichiometry and their relationships with changes in the food web and dominant pelagic fish species in San Francisco Estuary, California." Reviews in Fisheries Science **18**: 211-323.

Gilbert, P. M., J. Harrison, et al. (2006). "Escalating world wide use of urea: a global change contributing to coastal eutrophication." Biogeochemistry **77**(3): 441-463.

Gilbert, P. M., R. Magnien, et al. (2001). "Harmful algal blooms in the Chesapeake and coastal bays of Maryland, USA: Comparison of 1997, 1998, and 1999 events." Estuaries **24**(6A): 875-883.

Gillet, D. J. (2010). Effects of Habitat Quality on Secondary Production in Shallow Estuarine Waters and the Consequences for the Benthic-Pelagic Food Web. Biological Sciences. Glouchester Point, VA, College of William and Mary. **Ph.D.**

Gillet, D. J., A. F. Holland, et al. (2007). "On the ecology of olioghaetes: Monthly variation of community composition and environmental characteristic in two Southern Carolina tidal creeks." Estuaries and Coasts **30**: 238-252.

Gray, J. S., R. S. S. Wu, et al. (2002). "Effects of hypoxia and organic enrichmenton the coastal marine environment." Marine Ecology Progress Series **238**: 249-279.

Hammond, D. E., C. Fuller, et al. (1985). "Benthic fluxes in San Francisco Bay." Hydrobiologia **129**: 69-90.

Hargrave, B. T., M. Holmer, et al. (2008). "Towards classification of organic enrichment in marine sediments based on biogechemical indicators." Marine Pollution Bulletin **56**: 810-824.

Hargrave, B. T., M. Holmer, et al. (2008). "Towards classification of organic enrichment in marine sediments based on biogeochemical indicators." Marine Pollution Bulletin **56**: 810-824.

Hedges, J. I. and R. G. Keil (1995). "Sedimentary organic-matter preservation-- an assessment and speculative synthesis." Marine Chemistry **49**: 81-115.

Hedges, J. I. and R. G. Keil (1999). "Organic geochemical perspectives on estuarine processes: sorption reactions and consequences." Marine Chemistry **65**: 55-65.

Heggie, D. T., G. W. Skyring, et al. (1999). "Denitrification and denitrifying efficiencies in sediments of Port Phillip Bay: direct determinations of biogenic N2 and N-metabolite fluxes with implications for water quality." Marine and Freshwater Research **50**: 589-596.

Heijs, S. K., H. M. Jonkers, et al. (1999). "The buffering capacity towards free sulphide in sediments of a coastal lagoon (Bassin d'Arcachon, France)-- the relative importance of chemical and biological processes." Estuarine Coastal and Shelf Science **49**(1): 21-35.

Hentschel, B. (1996). "Ontogenic changes in particle-size selection by deposit-feeding spionid polychaetes: the influences of palp size on particle contact." Journal of Experimental Marine Biology & Ecology **206**: 1-24.

Herbert, R. A. (1999). "Nitrogen cycling in coastal marine sediments." FEMS Microbiology Review **23**: 563-590.

Hines, M., J. Faganeli, et al. (1997). "Sedimentary anerobic microbial biogeochemistry in the Gulf of Trieste, northern Adriatic Sea: Influcnes of bottom water oxygen depletion." Biogeochemistry **39**: 65-86.

Hogue, V. E., F. P. Wilkerson, et al. (2005). "Ultraviolet-B radiation effects on natural assemblages of central San Francisco Bay." Estuaries **28**(2): 190-203.

Hogue, V. E., F. P. Wilkerson, et al. (2001). "Phytoplankton and nutrient dynamics in Suisun, San Pablo, and Central Bays." IEP Newsletter **14**(4): 35-41.

Holmquist, J. G. (1994). "Benthic macroalgae as a dispersal mechanism for fauna: influence of a marine tumbleweed." Journal of Experimental Marine Biology & Ecology **180**: 235-251.

Howarth, R., F. Chan, et al. (2011). "Coupled biogeochemical cycles: eutrophication and hypoxia in temperate estuaries and coastal marine ecosystems." Frontiers in Ecology and the Environment **9**(1): 18-26.

Howarth, R. W. (1988). "Nutrient limitation of primary production in marine ecosystems." Annual Review of Ecology and Systematics **19**: 89-110.

Howarth, R. W. and J. W. B. Stewart (1992). The interactions of sulphur with other element cycles in ecosystems. Sulphur cycling on the continents: wetlands, terrestrial ecosystems and associated waterbodies: SCOPE 33. R. W. Howarth, J. W. B. Stewart and M. U. Ivanov. New York, NY, John Wiley and Sons**:** 67-84.

Howarth, R. W., D. P. Swaney, et al. (2000). "Climatic control on eutrophication of the Hudson River Estuary." Ecosystems **3**(2): 210-215.

Hull, S. C. (1987). "Macroalgal mats and species abundance: a field experiment." Estuarine, Coastal and Shelf Science **25**: 519-532.

Hulthe, G., S. Hulth, et al. (1998). "Effect of oxygen on degradation rate of refractory and labile organic matter in continental margin sediments." Geochimica et Cosmochimica Acta **62**: 1319-1328.

Isaksson, I. and L. Pihl (1992). "Structural changes in benthic macrovegetation and associated epibenthic faunal communities." Netherlands Journal of Sea Research **30**: 131-140.

Jahnke, R. A., J. R. Nelson, et al. (2000). "Benthic flux of biogenic elements on the Southeastern US continental shelf: influence of advective transport and benthic microalgae." Continental Shelf Research **20**(109-127).

Jones, M. and E. Pinn (2006). "The impact of a macroalgal mat on benthic biodiversity in Poole Harbour." Marine Pollution Bulletin **53**: 63-71.

Jorgensen, B. B. (1996). Material flux in sediments. Eurtophication in Coastal Marine Ecosystems, Coastal Marine Ecosystems, Coastal and Estuarine Studies, Vol. 2. B. B. Jorgensen and K. Richardson. Washington D.C., American Geophysical Union**:** 115-136.

Jorgensen, B. B. and K. Richardson (1996). Eutrophication in Coastal Marine Systems. Washington, D.C., American Geophysical Union.

Kaldy, J. E. and K. S. Lee (2007). "Factors controlling Zostera marina L. growth in the eastern and western Pacific Ocean: Comparisons between Korea and Oregon, USA." Aquatic Botany **87**: 116-126.

Kamer, K., K. Boyle, et al. (2001). "Macroalgal bloom dynamics in a highly eutrophic southern California estuary." Estuaries **24**(4): 623-635.

Kamer, K. and E. Stein (2003). Dissolved oxygen conentration as a potential indicator of water quality in Newport Bay: A review of scientific research, historical data, and criteria development. Westminster, California, Southern California Coastal Water Research Project.

Kana, T., C. Darkangelo, et al. (1994). "Membrane inlet mass spectrometer for rapid high-precision determination of N2, O2, and Ar in environmental water samples." Analytical Chemistry **66**(23): 4166-4170.

Kemp, W. M., W. R. Boynton, et al. (2005). "Eutrophication of Chesapeake Bay: historical trends and ecological interactions." Marine Ecology-Progress Series **303**: 1-29.

Kemp, W. M., P. A. Sampou, et al. (1990). "Ammonium recycling versus denitrification in Chesapeake Bay sediments." Limnology and Oceanography **35**: 1545-1563.

Kotta, J. and H. Orav (2001). "Role of benthic macroalgae in regulating macrozoobenthic assemblages in the Vainameri (north-eastern Baltic Sea)." Ann. Zool. Fennici **38**: 163-171.

Krause-Jensen, D., P. B. Christensen, et al. (1999). "Oxygen and nutrient dynamics within mats of the filamentous macroalga *Chaetomorpha linum*." Estuaries **22**(1): 31-36.

Kristensen, E., S. I. Ahmed, et al. (1995). "Aerobic and anaerobic decomposition of organic matter in marine sediment: which is fastest?" Limnology and Oceanography **40**: 1430-1437.

Kristiansen, K., E. Kristensen, et al. (2002). "The influence of water column hypoxia on the behaviour of manganese and iron in sandy coastal marine sediment." Estuarine Coastal & Shelf Science **55**(4): 645-654.

Kudela, R. M., J. Q. Lane, et al. (2008). "The potential role of anthropogenically derived nitrogen in the growth of harmful algae in California, USA." Harmful Algae **8**(1): 103-110.

Largier, J. L., C. J. Hearn, et al. (1996). Density structures in low-inflow "estuaries". Coastal and Estuarine Studies. D. G. Aubrey and C. T. Friederichs. **53:** 227-241.

Largier, J. L., J. H. Slinger, et al. (1991). The stratified hydrodynamics of the Palmiet-- A prototypical bar-built estuary. Dynamics and Exchanges in Estuaries and the Coastal Zone. D. Prandle. Washington D.C., American Geophysical Union**:** 135-153.

Lauringson, V. and J. Kotta (2006). "Influence of the thin drift algal mats on the distribution of macrozoobenthos in Koiguste Bay, NE Baltic Sea." Hydrobiologia **554**(97-105).

Lavery, P. S., R. J. Lukatelich, et al. (1991). Changes in the biomass and species composition of macroalgae in a eutrophic estuary, Elsevier. **33:** 1-22.

Lavery, P. S. and A. J. McComb (1991). Macroalgal-sediment nutrient interactions and their importance to macroalgal nutrition in a eutrophic estuary, Elsevier. **32:** 281-295.

Leventhal, J. and C. Taylor (1990). "Comparison of methods to determine degree of pyritization." Geochimica et Cosmochimica Acta **54**: 2621-2625.

Llanso, R. (1991). "Tolerance of low dissolved oxygen and hydrogen sulfide by the polychaete *Streblospio benedicti* (Webster)." Journal of Experimental Marine Biology and Ecology **153**: 165-178.

Lomstein, B. A., L. BonneGuldberg, et al. (2006). "Benthic decomposition of *Ulva lactuca:* A controlled laboratory experiment." Aquatic Botany **85**: 271-281.

Lung, W. S. and S. Bai (2003). "A water quality model for the Patuxent Estuary: current conditions and predictions under changing land-use scenarios." Estuaries **26**(2A): 267-279.

Magni, P., S. Micheletti, et al. (2005). "Relationships between chemical characteristics of sediment and macrofaunal communities in the Cabras Lagoon (Western Mediterranean, Italy)." Hydrobiologia **550**: 105-119.

Mann, K. H. (1988). "Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems." Limnology and Oceanography **33**: 910-930.

Mayer, L. M. (1994). "Surface area control of organic carbon accumulation in continental shelf sediments." Geochimica et Cosmochimica Acta **58**: 1271-1284.

McGlathery, K. (2001). "Macroalgal blooms contribute to the decline of seagrass in nutrient-enriched coastal waters." Journal of Phycology **37**(4): 453-456.

McGlathery, K., K. Sundbäck, et al. (2004). The importance of primary producers for benthic N and P cycling. The Influence of Primary Producers on Estuarine Nutrient Cycling. S. L. Nielsen, G. M. Banta and M. F. Pedersen, Kluwer Academic.

McGlathery, K. J., K. Sundback, et al. (2007). "Eutrophication in shallow coastal bays and lagoons: the role of plants in the coastal filter." Marine Ecology-Progress Series **348**: 1-18.

McLaughlin, K., M. Sutula, et al. (2011). Eutrophication and Nutrient Cycling in Famosa Slough: A Summary of Baseline Data for Monitoring Order R9-2006-0076. Costa Mesa, CA, Southern California Coastal Water Research Project.

McNichol, A., C. Lee, et al. (1988). "Carbon cycling in coastal sediments I: A quantitative estimate of the remineralization of organic carbon in the sediments of Buzzards Bay, Mass." Geochimica et Cosmochimica Acta **52**: 1531-1543.

Middelburg, J. J. and L. A. Levin (2009). "Coastal hypoxia and sediment biogeochemistry." Biogeosciences **6**: 1273-1293.

Middelburg, J. J. and F. J. R. Meysman (2007). "Ocean science-- Burial at sea." Science **316**: 1294-1295.

Middelburg, J. J., K. Soetaert, et al. (1996). "Denitrificationin marine sediments: A model study." Global Biogeochemical Cycles **10**: 661-673.

Miron, G. and E. Kristensen (1993). "Factors influencing the distribution of nereid polychaetes: the sulfide aspect." Marine Ecology Progress Series **93**: 143-153.

Moseman, S., L. Levin, et al. (2004). "Colonization, succession, and nutrition of macrobenthic assemblages in a restored wetland at Tijuana Estuary, California." Estuarine Coastal & Shelf Science **60**: 755-770.

Murias, T., J. Cabral, et al. (1996). "Short-term effects of intertidal macroalgal blooms on the macrohabitat selection and feeding behaviour of wading birds in the Mondego Estuary (West Portugal)." Estuarine Coastal & Shelf Science **43**: 677-688.

Nedwell, D. B., T. D. Jickells, et al. (1999). "Nutrients in estuaries." Advances in Ecological Research **29**: 43-92.

Neinhuis, P. H. (1992). "Eutrophication, water management, and the functioning of Dutch estuaries and coastal lagoons." Estuaries **15**: 538-548.

Neto, J. M., M. R. Flindt, et al. (2008). Modelling nutrient mass balance in a temperate meso-tidal estuary: Implications for management, Elsevier. **76:** 175-185.

Newton, T. J. and M. R. Bartsch (2007). "Lethal and sublethal effects of ammonia to juvenile lampsilis mussels (Unionidae) in sediment and water-only exposures." Environmental Toxicology and Chemistry **26**(10): 2057-2065.

Nezlin, N., K. Kamer, et al. (2009). "Dissolved oxygen dynamics in a eutrophic estuary, Upper Newport Bay, California." Estuarine, Coastal and Shelf Science **82**: 139-151.

Nixon, S. W. (1995). "Coastal marine eutrophication: A definition, social causes, and future concerns." Ophelia **41**(0): 199-219.

Norkko, A. and E. Bonsdorff (1996). "Rapid zoobenthic community responses to accumulations of drifting algae." Marine Ecology Progress Series **131**: 143-157.

NRC (2000). Clean Coastal Waters: Understanding and Reducing the Effects of Nutrient Pollution. C. o. G. Committee on the Causes and Management of Coastal Eutrophication. Ocean Studies Board and Water Science and Technology Board, Environment, and Resources. Washington D.C., National Research Council, National Academy Press.

Onuf, C. P. (2006). "Biofouling and the continuous monitoring of underwater light from a seagrass perspective." Estuaries and Coasts **29**: 511-518.

Osterling, M. and L. Pihl (2001). "Effects of filamentous green algal mats on benthic macrofaunal functional feeding groups." Journal of Experimental Marine Biology and Ecology **263**: 159-183.

Paerl, H. W. (2006). "Assessing and managing nutrient-enhanced eutrophication in estuarine and coastal waters: Interactive effects of human and climatic perturbations." Ecological Engineering **26**(1): 40-54.

Paerl, H. W., J. L. Pinckney, et al. (1998). "Ecosystem responses to internal and watershed organic matter loading: consequences for hypoxia in the eutrophying Neuse River Estuary, North Carolina, USA." Marine Ecology Progress Series **166**: 17-25.

Pearson, T. H. and R. Rosenberg (1978). "Macrobenthic succession in relation to organic enrichment and pollution of the marine environment " Oceanography and Marine Biology Annual Reviews **16**: 229-311.

Peckol, P. and D. Baxter (1986). "Population dynamics of the Onuphid polychaete *Diopatra cuprea* (Bosc) along a tidal exposure gradient." Estuarine Coastal & Shelf Science **22**: 371-377.

Peckol, P. and J. S. Rivers (1995). "Contribution by macroalgal mats to primary production of a shallow embayment under high and low nitrogen loading rates." Estuarine Coastal and Shelf Science **44**: 451-465.

Peterson, D. H., R. E. Smith, et al. (1985). "Interannual variability in dissolved inorganic nutrients in northern San Francisco Bay Estuary." Hydrobiologia **129**(37-58).

Pihl, L., I. Isaksson, et al. (1995). "Recent increase of filamentous algae in shallow Swedish bays: effects on the community structure of epibenthic fauna and fish." Netherlands Journal of Aquatic Ecology **29**(3-4): 349-358.

Porubsky, W. P., N. B. Weston, et al. (2009). "Benthic metabolism and the fate of dissolved inorganic nitrogen in intertidal sediments." Estuarine Coastal and Shelf Science **83**(4): 392-402.

Rabalais, N. N. and D. Harper, Eds. (1992). Studies of benthic biota in areas affected by moderate and severe hypoxia. Proceedings, Workshop on Nutrient Enhanced Coastal Ocean Productivity. College Station, Texas, NOAA Coastal Ocean Program, Texas A&M Sea Grant.

Rabalais, N. N., R. E. Turner, et al. (2002). "Gulf of Mexico hypoxia, A.K.A. "The dead zone"." Annual Review of Ecology and Systematics **33**: 235-263.

Raffaelli, D. (1999). "Nutrient enrichment and trophic organisation in an estuarine food web." Acta Oecologica **20**(4): 449-461.

Raffaelli, D., P. Balls, et al. (1999). "Major long-term changes in the ecology of the Ythan estuary, Aberdeenshire, Scotland; how important are physical factors?" Aquatic Conservation: Marine and Freshwater Ecosystems **9**(2): 219-236.

Raffaelli, D., J. Limia, et al. (1991). "Interactions between the amphipod *Corophium volutator* and macroalgal mats on estuarine mudflats." Journal of the Marine Biological Association of the United Kingdom **71**: 899-908.

Raiswell, R., F. Buckley, et al. (1987). "Degree of pyritization of iron as a palaeoenvironmental indicator of bottom-water oxygenation." Journal of Sedimentary Petrology **58**(5): 812-819.

Redfield, A. C. (1958). "The biological control of chemical factors in the environment." American Scientist **46**: 205-221.

Reise, K. (1977). "Predator exclusion experiments in an intertidal mudflat." Helgol Marine Research **30**: 263-271.

Rickard, D. and J. W. Morse (2005). "Acid Volatile Sulphide (AVS)." Marine Chemistry **97**: 141-197.

Risgaard-Petersen, N. and K. Jensen (1997). "Nitrification and denitrification in the rhizosphere of the aquatic macrophyte *Lobelia Dortmanna L.*" Limnology and Oceanography **42**: 1346-1354.

Rossi, F. (2007). "Recycle of buried macroalgal detritus in sediments: use of dual-labelling experiments in the field." <u>Marine Biology</u> **150**: 1073-1081.

Rossi, F., R. Forster, et al. (2007). "Human trampling on intertidal mudflats: effects on macrofauna biodiversity and population dynamics of bivalves." <u>Marine Biology</u> **151**: 2077-2090.

Rozan, T. F., M. Tallillefert, et al. (2002). "Ironsulphur-phosphorus cycling in the sediments of a shallow coastal bay: implcations for seiment nutrient release and bethic macroalgal blooms." <u>Limnology and Oceanography</u> **47**: 1346-1354.

Russell, M. J. and P. A. Montagna (2007). "Spatial and temporal variability and drivers of net ecosystem metabolism in western Gulf of Mexico estuaries." <u>Estuaries and Coasts</u> **30**(1): 137-153.

Rysgaard, S., P. Thastum, et al. (1999). "Effects of salinity on NH4+ adsorption capacity, nitrification, and denitrification in Danish estuarine sediments." <u>Estuaries</u> **22**(1): 21-30.

Salisbury, J., M. Green, et al. (2008). "Coastal acidification by rivers: a threat to shellfish." <u>EOS Transactions, American Geophysical Union</u> **89**: 513-528.

Salovius, S. and P. Kraufvelin (2004). "The filamentous green alga *Cladophora glomerata* as habitat for littoral macrofauna in the northern Baltic sea." <u>Ophelia</u> **58**(2): 65-78.

Sanchez-Moyano, J., F. Estacio, et al. (2001). "Effect of the vegetative cycle of *Caulerpa prolifera* on the spatio-temporal variation of invertebrate macrofauna." <u>Aquatic Botany</u> **70**(163-174).

Sand-Jensen, K. and J. Borum (1991). "Interactions among phytoplankton, periphyton, and macrophytes in temperate freshwater and estuaries." <u>Aquatic Botany</u> **41**: 137-175.

Seitzinger, S. P. (1988). "Denitrification in freshwater and coastal marine ecosystems: Ecological and geochemical significance." <u>Limnology and Oceanography</u> **33**(4): 702-724.

Sfriso, A., A. Marcomini, et al. (1987). "Relationships between Macroalgal Biomass and Nutrient Concentrations in a Hypertrophic Area of the Venice Lagoon." <u>Marine Environmental Research</u> **22**(4): 297-312.

Sfriso, A., A. Marcomini, et al. (1987). "Relationships between macroalgal biomass and nutrient concentrations in a hypertrophic area of the Venice Lagoon Italy." <u>Marine Environmental Research</u> **22**(4): 297-312.

Short, F. T., G. E. Jones, et al. (1991). <u>Seagrass decline: Problems and solutions</u>. Coastal Zone '91 Conference- ASCE, Long Beach, CA.

Stanley, D. W. and S. W. Nixon (1992). "Stratification and Bottom-Water Hypoxia in the Pamlico River Estuary." <u>Estuaries</u> **15**(3): 270-281.

Sundbäck, K. and K. J. McGlathery (2005). Interaction between benthic macro- and microalgae in the marine environment. <u>Interaction between benthic macro- and microalgae in the marine sediments</u>. E. Kristensen, J. E. Kostka and R. H. Haese, American Geophysical Union.

Sutula, M., K. Kamer, et al. (2004). Sediments as a non-point source of nutrients to Malibu Lagoon, California (USA). Westminster, California, Technical Report #441Southern California Coastal Water Research Project**:** 83.

Sutula, M., K. Kamer, et al. (2006). Sediments as an internal source of nutrients to Upper Newport Bay, California. Westminster, CA, Southern California Coastal Water Research Project**:** 151.

Thiel, M. and L. Watling (1998). "Effects of green algal mats on infaunal colonization of a New England mud flat-long lasting but highly localized effects." <u>Hydrobiologia</u> **375/376**: 177-189.

Thompson, J. K., J. R. Koseff, et al. (2008). "Shallow water processes govern system-wide phytoplnkon bloom dynamics: a field study." <u>Journal of Marine Systems</u> **74**: 153-166.

Thomsen, M. S. and K. McGlathery (2006). "Effects of accumulations of sediments and drift algae on recruitment of sessile organsims associated with oyster reefs." <u>Journal of Experimental Marine Biology and Ecology</u> **328**: 22-34.

Touchette, B. W. and J. M. Burkholder (2000). "Overview of the physiological ecology of carbon metabolim in seagrasses." <u>Journal of Experimental Marine Biology and Ecology</u> **250**: 169-205.

Touchette, B. W. and J. M. Burkholder (2000). "Review of nitrogen and phosphorus metabolism in seagrasses." <u>Journal of Experimental Marine Biology and Ecology</u> **250**: 133-167.

Touchette, B. W. and J. M. Burkholder (2007). "Carbon and nitrogen metabolism in the seagrass, Zostera marina L.: Environmental control of enzymes involved in carbon allocation and nitrogen assimilation." <u>Journal of Experimental Marine Biology and Ecology</u> **350**(1-2): 216-233.

Trainer, V., B. Hickey, et al. (2002). "Biological and physical dynamics of domoic acid production off the Wasington Coast." <u>Limnology and Oceanography</u> **47**(5): 1438-1446.

Tubbs, C. and J. Tubbs (1980). "Wader and Shelduck feeding distribution in Langstone Harbour, Hampshire." <u>Bird Study</u> **27**: 239-248.

Turner, R. E., N. Qureshi, et al. (1998). "Fluctuating silicate: nitrate ratios and coastal plankton food webs." <u>Proceedings of the National Academy of Sciences</u> **95**: 13,048-13.

Turpin, D. H. (1991). "Effects of inorganic N availability on algal photosynthesis and carbon metabolism." <u>Journal of Phycology</u> **27**: 14-20.

Twilley, R. R. (1985). "The exchange of organic carbon in basin mangrove forests in a southwest Florida estuary." <u>Estuarine Coastal and Shelf Science</u> **20**: 543-557.

Twilley, R. R., J. W. Cowan, et al. (1999). Benthic nutrient fluxes in selected estuaries in the Gulf of Mexico. Biogeochemistry of Gulf of Mexico Estuaries. T. S. Bianchi, J. R. Pennock and R. R. Twilley. New York, NY, Wiley-Liss**: 163-209.

Underwood, G. J. C. and J. Kromkamp (1999). Primary production by phytoplankton and microphytobenthos in estuaries. Advances in ecological research estuaries. D. B. Nedwell and D. G. Raffaell. London, Academic Press. **29:** 306.

USEPA (2003). Ambient Water Quality Criteria for Dissolved Oxygen, Water Clarity, and Chlorophyll a for Chesapeake Bay and Its Tidal Tributaries, U.S. Environmental Protection Agency, Washington D.C.

Valiela, I., K. Foreman, et al. (1992). "Couplings of Watersheds and Coastal Waters: Sources and Consequences of Nutrient Enrichment in Waquoit Bay, Massachusetts." Estuaries **15**(4): 433-457.

Valiela, I., J. McClelland, et al. (1997). "Macroalgal blooms in shallow estuaries: Controls and ecophysiological and ecosystem consequences." Limnology and Oceanography **42**(5): 1105-1118.

vanKatwijk, M. M., L. H. T. Vergeer, et al. (1997). "Ammonium toxicity in eelgrass Zostera marina." Marine Ecology Progress Series **157**: 159-173.

Viaroli, P., M. Bartoli, et al. (1996). "Macrophyte communities and their impact on benthic fluxes of oxygen, sulphide and nutrients in shallow eutrophic environments." Hydrobiologia **329**: 105-119.

Viaroli, P., M. Bartoli, et al. (1995). "Oxygen fluxes and dystrophy in a coastal lagoon colonized by *Ulva rigida* (Sacca Di Goro, Po River Delta, Northern Italy)." Fresenius Environmental Bulletin **4**: 381-386.

Viaroli, P., M. Bartoli, et al. (2004). "Biogeochemical indicators as tools for assessing sediment quality/vulnerability in transitional aquatic ecosystems." Aquatic Conservation: Marine and Freshwater Ecosystems **14**: S19-S29.

Viaroli, P., M. Bartoli, et al. (2008). "Community shifts, alternative stable states, biogeochemical controls and feedbacks in eutrophic coastal lagoons: a brief overview." Aquatic Conservation: Marine and Freshwater Ecosystems **18**: S105-S117.

Virnstein, R. (1979). "Predation on estuarine infauna: response patterns of component species." Estuaries **2**(2): 69-86.

Wankel, S. D., C. Kendall, et al. (2006). "Nitrogen sources and cycling in the San Francisco Bay Estuary: A nitrate dual isotopic composition approach." Limnology and Oceanography **51**(4): 1654-1664.

Wazniak, C. E., M. R. Hall, et al. (2007). "Linking water quality to living resources in a Mid-Atlantic lagoon system, USA." Ecological Applications **17 (supplement)**: s64-78.

Welsh, D. T. (2000). "Nitrogen fixation in seagrass meadows: regulation, plant-bacteria interactions and significance to primary productivity." Ecology Letters **3**: 58-71.

Wennhage, H. and L. Pihl (1994). "Substratum selection by juvenile plaice (Pleuronectes platessa L.): Impact of benthic microalgae and filamentous macroalgae." Netherlands Journal of Sea Research **32**(3-4): 343-351.

Wilkerson, F. P., R. C. Dugdale, et al. (2006). "Phytoplankton blooms and nitrogen productivity in San Francisco Bay." Estuaries and Coasts **29**(3): 401-416.

Williams, S. L. (1984). "Decomposition of the Tropical Macroalga Caulerpa-Cupressoides Field and Laboratory Studies." Journal of Experimental Marine Biology & Ecology **80**(2): 109-124.

Woodin, S. A. (1974). "Polychaete abundance patterns in a marine soft-sediment environment: importance of biological interactions." Ecological Monographs **44**(2): 171-187.

Zaldivar, J.-M., A. C. Cardoso, et al. (2008). "Eutrophication in transitional waters: an overview." Transitional Waters Monographs **1**: 1-78.

7. The Utility of the Macrobenthos as an Indicator of Estuarine Eutrophication

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7.1 Introduction

Macrobenthic fauna, or macrobenthos, are invertebrates living on and within the sediments of aquatic waterbodies. Macrobenthos are one of the primary tools used to assess the ecological condition of estuaries and coastal nearshore habitat because 1) they live in bottom sediments, where many stressors accumulate; 2) most macrobenthos are sedentary and therefore reflect the quality of their immediate environment (Pearson and Rosenberg 1978, Dauer 1993, Weisberg et al. 1997); 3) most communities are comprised of a diverse array of species with a variety of tolerances to stress, so the presence or absence of different taxa can provide information about the types of stressors present (Christman and Dauer 2003; Lenihan et al. 2003); and 4) they serve as food sources for many ecologically and economically important estuarine fish and birds (Virnstein 1979, Phil et al. 1992, Gillett 2010). Macrobenthic community-based assessment tools have traditionally been designed to assess overall habitat quality; integrating a variety of anthropogenic stressors (e.g., contaminants, eutrophication, or physical disturbance) while accounting for gradients in natural stressors/environmental conditions (e.g., salinity, sediment type, or depth).

More recently, interest has been increasing in the use of macrobenthos to distinguish and quantify the individual effects of multiple stressors in estuarine and marine environments (e.g., Christman and Dauer 2003, Lenihan et al. 2003). Measuring changes in macrobenthic community structure is thought to be particularly useful for this task, as the typical community is taxonomically diverse (especially at higher taxonomic levels). As a consequence of this diversity, there is an array of living positions, feeding modes, reproductive strategies, and tolerances/responses to different stressors, which will lead to differential responses in the community dominants mirroring changes in stressor concentration/severity. Furthermore, most macrobenthic fauna are relatively non-motile and live for many months to years and as such they are unable to avoid a disturbance like eutrophication, while integrating the exposure to a disturbance for longer periods of time than most planktonic or benthic primary producers (e.g., phytoplankton or microphytobenthos).

The purpose of this Chapter will be to assess the potential of macrobenthic fauna as indicators of eutrophication in California's estuarine waters. Following suit, we will provide a brief review of: 1) the general ecology of macrobenthos; 2) the importance of macrobenthos to the functioning of estuarine ecosystems and maintenance of estuarine beneficial uses; 3) how different components of the macrobenthic community respond to eutrophication and other stressors; and 4) potential macrobenthic-based indicators or metrics sensitive to eutrophication.

7.2 General Ecology of Macrobenthos

Strictly speaking, macrobenthic fauna are an operationally defined group of organisms. Definitions have varied historically, but for the purposes of this Chapter we will refer to the macrobenthos as any organism that would typically be retained on a 500-μm sieve; a definition used by most modern benthic ecologists (Gillett and Schaffner 2009). Given this relatively broad definition, it should not be surprising, that the macrobenthos encompass a large number of phyla. The most common phyla of estuarine macrobenthos include annelids, arthropods, cnidarians, ectoprocts, molluscs, and nemerteans. The biodiversity of estuarine macrobenthic communities is typically dominated by annelids (polychaetes, oligochaetes, and leeches) and arthropods (insects and crustaceans), whereas the biomass is dominated by molluscs (bivalves or gastropods). California spans different biogeographic regions and consequently the particular representatives of the different phyla are going to vary geographically. At present, there is not a comprehensive review of the macrobenthic fauna found throughout California's estuaries, but there are many reviews of the macrobenthos detailing their life history, distribution, and feeding habits (Fauchald and Jumars 1979, Gaston 1987, Sanchez-Mata et al. 1993, Gillett and Schaffner 2009) that are applicable to the macrobenthos of California.

The distribution of different macrobenthic taxa within an estuary is controlled by a number of biotic and abiotic factors, though the primacy of one versus the other appears to vary from system to system (Snelgrove and Butman 1994, Levinton and Kelaher 2004, Fleeger et al. 2008, Dethier 2010). Ultimately the regional larval pool determines what species populate and area, but the successful recruitment and growth of individuals (ignoring habitat quality and anthropogenic perturbations for the moment) is influenced post-settlement; primarily by sediment composition and salinity (Dethier 2010). Salinity affects the osmotic balance and ion regulation of most aquatic organisms. Although some benthic organisms have a wider salinity tolerance than others, there are very few species are capable of maintaining physiological function over the range of salinities observed in most estuaries (Kinne 1971). These physiological constraints create distinctive communities along the length of an estuary as the relative mixing of marine and freshwater changes (Boesch and Rosenberg 1981, Holland et al. 1987, Ranasinghe et al. 2010). Imposed upon salinity constraints are patterns in macrobenthic community composition related to sediment composition (i.e., silt, clay, and sand composition; e.g., Diaz and Schaffner 1990, Snelgrove and Butman 1994, Gillett and Schaffner 2009). Sediment preferences among the macrobenthos are largely related to living position and feeding mode. Sediments composed of smaller grain size particles tend to be dominated by mobile, non-selective deposit feeders that ingest sediment as they burrow (Lopez and Levinton 1987, Rice and Rhoads 1989). Sandier sediments tend to be dominated by filter feeders with passive collection mechanisms or limited ability to sort particles. In silt/clay dominated sediments, these types of feeding apparatus can be easily clogged the small sediment particles (Rice and Rhoads 1989).

The different species of the macrobenthic community can also be classified into functional groups based upon motility, living position in relation to the substrate-water interface, and their feeding habits. This approach, which we believe has a greater ecological relevance than taxonomic distinctions, typically spans taxonomic differences, with species from distinctly different phyla grouped together, but con-familial species potentially separated into different feeding or living position groups. Most macrobenthic

fauna are not nearly as mobile as nektonic finfish and crustaceans and are generally considered to be sessile organisms, but there are degrees of motility represented throughout the community. Truly sessile fauna are unable to actively change their location, often attached to the substrate onto which they settle from a mobile, larval stage (e.g., barnacles, oysters, hydrozoans). Mobile macrofauna in contrast, are able to move themselves through or along the surface of the sediment via peristaltic (e.g., nemerteans, or glycerid polychaetes) or ambulatory movement (e.g., xanthid mud crabs or isopods). Most macrobenthos, however, would be considered somewhere between the two endpoints: species that are able to move over small spatial scale in the sediment (e.g., California soft shell clam – Cryptomya californica, spionid polychaetes – Streblospio benedicti, or gammarid amphipods – Ampelisca abdita).

Macrofauna are typically also grouped by their living position in relation to the sediment. Epifauna are those organisms that live on or attached to the surface of the substrate (e.g., snails – Cerethidia spp. or tunicates – Molgula spp.). Demersal fauna are those fauna capable of swimming through the water column, though they spend considerable time associated with the bottom (e.g., mysids – Neomysis spp.). Infaunal macrobenthos are those organisms that live within soft sediments in singular tubes/burrows (e.g., Diopatra splendidissima or Corophium acherusicum), a gallery of connected burrows (e.g., nereid polychaetes – Neanthes arenaceodentata or capitellid polychaetes – Mediomastus californiensis), or actively burrowing through the sediment (e.g., tubificid oligochaetes – Tubificoides brownae, nemerteans –Tubulanus spp.). Infauna can be further subdivided by the depth in the sediment they live: interface fauna – those organisms that extend appendages or portions of their body into the water column or on the sediment surface (e.g., echiurians – Listriolobus spp., cumaceans –Diastylis spp.); shallow fauna that live relatively close to the sediment surface (<5 cm; e.g., polychaetes – Tharyx spp., or Polydora cornuta); and deep-dwelling fauna that live, in part at least, below (>5-cm) the sediment surface (e.g., maldanid polychaetes – Paraxillella affinis pacifica, or bivalves – Tagelus californianus).

The last common way to categorize the different species of macrofauna is into feeding guilds, which often combines both the method of food acquisition and living position. Most benthic ecologists recognize a number of different feeding guilds (Fauchald and Jumars 1979, Gaston 1987, Sanchez-Mata et al. 1993, Gillett and Schaffner 2009). Filter-feeders – those organisms that actively or passively remove food particles from the water column, which may or may not expose feeding appendages, such as palps, siphons, or tentacles above the sediment surface in the process of feeding; Grazers – typically motile organisms, which consume microphytobenthos or settled phytoplankton from the sediment surface; Interface-feeders – those relatively non-mobile organisms that are capable of feeding upon organic matter collected from the water column or on the sediment surface, many of which are capable of switching between the two depending upon hydrodynamics and food availability (Pohlo 1982, Taghon et al. 1980). This latter group can be subdivided into a bivalve component that are relatively large and can live deep in the sediment with siphons extending to the surface (e.g., Hines and Comtois 1985, Seitz et al. 2001) and a non-bivalve component that is typically smaller and living close to the sediment-water interface; Deposit-feeders – mobile or sessile fauna that feed upon microorganisms and organic matter in the sediment. This group can be subdivided into shallow deposit-feeders that typically live in the top

few centimeters of the sediment and deep deposit-feeders that live deeper in the sediment; Carnivores – relatively large, mobile organisms that feed solely upon meiofauna (benthic fauna between 500 μm and 63 μm) and other macrofauna; and Omnivores – relatively large, mobile fauna that feed upon other organisms, microphytobenthos, and sediment organic matter.

The community structure of macrobenthic infauna has been used as an indicator of ecosystem health and environmental stress for a number of years in a variety of estuarine habitats around the United States, including the US EPA Environmental Monitoring and Assessment Program (EMAP), National Coastal Assessment (NCA), Chesapeake Bay Benthic Monitoring Program, Southern California Bight Regional Monitoring Program, California Sediment Quality Objective, and internationally the European Union Water Framework Directive (WFD). Macrobenthic infauna and various aspects of their community structure are used in environmental monitoring programs because: 1) most infauna are relatively sessile in comparison to nekton and epifauna so they are unable to escape or avoid any stressors; 2) they are relatively long lived (months to years) compared to most aquatic 1° producers, integrating stressors over their lifespan; 3) they are more taxonomically diverse than any other non-microbial component of estuarine ecosystems and therefore encompass a variety of tolerances to a variety of stressors (Bilyard 1987, Warwick 1988, Dauer 1993, Weisberg et al. 1997, Casazza et al. 2002). Within the macrobenthos, there are a variety of aspects that can be used in environmental assessment, including individual responses (e.g., condition indices, cellular bioindicators, or contaminant loads; Ringwood and Keppler 1998, O'Conner 2002, Brylawski 2009), as well as community-level responses (e.g., abundance of sensitive/tolerant taxa, community composition changes; Weisberg et al. 1997, Borja et al. 2000, Smith et al. 2001, Llansó et al. 2002).

The macrobenthic infauna are also valuable tools for environmental assessment in estuaries because, in the absence of long-term hypoxic conditions (e.g., main-stem Chesapeake Bay), they can be found throughout all soft sediment habitats found in estuaries; from euhaline sandy sediments through tidal freshwater muds or from deep subtidal waters through the littoral zone. These macrobenthic communities, however, are not uniform across these gradients in physical habitat, with unique communities in each salinity and sediment regime (e.g., Sanders 1958, Holland et al. 1987, Attrill and Rundle 2002, Ranasinghe et al. 2010). Consequently, assessment tools developed to work across the entire spectrum of estuarine habitats use a categorical approach to assessment of condition that uses different aspects of community structure and/or different thresholds of community characters for the different salinity zones – typically following the Venice classification scheme (International Association of Limnology 1958) – and sediment types – typically either sands or muds – found in an estuary (e.g., Weisberg et al. 1997, Van Dolah et al. 1999, Llansó et al. 2002). Sub-dividing an estuarine system into different habitat classes for assessment helps to ensure that appropriate reference expectations and community character thresholds are used. For example, it would be inappropriate to expect a community from mesohaline-mud to ever have the diversity and community structure of a polyhaline-sand habitat; therefore different diversity expectations would have to be set if these disparate habitats were to assessed and compared equitably (Remane and Schlieper 1971, Boesch 1977, Attrill 2002).

The lower salinity (<5 psu) portions of estuaries are notoriously difficult systems for the application of macrobenthic community changes in assessing habitat quality due to the salinity fluctuations and high

turbidity, which act as stressors to the community, as well as the pervasive human perturbations typically found there throughout the United States and Europe (Draheim 1998, Alden et al. 2002, Attrill 2002, Diaz et al. 2004). Estuarine habitats that encompass a large amount of intertidal area additionally problematic, because at low tide the air exposure can create desiccation and large fluctuations in temperature that can impact community diversity, abundance, and biomass in comparison to adjacent subtidal habitats (Van Dolah et al. 2000, Holland et al. 2004). Considering these difficulties and a comparative lack of data in low salinity and intertidal habitats, the bulk of the discussion on the impacts of eutrophication on the macrobenthos will be constrained to subtidal habitats in higher salinities (>10 PSU).

7.3   Linkage of Macrobenthos to Ecosystem Function and Estuarine Beneficial Uses

Macrobenthos play a critical role in the biotic and abiotic functioning of the estuary; thus a diverse, fully functional macrobenthic community is an essential part of maintaining ecosystem services and related estuarine beneficial uses. The State of California has designated six "Estuarine Beneficial Uses" upon which to evaluate the estuarine natural resources (structure) and ecosystem services (function) (Chapter 2). These beneficial uses broadly address biodiversity and threatened/endangered species (rare [RARE], spawning [SPWN], and migratory [MIGR] uses), commercially valuable resources (commercial [COMM], shellfish [SHELL], and aquaculture [AQUA] uses), and the inherent value estuarine habitat for aquatic life (estuarine [EST] and wildlife [WILD] uses). The structure and function of the macrobenthic community encompass: 1) their contribution to estuarine and marine biodiversity; 2) direct recreational and fisheries harvest; 3) a food resource for a variety of estuarine aquatic life forms, including fish, birds, marine mammals; 4) a critical role in the maintenance of water column and sediment biogeochemical cycling; and 5) the consumption of a variety of organic matter sources and subsequent regeneration of nutrients to the water column.

From the estuarine beneficial use perspective, macrobenthos are part of diversity of aquatic life and as such a direct measure of EST beneficial uses. The State of California has recognized the intrinsic value of macrobenthos and as such, is currently developing a biocriteria program that includes macrobenthos as a primary indicator of aquatic life in streams (J. Bishop, SWRCB, Pers. Comm.). Development of macrobenthic-based assessment tools for California's estuaries will provide the State the same opportunity to establish biocriteria in estuaries.

In terms of commercial value, many species of macrobenthos are directly harvested (e.g., oysters, mussels, clams, shrimp, and lobsters) by humans, which would be classified as COMM and SHELL uses. Within California, commercial shellfish harvest represented approximately $100 million in fisheries landings in 2008 (NMFS pers. comm.), in addition to the creation of jobs and revenue related to harbor infrastructure, seafood processing and distribution, and tourism. The harvest of macrobenthos also provides recreational value. Beyond their direct commercial value, the macrobenthos provide an important source of food for estuarine and marine fish, birds and marine mammals (EST), including migratory fish and marine mammals (MIGR), spawning fish (SPAWN), and threatened/endangered species of fish and birds (RARE). Numerous commercially important nekton (e.g., Embiotaca jacksoni, Umbrina rancador, or Hypsopetta guttulata) from California's estuaries are dependent upon the

macrobenthos as a food source and thus provide indirect support for COMM beneficial uses (Allen et al. 2006).

The macrobenthos play a key role in sediment nutrient and contaminant cycling through bioturbation and bioirrigation (the mixing of sediment and advective exchange of sediment pore waters with surface waters) and thus are a key component of maintenance of good estuarine and marine habitat and water quality (EST and MAR). Active burrowing and the building of tubes or galleries in the sediment increases the penetration of oxygen into the sediment and the surface area of oxic/anoxic sediment horizons, which can enhance coupled nitrification/denitrification and ultimately remove nitrogen from the estuary (Aller 1982, Pelegrí et al. 1994, Mayer et al. 1995, Aller and Aller 1998). As infauna ventilate their burrows and tubes, there is an increase in the flux of pore water through the sediment and the exchange of porewater with overlying waters, which will carry dissolved nutrients and organic matter with it (Michaud et al. 2005, Michaud et al. 2006). In estuarine systems where the mixed layer extends to the bottom, filter-feeding benthos will enhance benthic-pelagic coupling by collecting water column production and depositing waste products at or below the sediment surface (Graf 1992, Gerritsen et al. 1994, Thompson and Schaffner 2001). Analogously, head-down deposit-feeders feed on bacteria and organic matter centimeters below the sediment surface and deposit waste at the surface, which exposes and recycles organic matter back to the water column (Lopez and Levinton 1987, Clough and Lopez 1993, Levin et al. 1997). Therefore, macrobenthos play an important role in processing organic matter, recycling nutrients, and sequestering contaminants, all of which support healthy estuarine and marine habitat.

Finally, from the biotic, food web perspective, a healthy, well-developed macrobenthic community consists of a diverse array of trophic levels and feeding guilds that utilize the variety of organic matter produced or deposited in the shallow waters of estuaries (e.g., Diaz and Schaffner 1990, Fauchald and Jumars 1979, Gaudênci and Cabral 2007). Much of this production though (e.g., microphytobenthos, bacteria/detritus, phytoplankton) is not directly available to these transient fauna. Macrofauna however, can directly consume most types of bacterial or primary production and via their own somatic growth, accumulate the energy and material in a form that can be consumed by fish or birds (Levin 1984, Iwamatsu et al. 2007, Neuman et al. 2008). In this respect, the macrobenthos serve as a conduit for the transfer of carbon from bacterial and primary production to higher trophic levels in estuaries, most of which cannot directly consume all of these types of organic matter (Gillett 2010). Thus macrobenthos play a key role in transfer of energy and carbon to higher trophic levels, a key ecosystem function.

7.4   Response of Macrobenthos to Eutrophication

7.4.1   Nutrient Loading, Eutrophication and Macrobenthos

Excessive amounts of nutrients that lead to excessive amounts of primary production and eutrophication typically do not have direct impacts on macrobenthic fauna, with the exception of harmful algal blooms (HABs; e.g., Anderson et al. 2002). Eutrophication primarily affects the macrobenthos via microbially mediated, indirect paths of water column hypoxia/anoxia or the

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accumulation of toxic reduced sulfides and ammonia in the sediment. As heterotrophic microbes consume the organic matter from the primary producers, oxygen is removed by aerobic microbes and reduced compounds are created as metabolic byproducts. Even in natural, non-eutrophic conditions, these processes occur in both muddy and sandy sediment environments and the fauna that live there are adapted to deal with low-oxygen, reducing environments. As the amounts of organic matter produced and accumulated in the system increases, low oxygen and reduced conditions begin to expand and either smother or poison the benthic fauna. These processes lead to progressive changes in the abundance, biomass, and composition of the macrobenthic community (Figure 7.1) and eventually lead to azoic conditions. We hypothesize that by looking at trajectories and magnitudes of these changes in macrobenthic communities, one should be able to distinguish between the effects of organic matter accumulation in the sediment/hypoxia compared to changes brought about by other common estuarine stressors (e.g., contaminants, physical disturbance, or salinity fluctuation).
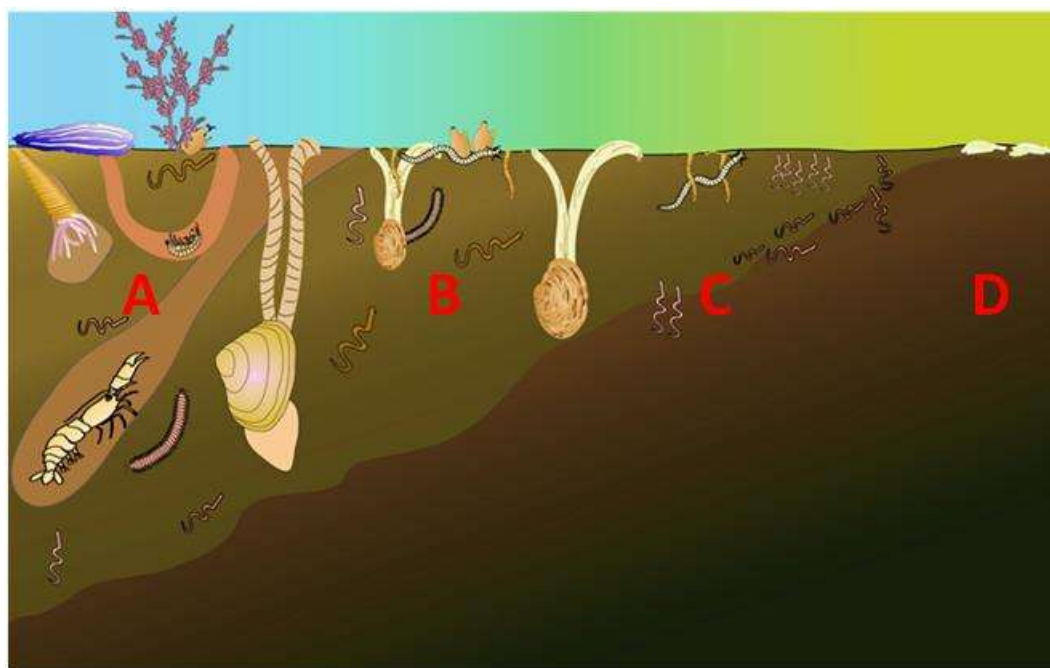
Figure 7.1. An illustration of the Pearson-Rosenberg (1978) conceptual model depicting changes in macrobenthic community structure with increasing eutrophication and organic matter accumulation in the sediment. For discussion purposes, the model has been subdivided to highlight four primary condition categories associated with such increases: A – Non-eutrophic, B – Intermediate Eutrophication; C – Severe Eutrophication; and D - Anoxic bottom water and azoic sediments.

7.4.2   Effects of Hypoxia

Most of the information detailing the response of macrobenthic fauna to eutrophication is related to the effects of low-oxygen (i.e., hypoxia or anoxia) on macrobenthic communities. Benthic sediments in estuaries are predominantly low-oxygen environments because of the large amounts of organic matter and large number of heterotrophic microbes there. As a consequence, most benthic fauna have evolved to deal with those conditions (Pearson and Rosenberg 1978, Hargrave et al. 2008), but hypoxic or anoxic

conditions in the overlying water can be an important factor structuring the composition of an ecosystem (e.g., Rosenberg et al. 1991, Diaz and Rosenberg 1995, Baustin and Rabalais 2009, Seitz et al. 2009). The excess organic matter produced under eutrophic conditions leads to hypoxic conditions when the organic matter is metabolized by heterotrophic organisms under conditions that do not allow for the replenishment of oxygen to the water column (Diaz and Rosenberg 1995, Hamden and Jonas 2006, Kemp et al. 2009).

The response of the macrobenthic community to hypoxic conditions is primarily negative. Increases in frequency and duration of hypoxic (<2.0 mg O_2 L^{-1}) or anoxic (<0.5mg O_2 L^{-1}) conditions lead to reduced community diversity, biomass, and productivity and eventually complete absence of macrofauna (Gray et al. 2002, Rakocinski 2009, Seitz et al. 2009). The degree of the response in these broad, community attributes and the trajectory of community changes from a species composition perspective will vary, depending upon the severity and duration of hypoxic conditions. Tolerance to low oxygen conditions varies widely among the taxonomically diverse macrobenthic community, though persistent anoxic conditions will eventually kill all metazoans (e.g., main stem Chesapeake Bay, Gulf of Mexico, coast of Oregon); Holland et al. 1977, Diaz and Rosenberg 2008, Rabalais et al. 2010). Among the most common types of estuarine macrofauna, crustaceans and gastropods are typically the most sensitive (LT_{50} anoxia <1 d), annelids the most tolerant (LT_{50} >5 d), and bivalve molluscs in-between, as different species have differing capabilities of sealing themselves off to the environment and waiting for better conditions (Llansó 1992, Sagasti et al. 2001, Gray et al. 2002, Calle-Delgado 2006).

Water column hypoxia can also have indirect effects on macrobenthic survival and community structure by altering behavior that increases the risk of being preyed upon. As oxygen concentrations in the benthic boundary layer decline, many species of infauna will start to move closer the sediment surface in an effort to extend appendages or siphons further up into the water column in search of oxygenated water (Rosenberg et al. 1991, Llansó 1992, Long et al. 2008). Eventually, continued exposure to low oxygen forces many infaunal species from the sediment entirely and they remain moribund on the sediment surface, which greatly increases their exposure to predation by benthivoric nekton (Nestlerode and Diaz 1992, Pihl et al. 1992, Seitz et al. 2003, Powers et al. 2005).

7.4.3   Effects of Increased Sediment Organic Matter Accumulation

Eutrophic conditions do not always lead to hypoxia and anoxia, but can still have impacts on the macrobenthic community of estuaries. Hypoxic conditions are, in part, a function of water column stratification and water residence time (Diaz and Rosenberg 1995, Hagy et al. 2004, Kemp et al. 2009) and many of California's estuaries that are always connected to the open ocean are not always prone to the formation of chronic hypoxic bottom waters. As such, understanding the effects of non-hypoxic eutrophication on the macrobenthos will be particularly relevant to California's estuaries.

Almost every modern work on the effects of eutrophication and the accumulation of organic matter on benthic fauna is based upon the conceptual model of Pearson and Rosenberg (1978). This paper summarizes one of the central tenets of benthic ecology: that there are relatively consistent and predictable changes in macrobenthic community structure with increasing accumulation of organic

matter in marine sediments (Figure 7.1). In short, the model proposes that: 1) under normal, non-eutrophic conditions, a benthic community should be composed of a trophically and functionally diverse array of species that span different body sizes and lifespans, as well as live at various depths through the sediment, often extending 10's of cm below the sediment-water interface[10]; 2) as organic matter begins to accumulate in the sediment and there will be changes in the community, shifting towards a less diverse community composed of smaller fauna with relatively short lifespans living near the sediment surface; until 3) eventually the sediments are devoid of macrofauna and are covered in mats of sulfur-oxidizing bacteria (i.e., Beggiatoa; Figure 7.2).
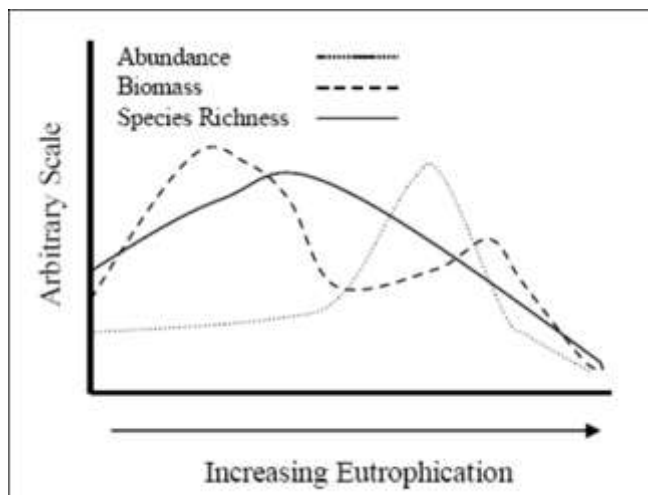
Figure 7.2. Conceptual patterns of abundance, biomass and species richness with increasing eutrophication. After Pearson and Rosenberg (1978).

Pearson and Rosenberg (1978) did not focus on low oxygen conditions in the water column above benthic communities, but on changes in the community structure were driven by the complex suite of biogeochemical processes in occurring in the sediments of marine and estuarine ecosystems experiencing eutrophication. All estuarine sediments receive oxygen via diffusion from the overlying water column (or from benthic autotrophs in shallow waters), so oxygen and oxidative processes will only penetrate relatively short distances, depending upon the grain size and porosity of the sediment, sediment mixing, as well as other hydrologic factors. As noted earlier, the presence of benthic infauna will typically enhance the depth of oxygen penetration due to tube building/ventilating and bioturbation. As a system becomes more eutrophic and organic matter begins to accumulate at greater rates in the sediment, bacterial production is stimulated and the demand for oxygen outstrips the rates of diffusion. This leads to anoxic, reducing processes dominating in formally oxygenated sediments,

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[10] In practice, this kind of community should only be expected in relatively high salinity environments (>10-15 PSU) with relatively little salinity fluctuation. The premise of community change is still appropriate in lower, more variable salinity environments, but the baseline community will likely be less trophically diverse and more tolerant of environmental stressors than higher salinity communities, as detailed in our subsequent discussion of the so called "estuarine quality paradox".

which leads to a variety of bacterial metabolic pathways that produce byproducts (primarily sulfide and ammonia in saline sediments) that are toxic to most metazoans (Pearson and Rosenberg 1978, Jørgensen 1996, Gray et al. 2002, Hargrave et al. 2008). These compounds and the reducing environment of the sediments are thought to be the mechanism behind the mortality leading to changes in community structure. Many of the species that are community dominants in disturbed habitats are always present at low densities and presumably at a competitive disadvantage to non-disturbed community dominants. Only when the non-disturbed dominants die off, are there available resources that allow tolerant fauna to flourish (e.g., Gillett et al. 2007).

As noted earlier, Pearson and Rosenberg (1978) only put forth a conceptual model (P-R model, hereafter); summarizing data to support their assertions, though not explicitly forming and testing any hypotheses (Gray et al. 2002). One of the first widespread codifications of this model for community change was proposed by Warwick (1986), comparing the k-dominance or cumulative-frequency curves of community abundance and biomass and their relative position to each other (referred to as Abundance-Biomass Comparisons [ABC]). If abundance plots above biomass (Figure 7.3a), it is indicative of a degraded community populated by large numbers of small organisms, if biomass plots above (Figure 7.3b), it is indicative of a non-degraded community where there are fewer, large-bodied organisms (implicitly long-lifespan is inferred), and if the plots cross (Figure 7.3c), it is indicative an intermediately degraded or transitional community. The difference in position between the abundance and biomass curves can be numerically quantified as W (below) (Clarke 1990) [S = total number of species, B = biomass of taxa *i* (% of total), and A = abundance of taxa *i* (% of total)], which allows for quantification of habitat quality beyond the good, bad, or intermediate classification of Warwick (1986).
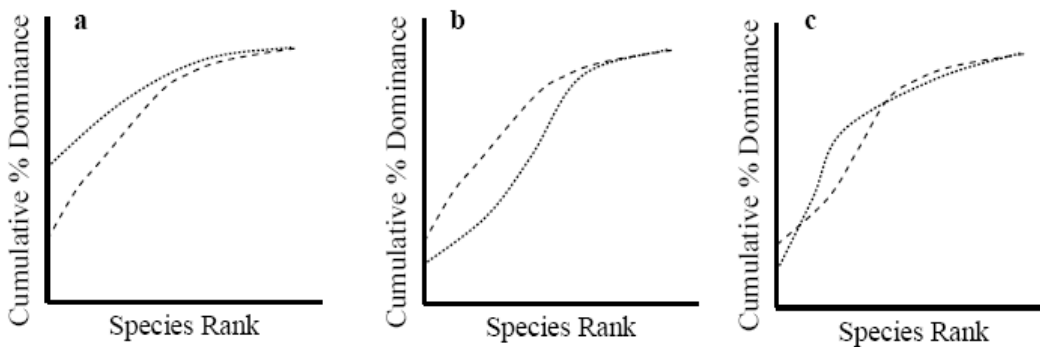
$$W = \sum_{i=1}^{S} (B_i - A_i)/(50\ S - 1\ )$$

Figure 7.3. Conceptual abundance-biomass (ABC) curves from degraded (a), non-degraded (b), and moderately degraded (c). Biomass is represented by the dashed line and abundance by the dotted line. After Warwick (1986).

The underlying assumptions in this assessment approach come directly out of the P-R model: that along a gradient of organic enrichment, a residential macrobenthic community will shift from one populated by larger, longer-lived species to that dominated by smaller, shorter-lived species. This characteristic interplay of community biomass and abundance, however, cannot be solely attributed to eutrophication and organic enrichment of a system. Physical disturbance and temporal successional dynamics in soft sediment benthic habitats can produce the same patterns in community abundance and biomass (Warwick et al. 1987; Beukema 1988), as many early successional or opportunistic species of estuarine macrobenthos exhibit the classic r-selected lifestyle of small body size, high reproductive output, and short lifespan (e.g., Levinton 1982; Zajac and Whitlach 1982a,b). That said, these processes should, depending upon the frequency of the disturbance, yield abundance-biomass patterns that change relatively quickly through time (e.g., Wilbur et al. 2008, Schaffner 2010) than those produced by systemic eutrophication, which produces a more persistent, impact leading to a long-term recovery (e.g., Rosenberg 1976, Borja et al. 2006, Diaz et al. 2008).

One of the drawbacks to the ABC method is the inability to distinguish between types and intensities of disturbance (i.e., eutrophication vs. physical or chemical disturbance; Warwick et al. 1987, Beukema 1988, Warwick and Clarke 1994). The root of this problem is because the ABC method does not account for the taxonomic composition of the benthic community and how it changes with different types of disturbance (Warwick and Clarke 1994). The P-R model not only focuses on the change in the biomass and abundance distributions of the macrobenthic community, but also the species that represent those changes. The physiology and life history differences among different macrobenthic taxa make some particularly sensitive or tolerant to changes in sediment chemistry that accompany eutrophication and excessive organic matter build-up in the sediment. Lists of sensitive and tolerant taxa from different locales have been compiled (e.g., Pearson and Rosenberg 1978, Gray et al. 2002) across the Northern Hemisphere and there can be some generalizations of these groups of species across habitats, but the identification of indicative taxa is likely a region-specific exercise.

Quantitative comparisons of the macrobenthic community across a gradient of organic matter enrichment that include information on abundance, biomass, and species composition are a more appropriate test of the P:R model. Weston (1990) looked at changes in polychaetes along a gradient of organic enrichment related to salmon-pen aquaculture. There were changes in feeding guild composition of the polychaete assemblage progressing from dominance by subsurface deposit-feeders in the most enriched sediments to a diversity of feeding guilds (i.e., carnivores, filter-feeders, herbivores) in non-enriched ambient sediments. This shift was even more pronounced when the biomass of the different feeding guilds was considered (Weston 1990). Lu and Wu (1998) and Edgar et al. (2005) also have demonstrated a very similar pattern when looking at the entire macrobenthic community. Sensitive taxa like echinoderms and crustaceans were much less abundant in the organically enriched sediments under aquaculture operations, while tolerant, deposit-feeding taxa (e.g., capitellid polychaetes or gastropods) dominated the community. In ambient sediments away from the aquaculture operations, the macrobenthic community was much more taxonomically diverse and had very few tolerant taxa present (Edgar et al. 2005). Similarly, Smith et al. (2001) used a multivariate approach to quantify stressor tolerance among the macrobenthos across a known pollution gradient

from sewage outfalls in the coastal waters of California. Using this approach allowed for the ranking of tolerance to pollution of a large number of the dominant fauna in the system that could be used to determine if a given sample was disturbed or not. Subsequently, this approach and the assignment of tolerance scores were applied to coastal bays and harbors of California and is a component of state-wide sediment quality assessment (Bay et al. 2009, Ranasinghe et al. 2009).

Most of the discussion presented so far, and the historical thread through the literature (summarized in Nixon 1995; Grall and Chauvaud 2002; Gray et al. 2002; Hyland et al. 2005), focuses on the negative impacts of eutrophication on biological resources. There has been recent interest, however, in how eutrophication can have both positive and negative effects on the functioning of macrobenthic communities in estuarine ecosystems (e.g., Beukema and Cadée 1997, Nixon and Buckley 2002, Rakocinski and Zapf 2005, Gillett 2010). By definition, eutrophication typically leads to an increase in the primary production of a system and this represents an increase in food availability for primary consumers, which has been linked to increases in benthic production, as well as fisheries yields (Nixon and Buckley 2002, Breitburg et al. 2009, Nixon 2009). This has been termed an "agricultural" model of eutrophication (Nixon and Buckley 2002), where increased nutrient inputs to an estuary stimulate overall system productivity like farmers apply fertilizer to their fields (e.g., Orr 1947, Sardá et al. 1996). The few examples of agricultural-style enrichment that have been illustrated, however, must be kept in the context of their ecosystem and of the fauna that were studied (Beukema and Cadée 1997, Nixon 2009, Gillett 2010), as well as the predominantly negative record of eutrophication on the structure and functioning of ecosystems.

Rakocinski and Zapf (2005) put forth a conceptual model of changes in macrobenthic function with increasing eutrophication that incorporates both the positive and negative aspects of eutrophication on benthic communities (Figure7.4). The predicted bi-directional response of the macrobenthic community is built upon aspects of the Pearson and Rosenberg (1978) model and intermediate disturbance theory (e.g., Connell 1978). In this model, as a system begins to become eutrophic, there is an increase in rate of macrobenthic function. This increase is related to increases in primary production, which provide a release from food limitation for existing fauna (e.g., Marsh and Tenore 1990, Sterner et al. 2002, Brylawski 2008), as well as beginning to alter the sediment biogeochemistry, allowing for the eutrophication-tolerant taxa to increase their proportion within the community before the sensitive taxa are severely impacted. As the degree of eutrophication progresses, the model predicts that there will be decline in community function due to the negative aspects of organic matter accumulation (reduced O_2 penetration and the build-up of toxic reduced compounds) outweighing the benefits of additional food. Consequently, the composition of the macrobenthic community changes, following the classic pattern of habitat degradation described in the preceding paragraphs.
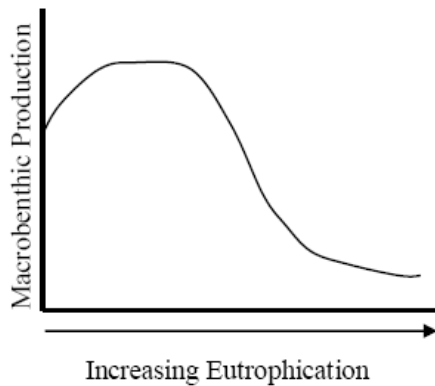
Figure 7.4. Conceptual relationship between macrobenthic production and eutrophication. After Gillett (2010).

This concept was further expanded upon by Gillett (2010), who tested the model and considered the response of macrobenthic community function (2° production) to increasing eutrophication in the context of different sub-habitats within estuaries and the macrofauna that live there. As conceptualized in Rakocinski and Zapf's (2005) model, Gillett (2010) showed an initial increase in secondary production with increasing eutrophication, followed by a decline in production as eutrophic conditions continued to intensify. There are two aspects of this eutrophication pattern that bear further detail: 1) macrobenthic community composition is important – the positive aspects of eutrophication (i.e., the fertilization effect) most strongly affected filter- and interface-feeding fauna that could directly utilize the increases in phytoplankton production in the water column (Gillett 2010), whereas other types of benthic fauna remained unchanged until the negative aspects became dominant; and 2) the benthic-pelagic setting is important – sandy, non-depositional habitats appear to have a greater buffer to eutrophication and the macrobenthic communities there experience negative impacts slower than their counterparts from depositional habitats, where the sediments are naturally rich with organic matter. These depositional habitats can be quickly oversaturated with organic matter and therefore habitat quality will start to degrade with only a small increase in eutrophication (Molinaroli et al. 2009).

The concepts of eutrophication having positive benefits to the macrobenthic community, while still representing a change in ecosystem condition from reference, has been incorporated into a small number of environmental monitoring programs that utilize the macrobenthos as their assessment tool (Chesapeake Bay Program – Weisberg et al. 1997; Mid-Atlantic US – Llansó et al. 2002; European Water Framework Directive – Lavesque et al. 2009). In these indices, which are largely built upon the Pearson and Rosenberg (1978) paradigm, macrobenthic abundance and biomass do not have a simple, positive linear relationship with habitat quality. Instead, they have a concave, unimodal relationship to habitat quality, where a sample can be assessed as degraded for having too much or too little biomass/abundance (Weisberg et al. 1997). It should be noted, however, that these indices were developed to assess overall habitat quality or integrity, not individual stressors on the macrobenthic community. However, there has been some work in recent years to use specific aspects of the macrobenthic community to assess multiple stressors impacting ecosystem quality. Christman and

Dauer (2003) and Dauer et al. (2000) were able to detect the differential response of the macrobenthic community in Chesapeake Bay to low oxygen stress and chemical contaminant stressors by looking at variation in benthic multi-metric index (Chesapeake Bay B-IBI [Weisberg et al. 1997]) scores in relation to environmental conditions. Furthermore, Dauer et al. (2000) were able to relate index score to different types of watershed development (urban, agricultural, and forested) and local water/sediment quality. Of greater to the importance of the situation in California's estuaries (i.e., limited hypoxia), Lenihan et al. (2003) were able to differentiate macrobenthic community responses to either organic matter enrichment or heavy metal contamination. In this study, they showed positive responses among annelids (i.e., increases in abundance and biomass) with organic matter enrichment, even when combined with increasing concentrations of heavy metals. Conversely, echinoderms had slightly positive responses to organic enrichment, but declined when exposed to heavy metals and arthropods declined with increased exposure to both types of stressor (Lenihan et al. 2003). This type of differential response by separate components of the macrobenthic community to different stressors could be used to delineate eutrophic impacts from the mix of co-occuring stressors typically found in estuarine ecosystems.

As we have presented, there are a number of reasonable conceptual models and experimental data to describe how eutrophication alters the composition and functioning of macrobenthic communities. Using the macrobenthic community for detection and quantification of eutrophic conditions in estuarine systems is, however, complicated. Most water bodies that experience eutrophication are also subject to a variety of other stressors (e.g., chemical contamination or physical disturbances) that have been shown to effect macrobenthic community structure (USEPA 2008). There is a wide array of different chemicals that that accumulate in estuarine sediments, including organic compounds, heavy metals, pesticide, pharmaceuticals (Sanger et al. 1999a,b; Kennish 2002). Many of the these chemicals can have toxic effects on the macrobenthos at the community level, reducing the number of sensitive taxa and overall community species richness, but without the potentially positive effects that the extra organic matter from eutrophication can create (Peterson et al. 1996, Gaston et al. 1998, Dauvin 2008). Additionally, the effects of many contaminants are taxonomically specific (organotins and gastropods, pesticides and crustaceans, or metals and annelids; Rand et al. 2000, Valiela 1995). Because of the diversity of chemical contaminants in estuarine sediments, there are not any generalized models of contaminant-driven changes in community structure like the P:R model. An overall loss in community diversity and disproportionate mortality among sensitive taxa with increasing chemical contamination should be expected (Peterson et al. 1996, Rakocinski et al. 1997, Gaston et al. 1998), but the impact on community abundance and biomass is unknown. That said, abundance and biomass should not increase, as they can with non-hypoxic eutrophication.

As alluded to earlier, physical disturbance of the benthic habitats, either natural (large storms or ice scour) or anthropogenic (dredging or benthic trawling), can have important influences on the structure of the macrobenthic community. These types of disturbance can defaunate a habitat and the recovery of the community will occur in a relatively predictable fashion through time (Rhoads and Boyer 1982, Rhoads and Germano 1986). At the beginning of the successional process, many of the same species that are pioneering, opportunistic organisms are also resistant to the stressors of eutrophication and the

accumulation of organic matter in sediments (Pearson and Rosenberg 1978, Rhoads and Boyer 1982, Gray et al. 2002). The model of macrobenthic community succession of Rhoads and Boyer (1982) is conceptually almost the mirror image of Pearson and Rosenberg's (1978) organic enrichment model. Physical disturbances severe enough to "restart" the successional process in estuarine systems are, however, much more stochastic than eutrophic stressors, which are persistent, systemic problems that even when corrected in the water column, have a legacy of organic matter in the sediments that will continue to negatively affect benthic fauna for a 5-10 years (e.g., Rosenberg 1976, Borja et al. 2006, Tett et al. 2007, Diaz et al. 2008). In contrast, recovery time from large-scale physical habitat disturbance like dredge-material disposal occurs over 2-3 years, with detectable changes in community structure in the short-term (e.g., Zajac and Whitlach 1982b, Wilbur et al. 2008, Schaffner 2010). Given these temporal differences, year-to-year comparisons of community data should allow for the separation of physical stress (significant year-to-year change in structure) and eutrophic stress (less year-to-year change) on the macrobenthic community. Additionally, like the chemical stressors, physical stressors should not have the positive biomass/production benefits to the macrobenthic community that accompany eutrophication of an ecosystem.

In just this short review, it should be apparent that there is a large amount of information available on the effects of eutrophication on the macrobenthic community. The major impediment to the development of community-based indicators specifically in estuaries is partially due to the variable nature of the estuarine environment and the physiological stress this places upon endemic estuarine fauna (Dauvin 2007, Dauvin and Ruellet 2009). The estuary represents an ecotone between the marine and freshwater systems and the fauna that inhabit this area are a mix of organisms invading (at geologic time scales) landward from the coastal ocean and seaward from riverine systems (Attrill and Rundle 2002). The osmotic stresses of fluctuating salinity, the physical stress of tidal erosion/deposition of surface sediments, and other natural stressors act in concert to select for fauna that are relatively predisposed to be tolerant of environmental stressors, which may make them better adapted to deal with eutrophic stressors than fauna from more stable marine or freshwater systems. This problem has been referred to as "the estuarine quality paradox" (Elliot and Quinto 2007); where the paradox is how to define or detect anthropogenic reductions in habitat quality on a community that is adapted to deal with changing physical conditions and high rates of primary production naturally occurring in estuaries (Dauvin 2007, Dauvin and Ruellet 2009). This problem is even further complicated when looking at eutrophic impacts, particularly at the beginning of the eutrophication process where impacts may be more subtle. Though this paradox makes it a challenge to use macrobenthos as an indicator of eutrophication in estuaries, it is not impossible given the use of the macrobenthos as a monitoring tool in estuaries around the world (Diaz et al. 2004). If the community characteristics that are chosen to be used as indicators are sensitive/or unique only to eutrophication and if the choice of reference condition(s) incorporates the environmental variation of the estuarine ecosystem by stratifying sampling and assessment tools by environmental gradients (e.g., Weisberg et al. 1997, Llansó et al. 2002) then the problems associated with the estuarine quality paradox can be reduced.

7.3.4 Predicting Response of Macroinvertebrates to Hypoxia and Increased Sediment Organic Matter Accumulation

As previously noted, the conceptual model of Pearson and Rosenberg (1978) summarizes the different patterns typically observed in marine/estuarine systems in the response of the macrobenthic community to eutrophication, organic matter accumulation, and hypoxia. This framework has been used to inform a variety of experiments in benthic ecology and to evaluate habitat structure/quality at broad spatial and temporal scales. A universally accepted, mechanistic model (like nutrient-phytoplankton-zooplankton [NPZ] models) detailing why the changes in macrobenthic communities in relation to eutrophication occur the way they do is, however, still lacking. This gap in our knowledge is the product of a number of complications: 1) the complex nature of macrobenthic community organization and succession; 2) the sheer diversity of macrobenthic communities throughout the World; 3) a lack of detailed understanding of most benthic species' physiological response to inputs of organic matter; and 4.) the complex hydrodynamic and biological processes that translates eutrophication to the benthos. As an example, there is still an active debate in the scientific literature about the primacy of top-down (predation or recruitment dynamics) and bottom-up processes (food supply or environmental stressors) in the structuring and controlling of macrobenthic communities (e.g., Sardá et al. 1996, Posey et al. 2006, Fleeger et al. 2008). Understanding the interaction of these kinds of very basic processes would be an important step in the modeling of the benthic community.

This is not to say that there have not been attempts to create dynamic, predictive models of the macrobenthos in response to eutrophication and organic matter accumulation/oxygen dynamics. Most of the available models, however, lack generality. Sophisticated, ecosystem-scale models have been developed for the Chesapeake Bay (Hopkins et al. 2000, US ACE 2000) and the North Sea (Blackford 1997, Allen et al. 2001) among other systems. These projects incorporate benthic sub-models that interact with hydrodynamic and land use models of eutrophication. In both of these models, the benthic community is primarily modeled as a sink for organic matter, but one that is also impacted by the accumulation of organic matter and how it changes oxygen dynamics in the water column and the sediment. These kinds of modeling efforts tend to lack fine-scale taxonomic/functional details, separating the macrobenthos only into filter- or deposit-feeding compartments. Consequently, the outputs are modeled predictions of biomass for the two types of macrobenthos that rarely match field observations and are unable to predict shifts in the composition of the macrobenthic community that are observed in the ecosystem (Schaffner et al. 2002).

Statistical models have been developed to predict macrobenthic community structure as a function of environmental parameters including sediment total organic carbon (TOC; e.g., Austen and Widdicombe 2006, Magni et al. 2009). These kinds of models do not explicitly focus on the mechanisms of how organic matter accumulation structures the macrobenthic community and can, therefore, bypass the absence of detailed physiological information. Furthermore, the model output can yield detailed community information about changes in species composition, abundance, biomass, etc, but given their taxonomic specificity, the likelihood that published statistical models from other ecosystems could be directly used in California's estuaries and coastal ocean is unlikely. That said, the general approach is appealing (i.e., potentially detailed output without detailed mechanistic data) and may provide

guidelines for the construction of similar models for California's macrobenthic communities and in turn producing useful macrobenthic-based indicators of systemic eutrophication.

7.4   Review of Potential Macrobenthic Indicators

From the viewpoint of study design, the macrobenthos are relatively easy to quantitatively sample, especially in soft sediments. Samples of sediment and macrobenthos can be collected with a variety of grabs (e.g., Smith-MacIntyre grab, Van Veen grab, Young grab) or cores (e.g., box cores, push cores, or vibrating cores) that can be deployed from various sized vessels, by divers, or by wading in shallow water (see review in Holme and McIntyre 1984). Once collected, organisms can be separated from the sediment using sieves with a variety of mesh sizes. Macrobenthic fauna are typically collected with a 500-μm sieve, though larger sized meshes can be used to simplify sample processing or to establish size-spectra within the community, while smaller sized meshes are used to sample juvenile macrobenthic fauna (e.g., Edgar 1990). The selection of sampling gear and sieve size is an important consideration, as they will both influence the characterization of the macrobenthic community. Different gear types sample to different depths in the sediment and larger sample areas will have a greater likelihood of collecting rarer taxa. Different sieve sizes will retain or exclude different size classes of organisms, which will influence abundance and biomass measurements – especially for small fauna like oligochaetes and polychaetes (Gillett et al. 2005). Most macrobenthic monitoring programs in California have refined their protocols to using a Van Veen grab for sample collection and a 1-mm mesh sieve for sample processing to balance community characterization and ease of sample processing (Smith et al. 2001, Bay et al. 2009, Ranasinghe et al. 2009).

The State of California's Sediment Quality Objectives (CASQO) monitoring program currently conducts a survey of the macrobenthic fauna that are used as part of a sediment triad approach to determine the chemical contamination of sediments in the State's waters, including estuaries (Bay et al. 2009). Ideally, collection of macrobenthic-based indicators for eutrophication assessment in these same estuaries could be incorporated into the current CASQO sampling protocol without adding the need for additional sample collection or processing. A draft sampling protocol for the macrobenthic component of the CASQO program is presented Bay et al. (2009). Briefly, this approach entails collecting subtidal sediments ≥5 cm deep with a either a 0.1- or 0.05-m^2 Van Veen grab, sieving organisms with a 1-mm screen (500-m in San Francisco Bay), preserving retained material with formalin, from which all fauna are identified to the lowest possible taxonomic level. The macrobenthic component of the CASQO assessment procedure is based only upon species composition and abundance of the macrobenthic community, which are summarized with four different indices: the index of biotic integrity (IBI); relative benthic index (RBI); benthic response index (BRI); and the river invertebrate prediction and classification system (RIVPACS; reviewed in Ranasinghe et al. 2009). As such, any proposed metrics for assessment of eutrophication that include a biomass component would require at least additional processing of the macrobenthic samples used in index calculation (i.e., measuring biomass), if not collection and processing of totally unique samples.

Our proposed candidate list of macrobenthic-based indicators of estuarine eutrophication will be based upon the current state of knowledge on the response of the macrobenthic community to the production

and accumulation of excessive amounts of organic matter in soft sediment habitats of California's enclosed bays and perennially tidal lagoons. Candidate metrics will have to be evaluated on: 1) their flexibility among the different biogeographic regions of the California coast; 2) their flexibility for application in different salinity and sediment zones of estuaries; 3) the ease and degree of expertise need to collect data and calculate the metric; and 4) their accuracy in predicting eutrophic condition determined from an independent, non-biotic indicator (e.g., historical water quality, sediment organic content, or sediment redox state). Considering the preexisting CASQO program, we will also assess metrics in light of their compatibility with the CASQO benthic monitoring program. Our goal, however, is to develop the best macrobenthic community-based metrics that will be capable of predicting eutrophic conditions in California's estuaries. If they are not compatible with the CASQO protocols, then further alternatives in sampling or metric selection will have to be considered upon consultation with experts in the field of benthic ecology and habitat assessment.

As detailed above, there is a long history among benthic ecologists looking at the effects of eutrophication on macrobenthic infauna, but there have been relatively few attempts to mathematically quantify these effects and fewer still implemented in benthic monitoring and assessment programs. The most well known and established programs measure total impact of all types of anthropogenic stressors on the macrobenthic community and therefore there are not any tested and validated measures of eutrophication and organic matter accumulation in the sediment to directly apply to California's estuaries. That being said, the concepts used for detecting total stress in the macrobenthic community are still likely applicable for focusing only on eutrophic stressors with some modifications derived from the conceptual models presented above. At present, most of the available macrobenthic community data for California's estuaries are from subtidal sediments in waters with salinities greater than 18 PSU (practical salinity units), so any specific indicators that will be developed should be constrained to these kinds of habitats as starting point; possibly expanding to other estuarine habitats once these concepts have been demonstrated in those areas where there is abundant data. Additionally, many of California's estuaries become seasonally closed to the ocean, which leads to changes in circulation, salinity, and other water quality aspects that impact the macrobenthic community and likely obscure the impacts of eutrophication. Consequently, these types of estuaries will also have to be excluded from this initial characterization of macrobenthic indicators of eutrophication.

Based upon the conceptual models discussed earlier, we would propose that three aspects of the macrobenthic community could be the most successful in detecting the effects of eutrophication in California's estuarine ecosystems: 1) taxonomic composition of the community, 2) abundance, and 3) biomass. Given the physical heterogeneity of estuaries, any of the approaches to assessing eutrophication will likely have to be separately calibrated for different habitats (e.g., salinity zones, sediment types, or hydrology) (e.g., Weisberg et al. 1997, Llansó et al. 2002).

7.4.1 Taxonomy

Given the diversity in species found in the estuarine macrobenthic communities there will likely be a variety of species that are either sensitive or tolerant of eutrophic stressors and the accompanying accumulation of organic matter in the sediment. This concept is part of nearly every macrobenthic-based assessment tool (e.g., Chesapeake Bay B-IBI, USEPA MAIA index, EU AMBI index, or the State of California BRI), which either have measures of tolerant/sensitive taxa or assign all dominant taxa a tolerance score. All of these approaches have broad species lists, often for different salinity, sediment, or depth strata, which have been selected because they respond to a combination of multiple stressors. In focusing on eutrophic stressors, the lists of sensitive/indicative taxa will have to be smaller. Taxonomic reviews by Pearson and Rosenberg (1978) and Gray et al. (2002) list a wide variety of candidate species for different types of estuarine habitats that have been demonstrated to be sensitive to organic matter enrichment in other systems. There are a number of congeneric and confamiliar species in California's estuaries and consultation with regional experts (e.g., Southern California Association of Marine Invertebrate Taxonomists [SCAMIT]) will aid in the selection of appropriate macrobenthic species, feeding guilds, or living positions to use as indicators. Tolerant taxa are commonly found in most habitats, but become dominant parts of the community when other species are excluded. If abundance and biomass of the sensitive/tolerant taxa are not considered, then the absence of sensitive taxa is likely a better indicator of eutrophication than the presence of any tolerant species. Another potential option would be to compare species richness ratios of different groups of taxa or ecologically similar species between samples. Macrobenthic species composition data are already being collected as part of ongoing monitoring efforts in California enclosed bays and estuaries, so using taxonomic-based metrics would not entail any additional sample collection or data processing.

7.4.2 Abundance

Following the P-R model relating macrobenthic community characteristics to increases in sediment organic matter loads (Figure 7.2), total community abundance is not a simple linear relationship, but a unimodal response with peak levels at moderate-levels of enrichment. The response is a product of the more tolerant taxa, which tend to have r-selected life histories, start to dominate the benthic community, but before the accumulating organic matter and reduced compounds in the sediment make conditions inhospitable for a greater portion of the community. This pattern of total community abundance makes a poor diagnostic of eutrophication because physical disturbances and successional recovery of the community will produce similar changes in abundance, because many species of that are tolerant to eutrophic stressors are also pioneer/early successional taxa. Conversely, abundance ratios of certain key taxa or feeding guilds may be useful as a measure of eutrophication in Californian estuaries. There still could be some confusion in distinguishing eutrophic impacts from successional/physical disturbance processes on the community, but looking at temporal or spatial variation should allow for the separation of eutrophic stresses. Physical disturbances are typically comparatively episodic and spatially-constrained, whereas eutrophication-driven stressors are persistent and tend to system-wide phenomena. Abundance-based metrics could be practically useful as abundance data are already collected by existing benthic monitoring programs in California and likely would not require additional changes to data collection procedures, though it would require additional analyses/classifications.

7.4.3 Biomass

Biomass-based metrics, measured in ash free dry mass (AFDM) show the greatest promise for detecting eutrophic stressors in estuarine ecosystems because the biomass-organic matter relationship (Figure7.2) is shaped differently than the relationships between biomass and other stressors. However, after the community reaches a biomass maximum and then starts to decline as eutrophic conditions increase, eutrophic-driven trends are less distinguishable from those of other stressors. Consequently, single-point measure of community biomass may not be sufficient for diagnosis of eutrophic conditions. Like potential abundance-based metrics, assessing spatial and temporal variation in community biomass could be used to distinguish eutrophication from other stressors given the temporal differences between the different types of stressors. A better solution may be to assess the community in terms of the biomass ratios of different taxa or feeding guilds (Section 7.2) to capture the changes in the community with differing degrees of eutrophic stress, even beyond the biomass maximum where the community biomass declines. Biomass data are not currently collected in any state-wide macrobenthic monitoring effort and incorporating these measurements of AFDM would represent a potentially significant increase in work load, particularly if species-specific biomass is required. An alternative approach to measuring biomass may be to sieve samples on a nested series of sieves – an array of 5-mm, 2-mm, 1-mm, 500-μm, and 250-μm screens is standard – (e.g., Edgar 1990). This would mechanically group organisms into size classes (a crude approximation of biomass) and the species distribution and abundance/dominance within each of these size classes should change with along a gradient of eutrophication/organic matter accumulation. The nested sieve approach would entail some additional work in the sample processing stage of the current macrobenthic monitoring programs, but less so than the measurement of AFDM.

7.5 Summary, Data Gaps, and Recommendations

Table 7.1 provides a summary of macrobenthic indicators and recommended next steps. Macrobenthic taxonomic composition, abundance and biomass have the potential to be part of NNE assessment framework in enclosed bays and estuaries with salinities of >18 PSU. Using only singular aspects of macrobenthic community structure (i.e., taxonomy, abundance, or biomass) will likely not be a robust method to assess eutrophication or, more generally, the trophic-state of an estuary. Though simpler metrics should be tested as well, it is most likely that a combination of all three aspects of macrobenthic community structure will prove to yield the best assessment tool. Measures of mean per capita biomass (community biomass ÷ community abundance), relative biomass distribution among different taxonomic or ecological groups, or the species-specific abundance in different size classes of organisms are slightly more complex measures than total abundance or a species list, but they also have the potential to capture more subtle changes in community structure brought upon by eutrophic stressors.

Several key data gaps exist and resolving them will be required to determine the ultimate utility of macrobenthos in assessing the eutrophic state of California's bays and estuaries. First, we recommend assembling a workgroup to identify potential species or metrics based on taxonomic composition and abundance, then use existing data collected through EPA Environmental Monitoring and Assessment

Program (EMAP) and regional monitoring programs to test out the utility of these metrics as a tool for eutrophication.

Second, though it is not currently collected in California's existing, state-wide monitoring programs, biomass data (or a reasonable approximation thereof) are probably going to have to be collected to successfully distinguish eutrophication from other stressors. We recommend a small pilot project to include biomass in new ambient monitoring of macrobenthos, in order to test out the applicability of this metric to detect eutrophication.

Finally, looking at spatial variation will also likely be necessary to separate different stressors: where measures of poor/impacted community structure with less spatial variation would be indicative of eutrophic stress on the community, compared to those with larger spatial variation, which would be indicative of other types of physical disturbance or successional changes in the community. Different combinations of metrics or differential thresholds will likely have to be implemented to tailor any assessment tool to the different sediment, salinity, or flow regimes within and among California's varied estuarine systems.

Table 7.1. Summary of literature review for candidate macrobenthos indicators for the E-NNE.

Indicator	Methods	Information	Summary of Review
Taxonomic composition	Field/lab identification	Changes in community composition	Macrobenthic fauna meet review criteria for subtidal habitats with fine-grained sediments of >18 ppt and indicators are being routinely collected as a part of the SWAMP and Sediment Quality Objectives monitoring programs. Data exist statewide to evaluate the utility of this indicator to detect eutrophication. **Recommend analysis of existing data as next steps to further considering taxonomic composition and abundance in the NNE.**
Abundance	Field/lab counts	Changes in relative abundance of tolerant versus sensitive taxa	
Biomass	Field sampling and laboratory picking and analysis of ash-free dry mass or biovolume	Ash-free dry mass of macrobenthos biomass (destructive) or biomass based on biovolume (non-destructive)	Biomass has potential to provide improved information on macrobenthos community response to eutrophication. **Recommend pilot study in Southern California Bight Regional Monitoring Program or SF Bay RMP to collect biomass data along with taxonomic composition, abundance, and sediment chemistry in order to further consider this metric.**

7.6 References

Alden, RW III, DM Dauer, JA Ranasinghe, LC Scott, and RJ Llansó. 2002. Statistical verification of the Chesapeake Bay benthic index of biotic integrity. *Environmetrics*. 13:473-498.

Allen et al. 2006 ***Martha's citation***

Allen, JI, J Blackford, J Holt, R Proctor, M Ashworth and J Siddorn. 2001. A highly spatially resolved ecosystem model for the North West European Continental Shelf. *Sarsia*. 86:423-440.

Aller, RC. 1982. The effects of macrobenthos on chemical properties of marine sediment and overlying water. pp. 53-102. *In*: P. L. McCall and M. J. S. Tevesz (*eds*), Animal-Sediment Relations The Biogenic Alteration of Sediments. Plenum Press, New York.

Aller, RC and JY Aller. 1998. The effect of biogenic irrigation intensity and solute exchange on diagenetic reaction rates in marine sediments. *Journal of Marine Research*. 56:905-936.

Aller, RC, and JY Yingst. 1985. Effects of the marine deposit-feeders *Heteromastus filiformis* (Polychaeta), *Macoma bathica* (Bivalvia), and *Tellina texana* (Bivalvia) on averaged sedimentary solute transport, reaction rates, and microbial distributions. *Journal of Marine Research*. 43:615-645.

Anderson, DM, PM. Glibert, and JM. Burkholder. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. *Estuaries*. 25:704-726.

Attrill, MJ, and SD Rundle. 2002. Ecotone or ecocline: ecological boundaries in estuaries. *Estuarine, Coastal and Shelf Science*. 55:929-936.

Attrill, MJ. 2002. A testable linear model for diversity trends in estuaries. *Journal of Animal Ecology*. 71:262-269.

Austen, MC and S Widdicombe. 2006. Comparison of the response of meio- and macrobenthos to disturbance and organic enrichment. *Journal of Experimental Marine Biology and Ecology*. 330:96-104.

Baustian, MM and NN Rabalais. 2009. Seasonal composition of benthic macroinfauna exposed to hypoxia in the Northern Gulf of Mexico. *Estuaries and Coasts*. 32:975-983.

Bay, SM, DJ Greenstein, JA Ranasinghe, DW Diehl and AE Fetscher (2009). Sediment Quality Assessment Draft Technical Support Manual. Costa Mesa, CA, Southern California Coastal Water Research Project**:** 114.

Beukema, JJ, and GC. Cadeé. 1997. Local differences in macrozoobenthic response to enhanced food supply caused by mild eutrophication in a Wadden Sea area: food is only locally a limiting factor. *Limnology and Oceanography*. 42:1424-1435.

Beukema, JJ. 1988. An evaluation of the ABC-method (abundance/biomass comparison) as applied to macrozoobenthic communities living on tidal flats in the Dutch Wadden Sea. *Marine Biology*. 99:425-433.

Bilyard, GR. 1987. The value of benthic infauna in marine pollution studies. *Marine Pollution Bulletin*. 18:581-585.

Blackford, JC. 1997. An analysis of benthic biological dynamics in a North Sea ecosystem model. *Journal of Sea Research*. 38:213-230.

Boesch, DF. 1977. A new look at the zonation of benthos along the estuarine gradient. pp. 245-266. *In*: B. C. Coull (*eds*), Ecology of Marine Benthos. University of South Carolina Press, Columbia, SC.

Boesch, DF and R Rosenberg. 1981. Response to stress in marine benthic communities. pp. 179-200. *In*: G. Barrett and R. Rosenberg (*eds*), Stress Effects on Natural Ecosystems. J. Wiley and Sons, New York.

Borja, A, J Franco and V Pe'rez. 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin*. 40:1100-1114.

Borja, A, I Muxika, and J Franco. 2006. Long-term recovery of soft-bottom benthos following urban and industrial sewage treatment in the Nerviń estuary (southern Bay of Biscay). *Marine Ecology Progress Series*. 313:43-55.

Breitburg, DL, JK Craig, RS Fulford, KA Rose, WR Boynton, DC Brady, BJ Ciotti, RJ Diaz, KD Friedland, JD Hagy, DR Hart, AH Hines, ED Houde, SE Kolesar, SW Nixon, JA Rice, DH Secor and TE Targett. 2009. Nutrient enrichment and fisheries exploitation: interactive effects on estuarine living resources and their management. *Hydrobiologia*. 629:31-47.

Brylawski, BJ (2008). Cultural Eutrophication and the Clam *Macoma balthica*: evidence for trophic disruption and effects on blue crabs. Gloucester Point, VA, The College of William and Mary. Ph.D Dissertation.

Calle-Delgado, KP (2007). Tolerance of Tidal Creek Macrobenthic Organisms to Multiple Stressors: Implications on Distributional Patterns. Columbia, SC, University of South Carolina. Ph.D Dissertation

Casazza, G, C Silvestri, and E Spada. 2002. The use of bio-indicators for quality assessments of the marine environment: examples from the Mediterranean Sea. *Journal of Coastal Conservation*. 8:147-156.

Christman, CS, and D Dauer. 2003. An approach for identifying the causes of benthic degradation in Chesapeake Bay. *Environmental Monitoring and Assessment*. 81:187-197.

Clarke, KR. 1990. Comparisons of dominance curves. *Journal of Experimental Marine Biology and Ecology*. 138:143-157.

Clough, LM, and GR Lopez. 1993. Potential carbon sources for the head-down deposit-feeding polychaete *Heteromastus filiformus*. *Journal of Marine Research*. 51:595-616.

Connell, JH. 1972. Community interactions on marine rocky intertidal shores. *Annual Review of Ecological Systems*. 3:169-192.

Dauer, DM, JA Ranasinghe, and SB Weisberg. 2000. Relationships between benthic community condition, water quality, nutrient loads, and land use patterns in Chesapeake Bay. *Estuaries*. 23:80-96.

Dauer, DM. 1993. Biological criteria, environmental health, and estuarine macrobenthic community structure. *Marine Pollution Bulletin*. 26:249-257.

Dauvin, J. 2007. Paradox of estuarine quality: benthic indicators and indices, consensus or debate for the future. *Marine Pollution Bulletin*. 55:271-281.

Dauvin, J, and T Ruellet. 2009. The estuarine quality paradox: is it possible to define and ecological quality status for specific modified and naturally stressed estuarine systems. *Marine Pollution Bulletin*. 59:38-47.

Diaz, RJ, DC Rhoads, JA Blake, RK Kropp and KE Keay. 2008. Long-term trends of benthic habitats related to reduction in wastewater discharge to Boston Harbor. *Estuaries and Coasts*. 31:1184-1197.

Diaz, RJ, and R Rosenberg. 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioral responses of benthic macrofauna. *Oceanography and Marine Biology an Annual Review*. 33:245-303.

Diaz, RJ, and R Rosenberg. 2008. Spreading dead zones and consequences for marine ecosystems. *Science*. 321:926-929.

Diaz, RJ, M Solan, and RM Valente. 2004. A review of approaches for classifying benthic habitats and evaluating habitat quality. *Journal of Environmental Management*. 73:165-181.

Diaz, RJ. 1994. The response of tidal freshwater macrobenthos to sediment disturbance. *Hydrobiologia*. 278:201-212.

Diaz, RJ, and LC Schaffner. 1990. The Functional Role of Estuarine Benthos. pp. 25-56. *In*: M. Haire and E.C. Krome (*eds*), Perspectives on the Chesapeake Bay, 1990. Advances in Estuarine Sciences. United States Environmental Protection Agency, Gloucester Point, VA.

Draheim, RC. 1998. Tidal Freshwater and Oligohaline Benthos: Evaluating the Development of a Benthic Index of Biological Integrity for Chesapeake Bay, Masters Thesis. Gloucester Point, VA, The College of William and Mary.

Edgar, GJ, CK. Macleod, RB. Mawbey, and D Shields. 2005. Broad-scale effects of marine salmonid aquaculture on macrobenthos and the sediment environment in southeastern Tasmania. *Journal of Experimental Marine Biology and Ecology*. 327:70-90.

Edgar, GJ. 1990. The use of the size structure of benthic macrofaunal communities to estimate faunal biomass and secondary production. *Journal of Experimental Marine Biology and Ecology*. 137:195-214.

Elliott, M, and V Quintino. 2007. The estuarine quality paradox, environmental homeostasis and the difficulty of detecting anthropogenic stress in naturally stressed areas. *Marine Pollution Bulletin*. 54:640-645.

Elliott, MD, Burdon, KL. Hemingway, and SE Apitz. 2007. Estuarine, coastal and marine ecosystem restoration: Confusing management and science - a revision of concepts. *Estuarine Coastal and Shelf Science*. 74:349-366.

Evrard, V, K Soetaert, CHR Heip, M Huettel, MA Xenopoulos and JJ Middelburg. 2010. Carbon and nitrogen flows through the benthic food web of a photic subtidal sandy sediment. *Marine Ecology Progress Series*. 416:1-16.

Fauchald, K, and PA Jumars. 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanography and Marine Biology an Annual Review*. 17:193-284.

Fleeger, JW, DS Johnson, KA Galván, and LA Deegan. 2008. Top-down and bottom-up control of infuana varies across the saltmarsh landscape. *Journal of Experimental Marine Biology and Ecology*. 357:20-34.

Gaston, GR, CF Rakocinski, SS Brown, and CM Cleveland. 1998. Trophic function in estuaries: response of macrobenthos to natural and contaminant gradient. *Marine and Freshwater Research*. 49:833-846.

Gaston, GR. 1987. Benthic polychaeta of the Middle Atlantic Bight: feeding and distribution. *Marine Ecology Progress Series*. 36:251-262.

Gaudeñcio, MJ, and HN Cabral. 2007. Trophic structure of macrobenthos in the Tagus estuary and adjacent coastal shelf. *Hydrobiologia*. 587:241-251.

Gerritsen, J, AF Holland, and DE Irvine. 1994. Suspension-feeding bivalves and the fate of primary production: an estuarine model applied to Chesapeake Bay. *Estuaries*. 17:403-416.

Gillett, DJ (2010). Effects of Habitat Quality on Secondary Production in Shallow Estuarine Waters and the Consequences for the Benthic-Pelagic Food Web. <u>Biological Sciences</u>. Gloucester Point, VA, The College of William and Mary. Ph.D Dissertation.

Gillett, DJ, AF Holland, and DM Sanger. 2005. Secondary production of a dominant oligochaete (*Monopylephorus rubroniveus*) in the tidal creeks of South Carolina and its relation to ecosystem characteristics. *Limnology and Oceanography*. 50:566-577.

Gillett, DJ, AF Holland, and DM Sanger. 2007. On the ecology of oligochaetes: Monthly variation of community composition and environmental characteristics in two South Carolina tidal creeks. *Estuaries and Coasts*. 30:238-252.

Gillett, DJ, and LC Schaffner. 2009. Benthos of the York River. *Journal of Coastal Research*. 57:80-98.

González-Oreja, JA, and JI Saiz-Salinas. 1999. Loss of heterotrophic biomass structure in an extreme estuarine environment. *Estuarine, Coastal and Shelf Science*. 48:391-399.

Graf, G. 1992. Benthic-pelagic coupling: a benthic view. *Oceanography and Marine Biology an Annual Review*. 30:149-190.

Grall, J, and L Chauvaud. 2002. Marine eutrophication and benthos: the need for new approaches and concepts. *Global Change Biology*. 8:813-830.

Gray, JS, RS Wu, and YY Or. 2002. Effects of hypoxia and organic enrichment on the coastal marine environment. *Marine Ecology Progress Series*. 238:249-279.

Hagy, JD, WR Boynton, CW Keefe, and KV Wood. 2004. Hypoxia in Chesapeake Bay, 1950-2001: long-term change in relation to nutrient loading and river flow. *Estuaries*. 27:634-658.

Hamdan, LJ and RB Jonas. 2006. Seasonal and interannual dynamics of free-living bacterioplankton and microbially labile organic carbon along the salinity gradient of the Potomac river. *Estuaries and Coasts*. 29:40-53.

Heip, CHR, NK Goosen, PMJ Herman, J Kromkamp, JJ Middleburg, and K Soetaert. 1995. Production and consumption of biological particles in temperate tidal estuaries. *Oceanography and Marine Biology an Annual Review*. 33:1-149.

Hinchey, EK, LC Schaffner, CC Hoar, BW Vogt, and LP Batte. 2006. Responses of estuarine benthic invertebrates to sediment burial: the importance of mobility and adaptation. *Hydrobiologia*. 556:85-98.

Hines, AH, and KL Comtois. 1985. Vertical distribution of infauna in sediments of a subestuary of central Chesapeake Bay. *Estuaries*. 8:296-304.

Holland, AF, DM Sanger, CP Gawle, SB Lerberg, MS Santiago, GHM Riekerk, LE Zimmerman, and GI Scott. 2004. Linkages between tidal creek ecosystems and the landscape and demographic attributes of their watersheds. *Journal of Experimental Marine Biology and Ecology*. 298:151-178.

Holland, AF, AT Shaughnessy, and MH.Heigel. 1987. Long-term variation in mesohaline Chesapeake Bay Macrobenthos: spatial and temporal patterns. *Estuaries*. 10:227-245.

Holland, AF, NK Mountford, and JA Mihursky. 1977. Temporal variation in Upper Bay mesohaline benthic communities: I. The 9-m mud habitat. *Chesapeake Science*. 18:370-378.

Holme, NA and AD McIntyre. 1984. Methods for the Study of Marine Benthos 2nd Ed, 2nd. Blackwell Scientific, Oxford

Hopkins, K, B Brown, LC Linker and RL Mader_Jr. (2000). Chesapeake Bay Watershed Land Uses and Linkages to the Airshed and Estuarine Models A Report of the Modeling Subcommittee. Annapolis, MD, Chesapeake Bay Program Office.

Hyland, J, L Balthis, I Karakassis, P Magni, A Petrov, J Shine, O Vestergaard, and R Warwick. 2005. Organic carbon content of sediments as an indicator of stress in the marine benthos. *Marine Ecology Progress Series*. 295:91-103.

International Association of Limnology. 1958. The Venice system for the classification of marine waters according to salinity. *Limnology and Oceanography*. 3:346-347.

Iwamatsu, S, A Suzuki and M Sato. 2007. Nereidid polychaetes as the major diet of migratory shorebirds on the estuarine tidal flats at Fujimae-Higata in Japan. *Zoological Science*. 24:676-685.

Jørgensen, BB. 1996. Material flux in Sediments. pp. 115-136. *In*: BB Jørgensen and K. Richardson (*eds*) Eutrophication in Coastal Marine Ecosystems, Coastal and Estuarine Studies, Vol 2. American Geophysical Union, Washington, DC.

Kemp, WM, JM Testa, DJ Conley, D Gilbert and JD Hagy. 2009. Temporal responses of coastal hypoxia to nutrient loading and physical controls. *Biogeosciences*. 6:2985-3008.

Kennish, MJ. 1986. Ecology of Estuaries, Volume I Physical and Chemical Aspects. CRC Press,, Boca Raton, FL.

Kennish, MJ. 2002. Environmental threats and environmental future of estuaries. *Environmental Conservation*. 29:78-107.

Kinne, O. 1971. Salinity. pp 821-1023 *In*: O. Kinne (*ed*) Marine Ecology A Comprehensive, Integrative Treatise on Life in Oceans and Coastal Waters. Volume I Environmental Factors Part 2. Wiley-Interscience, New York.

Lavesque, N, H Blanchet, and X de Montaudouin. 2009. Development of a multimetric approach to assess perturbation of benthic macrofauna in *Zoster noltii* beds. *Journal of Experimental Marine Biology and Ecology*. 368:101-112.

Lenihan, HS, CH Peterson, SL Kim, KE Conlan, R Fairey, C McDonald, JH Grabowski and JS Oliver. 2003. Variation in marine benthic community composition allows discrimination of multiple stressors. *Marine Ecology Progress Series*. 261:63-73.

Levin, L, N Blair, D DeMaster, G Plaia, W Fornes, C Martin, and C Thomas. 1997. Rapid subduction of organic matter by maldanid polychaetes on the North Carolina slope. *Journal of Marine Research*. 55:595-611.

Levin, LA. 1984. Multiple patterns of development in *Streblospio benedicti* Webster (Spionidae) from three coasts of North America. *Biological Bulletin*. 166:434-508.

Levinton, JS. 1982. Reproductive Strategies. pp. 123-137. *In*: (*eds*), Marine Ecology. Prentice Hall, New Jersey.

Levinton, J. and B Kelaher. 2004. Opposing organizing forces of deposit-feeding marine communities. *Journal of Experimental Marine Biology and Ecology*. 300:65-82.

Llansó, RJ, LC Scott, JL Hyland, DM Dauer, DE Russell, and FW Kutz. 2002. An estuarine benthic index of biotic integrity for the Mid-Atlantic region of the United States. II Index development. *Estuaries*. 25:1231-1242.

Llansó, R.J. 1992. Effects of hypoxia on estuarine benthos: the Lower Rappahannock River (Chesapeake Bay), a case study. *Estuarine, Coastal and Shelf Science*. 35:491-515.

Long, WC, BJ Brylawski, and RD Seitz. 2008. Behavioral effects of low dissolved oxygen on the bivalve *Macoma balthica*. J*ournal of Experimental Marine Biology and Ecology*. 359:34-39.

Lopez, GR. and JS. Levinton. 1987. Ecology of deposit-feeding animals in marine sediments. *The Quarterly Review of Biology*. 62:235-260.

Lu, L. and RS. S Wu. 1998. Recolonization and succession of marine macrobenthos in organic enriched sediment deposited from fish farms. *Environmental Pollution*. 101:241-251.

Marsh, AG, and KN. Tenore. 1990. The role of nutrition in regulating the population dynamics of opportunistic, surface deposit feeders in a mesohaline community. *Limnology and Oceanography*. 35:710-724.

Michaud, E, G Desrosiers, F Mermillod-Blondin, B Sundby, and G Stora. 2005. The functional group approach to bioturbation: the effects of biodifusers and gallery-diffusers of the *Macoma balthica* community on sediment oxygen uptake. *Journal of Experimental Marine Biology and Ecology*. 326:77-88.

Michaud, E, G. Desrosiers, F. Mermillod-Blondin, B. Sundby, and G. Stora. 2006. The functional group approach to bioturbation: II. The effects of the Macoma balthica community on fluxes of nutrients and dissolved organic carbon across the sediment-water interface. *Journal of Experimental Marine Biology and Ecology*. 337:178-189.

Molinaroli, E, S Guerzoni, G De Falco, A Sarretta, A Cucco, S Como, S Simeone, A Perilli and P Magni. 2009. Relationships between hydrodynamic parameters and grain size in two contrasting transitional environments: The Lagoons of Venice and Cabras, Italy. *Sedimentary Geology*. 219:196-207.

Nestlerode, JA and RJ Diaz. 1998. Effects of periodic environmental hypoxia on predation of a tethered polychaete, *Glycera americana*: implications for trophic dynamics. *Marine Ecology Progress Series*. 172:185-195.

Neuman, KK, LA Henkel and GW Page. 2008. Shorebird use of sandy beaches in central California. *Waterbirds*. 31:115-121.

Nixon, SW. 1995. Coastal marine eutrophication: a definition, social causes, and future concerns. *Ophelia*. 41:199-219.

Nixon, SW. 2009. Eutrophication and the macroscope. *Hydrobiologia*. 629:5-19.

Nixon, SW, and BA Buckley. 2002. "A strikingly rich zone" - nutrient enrichment and secondary production in coastal marine ecosystems. *Estuaries*. 25:782-796.

Nixon, SW, RW Fulweiler, BA Buckley, SL Granger, BL Nowicki and KM Henry. 2009. The impact of changing climate on phenology, productivity, and benthic-pelagic coupling in Narragansett Bay. *Estuarine Coastal and Shelf Science*. 82:1-18.

O'Connor, TP. 2002. National distribution of chemical concentrations in mussels and oysters in the USA. *Marine Environmental Research*. 53:117-143.

Peterson, CH, MC Kennicutt, II, RH Green, P Montagna, DE Harper, Jr, EN Powell, and PF Roscigno. 1996. Ecological consequences of environmental perturbations associated with offshore hydrocarbon production: a perspective on long-term exposures in the Gulf of Mexico. *Canadian Journal of Fisheries and Aquatic Science*. 53:2637-2654.

Pihl, L, SP Baden, RJ Diaz, and LCSchaffner. 1992. Hypoxia-induced structural changes in the diet of bottom-feeding fish and crustacea. *Marine Biology*. 112:349-361.

Pohlo, R. 1982. Evolution of the tellinacea (Bivalvia). *Journal of Molluscan Studies*. 48:245-256.

Posey, MH, TD. Alphin, and L Cahoon. 2006. Benthic community responses to nutrient enrichment and predator exclusion: influence of background nutrient concentrations and interactive effects. *Journal of Experimental Marine Biology and Ecology*. 330:105-118.

Powers, SP, CH Peterson, RR Christian, E Sullivan, MJ Powers, MJ Bishop, and C Buzzelli. 2005. Effects of eutrophication on bottom habitat and prey resources of demersal fishes. *Marine Ecology Progress Series*. 302:233-243.

Rabalais, NN, RJ Diaz, LA Levin, RE Turner, D Gilbert and J Zhang. 2010. Dynamics and distribution of natural and human-caused hypoxia. *Biogeosciences*. 7:585-619.

Rakoncinski, CF. 2009. Linking allometric macrobenthic processes to hypoxia using the Peters mass balance model. *Journal of Experimental Marine Biology and Ecology*. 381:S13-S20.

Rakocinski, CF, and GA Zapf. 2005. Macrobenthic process-indicators of estuarine condition. pp. 315-331. *In*: SA Bortone (*eds*), Estuarine Indicators. CRC Press, New York.

Rakocinski, CF, SS Brown, GR Gaston, RW Heard, WW Walker, and JK Summers. 1997. Macrobenthic responses to natural and contaminant-related gradients in nothern Gulf of Mexico estuaries*. Ecological applications*. 7:1278-1298.

Ranasinghe, JA, SB Weisberg, RW Smith, DE Montagne, B Thompson, JM Oakden, DD Huff, DB Cadien, RG Velarde and KJ Ritter. 2009. Calibration and evaluation of five indicators of benthic community condition in two California bay and estuary habitats. *Marine Pollution Bulletin*. 59.

Ranasinghe, JA, KI Welch, PN Slattery, DE Montagne, DE Huff, H Lee_II, JL Hyland, B Thompson, SB Weisberg, JM Oakden, DB Cadien and RG Velarde. 2010. Habitat-related benthic macrofaunal assemblages of bays and estuaries of the western United States. *Integrated Environmental Assessment and Management*.DOI: 10.1897/IEAM 62.

Rand, GM, PG Wells, and LS McCarty. 1995. Introduction to aquatic toxicology. pp. 3-70. In: G. M. Rand (eds), Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment, 2nd Edition. Taylor and Francis, Washington DC.

Remane, A. and C Schlieper. 1971. Biology of Brackish Water 2nd Edition. John Wiley and Sons, New York.

Rhoads, DC and LF Boyer. 1982. The effects of marine benthos on physical properties of sediments a successional perspective. pp. 3-52. *In*: P. L. McCall and M. J. S. Tevesz (*eds*), Animal-Sediment Relations the Biogenic Alteration of Sediments. Plenum Press, New York.

Rhoads, DC and JD Germano. 1986. Interpreting long-term changes in benthic community structure: a new protocol. *Hydrobiologia*. 142:291-308.

Rice, DL, and DC Rhoads. 1989. Early diagenesis of organic matter and the nutritional value of sediment. pp. 59-97. *In*: G Lopez, G Taghon, and J Levinton (*eds*), Ecology of Marine Deposit Feeders. Springer-Verlag, New York.

Ringwood, AH. and CJ Keppler. 1998. Seed clam growth: an alternative sediment bioassay developed during EMAP in the Carolinian Province. *Environmental Monitoring and Assessment*. 51:247-257.

Rosenberg, R. 1976. Benthic faunal dynamics during succession following pollution abatement in a Swedish estuary. *Oikos*. 27:414-427.

Rosenberg, R, B Hellman, and B Johansson. 1991. Hypoxic tolerance of marine benthic fauna. *Marine Ecology Progress Series*. 79:127-131.

Sagasti, A, LC Schaffner, and JE Duffy. 2001. Effects of periodic hypoxia on mortality, feeding, and predation in an estuarine epifaunal community. *Journal of Experimental Marine Biology and Ecology*. 258:257-283.

Sanchez-Mata, A, M Lastra and J Mora. 1993. Macrobenthic crustacean characterization of an estuarine area. *Crustaceana*. 64:337-355.

Sanders, HL. 1958. Benthic studies in Buzzards Bay. I Animal-sediment relationships. *Limnology and Oceanography*. 3:245-258.

Sanger, DM, AF Holland, and GI Scott. 1999a. Tidal creek and salt marsh sediments in South Carolina coastal estuaries: I. distribution of trace metals. *Archives of Environmental Contamination and Toxicology*. 37:445-457.

Sanger, DM, AF Holland, and GI Scott. 1999b. Tidal creek and salt marsh sediments in South Carolina coastal estuaries: II. distribution of organic contaminants. *Archives of Environmental Contamination and Toxicology*. 37:458-471.

Sardá, R, K Foreman, CE Werme, and I Valiela. 1998. The impact of epifaunal predation on the structure of macroinfaunal communities of tidal salt marsh creeks. *Estuarine, Coastal and Shelf Science*. 46:657-669.

Schaffner, LC. 1990. Small-scale organism distributions and patterns of species diversity: evidence for positive interactions in an estuarine benthic community. *Marine Ecology Progress Series*. 61:107-117.

Schaffner, LC. 2010. Patterns and rates of recovery of macrobenthic communities in a polyhaline temperate estuary following sediment disturbance: Effects of disturbance severity and potential importance of non-local processes. *Estuaries and Coasts* 33: 1300-1313

Schaffner, LC, CT Friedrichs and DM Dauer (2002). Review of the Benthic Process Model with Recommendations for Future Modeling Efforts A Report From the Benthic Process Model Review Team. Annapolis, MD, Chesapeake Bay Program**:** 38.

Seitz, RD, DM Dauer, RJ Llanso' and WC Long. 2009. Broad-scale effects of hypoxia on benthic community structure in Chesapeake Bay, USA. *Journal of Experimental Marine Biology and Ecology*. 381:S2-S12.

Seitz, RD, RN Lipcius, AH Hines, and DB Eggleston. 2001. Density-dependent predation, habitat variation, and the persistence of marine bivalve prey. *Ecology*. 82:2435-2451.

Smith, RW, M Bergen, SB Weisberg, D Cadien, A Dalkey, D Montagne, JK Stull and RG Velarde. 2001. Benthic response index for assessing infuanal communities on the Southern California mainland shelf. *Ecological Applications*. 11:1073-1087.

Snelgrove, PVR, and CA Butman. 1994. Animal-sediment relationships revisited: cause versus effect. *Oceanography and Marine Biology an Annual Review*. 32:111-177.

Soetaert, K and JJ Middelburg. 2009. Modeling eutrophication and oligotrophication of shallow-water marine systems: the importance of sediments under stratified and well-mixed conditions. *Hydrobiologia*. 629:239-254.

Sterner, RW. and JJ Elsner. 2002. Ecological Stoichiometry: The biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton, NJ.

Tagon, GL, ARM Nowell, and PA Jumars. 1980. Induction of suspension feeding in spionid polychaetes by high particle fluxes. *Science*. 210:562-564.

Tett, P, R Gowen, D Mills, T Fernandes, L Gilpin, M Huxham, K Kennington, P Read, M Service, M Wilkinson and S Malcom. 2007. Defining and detecting undesirable distrubance in the context of marine eutrophication. *Marine Pollution Bulletin*. 55:282-297.

Thompson, ML. and LC Schaffner. 2001. Population biology and secondary production of the suspension feeding polychaete *Chaetopterus* cf. *variopedatus*: Implications for benthic-pelagic coupling in lower Chesapeake Bay. *Limnology and Oceanography*. 46:1899-1907.

United_States_Army_Corps_of_Engineers (2000). Development of a Suspension Feeding and Deposit Feeding Benthos Model for Chesapeake Bay Project No. USCE0410. Annapolis, MD, Chesapeake Bay Program Office**:** 116.

United_States_Environmental_Protection_Agencey (2008). National Coastal Condition Report III. Washington, D.C, Office of Research and Development/Office of Water**:** 324.

Valiela, I. 1995. Marine Ecological Processes. 2nd edition. Springer-Verlag, New York.

Van Dolah, RF, DE Chestnut, and GI Scott. 2000. A Baseline Assessment of Environmental and Biological Conditions in Broad Creek and the Okatee River, Beaufort County, South Carolina. Final Report. South Carolina Department of Health and Environmental Control, Columbia, SC.

Van Dolah, RF, JL Hyland, AF Holland, JS Rosen, and TR Snoots. 1999. A benthic index of biological integrity for assessing habitat quality in estuaries of the southeastern USA. *Marine Environmental Research*. 48:269-283.

Virnstein, RW. 1979. Predation on estuarine infauna: response patterns of component species. *Estuaries*. 2:69-86.

Warwick, RM, and KR Clarke. 1994. Relearning the ABC: taxonomic changes and abundance/biomass relationships in disturbed benthic environments. *Marine Biology*. 118:739-744.

Warwick, RM, TH Pearson, and Ruswahyuni. 1987. Detection of pollution effects on marine macrobenthos: further evaluation of the species abundance/biomass method. *Marine Biology*. 95:193-200.

Warwick, R.M. 1986. A new method for detecting pollution effects on marine macrobenthic communities. Marine Biology. 92:557-562.

Warwick, R.M. 1988. Effects on community structure of a pollutant gradient - summery. Marine Ecology Progress Series. 46:207-211.

Weisberg, SB, JARanasinghe, DM Dauer, LC Schaffner, RJ Diaz, and JB Frithsen. 1997. An estuarine benthic index of biotic integrity (B-IBI) for Chesapeake Bay. *Estuaries*. 20:149-158.

Weston, DP. 1990. Quantitative examination of macrobenthic community changes along an organic enrichment gradient. *Marine Ecology Progress Series*. 61:233-244.

Wilbur, DH, GI Ray, DG Clarke and RJ Diaz. 2008. Responses of benthic infauna to large-scale sediment disturbance in Corpus Christi Bay, Texas. *Journal of Experimental Marine Biology and Ecology*. 365:13-22.

Zajac, RN, and RB Whitlatch. 1982. Responses of estuarine fauna to disturbance. I. Spatial and temporal variation of initial recolonization. *Marine Ecology Progress Series*. 10:1-14.

Zajac, RN, and RB Whitlatch. 1982. Responses of estuarine infauna to disturbance. II Spatial and temporal variation of succession. *Marine Ecology Progress Series*. 10:15-27.

8. Synthesis of Candidate Indicator Review, Data Gaps, and Recommendations

Martha Sutula

The goal of the candidate indicator review was to evaluate appropriate indicators to assess eutrophication and other adverse effects of anthropogenic nutrient loading in California estuaries. This section summarizes the results of the review, proposes specific indicators by estuarine class and habitat type, identifies data gaps, and recommends next steps.

8.1 Context for Evaluating and Selecting Candidate Indicators

8.1.1 Estuarine Classes and Habitat Types

Selected indicators for the estuarine NNE framework will vary by estuarine class and habitat type. In order to provide a synthesis on what indicators met the review criteria and which to include the NNE assessment framework, it is important to reiterate the relevant classes and habitat types (presented in Chapter 2). For the purposes of developing an NNE, California estuaries can be separated into two main groups according to the status of the tidal exchange with the coastal ocean:

- "Open" to surface water tidal exchange
- "Closed" to surface water tidal exchange

Estuarine classes that fall entirely into this "open" category include enclosed bays and perennially tidal lagoons and river mouth estuaries. In addition, intermittently tidal lagoons and river mouths that open at least once per year may be assessed using indicators applicable to this category when the mouth of the estuary is open to surface water tidal exchange. Estuaries that are "closed" to surface water tidal exchange include intermittently and ephemerally tidal lagoons and river mouth estuaries when the mouth is closed.

8.1.2 Context for Selection of "Primary" and "Supporting Indicators" for the NNE Diagnostic Assessment Framework

Within the regulatory context, waterbody assessments are made in order to determine compliance with permits, TMDL implementation plans, or to make determination of whether the waterbody is meeting beneficial uses or impaired, as an example, for nutrients. In this context, a diagnostic assessment framework is the structured set of decision rules and guidance for interpretation that helps to classify the waterbody in categories of minimally disturbed, to moderately disturbed, to very disturbed. Although scientists can provide guidance and data synthesis to illustrate how the assessment framework could be formed, ultimately the decision of what levels to set thresholds (i.e., NNEs) that separate the categories (e.g., minimally versus moderately and very disturbed) is a policy decision.

Development of the diagnostic assessment framework begins by choosing indicators that would be measured and used to determine waterbody status. It is important to distinguish between three types of

indicators for an NNE assessment framework: 1) Primary indicators, 2) Supporting indicators, and 3) Co-factor indicators required for data interpretation.

Primary indicators are those for which regulatory endpoints could be developed. Designation of these indicators as "primary" implies a higher level of confidence in these indicators to be used to make an assessment of adverse effects, based on a wealth of experience and knowledge about how this indicator captures and represents ecological response. Primary indicators are those which met all explicit criteria (see Section 2.5) established to evaluate candidate NNE indicators. Supporting indicators which did not meet all the criteria, but which could be collected to provide supporting lines of evidence, though they should not be used alone to make determination of whether the waterbody was meeting beneficial uses. These indicators may have met many, though not all evaluation criteria, but are considered important because they are commonly used to assess eutrophication in scientific studies. Establishment of NNE endpoints for these indicators is not required nor anticipated, though use of the indicator as supporting evidence over time may increase confidence and cause it to be promoted to "primary." Finally, co-factors are indicators that could be part of a routine monitoring program and important for data interpretation and trends analysis.

8.2   Synopsis of Review of Candidate Indicators

Tables 8.1 – 8.3 present a brief synopsis of which individual indicators met review criteria and which are designated as primary or supporting indicators for the NNE assessment framework for estuaries. Applicability of indicators varies by habitat, so a detailed explanation of recommended indicators, data gaps and next steps is given in Section 8.3 below.

Table 8.1. Summary of review of primary producer indicators.

Group	Indicator or Metric	Met Criteria?	Recommended for NNE?
Macroalgae	Percent Cover	Four of Four	**Recommend as primary indicator.**
	Biomass	Four of Four	
Phyto-plankton	Chlorophyll-a conc. and productivity	Four of four	**Recommend as primary indicator.**
	Taxonomic composition	Three of four	Lack of experience predicting phytoplankton assemblages. **Recommend as supporting indicator.**
	Harmful algal bloom species abundance	Three of four	Limited data and understanding of controls on HAB occurrence and toxin production, *with exception of fresh and brackish water cyanobacteria.* **Recommend as cyanobacteria cell counts and toxin concentrations as primary indicator and other HAB species as secondary indicator.**
	Harmful algal bloom species -- toxin concentration		
Seagrass and Brackish Water SAV	SAV Areal Distribution	Two of four	These indicators are a core component of seagrass monitoring programs, but the indicators themselves are not indicative of eutrophication per se, but integrative of all stressors. **Recommend as supporting indicator in seagrass habitats: areal distribution, % cover, light attenuation, and density. Recommend as supporting indicator in brackish water SAV: and areal distribution, % cover, light attenuation, and biomass in brackish SAV habitats.**
	SAV Taxonomic Composition	One of four	
	SAV Biomass	Two of four	
	SAV Density	Two of four	
	Water Column Light Attenuation	Two of four	
	Epiphyte Load on SAV	Three of four	Standard method does not exist and load is difficult to quantify. **Recommend as supporting indicator or in combination with macroalgal biomass/cover, phytoplankton biomass in a "multiple lines of evidence" assessment framework for seagrass.**
	Macroalgal Biomass/Cover on SAV	Four of four	**Recommend as a primary indicator.**
	Water Column Chlorophyll-a	Four of four	**Recommend as primary indicator.**

Table 8.2. Summary of review of sediment and water chemistry indicators.

Group	Indicator or Metric	Met Criteria?	Recommended Action
Nutrients	Ammonia	Three of four	Evidence of ammonium inhibition of diatom nitrate assimilation, but ecological importance of process not well understood for estuaries. **Recommend inclusion as supporting indicator for phytoplankton-dominated enclosed bays**
	Urea	Three of four	Causative link between urea and HABs has not been established and little data are available on urea concentrations in California estuaries. **Recommend inclusion as supporting indicator for phytoplankton-dominated enclosed bays**
	N:P Ratio	Three of four in brackish habitat	Linkage to beneficial uses and signal:noise ratio only strong in brackish water estuaries (e.g., coastal lakes). **Recommend inclusion as a supporting indicator for "closed" estuaries or brackish water regions of "open" estuaries**
Water Clarity	Secchi Depth	Two of Four	Phytoplankton biomass a component of light attenuation, but other factors unassociated with eutrophication can contribute (e.g., suspended sediment), so poor linkage to BUs and signal:noise. **Include as co-factor or supporting indicator for seagrass habitat. Otherwise not recommended.**
	Kd (Light extinction)		
	Turbidity		
Dissolved Oxygen	Dissolved Oxygen Conc.	Four of Four	**Recommend as primary indicator in NNE framework**
	Biological or Chemical Oxygen Demand	Two of four	Indirect linkage to beneficial uses and poor signal: noise ratio. **Can include as a co-factor in monitoring, but not recommended for inclusion in NNE framework.**
	Sediment oxygen demand	Two of four	
Benthic Meta-bolism	Benthic production: respiration ratio	Two of four	Indirect linkage to beneficial uses and poor signal: noise ratio. **Provides useful information as part of a special study, but not recommended for inclusion in NNE framework**
	Benthic TCO2 flux	Two of four	
Organic Matter Accum. & Sediment Redox	Sediment %OC, N, P	Two of four	Indirect linkage to beneficial uses and poor signal: noise ratio. **Recommended as a supporting indicator for shallow subtidal.**
	Sediment C:N:P ratio	Two of four	
	Sediment TOC:TS & DOP	Three of four	Moderate signal:noise ratio, that may be improved with additional work on expectations given geology and habitat type. **Recommend inclusion as supporting indicator to further evaluate performance.**
Nitrogen Cycling	Denitrification Efficiency	Two of four	Technically difficult to measure and poor-moderate signal:noise. **Not recommended for inclusion as supporting indicator**

Table 8.3. Summary of review of macrobenthos indicators.

Group	Indicator	Met Criteria?	Recommended Action
Macrobenthos	Taxonomic composition and abundance	Two of four	Macrobenthic taxonomic composition and abundance response to a variety of stressors and are not specific to eutrophication. In addition, it is difficult to develop predictive models for any of the three indicators. However, in combination with biomass, these indicators have the potential to be a supporting line of evidence for eutrophication, particularly in habitats > 18 ppt. Because they are already employed in sediment quality objectives and will be a core component of estuarine ambient monitoring programs in the future, there is potential to gain additional information on eutrophication, with minor modification of existing protocol. **Recommend as supporting indicator, pending analysis of existing data as next steps to further considering taxonomic composition and abundance in the NNE and pilot study to collect biomass data along with taxonomic composition, abundance, and sediment chemistry in order to further consider this indicator.**
	Biomass	Three of four	

8.3   Recommended Indicators for the Estuarine NNE Framework

8.3.1   *Recommended Indicators for "Open" Estuaries*

A suite of recommended "primary" (those for which regulatory endpoints would be developed) and "supporting" indicators (those for which no regulatory endpoints would be developed in the near term, but could be used as supporting lines of evidence) are recommended the three major habitat types within "open" estuaries:

- Unvegetated intertidal flats and shallow subtidal sediments
- Unvegetated moderate to deepwater subtidal habitat
- Seagrass habitat (intertidal and subtidal) and brackish SAV habitat (subtidal)

Table 8.4. Table of proposed primary and supporting NNE indicators by habitat type. Note that primary and supporting indicators recommended for unvegetated subtidal habitat are also applicable for seagrass habitat.

Habitat	Primary Indicators	Supporting Indicators
All Subtidal Habitat	Phytoplankton biomass and productivity Cyanobacteria cell counts and toxin concentration[11] Dissolved oxygen	Water column nutrient concentrations and forms[12] (C, N, P, and Si) Phytoplankton assemblages HAB species cell count and toxin concentrations Macrobenthos taxonomic composition, abundance and biomass Sediment C, N, P, S, particle size (and ratios therein) and degree of pyritization
Seagrass and Brackish SAV Habitat	Phytoplankton biomass and productivity Macroalgal biomass & cover	Light attenuation, suspended sediment conc. or turbidity Seagrass areal distribution,% cover, density Epiphyte load Brackish SAV areal distribution,% cover, biomass
Intertidal Flats	Macroalgal biomass and cover	Sediment % OC, N, P, S, particle size, degree of pyritization Microphytobenthos taxonomic composition and benthic chl a biomass

Moderate to Deepwater Subtidal Habitats

In moderate to deepwater unvegetated subtidal habitat, phytoplankton and microphytobenthos dominate primary production. Little is known about biomass and taxonomy of microphytobenthos. Therefore, the primary NNE indicators to assess eutrophication focus on measures of phytoplankton (biomass, productivity) and harmful algal species cell counts (specifically cyanobacteria counts and toxin concentration), and dissolved oxygen. Note that cyanobacteria species such as Microsystis spp. would not typically be expected to flourish in mid-high salinity habitats, but would included as a primary indicator in the event that cyanobacteria from freshwater sources upstream are a concern. Thus, there is an expressed intent to capture potential adverse effects of cyanobacteria that may be transported downstream from freshwater habitats, as whole cells or as toxin.

Supporting indicators for this habitat types include information on the phytoplankton assemblage, HAB species cell count and toxin concentrations. For shallow to moderately deep water subtidal habitats in which hypoxia may not be occurring, but in which organic matter loading can be causing a decline in benthic habitat quality, macrobenthos taxonomic composition, abundance and biomass and sediment organic matter accumulation (sediment C, N, P, S, particle size (and ratios therein) and degree of pyritization) may be useful supporting indicators.

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[11] Note that cyanobacteria cell counts and toxin concentrations are not anticipated to be proliferate in marine environment. Inclusion in polyhaline and euhaline habitats is an attempt to capture effects of cyanobacteria blooms transported from freshwater and oligohaline environments.

[12] Forms referred to relative distribution of dissolved inorganic, dissolved organic, and particulate forms of nutrients, including urea and ammonium

Intertidal and Subtidal Seagrass and Brackish Water SAV Habitat

The primary NNE indicators for seagrass and brackish water SAV habitat represent a combination of factors that result in reduced light attenuation to the bed, resulting in reduced photosynthetic activity by the plants. Thus phytoplankton biomass, macroalgal biomass and percent cover and epiphyte load are the primary NNE indicators for this habitat type. Other primary indicators of subtidal habitat (see above) could also apply to these habitats.

Supporting indicators for seagrass habitat should include information on the overall health of the seagrass habitat, including areal distribution, density, water column light attenuation, and epiphyte load on seagrass leaves.

Supporting indicators for brackish water SAV habitat should include information on the overall health of the SAV bed, including areal distribution, biomass, water column light attenuation, and epiphyte load.

Unvegetated Intertidal Flats and Shallow Subtidal Habitat

The primary NNE indicator for unvegetated intertidal and shallow subtidal habitat is macroalgal biomass and cover. Supporting indicators for this habitat type include sediment particle size, %OC, N, P and S, degree of pyritization and microphytobenthos biomass and taxonomic composition.

Note that macroalgal biomass and percent cover is most applicable to fine-grained intertidal and shallow subtidal habitats and may be only partially effective in sandy substrates that can dominate in river mouth estuaries. For this reason, microphytobenthos biomass and taxonomic composition, though considered a supporting indicator, is strongly recommended in sandy intertidal and shallow subtidal habitats.

8.3.2   Recommended Indicators for "Closed" Estuaries

There are two principle habitat types when intermittently or ephemerally tidal estuaries are "closed".

- Unvegetated subtidal habitat
- Brackish SAV habitat (subtidal)

Table 8.5 summaries the primary and supporting indicators by these two habitat types.

Table 8.5. Table of proposed primary and supporting NNE indicators by habitat type. Note that primary and supporting indicators recommended for unvegetated subtidal habitat are also applicable for brackish SAV habitat.

Habitat	Primary Indicators	Supporting Indicators
All Subtidal Habitat	Phytoplankton biomass and productivity Cyanobacteria cell counts and toxin concentration Dissolved oxygen Rafting or floating macroalgae biomass and % cover	Phytoplankton assemblages, including HAB species cell count and toxin concentrations Sediment C, N, P, S, particle size (and ratios therein) and degree of pyritization Microphytobenthos taxonomic composition and benthic chl a biomass Water column nutrient concentrations and forms[13] (C, N, P, and Si)
Brackish SAV	Phytoplankton biomass and productivity Macroalgal biomass & cover Dissolved oxygen	Light attenuation, suspended sediment conc. Epiphyte load Brackish SAV areal distribution, % cover, biomass

Subtidal Habitats

As with "open" estuaries, the primary indicators of eutrophication in the moderate to deepwater unvegetated subtidal habitat of intermittently or ephemerally tidal estuaries with a "closed" inlet include phytoplankton (biomass, productivity) and harmful algal species cell counts (specifically cyanobacteria counts and toxin concentration), and dissolved oxygen. In addition, it is common to observe rafting or floating macroalgae, particularly in oligohaline habitats.

Supporting indicators for this habitat types include information on the phytoplankton assemblage, cell count, toxin concentrations of other HAB species, and total N: total P ratios. In these environments, macrobenthos are not a reliable indicator of eutrophication, but sediment organic matter accumulation (sediment C, N, P, S, particle size (and ratios therein) and degree of pyritization) may be useful supporting indicators. As with open estuaries, microphytobenthos biomass and taxonomic composition is an important supporting indicator recommended in sandy subtidal habitats.

Brackish Water SAV Habitat

The primary NNE indicators for brackish water SAV habitat represent a combination of factors that result in reduced light attenuation to the canopy or bed, resulting in reduced photosynthetic activity by the plants. Thus phytoplankton biomass, macroalgal biomass and percent cover and epiphyte load are the

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[13] Forms referred to relative distribution of dissolved inorganic, dissolved organic, and particulate forms of nutrients, including urea

primary NNE indicators for this habitat type. Other primary indicators of subtidal habitat (see above) could also apply to these habitats.

Supporting indicators for this habitat should include information on the overall health of the brackish SAV habitat, including areal distribution, biomass, water column light attenuation and suspended sediment load, and epiphyte load on SAV leaves.

8.4 Data Gaps and Recommended Next Steps

Development of an NNE assessment framework for California estuaries begins by specifying how primary and supporting indicators would be used as multiple lines of evidence to diagnose adverse effects of eutrophication. This section identifies the data gaps and recommended next steps to use the identified primary and supporting indicators in development of an assessment protocol to assess eutrophication.

Assessment frameworks would need to be created for habitat types identified in this review, with some differences specified by estuarine inlet status (closed or open). Table 8.6 -8.10 summarizes data gaps and recommended next steps for development of the NNE assessment framework by habitat type.

Note that no attempt is made to neither prioritize nor reduce/eliminate "next steps" in any habitat types, despite acknowledged limitation in available resources. The NNE technical team assumes this prioritization and focusing of resources would be done by the SWRCB, with advice from its advisory groups.

8.4.1 Dissolved Oxygen in All Subtidal Habitat

All six coastal Regional Boards have numeric dissolved oxygen objectives applicable to estuaries. However, there is generally a lack of consistency among RWQCBs in their approach. This lack of consistency resulted in the review of science supporting estuarine dissolved oxygen objectives, with the goal of developing a consistent approach statewide that protects specific designated uses and aquatic habitats. The approach taken is similar to that of the US EPA Virginia Province Salt Water Dissolved Oxygen Criteria document (US EPA 2003). The goal of the Virginia Province Approach is to maintain and support aquatic life communities and their designated uses. The approach relies primarily on data generated at the organism rather than the population level, and are designed to protect the most sensitive life stage of organisms which spend part of all of their life history within an estuary. The approach was developed for the region of the east coast of the US from Cape Cod, MA, to Cape Hatteras, NC and has been applied in the Chesapeake Bay (Batiuk et al. 2009), and other coastal regions of the US including Maine and Alabama.

The technical report summarizing the findings of the literature review will be available in the summer of 2011. This report highlights data gaps and summarizes the science that can be used to set dissolved oxygen objectives for selected estuarine classes.

8.4.2   Phytoplankton and Water Column Nutrient Indicators in Unvegetated Subtidal Habitat

Within the realm of phytoplankton indicators, biomass, productivity, cyanobacterial cell counts and toxin concentration are designated as primary indicators for all subtidal habitats. The intent with primary indicators is to establish NNE endpoints, while supporting indicators can be used as additional lines of evidence, but endpoints would not be developed in the near term. To establish numeric thresholds for these primary indicators, a number of data gaps and next steps must be addressed (Table 8.6).

For phytoplankton biomass and productivity, there is a large amount of experience and studies that exist globally, but a lack of data for most California estuaries, with the exception of San Francisco Bay (see review by McKee et al. 2011), where a water quality data set of nearly 40 years exists. It is recommended that a working group of experts be assembled to develop an assessment framework for biomass and productivity that takes into account the high spatial and temporal variability of phytoplankton, using San Francisco Bay as a "test case."

Endpoints for phytoplankton biomass in seagrass and brackish SAV habitat may differ from that established for unvegetated subtidal habitat and will be established through a separate process (see Section 8.4.3 on phytoplankton biomass, macroalgal biomass and cover and epiphyte load in seagrass and brackish SAV).

For cyanobacteria cell counts and toxins concentrations, guidelines exist to establish NNE endpoints in fresh habitats, based on human and faunal exposure to toxin concentrations. The applicability of these endpoints should be examined for translation to estuarine habitats (see Section 8.4.6).

Phytoplankton assemblage information, other HAB species cell counts and toxin concentration, and nutrient stoichiometry are designated as supporting indicators for phytoplankton dominated subtidal habitat. It is recommended that these indicators be included in routine monitoring program and basic research be supported in order to better understand how to use this information to diagnose eutrophication in the future.

Table 8.6. Summary of Data Gaps and Recommended Next Steps for Phytoplankton and Water Column Nutrient Indicators in Unvegetated Subtidal Habitat.

Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Phytoplankton biomass and productivity	Primary indicator	Wealth of experience and studies exists globally, but lack of data for most California estuaries and lack of specific studies to establish thresholds. Precise thresholds may vary from estuary to estuary, depending on co-factors.	Recommend development of a white paper and a series of expert workshops to develop NNE assessment framework for phytoplankton biomass, productivity, taxonomic composition/assemblages, abundance and/or harmful algal bloom toxin concentrations in "open" and "closed" estuaries. Include review of relevant thresholds for nutrient stoichiometry as relevant for "closed" estuaries.	No work under-taken
Cyanobacteria cell count and toxin conc.	Primary indicator	Data and precedent exist to establish NNE thresholds.		
Nutrient stoichiometry	Supporting indicator	Lack of data in California estuaries on use of nutrient stoichiometry to predict cyanobacteria dominance in oligohaline to mesohaline habitats.		
Ammonium	Supporting	Ammonium inhibition of nitrate uptake by diatoms document, although importance of this effects vis-à-vis other controls on production and species dominance not well understood	Future investigations on utility of ammonium as an indicator should be focused first on San Francisco (SF) Bay, where debate on ammonium is a priority issue. Formulate a working group of scientists to synthesize available data on factors known to control primary productivity in different regions in SF Bay, develop consensus on relative importance of ammonium inhibition of phytoplankton blooms, and evaluate potential ammonium endpoints (see McKee et al. 2011 for further details).	No work undertaken
Phytoplankton assemblages, HAB species cell count, toxin conc.	Supporting indicator	Controls on phytoplankton assemblages, euhaline and marine HAB bloom occurrence and toxin production not well understood	Include as indicator in monitoring program and support basic research to increase understanding of drivers.	Not applicable
Urea	Supporting	Lack of data on urea concentrations in estuaries		

8.4.3   Phytoplankton, Macroalgae, Epiphyte Load and Light Attenuation in Seagrass Habitats

For seagrass habitats, macroalgal biomass and cover and phytoplankton biomass are designated as primary indicators, while light attenuation and epiphyte load are designated as supporting indicators (Table 8.7). Development of an assessment framework for seagrass based on these indicators will require addressing the following studies: 1) conduct studies identifying thresholds associated with adverse effects of macroalgal biomass and cover on seagrass growth, 2) collect data on light requirements of California seagrass and determine combinations of phytoplankton biomass and turbidity that result in light attenuation beyond levels of tolerance of seagrass, and 3) assemble a workshop of experts to construct assessment framework for seagrass habitat that uses macroalgae, phytoplankton, epiphyte load in a multiple lines of evidence fashion. Studies to identify thresholds associated with adverse effects of macroalgal biomass and cover are funded and will begin the summer 2011.

Table 8.7. Data gaps and recommended next steps for development of an NNE assessment framework for seagrass habitat.

Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Macroalgal biomass and cover	Primary indicator	Data lacking on dose and response of macroalgal biomass on seagrass growth	Conduct experiments on biomass, cover and duration of macroalgae that results in reduced seagrass growth. Survey ranges of biomass, duration and cover associated with macroalgae on seagrass	Funded. Study to begin summer 2011
Phytoplankton biomass	Primary indicator	Data lacking on light requirements for California seagrass.	Determine light requirements for California seagrass and survey range of epiphyte loads on seagrass beds. Develop assessment framework as a function of light attenuation, macroalgal biomass and epiphyte load	No work undertaken
Light attenuation	Supporting indicator			
Epiphyte load	Supporting indicator	Scientific foundation exists, but epiphyte load difficult to quantify.		

8.4.4   Macroalgae, Sediment C:N:P:S Ratio, Degree of Pyritization and Microphytobenthos on Intertidal Flats

Discussion of data gaps in intertidal flat habitat in "open" estuaries distinguishes between fine-grained (mud flats) and course grained (sand-flats) habitat types (Table 8.8).

In mud flats of "open" estuaries, macroalgal biomass and percent cover are the primary NNE indicators. In these habitat types, data are lacking on the thresholds of effects of macroalgae on benthic infauna as well as documentation of the range of duration of biomass and cover associated with macroalgae on intertidal flats. To address these data gaps, recommended next steps include: 1) conducting experiments and field surveys to address these data gaps, and 2) synthesis of these data into an assessment framework. In sand flats, use of macroalgae as an indicator is questionable, as it is more common to see high biomass of microphytobenthos in eutrophic conditions. Therefore, in "open" estuaries dominated

by sandy intertidal flats, as is the case in river mouth estuaries, a more important indicator may be microphytobenthos biomass and taxonomic composition. Since little is known about controls on microphytobenthos, it recommended that research be supported to improve understanding of appropriate indicators of eutrophication in river mouth estuaries in the "open" condition.

In addition to microphytobenthos biomass and taxonomic composition, sediment C:N:P:S ratio and degree of pyritization are considered to be supporting indicators. As an indicator, these measures may provide useful information about changes in sediment redox chemistry that are directly linked to toxic levels of sulfide and ammonium in sediment pore waters. However, it is not clear the degree to which this is a useful or sensitive indicator in tidal flats. Therefore, it is recommended that sediment C:N:P:S and degree of pyritization be included in monitoring, experiments and field studies recommended above for macroalgae to further evaluate the utility of this indicator.

Table 8.8. Data gaps and recommended next steps for development of an NNE assessment framework for intertidal flats in "open" estuaries.

Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Macroalgal biomass and cover	Primary	Data lacking on dose and response of macroalgal biomass on benthic infauna in intertidal flats	Conduct experiments on biomass, cover and duration of macroalgae that results in reduced diversity and abundance of benthic infauna in tandem with sediment C:N:P:S and degree of pyritization. Survey ranges of biomass, duration and cover associated with macroalgae on seagrass	Funding and study in progress
Sediment C:N:P:S and degree of pyritization	Supporting indicator	Data lacking on the sensitivity of this indicator vis-à-vis primary producers		
Microphyto-benthos biomass and taxonomic composition	Supporting	Data lacking effects of eutrophication on biomass and taxonomic composition across gradients of particle size and salinity	Conduct field studies that document change in biomass and taxonomic composition of microphytobenthos along disturbance gradient in sandy intertidal flats and shallow subtidal habitat of "open" estuaries.	No work undertaken

8.4.5   Macrobenthos Biomass, Taxonomic Composition, and Abundance, Sediment C:N:P:S and Degree of Pyritization in Subtidal Habitats >18 ppt

In subtidal habitats of "open" estuaries with salinities greater than 18 ppt, macrobenthos biomass, taxonomic composition and abundance may provide additional information on eutrophication. As macrobenthos taxonomic composition and sediment %C and %N are already being used in the assessment of sediment quality objectives, the addition of macrobenthic biomass and sediment P, S and degree of pyritization represents an attempt to enhance information collected through the SQO protocol to assess effects of eutrophication. Recommended next steps to further explore the utility of these indicators for this purpose includes (Table. 8.9): 1) analysis of existing regional monitoring datasets for useful taxonomic indicators of eutrophication and 2) conducting a pilot study in a future

regional monitoring program study to test the utility of including biomass, sediment C:N:P:S ratios, and degree of pyritization as a standard part of this protocol.

Table 8.9. Data gaps and recommended next steps for use of macrobenthos and indicators of sediment organic matter accumulation in "open" estuaries with salinities > 18 ppt.

Indicator	Designation	Status of Science	Recommended Next Steps	Status of work
Macrobenthos taxonomic composition, abundance, biomass	Supporting	Lack of data on the degree to which macrobenthos biomass, in combination with taxonomic composition and abundance, may provide specific diagnosis of eutrophication and how this would differ by salinity regime.	Analyze existing regional monitoring datasets for taxonomic indicators of eutrophication Conduct pilot study in future regional monitoring program study to test utility of including biomass in macrobenthos assessment protocol.	No work undertaken
Sediment C:N:P:S and degree of pyritization	Supporting	Lack of understanding of the sensitivity of sediment C:N:P:S ratio or degree of pyritization in diagnosing eutrophication	Analyze existing regional monitoring datasets for utility of C:N:P:S or degree of pyritization Include indicator in pilot study (polyhaline-euhaline) or field studies (oligohaline-mesohaline) to determine sensitivity and utility for NNE framework Include as indicator in experiments on effects of macroalgae on benthic infauna on intertidal flats (see below)	No work undertaken

8.4.6 Phytoplankton, Macroalgae and Epiphyte Load in Vegetated (Brackish SAV) and Unvegetated Subtidal Habitats of "Closed" Estuaries

In intermittently and ephemerally tidal estuaries during a "closed" tidal inlet condition, primary NNE indicators include macroalgal biomass and cover, phytoplankton biomass, cyanobacterial cell counts and toxin concentrations. Table 8.10 gives a summary of data gaps and recommended next steps for these indicators both vegetated (brackish SAV) and unvegetated subtidal habitats.

Table 8.10. Data gaps and recommended next steps for development of an NNE assessment framework for unvegetated and vegetated (brackish SAV habitat) in closed estuaries.

Habitat Type	Indicator	Designation	Status of Science	Recommended Next Steps	Status of Work
Unvegetated Oligohaline to Mesohaline Habitat	Macroalgal biomass and cover	Primary	Lack of data on thresholds of effects of macroalgal biomass/cover associated with effects on dissolved oxygen, microphytobenthos and pelagic invertebrates	Modeling studies and/or experiments to investigate linkage between macroalgae biomass/cover and dissolved oxygen, microphytobenthos and pelagic invertebrates	No work undertaken
	Cyanobacteria cell count and toxin concentrations	Primary	Studies exist to establish thresholds for freshwater lakes.	Evaluate applicability of freshwater lakes NNE thresholds and WHO guidelines for "closed" estuaries	No work undertaken
	Phytoplankton biomass	Primary			
	Microphytobenthos biomass and taxonomic composition	Secondary	Lack of information on controls on biomass and taxonomic composition	Conduct experiments on degree to which floating macroalgae, phytoplankton and epiphyte loads adversely affect brackish SAV and microphytobenthos	No work undertaken
Brackish SAV	Macroalgal biomass and cover	Primary indicator	Data lacking on response of canopy-forming brackish SAV to factors that result in greater water column light attenuation: floating macroalgae, phytoplankton biomass and epiphyte load.	Conduct field studies documenting biomass, areal extent and % cover of brackish SAV relative to gradients of nutrient loading	
	Phytoplankton biomass	Primary indicator			
	Light attenuation	Supporting indicator			
	Epiphyte load	Supporting indicator			

Floating or rafting mats of macroalgae can have a significant effect on other primary producers in "closed" estuaries. No data or studies are available to document what levels of floating algae result in adverse effects neither on brackish SAV (see below) nor on microphytobenthos. To address these data gaps, two types of studies are recommended: 1) modeling or experiments to document thresholds of effects of floating or rafting macroalgae on microphytobenthos and 2) field studies or experiments that documents linkage between macroalgae, microphytobenthos, dissolved oxygen and pelagic invertebrates.

For brackish SAV habitat, a set of experiments similar to that envisioned for seagrass could be envisioned. However, significant data gaps exist with respect to the sensitivity of brackish SAV habitat to light attenuation from macroalgae and phytoplankton, so the flavor of these studies must be adapted to the particulars of brackish SAV, for several reasons (Table 8.7). First, brackish SAV is typically forms canopies rather than beds, so brackish SAV has the ability to present leaves at the surface and is much less susceptible to effects of light attenuation. Second, despite studies in the European Mediterranean showing that brackish SAV of genus Ruppia spp. can be adversely affected by eutrophication, anecdotal

evidence in California suggests that Ruppia spp. and other species of SASV thrive under hypereutrophic conditions. Therefore, recommended next steps includes two types of studies: 1) experiment to determine the susceptibility of brackish SAV to light attenuation or smothering from phytoplankton and macroalgae and 2) field studies to document the biomass of brackish SAV as a function of nutrient loading and other co-factors.

For unvegetated subtidal habitat in closed estuaries, the lentic conditions under which phytoplankton grow are similar to that of freshwater lakes. Little data is available on the concentrations of phytoplankton biomass and speciation in California estuaries in this condition. However, it is recommended that the numeric endpoints for the California lakes NNE (phytoplankton biomass, cyanobacteria cell counts and toxin concentrations) be evaluated for applicability to unvegetated subtidal habitats.

Appendix 1 - Definitions of Terms for Preliminary Classification of Estuaries

Enclosed bay: This class of estuary is bounded by enclosing landforms, forming a topographic depression. By the SWRCB definition, these estuaries are distinguished from open embayments by the criteria that the width of their inlet is 75% less than the largest width of the estuary. They are perennially open to tidal exchange with a large ocean inlet and, as consequence, are well-flushed, often deep and subject to potentially high energy input from tides and currents. These estuaries have enclosure ratios (100*CA mouth/Area estuary) > 0.1 and subtidal habitat > 50% of the total estuarine area.

Coastal Lagoon: This class of estuary tends to be nearly or completed enclosed by a sand bar, forming a topographic depression. Lagoons are shallow in depth, with reduced exchange with the ocean, and quiescent in terms of wind, current and wave energy. They have an enclosure rations of <0.1 and subtidal habitat of <50%. The flushing times tend to be long relative to riverine estuaries and even embayments, as the restricted exchange with the marine end member and reduced river input lengthen residence times (cut-offs to be defined). They can be perennially, intermittently, ephemerally open to surface water tidal exchange, or permanently closed, but receive exchange with the coastal ocean through the sand berm.

River Mouth: This class of estuary tends to be linear in form (no well defined topographic depression) with a well-defined channel and fresh and brackish water vegetation and/or riparian vegetation occurring near the mouth. These estuaries are typically characterized by high rates of deposition and erosion, with sediments consisting of poorly sorted materials. They can be associated with a delta, bar or barrier island and other depositional features. These estuaries have high flushing rates (cut-offs to be defined) and enclosure ratios >0.1. They can be perennially, intermittently or ephemerally open to surface water tidal exchange.

Surface Water Tidal Connection

Perennially tidal = Perennial surface water connection to tidal water source (>11 months per year)

Intermittently tidal = Surface water connection to tidal water source occurs every year up to 11 months per year.

Ephemerally tidal = Surface water connection to tidal water source does not occur every year, but only 1 or more 2 times per decade, usually during large storm events.