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# Thresholds of adverse effects of macroalgal abundance and sediment organic matter on benthic habitat quality in estuarine intertidal flats

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Martha Sutula, Lauri Green<sup>1</sup>, Giancarlo Cicchetti<sup>2</sup>,  
Naomi Detenbeck<sup>2</sup> and Peggy Fong<sup>1</sup>

## ABSTRACT

Confidence in the use of macroalgae as an indicator of estuarine eutrophication is limited by the lack of quantitative data on the thresholds of adverse effects of macroalgae on benthic habitat quality. In the present study, we utilized sediment profile imagery (SPI) to identify thresholds of adverse effect of macroalgal biomass, sediment organic carbon (%OC) and sediment nitrogen (%N) concentrations on the apparent Redox Potential Discontinuity (aRPD), the depth that marks the boundary between oxic near-surface sediment and the underlying suboxic or anoxic sediment. We surveyed 16 sites in eight California estuaries. At each site, SPI, macroalgal biomass and sediment cores were collected at 20 locations along an intertidal transect; cores were analyzed for %OC and %N. Classification and Regression Tree (CART) analysis was used to identify step thresholds associated with a transition from “reference” or natural background levels of macroalgae, defined as that range in which no effect on aRPD was detected. Ranges of 3 to 15 g dw macroalgae m<sup>-2</sup>, 0.4 to 0.7%OC and 0.05 to 0.07% N were identified as transition zones from reference conditions across these estuaries. Piecewise regression analysis

was used to identify thresholds of adverse effects, associated with the transition from a steep decline in aRPD to a consistent minimum value. Levels of 175 g dw macroalgae m<sup>-2</sup>, 1.1% OC and 0.1% N were identified as thresholds of adverse effects, associated with a shallowing of aRPD to near zero depths. As an indicator of ecosystem condition, shallow aRPD has been related to reduced volume and quality for benthic infauna and alteration in community structure. These effects have been linked to reduced availability of forage for fish, birds and other invertebrates, as well as to undesirable changes in sediment biogeochemical processing of nutrients.

## INTRODUCTION

Marine macroalgae form an important component of productive and highly diverse ecosystems in estuaries worldwide and, in moderate abundances, provide vital ecosystem services (for a review see Fong 2008). However, some species of macroalgae thrive in nutrient-enriched waters and cause extensive blooms in intertidal and shallow subtidal habitats. These macroalgal blooms outcompete other primary producers, at times completely blanketing the seafloor and intertidal flats. This results in hypoxia and reduced abundance and diversity of benthic

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<sup>1</sup>University of California, Department of Evolutionary Biology and Ecology, Los Angeles, CA

<sup>2</sup>US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Atlantic Ecology Division, Narragansett, RI

invertebrates, leading to trophic level effects on birds and fish and disruption of biogeochemical cycling (Sfriso *et al.* 1987; Raffaelli *et al.* 1989; Valiela *et al.* 1992, 1997; Young *et al.* 1998; Bolam *et al.* 2000). The causal mechanisms for adverse effects on benthic invertebrates have been well studied; labile organic matter associated with macroalgal blooms stimulates the bacterial communities in sediments, increasing benthic oxygen demand (Sfriso *et al.* 1987, Lavery and McComb 1991), and decreasing sediment redox potential (Cardoso *et al.* 2004). Zones of sediment anoxia and sulfate reduction become shallow, often extending throughout the sediment under the algal mat (Dauer *et al.* 1981, Hentschel 1996). This leads to porewater ammonia and sulfide concentrations that are toxic to surface deposit feeders (Gianmarco *et al.* 1997, Kristiansen *et al.* 2002),

While many studies have documented these effects, few have been conducted with the expressed intent of informing thresholds of adverse effects of macroalgae. Several studies have used controlled field experiments to show causal effects of manipulated macroalgal biomass and duration on benthic infaunal abundance and diversity (Green 2010, Green *et al.* Submitted, Norkko and Bonsdorff 1996, Cummins *et al.* 2004). While these studies provide well-documented “benchmarks” of adverse effects, collectively they have the drawback that the findings are most applicable in the estuaries in which the experiments were conducted. It is difficult to extrapolate these experimental results to other estuaries that may vary with respect to climate, hydrology, and sediment bulk characteristics, all of which could influence the susceptibility of benthic habitat to macroalgal blooms. Further, even in the most comprehensive of these studies, a large gap exists among biomass treatments in which observed no-effect and effect levels occurred (0 - 125 g dry weight (dw) m<sup>-2</sup>; Green *et al.* Submitted) that leaves room for refinement in understanding of where the actual thresholds may be occurring. Field survey allows us to capture a wider gradient of condition and can help to fill this gap. The one example of this that is relevant for threshold investigation is Bona (2006), who conducted a field survey of effects of macroalgal abundance on benthic habitat quality using sediment profile imaging. However, this work was conducted in one estuary, and was not intended for applicability across a wide range of estuarine gradients. Use of macroalgal indicators is increasing in regional and national assessments of estuarine eutrophication

(McLaughlin *et al.* Submitted, Bricker *et al.* 2007). Consequently, an improved understanding of thresholds across estuaries will help to refine the diagnostic frameworks with which these assessments are made (Bricker *et al.* 2003, Scanlan *et al.* 2007, Zaldivar *et al.* 2008, Borja *et al.* 2011).

The objectives of this study were to: 1) document the relationships between macroalgal biomass and cover, sediment organic matter and nutrients, and benthic habitat quality measured as apparent Redox Potential Discontinuity (aRPD) across a range of eight enclosed bays and coastal lagoons in California and 2) identify thresholds or tipping points in benthic habitat quality in these data as well as the reference envelope where the likelihood of adverse effects are low.

Ecological thresholds have been defined as “the point at which there is an abrupt change in an ecosystem quality, property or phenomenon, or where small changes in an environmental driver produce large responses in the ecosystem” (Grossman *et al.* 2006). Cuffney *et al.* (2010) further distinguish between resistance thresholds (e.g., a sharp decline in ecosystem condition following an initial no effect zone) and exhaustion thresholds (a sharp transition to zero slope at the end of a stressor gradient at which point the response variable reaches a natural limit). As defined by Cuffney *et al.* (2010), resistance and exhaustion thresholds are both examples of slope thresholds. Change point or step-like thresholds have also been described, denoting an abrupt discontinuity in magnitude of a response variable along a stressor gradient, but not necessarily associated with a change in slope (Qian *et al.* 2003). Finally, others have associated ecological thresholds with the concept of resilience and a transition between alternate stable states (Resilience Alliance and Sante Fe Institute 2004). These state-changes may be associated with either abrupt changes in one or more response variable as a key driver crosses a threshold value, or with smooth gradual changes in response variables. In contrast to resistance or exhaustion thresholds, we define “reference envelope” as the physical, chemical or biological characteristics of sites found in the best available condition according to the variable of interest (i.e., aRPD; Stoddard *et al.* 2006), since no California estuaries are without some form of human disturbance. In concept, the distance between the reference and threshold of adverse effects reflects the biogeochemical mechanisms of response, as well as site-specific differences among or within estuaries

and other sources of variation. Different statistical methods are available to quantify step thresholds (CART or changepoint analysis) or slope thresholds (piecewise regression analysis) associated with reference and non-reference populations.

## METHODS

### Conceptual Approach

For this study, we used sediment profile imaging (SPI) technology (Rhoads and Cande 1971; Rhoads and Germano 1982, 1986; Bona 2006) to rapidly evaluate benthic habitat quality directly associated with macroalgal abundance. SPI has previously been used to document macroalgal effects on subtidal benthic habitat quality (Bona 2006) in Venice Lagoon. We chose to focus on estuarine intertidal flats as the targeted habitat type for this study, as monitoring of macroalgae is most cost-effective in this zone (Scanlan *et al.* 2007). The camera can be deployed rapidly, which allowed us to survey a wide range of conditions within and across estuaries.

SPI technology uses a specialized camera system with a wedge-shaped prism that penetrates into soft substrates to image a cross-section or profile of the sediment with depth below the surface (Rhoads and Cande, 1971). The typical use of SPI data in subtidal sediments involves the calculation of multi-metric indices based on indicators ranging from presence/absence of reduced gas bubbles, presence/absence of low DO at the benthic boundary layer, stage of benthic colonization, presence or absence of various faunal features, and the apparent Redox Potential Discontinuity (aRPD) depth (cm), i.e., the boundary between the lighter tan, brown, or reddish oxic or sub-oxic near-surface sediment and the underlying darker grey or black hypoxic or anoxic sediment (Rhoads and Germano 1982, Nilsson and Rosenberg 1997). Sediment profile imaging has rarely been conducted in intertidal habitat, and thus many of these indicators and the multi-metric indices upon which they are built on are not applicable in this habitat type. Preliminary SPI of estuarine intertidal flats showed a distinct lack of gas bubbles and burrows that could be used to identify stage of colonization. Instead, we chose to focus on aRPD as the univariate response variable, which approximates the extent of oxygen penetration into the sediment and the vertical extent of infaunal activity within the sediment. Macroalgal biomass and cover, sediment percent organic carbon (%OC)

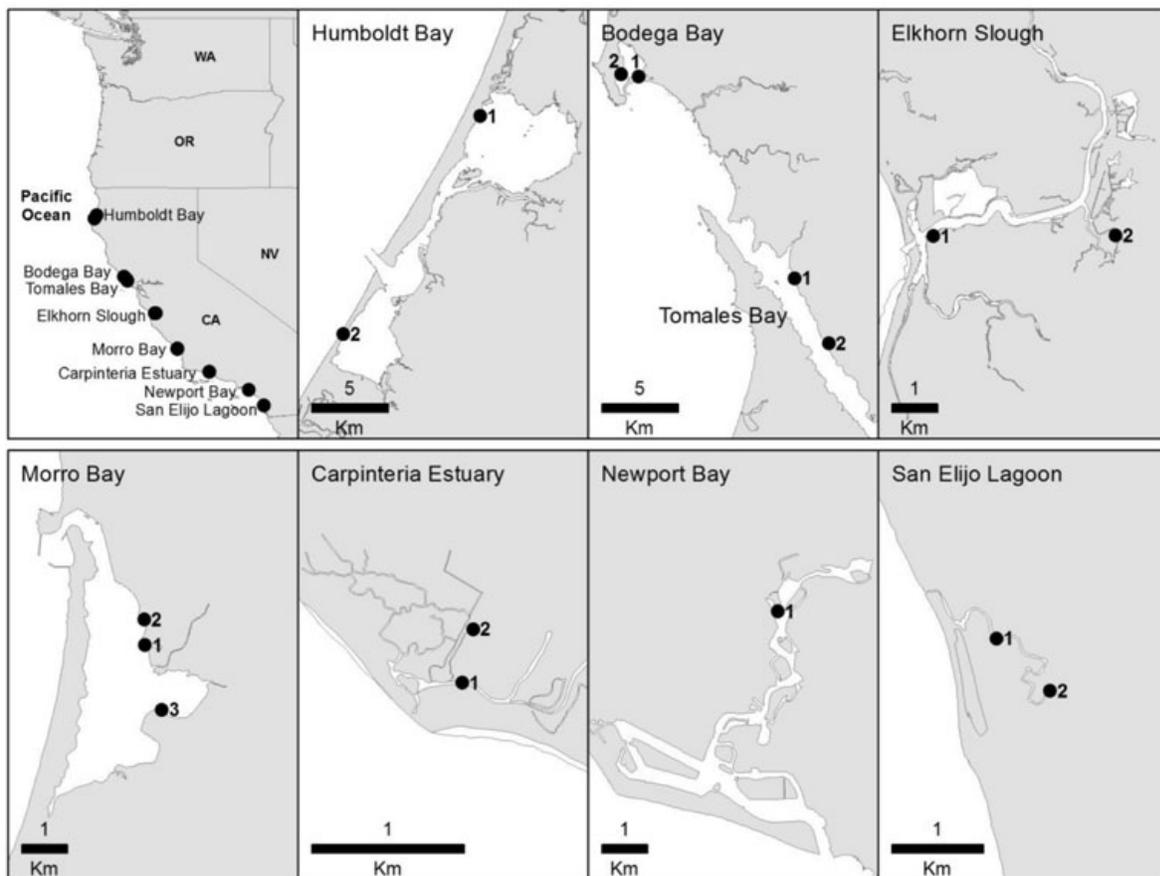
and nitrogen (%N) content served as independent variables in our analyses.

The aRPD in estuarine sediments, in the most basic interpretation, represents the depth zone in which iron remains oxidized and insoluble, in the form of characteristically tan, brown, or red colored ferric hydroxides (Teal *et al.* 2009). Accordingly, the lower limit of the aRPD represents the Fe redox boundary, a reduced environment where reductive dissolution of iron occurs (Teal *et al.* 2009). While the underlying mechanisms and functions associated with the aRPD may be complex and intertwined (Teal *et al.* 2010), it has been clearly shown that the aRPD depth represents a separation of the sediment into two chemically distinct layers (Teal *et al.* 2010), and that this depth is well correlated with a number of co-varying factors including bottom-water DO concentrations (Diaz *et al.* 1992, Cicchetti *et al.* 2006, Shumchenia and King 2010), faunal successional stage (Pearson and Rosenberg 1978, Nilsson and Rosenberg 1997, Rosenberg *et al.* 2003), bioturbation (Pearson and Rosenberg 1978, Rhoads and Germano 1982), sediment type (Rosenberg *et al.* 2003), %OC (Rosenberg *et al.* 2003), physical energy (Rhoads and Germano 1982). We interpret aRPD for this paper as “a reasonable approximation of benthic ecosystem functioning ... [that] is highly context driven” (Teal *et al.* 2010), within our context of estuarine intertidal flats on the west coast of the United States.

### Study Area and Site Selection

The California Coast ranges from the Smith River (41.46°N) to the U.S.-Mexico border (32.53°N; Figure 1). Along this 1700 km coastline, the cool California Current offshore, enhanced by upwelling of cold sub-surface waters, creates a temperate climate north of Cape Mendocino and a moderate Mediterranean climate to the south. Average annual air temperatures and rainfall range from 15°C and 967 mm of rainfall in the north to 19°C and 262 mm in the south. Rainfall along the coast is concentrated largely over the fall through spring and, north of Cape Mendocino, extends through early summer months.

California has a diverse array of enclosed bays and “bar-built” lagoons and river mouth estuaries, resulting from variable geomorphology, climate and oceanic influences (Ferren 1996). In California, enclosed bays represent the largest percent of estuarine habitat by area (90%), typically featuring



**Figure 1. Map of showing location of estuaries and sampling sites for study.**

more subtidal habitat, stronger tidal prisms and thus better flushing; bar-built estuaries represent the greatest number (75%), a type of estuary named for the formation of sandbars that build up along the mouth as a consequence of the longshore transport of sand (Sutula 2011). Bar-built estuaries are usually shallow (<2 m), with reduced tidal action during time periods when the sand bar restricts tidal exchange, typically during periods of low freshwater input (Largier *et al.* 1996)

We selected 16 sites in 8 enclosed bays and bar-built estuaries along the California coast using the following criteria: 1) promoted a balance of enclosed bays and bar-built estuaries, 2) represented a geographic gradient along the coast, 3) estuary was open to surface water tidal exchange at time of sampling (Table 1). Within each estuary, one to three sites were selected along the longitudinal axis of the estuary, based on: 1) presence of an intertidal flat of minimum length and width of 30 meters by 3 meters respectively at low tide, and 2) accessibility (Figure 1; Table 2).

### Field Sampling and Laboratory Methods

Field measurements were conducted in the months of August -September 2011. At each site within an estuary, a 20 m transect was laid out in the intertidal area, parallel to the water's edge, and along the same elevational contour at approximately 0.3 - 0.6 m above MLLW. Sampling of these areas has been demonstrated to be representative of macroalgae on intertidal channels and mudflats (Kennison *et al.* 2003). Along the transects, percent cover and biomass of macroalgae were estimated at 20 randomly chosen 0.0625-m<sup>2</sup> quadrats, hereto referred to as plots, using the point intercept method. Biomass was harvested by cutting through the mats with a razor blade along the inner edge of a quadrat of identical size and placement as for cover estimates. Samples were stored in labeled plastic bags on ice in the dark until delivery to the lab. At each point where biomass was collected, an 8 megapixel sediment profile camera with a 15 cm prism width was inserted manually into the sediment to a depth of approximately 15 cm, and a digital image of the sediment cross section was taken. The

**Table 1. Estuary name, locations, class and size, and the latitude and longitudes of sites sampled in the study.**

Estuary Name	Region	Type	Size (km <sup>2</sup> )	Site Number	Latitude, Longitude
Humboldt Bay (HB)	North Coast	Enclosed Bay	66.10	1	N 40 51.019, W 124 9.559
				2	N 40 43.067, W 124 15.500
Bodega Bay (BB)	North Coast	Enclosed Bay	3.72	1	N 38 18.935, W 123 2.601
				2	N 38 18.9931, W 123 3.401
Tomales Bay (TB)	North Coast	Enclosed Bay	31.15	1	N 38 11.970, W 122 55.280
				2	N 38 9.696, W 122 53.660
Elkhorn Slough (ES)	Central Coast	Bar-built Estuary	4.17	1	N 36 48.566, W 121 46.972
				2	N 36 48.611, W 121 44.284
Morro Bay (MB)	Central Coast	Bar-built Estuary	10.21	1	N 35 20.7201, W 120 50.636
				2	N 35 21.021, W 120 50.652
				3	N 35 19.959, W 120 50.384
Carpinteria Estuary (CE)	South Coast	Bar-built Estuary	0.85	1	N 34 23.900, W 119 32.081
				2	N 34 24.057, W 119 32.041
Newport Bay (NB)	South Coast	Bar-built Estuary	6.70	1	N 33 38.478, W 117 53.374
San Elijo Lagoon (SL)	South Coast	Bar-built Estuary	2.15	1	N 33 00.679 W-117 16.443
				2	N 33 00.358 W -117 16.076

camera, constructed by the USEPA, incorporates a Konica-Minolta Dimage A2.e. Further, at each point a grab sample (cores of 12.5 cm inner diameter, 2 cm deep) of surface sediments was taken for analysis of grain size, %OC, and %N.

In the laboratory, macroalgal biomass samples were cleaned of macroscopic debris, mud and animals, and sorted to genus level. Samples were spun in a salad spinner for 30 seconds to shed excess water, weighed wet, dried at 60°C to a constant weight, and weighed dry. For data analysis, weights of all macroalgal genera were summed for each quadrat and normalized over the area of the biomass sampled to give a total macroalgae wet weight, dry weight, and percent composition in each quadrat. A least squares regression between wet weight and dry weight biomass was calculated for each algal genus or

group in order to compare our results, presented in dry weight, to other studies, many of which report findings in wet weight (Table 2).

The sediment collected at each quadrat was transferred to an aluminum dish, weighed wet, dried at 60°C to a constant weight, and weighed dry. A subsample of dried sediment was ground with a mortar and pestle for analysis of percent total nitrogen (%N) and % organic carbon (%OC). Samples for %OC were acidified to remove carbonates; %OC and %N were measured by high temperature combustion on a Control Equipment Corp CEC 440HA elemental analyzer at the University of California Marine Science Institute, Santa Barbara. The remainder of the sediment was reweighed dry, wet sieved through a 65 µm sieve, dried at 60°C to a constant weight, and weighed dry to determine grain size. Percent fines were

**Table 2. Wet-dry biomass least square relationships by algal species or genus. n = sample size. % Solids is given at the median biomass value across plots.**

Genus or Species	Least Squares Regression Equation	n	Fit (R <sup>2</sup> )	% Dry Weight
<i>Ulva spp.</i>	DryWt = -7.5641 + 0.1364*WetWt	243	0.92	12.9
<i>Gracillaria spp.</i>	DryWt = 1.3525 + 0.1874* WetWt	41	0.98	15.9
<i>Ceramium spp.</i>	DryWt = 0.7239 + 0.1368* WetWt	20	0.85	18.3
<i>Lola spp.</i>	DryWt = - 8.5713 + 0.1887* WetWt	17	0.80	17.3
All	DryWt= 11.939 + 0.0685*WetWt	321	0.45	13.1

calculated through the difference between the total weight and the weight of the sieved portion.

SPI imagery was transferred to a computer and the lighter tan, brown, or red aRPD area was digitized using Adobe Photoshop CS Version 8 2003 (Figure 2). The aRPD depth was calculated as the digitized area divided by the width of the image to provide an average depth across the width of the image.

## Statistical Methods

Quantile regression was used to investigate the conditional median or other quantiles of the macroalgal biomass as a function of percent cover using PROC QUANTREG procedure. Least squares regression was used to quantify the relationship between grain size, sediment %OC and %N using the PROC REG procedure. These

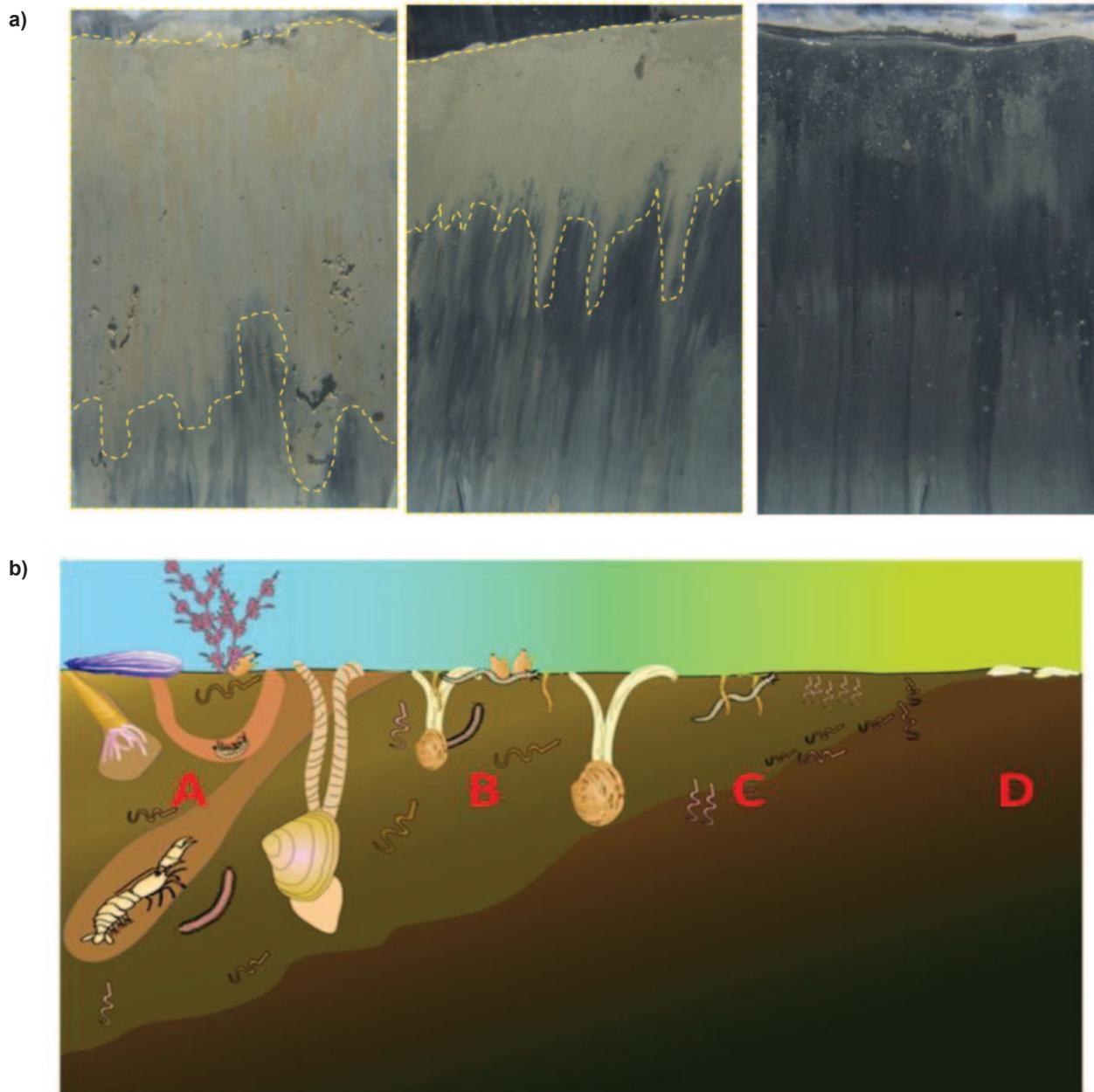


Figure 2. Example of sediment profile images (a) from showing aRPD varying from 8.3 cm, 3.7 cm and 0 cm (left to right respectively). Vertical length of image represents 10 cm in depth. Dotted line represents digitized area of aRPD. Images are contrasted against an illustration (b) of the Pearson-Rosenberg (1978) conceptual model depicting changes in macrobenthic community structure with increasing organic matter accumulation in the sediment. 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. From Gillette and Sutula in Sutula (2011).

analyses were performed using SAS Statistical Software Version 9.3.

Two types of ecological response thresholds for aRPD were investigated. The first type, a “step” threshold, was evaluated as a statistically significant change in magnitude of aRPD along gradients of sediment %N, sediment %C, and macroalgal dry weight biomass. In this case, the step threshold does not necessarily represent a resistance threshold (Cuffney *et al.* 2010), i.e., a transition from a no-effect zone with zero response slope to a zone of accelerated change in aRPD. Instead, this threshold answers the question “At what level of stressor can you detect an overall reduction in aRPD between reference and impacted classes?” The second type, a “slope” threshold, was evaluated as a detectable change in slope of aRPD to each of the three stressors. The slope threshold can be interpreted as the point at which one could expect to see an improvement in benthic condition as stressor levels are reduced, or conversely, the point at which maximum benthic degradation is achieved because the sediments have become anoxic to the surface (Figure 2). The latter is analogous to an exhaustion threshold based on the Cuffney *et al.* (2010) definition.

Step thresholds were analyzed using Classification and Regression Tree (CART) analysis with SYSTAT software (Brieman *et al.* 1984). A maximum split number of 2 was set, with  $p < 0.05$  as the stopping criteria. One thousand bootstrapping iterations were run with 10% replacement to generate confidence intervals for step thresholds. Step thresholds were evaluated both at the plot scale ( $n = 305$ ) and at the site scale, the latter using site averages. The former allows a more accurate assessment of the level of stressor associated with an impact because of the variation in stressor levels within sites. However, because macroalgal biomass is typically averaged at the transect or site-scale, site-level thresholds were also of interest. Potential effects of spatial autocorrelation on results of plot-scale analyses were evaluated using partial Mantel tests of residuals from CART analysis in R with the ECODIST package (Goslee and Urban 2007). Step thresholds were calculated for each dominant algal genus individually at the plot level (*Ulva spp.*, *Ceramium spp.*, *Gracilaria spp.*, and *Lola spp.*) and all algal species together.

Slope thresholds for average and median response were evaluated through piecewise

regression analysis using the NONLIN (nonlinear curve fitting) procedure in SYSTAT (Systat Software, Inc., Chicago, IL). Piecewise regression analysis allows evaluation of a segmented linear response with a change in slope at one or more points. To facilitate convergence, models were fit in two stages. First, models were fit with fixed thresholds based on a series of ten potential values chosen at equal intervals along a log<sub>10</sub> scale of each stressor variable (sediment N, sediment C, or macroalgal biomass dry weight). The model with the best fit in each series then was used as an initial estimate for the slope break variable in a model fitting procedure in which all three parameters were optimized (y-intercept (b<sub>0</sub>), initial slope (b<sub>1</sub>), and break), e.g.:

$$\text{aRPD} = (\text{Sediment}\%N < \text{break}) * (b_0 + (b_1 * \text{Sediment}\%N)) + (\text{Sediment}\%N \geq \text{break}) * (b_0 + (b_1 * \text{break}))$$

This model form assumes that aRPD decreases until it reaches a low value and then remains constant with increasing sediment N. Models were fit using both the least-squares minimization and robust regression techniques (based on least absolute deviation). The latter technique is robust to outliers both in the response variable and in covariates (Birkes and Dodge 1993). Final models were evaluated based on Aikake’s Information Criterion corrected for small sample size (AICc; Burnham and Anderson 2002). Three alternative models were evaluated – one with no slope, one with a slope but no break point, and one with an initial slope and break point. Slope models performed better at the site-averaged rather than plot scale because not all sites had sediment conditions spanning the slope threshold; site-scale also has the advantage that macroalgal biomass is typically reported as a transect-average, so site-averaged thresholds are more relevant for this type of data. In cases where the slope and no-slope break models provided the best fit, the x-intercept was calculated as an indicator of the point of adverse effect and confidence intervals were generated in SYSTAT using bootstrap analysis.

Percent cover was not a good indicator of effects on aRPD; thus, results for % cover are not shown. At the site scale, only 59 to 69% of bootstrap CART trials yielded one or more significant cut point values. Outlier sites (Elkhorn Slough Site 1 and Humboldt Bay Site 2, hereto referred to as ES-1 and HB-2 respectively, with very high macroalgal

biomass, low %fines, sediment %OC and %N, were removed from the analysis of step thresholds for biomass because it is suspected that these transects represent high energy sites where it is likely that macroalgae was rafted up (Rhoads and Germano 1982).

## RESULTS

### Range of Conditions within and across Estuaries

Taken collectively across all estuaries (Figure 1), the sites we sampled represented a wide range in condition with respect to sediment bulk characteristics (0.01 - 22.6% OC, 0.02 - 1.57 %N, and 0 - 96% fines), aRPD depth (0 - 17 cm), macroalgal biomass (0 - 1717 g dw m<sup>-2</sup>), and macroalgal cover (0 - 100%; Table 3). Many sites showed a broad distribution of these properties as well; notable exceptions to this included Elkhorn Slough site 2, which consistently had very low aRPD and very high sediment %OC, %N and macroalgal abundance, and Carpinteria Estuary site 2, which had no macroalgae present during the time of sampling.

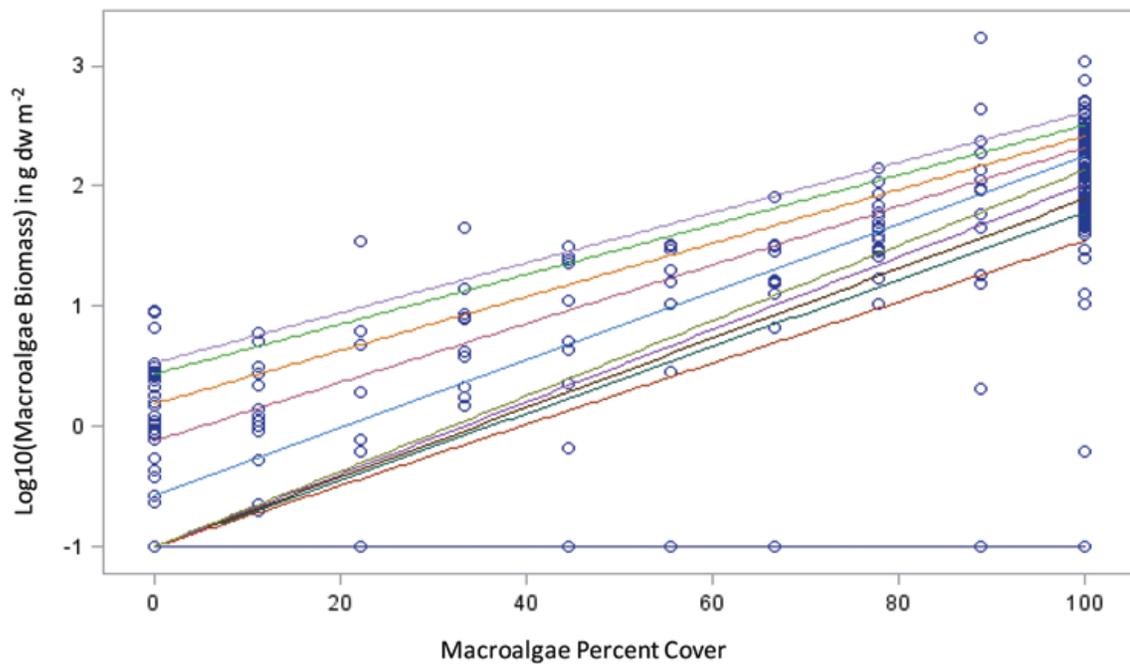
Four of eight estuaries (Elkhorn Slough, Morro Bay, Carpinteria Estuary, and San Elijo Lagoon) were completely dominated by *Ulva* spp. (*U. intestinalis*, *U. expansa*, or *U. lactuca*). An additional three were co-dominated by *Ulva* spp. and other species of red (*Gracilaria* spp. in Bodega Bay and Tomales Bay) or green algae (*Lola* spp. in Humboldt Bay). Newport Bay was the only system in which the red algal genus *Ceramium* spp. was found and it completely dominated biomass in this estuary.

### Relationships between Macroalgal Biomass and Macroalgal Cover

Across estuaries, algal biomass generally increased with increasing % cover (Figure 3). Both low and high biomass were possible at high % cover; for example, at >80% cover, 20% of plots were below 16 g dw m<sup>-2</sup> while 20% of plots were above 93 g dw m<sup>-2</sup> (Figure 3). At 100% cover, 60% of plots exceeded 100 g dw m<sup>-2</sup>. However, high biomass generally did not occur at low % cover; at <30% cover, only 5% of plots exceeded a biomass of 14 g dw m<sup>-2</sup>.

**Table 3. Mean and standard deviation (SD) of %OC, %N, aRPD (cm), and macroalgal biomass and cover of 20 plots per estuary site.**

Estuary	Site No.	aRPD		Sediment %OC (g dw)		Sediment %N (g dw)		Macroalgal Biomass (g dw m <sup>-2</sup> )		Macroalgal Cover (%)	
		Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range	Mean±SD	Range
Humboldt Bay (HB)	1	3.0 ± 1.5	0.6 - 7.3	0.5 ± 0.2	0.2 - 1.0	0.05 ± 0.02	0.03 - 0.09	85.0 ± 57.8	0.0 - 235.0	77 ± 35	0 - 100
	2	3.8 ± 3.4	0.2 - 10.1	0.2 ± 0.0	0.1 - 0.3	0.03 ± 0.01	0.02 - 0.04	226.0 ± 148.1	0.0 - 507.9	88 ± 30	0 - 100
Bodega Bay (BB)	1	3.6 ± 2.4	0.2 - 11.3	0.1 ± 0.0	0.0 - 0.2	0.02 ± 0.01	0.01 - 0.03	7.3 ± 14.9	0.0 - 51.5	17 ± 33	0 - 100
	2	1.3 ± 1.9	0.1 - 6.6	0.3 ± 0.1	0.2 - 0.6	0.06 ± 0.01	0.04 - 0.09	228.4 ± 407.8	29.2 - 1716.9	83 ± 16	56 - 100
Tomales Bay (TB)	1	7.8 ± 3.5	4.2 - 17.0	0.3 ± 0.0	0.2 - 0.4	0.03 ± 0.01	0.02 - 0.04	5.7 ± 16.1	0.0 - 74.7	27 ± 32	0 - 100
	2	7.9 ± 3.6	0.6 - 12.8	0.4 ± 0.1	0.3 - 0.5	0.05 ± 0.01	0.03 - 0.06	3.5 ± 12.1	0.0 - 54.0	8 ± 23	0 - 100
Elkhorn Slough (ES)	1	8.7 ± 2.6	1.1 - 12.2	0.3 ± 0.1	0.2 - 0.6	0.04 ± 0.01	0.03 - 0.07	165.9 ± 91.5	0.5 - 335.5	96 ± 9	67 - 100
	2	0.6 ± 1.0	0.1 - 4.1	9.5 ± 4.9	3.8 - 22.6	0.77 ± 0.31	0.43 - 1.57	264.5 ± 159.8	4.1 - 750.0	95 ± 16	33 - 100
Morro Bay (MB)	1	2.3 ± 3.0	0.0 - 8.6	0.6 ± 0.2	0.3 - 0.9	0.08 ± 0.02	0.05 - 0.12	83.0 ± 33.8	0.0 - 138.0	98 ± 6	78 - 100
	2	5.4 ± 1.4	2.1 - 7.2	0.1 ± 0.0	0.1 - 0.2	0.02 ± 0.01	0.02 - 0.03	65.3 ± 125.4	0.0 - 493.9	47 ± 42	0 - 100
Carpinteria Estuary (CE)	3	2.6 ± 2.3	0.0 - 8.1	0.8 ± 0.4	0.3 - 1.9	0.12 ± 0.05	0.05 - 0.28	53.3 ± 30.8	0.0 - 107.8	83 ± 32	0 - 100
	1	1.0 ± 0.8	0.0 - 2.9	1.7 ± 0.2	1.3 - 2.1	0.18 ± 0.03	0.13 - 0.24	23.5 ± 29.0	0.0 - 103.2	41 ± 40	0 - 100
Newport Bay (NB)	2	3.1 ± 1.2	1.3 - 5.6	0.7 ± 0.2	0.4 - 1.1	0.08 ± 0.01	0.05 - 0.14	0.0 ± 0.0	0.0 - 0.0	0 ± 0	0 - 0
	1	3.4 ± 2.0	0.7 - 9.3	0.6 ± 0.1	0.4 - 0.9	0.07 ± 0.01	0.05 - 0.10	4.4 ± 7.0	0.0 - 29.8	17 ± 28	0 - 100
San Elijo Lagoon (SL)	2	4.1 ± 2.7	0.7 - 12.0	0.6 ± 0.1	0.4 - 0.8	0.07 ± 0.02	0.04 - 0.12	17.4 ± 21.6	0.0 - 84.3	41 ± 34	0 - 100
	1	5.3 ± 3.1	0.0 - 10.4	0.5 ± 0.5	0.2 - 2.4	0.06 ± 0.04	0.02 - 0.21	8.0 ± 11.2	0.0 - 45.9	31 ± 42	0 - 100
	2	2.7 ± 2.3	0.3 - 6.5	2.4 ± 0.4	1.7 - 3.2	0.29 ± 0.05	0.19 - 0.39	69.9 ± 68.8	0.0 - 220.0	67 ± 37	0 - 100



**Figure 3.** Plot of quantile regression results of macroalgal percent cover (x axis) and log<sub>10</sub> of macroalgal biomass (g dw m<sup>-2</sup>). Lines represent individual quantiles (0.95, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.2, 0.1, and 0.05) from top to bottom. Note that it is possible to have 0 biomass at a range of percent cover as well as measureable biomass at 0% cover.

### Relationships between Sediment Percent Organic Carbon, Nitrogen and Grain Size

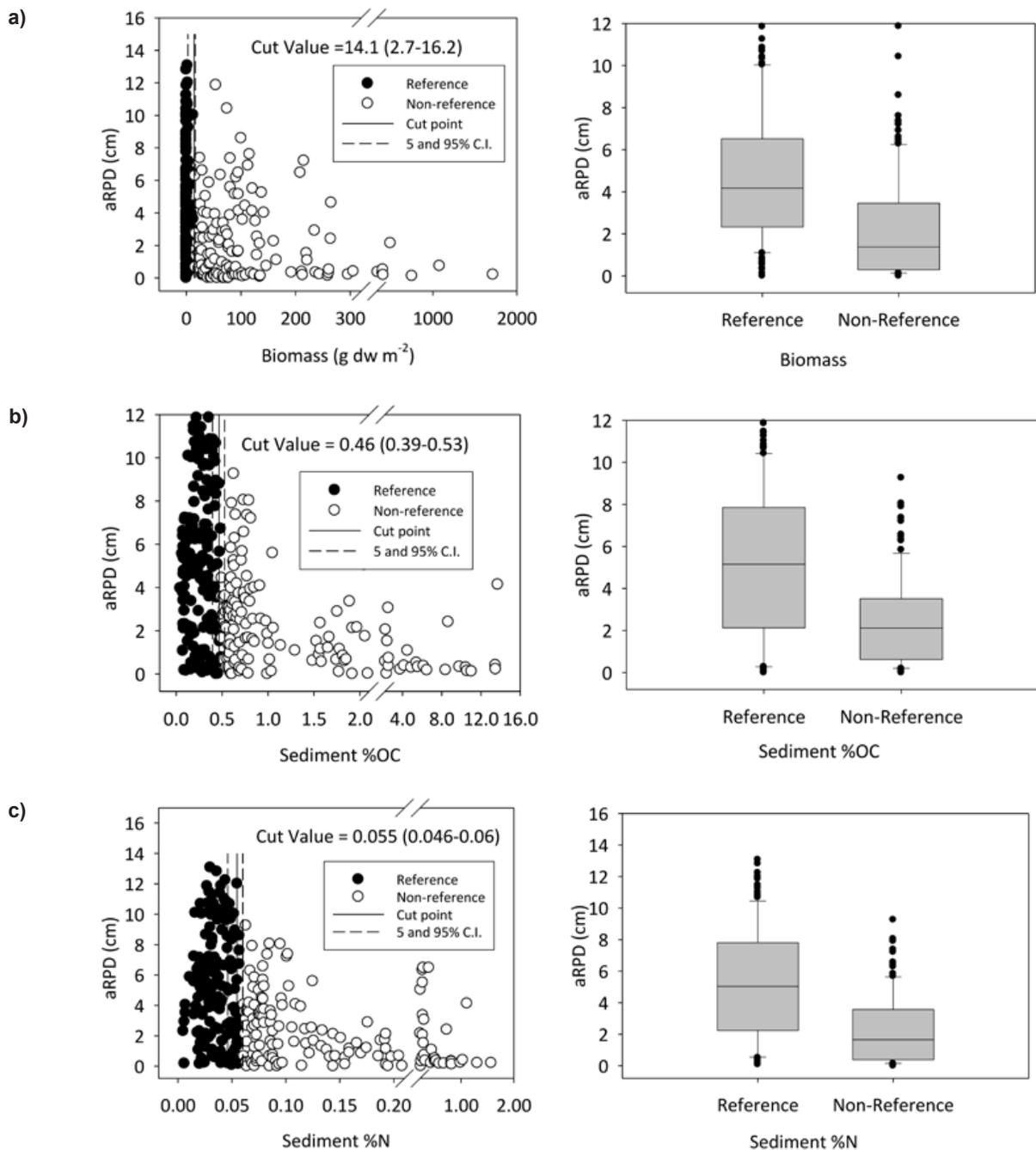
Across estuaries, sediment %OC and %N were highly positively correlated with % fines (p-value <0.0001 for both correlations); a least squares regression of % fines with the square root of %OC and square root of %N resulted in a linear fit of  $R^2 = 0.53$  and  $0.55$  respectively. Sediment %OC and %N showed a high degree of covariance (p-value <0.0001,  $R^2 = 0.98$ ).

### Thresholds for Macroalgal Biomass, Sediment %OC, %N Relative to aRPD

Grouping data across estuaries and algal genera, CART analysis allowed us to identify relatively tight step thresholds based on plot-scale data for sediment %N, sediment %OC, and macroalgal biomass; Figure 4a through c). Similar thresholds were found on site-scale averages for sediment N (0.064 %N) and sediment C (0.70 %C; Figure 5b through c), but were slightly higher and less certain for site-scale as compared to plot-scale sediment %OC (Figures 4c and 5c). Although CART analysis identified a step threshold for site-averaged macroalgal biomass (52.6 g dw m<sup>-2</sup>), it was very diffuse (wide confidence interval), even after the ES-1 and HB-2 outlier site data had been removed (Figure 5a). Partial Mantel

tests showed no evidence of spatial autocorrelation in residuals from CART analyses of the plot data using either sediment %OC or %N ( $p > 0.05$ ) and only marginal evidence for spatial autocorrelation of residuals from CART analysis based on macroalgal biomass dry weight ( $p = 0.05$ ).

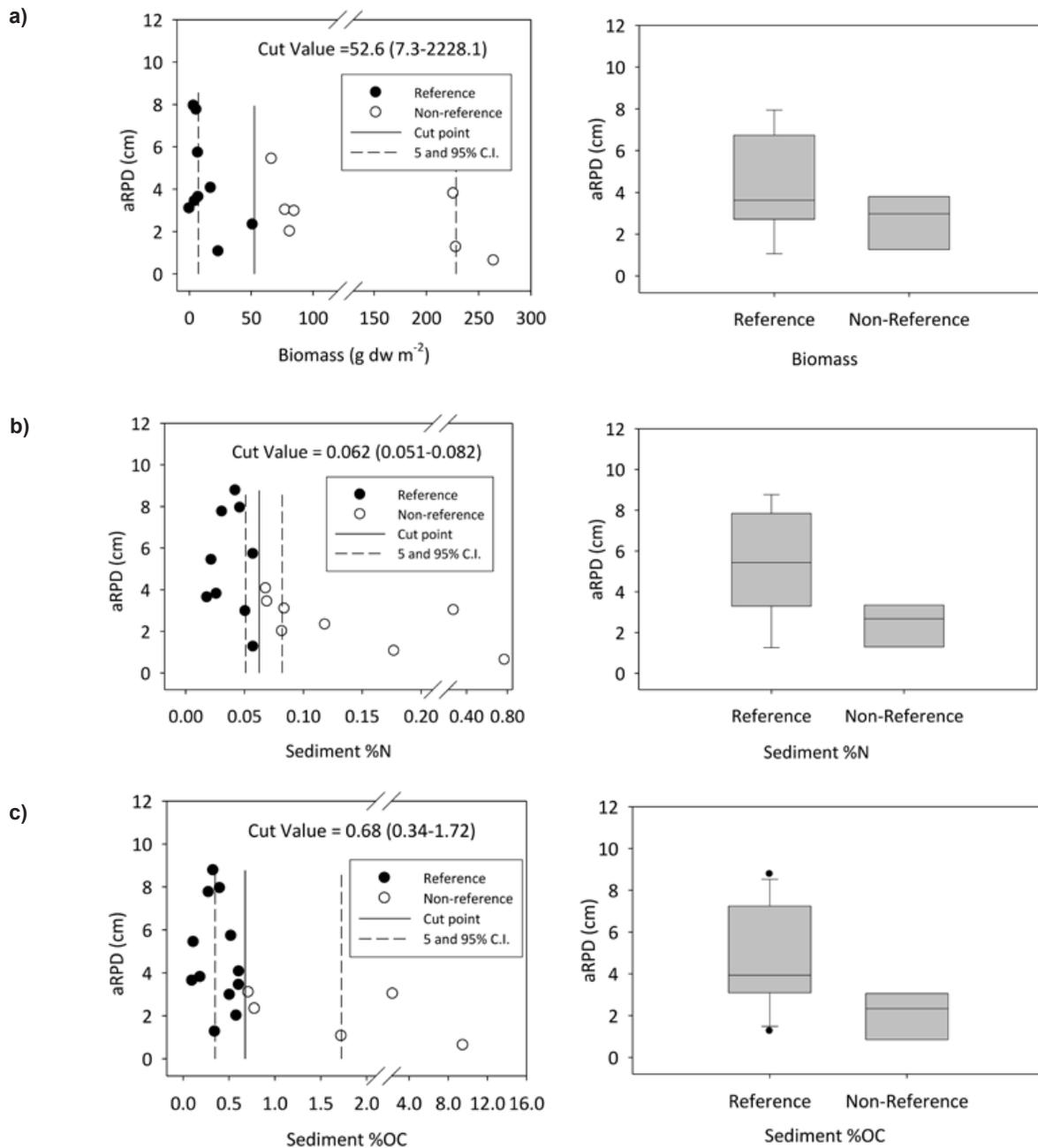
With respect to slope thresholds, the best site-average model fits for aRPD versus sediment %N were those that incorporated both an initial slope term and a break in slope, based on AICc criteria (Table 4; Figure 6a). Removing outliers ES-1 and HB-2 improved fits for %N, %OC and biomass models. The slope threshold was similar between least-squares and robust regression results, at ~0.11 and ~0.14 %N, respectively, approximately double that of the step thresholds presented above. The best site-average model fits for aRPD versus sediment %OC did not include the models with a slope change, although relative likelihoods were still relatively high for the latter, and the robust regression model fit with a slope break parameter was better than the model with only intercept and slope terms. The least squares and robust regression slope thresholds were 1.08 and 1.22 %OC respectively, again, higher than for the corresponding step threshold (Figure 6b). The best site-average model fits for aRPD versus macroalgal biomass did not include the models with a slope change; a “no



**Figure 4.** aRPD as a function of biomass (a), sediment %N (b) and sediment %C (c) with X axis delineating low and high aRPD groups as defined by bootstrapped CART analysis for plot level data. The cut value is the mean step threshold, shown as the solid vertical line, with 5<sup>th</sup> and 95<sup>th</sup> percentiles as dashed lines. Box plots (right) for two groups are next to scatter plot (left hand). Biomass thresholds shown reflect elimination of ES-1 and HB-2 outliers.

break” slope model was more significant than one with a slope break, regardless of whether ES-1 and HB-2 were excluded (Table 4). After removing these outliers, the least squares model median X-intercept (representing the macroalgal biomass at which aRPD approaches zero) was 319 g dw m<sup>-2</sup>, with 5<sup>th</sup> and 95<sup>th</sup> percentiles ranging from 175 - 358 g m<sup>-2</sup> (Table 5). The 5<sup>th</sup> percentile (175 g dw m<sup>-2</sup>)

of this X intercept a more conservative estimate of an effects threshold than the 50<sup>th</sup> or 95<sup>th</sup> percentile. The robust model gave results similar to the least squares model results for the X intercept (189 - 358 g m<sup>-2</sup>). In this case, the effect of trailing data points at near zero aRPD values with increasing biomass causes an increase in the median value of the X intercept and a widening of this confidence interval.



**Figure 5.** aRPD as a function of biomass (a), sediment %N (b) and sediment %OC (c) with X axis delineating low and high aRPD groups as defined by bootstrapped CART analysis for site-averaged data. The cut value is the mean step threshold, shown as the solid vertical line, with 5<sup>th</sup> and 95<sup>th</sup> percentiles as dashed lines. Box plots (right) for two groups are next to scatter plot (left). Biomass thresholds shown reflect elimination of ES-1 and HB-2 outlier.

Including these outliers caused a further widening of confidence intervals.

Analysis of data at the algal genus level gave step thresholds for %C and %N that were relatively consistent with those identified for data grouped across algal genus (0.18 - 0.63 %C and 0.03 - 0.07 %N; Table 6). For biomass, there was more variability. Step thresholds for *Ulva* spp., *Ceramium*

spp. and *Gracilaria* spp. ranged from a low of 9.4 to 46.1 g dw m<sup>-2</sup>, while the threshold for *Lola* spp. was substantially higher (261 g m<sup>-2</sup>). Step thresholds for biomass grouping algal genus (7.2 g m<sup>-2</sup>) was lower than that which excluded only *Lola* spp. (13.1 g m<sup>-2</sup>), though the confidence intervals were virtually identical (2.7 - 16.2 g m<sup>-2</sup>). Interestingly, the comparison of %N and %C thresholds for plot-level

**Table 4. Piecewise regression analysis of site-averaged aRPD response as a function of sediment %N, sediment %C, and macroalgal biomass. Models with and without break point and with and without slope are compared using AIC criterion corrected for small sample size (AICc). Best (lowest) AICc is shown in bold. Relative likelihood of a significant slope or of having a break in slope are bolded for values greater than 1. Results shown for models with and without ES-1 and HS-2 outliers removed. n = sample size.**

Fit Method	Predictor	Parameter Estimates (Wald 95% CI)		n	Residual SS	AICc		Relative Likelihood of Significant Break		Relative Likelihood of Significant Slope
		Break	Y-Intercept			Initial Slope	Full Model	No Break	No Slope	
Least Squares	%N	0.11 (0.017-0.21)	6.7 (3.8-10.3)	17	58.2	80.5	<b>77.1</b>	82.3	0.19	<b>13.38</b>
Robust Regression	%N	0.14	6.3	17	60.0	81.0	<b>77.8</b>	83.1	0.20	<b>14.21</b>
Least Squares <sup>†</sup>	%N	0.11 (0.029 - 0.18)	7.4 (4.0-1.0)	16	54.9	<b>76.8</b>	77.8	78.9	<b>1.71</b>	<b>1.67</b>
Robust Regression <sup>†</sup>	%N	0.14	6.2	16	58.6	<b>77.8</b>	78.9	79.6	<b>1.73</b>	<b>1.38</b>
Least Squares	%C	1.17 (-0.17-2.51)	6.1 (3.3-9.0)	17	65.2	82.4	<b>81.7</b>	82.3	0.71	<b>1.35</b>
Robust Regression	%C	1.20	5.9	17	67.2	<b>82.9</b>	83.1	83.1	<b>1.07</b>	<b>1.00</b>
Least Squares <sup>†</sup>	%C	1.08 (-0.011 -2.16)	6.5 (3.4-9.8)	16	62.9	78.9	<b>78.5</b>	78.9	0.82	<b>1.18</b>
Robust Regression <sup>†</sup>	%C	1.22	5.8	16	66.5	79.8	80.3	<b>79.6</b>	<b>1.24</b>	0.70
Least Squares	Biomass	23.6 (-219.5-266.6)	5.5 (2.6-8.7)	17	89.4	87.8	83.6	<b>82.3</b>	0.12	0.53
Robust Regression	Biomass	96.60	3.8	17	81.4	86.2	86.4	<b>83.1</b>	<b>1.09</b>	0.19
Least Squares <sup>†</sup>	Biomass	23.58 (-11.4-58.6)	5.8 (3.2-8.4)	15	44.4	70.9	<b>67.5</b>	70.7	0.19	<b>5.04</b>
Robust Regression <sup>†</sup>	Biomass	23.58	6.3	15	44.8	71.0	<b>69.8</b>	71.6	0.54	<b>2.48</b>

<sup>†</sup>Outlier ES-1 and HS-2 removed

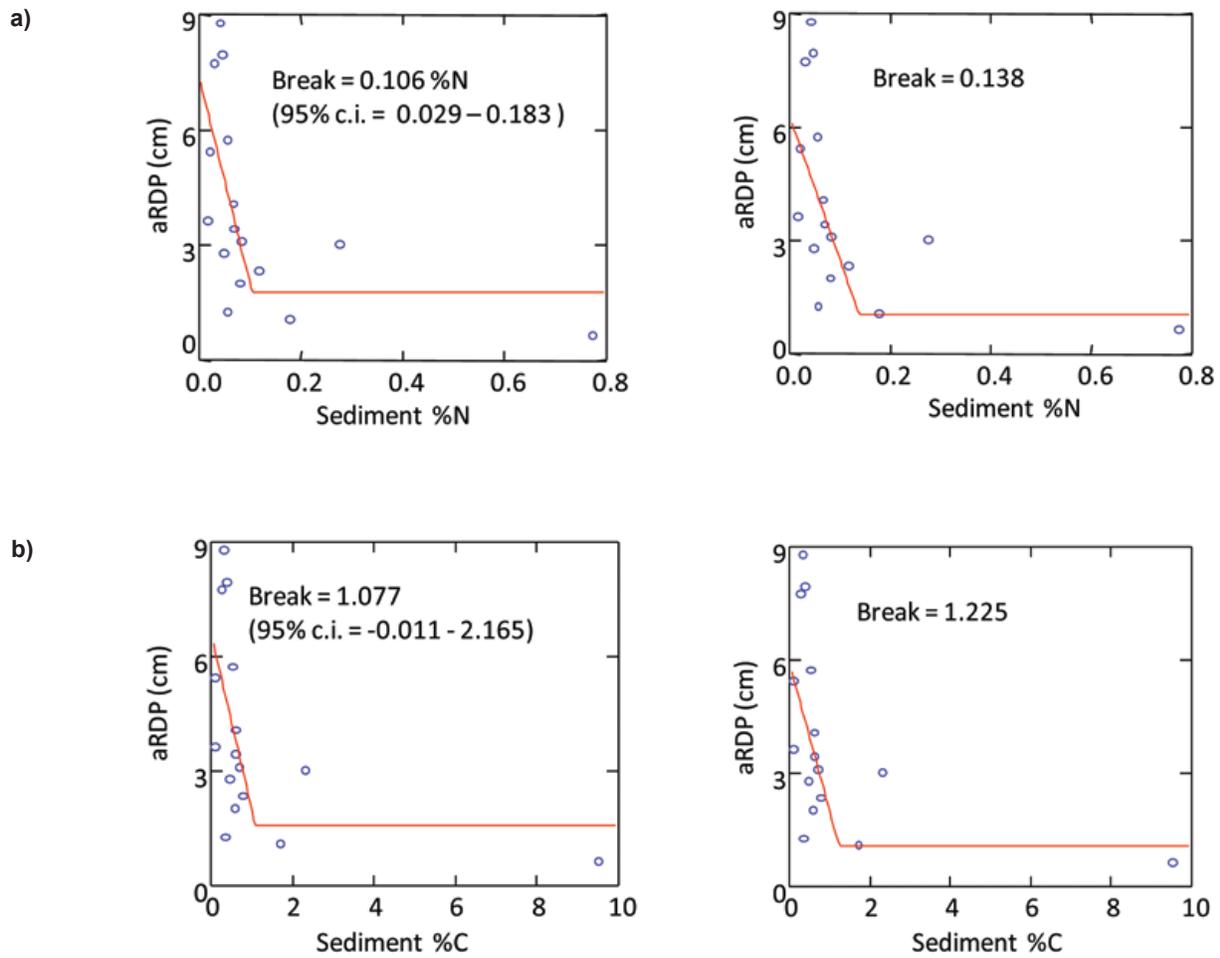


Figure 6. Piecewise regression analysis of relationship between aRDP (cm) and site-average sediment %N (a) and sediment %C (b) by least-squares (left) and robust regression (right).

Table 5. X-intercept corresponding to simple linear model is shown based on bootstrap analysis (n = 1000, median, 95% confidence interval), given for no-break models (see Table 4).

Fit Method	Predictor	Y-Intercept	Slope	X-Intercept	X-Intercept Parameter Estimates (Bootstrap 95% CI)		
					Median	5th	95th
Least Squares	Sediment %N	4.7	-6.36	0.7	0.73	0.18	0.87
Robust regression	Sediment %N	3.9	-4.23	0.9	0.91	0.20	1.24
Least Squares	Sediment %C	3.8	-0.33	11.4	9.39	1.87	10.96
Least Squares	Biomass	4.6	-0.01	537.8	445.70	0.00	1931.40
Robust regression	Biomass	3.9	-0.012	336.8	323.60	0.00	2177.60
Least Squares <sup>†</sup>	Biomass	4.5	-0.013	362.5	318.60	175.80	358.80
Robust regression <sup>†</sup>	Biomass	3.9	-0.011	339.1	318.00	189.40	358.70

<sup>†</sup>Outlier ES-1 and HS-2 removed

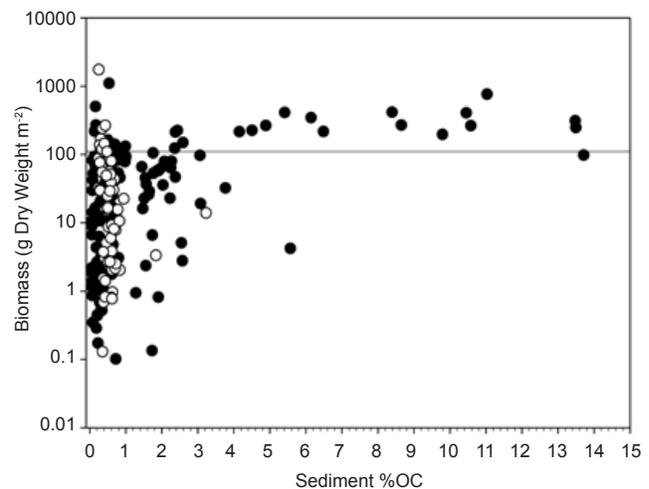
**Table 6. Mean (5<sup>th</sup> - 95<sup>th</sup> percentile) cut values for step thresholds, based on results of aRPD as a function of biomass, sediment %N and sediment %C as defined by bootstrapped CART analysis for plot level data.**

Parameter	Dominant Algae Group	Mean (5 <sup>th</sup> - 95 <sup>th</sup> percentile) Cut Value	Model Fit (R <sup>2</sup> )
Macroalgal Biomass (g dw m <sup>-2</sup> )	<i>Ulva spp.</i>	28.9 (2.1-296.0)	0.12
	<i>Ceramium spp.</i>	46.1 (3.3-85.5)	0.38
	<i>Gracilaria spp.</i>	9.1 (2.0-15.2)	0.18
	<i>Lola spp.</i>	261.3 (156.1-443.5)	0.28
	All	7.2 (2.7-15.2)	0.09
Sediment %N	<i>Ulva spp.</i>	0.06 (0.05-0.09)	0.35
	<i>Ceramium spp.</i>	0.05 (0.04-0.06)	0.36
	<i>Gracilaria spp.</i>	0.07 (0.05-0.10)	0.13
	<i>Lola spp.</i>	0.03 (0.02-0.03)	0.29
	All	0.06 (0.05-0.07)	0.22
Sediment %OC	<i>Ulva spp.</i>	0.44 (0.40-0.49)	0.36
	<i>Ceramium spp.</i>	0.34 (0.29-0.43)	0.19
	<i>Gracilaria spp.</i>	0.63 (0.47-0.79)	0.13
	<i>Lola spp.</i>	0.18 (0.16-0.21)	0.26
	All	0.49 (0.39-0.78)	0.19

data with and without *Lola spp.* found no significant differences, suggesting that plots dominated by *Lola spp.* did not have a strong feedback loop with sediments. Mean sediment %OC at *Lola spp.*-dominated sites was low ( $0.18 \pm 0.04$  %OC), despite high biomass ( $251 \pm 138$  g dw m<sup>-2</sup>). In contrast, plots with high *Ulva spp.* biomass (>100 g m<sup>-2</sup>) were associated with very high sediment %OC (>1% OC; Figure 7).

## DISCUSSION

Our study of aRPD in the intertidal flats of eight California estuaries identified two types of statistically-defined thresholds for macroalgal biomass, sediment %C and %N as stressors to benthic habitat: 1) a step threshold which identifies the range in concentration at which there is a detectable overall reduction in aRPD between reference and non-reference sites (reference envelope) and 2) a slope break threshold, which is the point at which maximum benthic degradation is achieved because anoxic sediments extend to the sediment surface. Ecologically, this slope break threshold is equivalent to an “exhaustion threshold” (Cuffney *et al.* 2010). Teal *et al.* (2010) suggested that aRPD formation is complex, is affected by a number of other constituents, and responds to a variety of driving factors (including oxygen concentrations, bioturbation, total organic carbon,



**Figure 7. Relationship between sediment %OC and macroalgal biomass. Color of symbol indicates algal genus, where *Ulva spp.* = black circle, *Ceramium spp.* and *Gracilaria spp.* = white circle.**

physical energy, and a host of other physical and sediment attributes) that all vary temporally and spatially within estuarine sediments. In our study of estuarine intertidal flats, aRPD was highly variable, reflective of a broad range of conditions captured among these eight estuaries. However, this slope break (sediment %OC and %N) or 5<sup>th</sup> percentile of the X intercept (macroalgae) in the no-slope-break models, represents an exhaustion threshold in organic matter accumulation that appears to override other factors controlling aRPD, driving it to near zero

levels. As an indicator of ecosystem condition, a shallowing of aRPD translates to reduced habitat volume and quality for benthic infauna and alteration in their community structure (Pearson and Rosenberg 1978, Nilsson and Rosenberg 1997, Rosenberg *et al.* 2003). These effects have been linked to reduced availability of forage for fish, birds and invertebrates (Raffaelli *et al.* 1989, 1991; Bolam *et al.* 2000). Thus the ranges associated with “reference” and near zero aRPD represent bookends of a gradient of increasing organic matter loading along which increasing adverse effects can be documented. Site-specific differences in response to stressors among or within estuaries and other sources of variation produce a widening of the gap between as well as the confidence intervals around these “bookends” of stress levels.

We found that biomass of 3 to 15 g dw m<sup>-2</sup> represented a statistically-defined reference envelope or background level of macroalgal abundance in these eight California estuaries, interpreted as the range at which no detectable effect on aRPD is evident. This reference envelope was identified through detection of step thresholds, the point at which overall reduction occurs in aRPD between reference and impacted classes. We found no previous studies that have reported reference levels of macroalgae in intertidal flats. However, the proposed macroalgal assessment framework for the European Union Water Framework Directive (EU WFD; Scanlan *et al.* 2007), which was created based on best professional judgment of experts from several EU member states, categorized estuaries with 0 to 100 g ww m<sup>-2</sup> (~0 - 10 g dw m<sup>-2</sup>) as in high ecological condition. This expert-defined “reference envelope” agrees well with our statistically-derived range of 3 to 16 g dw m<sup>-2</sup>. (Scanlan *et al.* 2007). This reference envelope, representing the characteristics of ‘least disturbed’ sites with respect to aRDP, can be distinguished from benchmarks of “no observed effects levels,” in which some effects of the stressor may be apparent, but not adverse effects. A field experiment by Cardoso *et al.* (2004) found a positive effect on invertebrate diversity and abundance at approximately 30 g dw m<sup>-2</sup> (300 g ww m<sup>-2</sup>; Cardoso *et al.* 2004); our confidence that this finding of 30 g dw m<sup>-2</sup> represents a no-effect benchmark is low, as the treatment was a single rather than continuous application.

In contrast to reference, a macroalgal biomass of 175 g dw m<sup>-2</sup> appears to be an exhaustion threshold

or tipping point where aRPD depth approaches zero, which we interpret as levels corresponding to strong adverse effects to benthic habitat quality. A survey of field experiments reporting effects of macroalgal biomass on benthic infaunal community structure in the literature generally supports these findings, with adverse effects reported at ranges from 110-863 g dw m<sup>-2</sup> (840 - 6000 g ww m<sup>-2</sup>; Table 7). Most of these studies were based on a single treatment level and a one-time application of algae; therefore the utility of many of these studies to identify benchmarks of adverse effects associated with macroalgae biomass is limited. However, three studies are of particular interest: Green *et al.* (Submitted), Bona (2006) and Green (2010). Green *et al.* (Submitted) conducted experiments at four sites in two California estuaries with five treatment levels of macroalgal biomass, controlling for duration. They documented significant adverse effects to benthic infaunal diversity at 110 to 120 g dw m<sup>-2</sup> (840 - 940 g ww m<sup>-2</sup>); at this level, total macrofaunal abundance decreased by at least 67% and species richness declined at least 19% within two weeks at three of the four sites in the two estuaries. At this benchmark, surface deposit feeders significantly declined, a functional group important as a forage for fish and birds (Posey *et al.* 2002). Similarly, Bona (2006) found an adverse effect level of 700 g ww m<sup>-2</sup> to (90 g dw m<sup>-2</sup>) with a study in Venice Lagoon that employed sediment profile imagery to identify thresholds of macroalgal biomass associated with a significant decline in large filter feeders. We interpret the thresholds identified by these two studies to represent a lowest observed effect levels (LOEL), representing intermediate adverse effects to benthic community structure. At higher abundances, effects on benthic habitat quality are more significant, including sharp declines in abundance of infauna, and the absence of an aRPD, coincident with the production of high porewater sulfide and ammonium concentrations. For example, Green (2010) demonstrated that macroalgal mats of 190 g dw m<sup>-2</sup> (1373 g ww m<sup>-2</sup>) produced porewater sulfide in surficial sediments (0 - 4 cm) at concentrations known to be toxic to infauna after 8 weeks (60 mM S<sup>-2</sup>). This work agrees with our observed threshold of 175 g dw m<sup>-2</sup> associated with near zero aRPD.

Unlike previous studies (Bona 2006, Jones and Phinn 2006, Pihl *et al.* 1995), our study did not find a strong relationship between macroalgal %cover and aRPD. In the previous two studies, no

**Table 7. Summary of observed effects of macroalgal abundance on infauna and resident epifauna on intertidal flats (from Sutula (2011)).**

Location	Source	Treatment Level/ Abundance	Observed Duration	Observed	Comments
Baltic Sea	Norko and Bonsdorf 1996	2 kg ww m <sup>2</sup>	34 days	Reduced abundance of most macrobenthic invertebrates but not all	~280 g dw m <sup>2</sup> ; Single treatment level - single algal application
Australia	Cummins <i>et al.</i> 2004	4.5 kg ww m <sup>2</sup>	12 weeks	Reduced macrobenthos species abundance	~640 g dw m <sup>2</sup> ; Single treatment level -single algal application
Portugal	Cardoso <i>et al.</i> 2004	0.3 kg ww m <sup>2</sup> no effect 3 kg ww m <sup>2</sup> adverse effect	4 weeks	Reduced macrobenthos species abundance species specific response	~30 g dw m <sup>2</sup> ; Multi treatment levels -single algal application
California	Green 2010	0.5 cm (60 g dw m <sup>2</sup> ); no adverse effect after 2-8 weeks 8 weeks; 1.5 cm (186 g dw m <sup>2</sup> ); adverse effect after 4 weeks; 3.0 cm (416 g dw m <sup>2</sup> ); adverse effect after 2 weeks	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 <sup>2</sup> adverse effects were species specific	22 weeks	After 10 weeks some surface deposit feeders decreased while some subsurface feeders increased; After 22 weeks patterns similar	~420 g dw m <sup>2</sup> ; Multi treatment levels -single algal application
Scotland	Raffaelli 2000	No biomass treatment after 10 weeks increase is species specific. 3 kg ww m <sup>2</sup> 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	~420 g dw m <sup>2</sup> ; Multi treatment levels-single algal application
Sweden	Osterling and Pihl 2001	1.2 kg ww m <sup>2</sup> 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	~160 g dw m <sup>2</sup> ; Single treatment level -single algal application
California	Everett 1991	~6 kg ww m <sup>2</sup> adverse effects after 2 months and six months	6 months	Clams and shrimp abundance increased in plots where macroalgae was removed	~ 863 g dw m <sup>2</sup> ; Removal experiment
Scotland	Bolam <i>et al.</i> 2000	~1 kg ww m <sup>2</sup> 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	~131 g dw m <sup>2</sup> ; Single treatment level -single algal application
England	Jones and Pinn 2006	Adverse effects >70% cover	not recorded	Species diversity declined when % cover increased from 5 - 70% in one month	Correlative field study; Low cover did not always = high diversity
Sweden	Pihl <i>et al.</i> 1995	Some negative effects with 1% cover, greatest effects >30% cover	not recorded	Crabs negatively affected by moderate and high percent cover	Correlative field study; 1-day sampling events
Baltic Sea	Lauringson and Kotta 2006	No clear relationship with mat depth and infaunal abundance	Not recorded	Herbivores more prominent within mats; Detritivores more prominent in sediment	Correlative field study; Subtidal
Italy	Bona 2006	0.7 kg ww m <sup>2</sup> and > 70% cover	Not recorded	Loss of Stage III benthic colonization by filter feeders	~90 g dw m <sup>2</sup> ; Use SPI camera for correlative field study
California	Green <i>et al.</i> submitted	Identified 110-120 g dw m <sup>2</sup> at 4 wks as benchmark for adverse effects	10 weeks	Reduced diversity and abundance of surface deposit feeders	Manipulative field experiment with 5 treatment levels and biweekly monitoring of duration

documentation of biomass was made, only cover, so it is not possible to understand how cover related to organic matter loading (biomass). In Bona (2006), cover greater than 70% was generally associated with absence of large filter feeders. Furthermore, during the preliminary growth phase, macroalgae will typically exhibit a very thin layer of biomass at high cover. Our data as well as other studies have demonstrated that it is possible to document high % cover with little measurable biomass (McLaughlin *et al.* Submitted). Cover is an important variable in estimating the spatial patchiness or extent of an effect (Scanlan *et al.* 2007). Our study found that high biomass generally did not occur at <30% cover. Thus, % cover has the potential to be used as a screening indicator to identify areas of potential risk to macroalgal blooms, because measurement of biomass is more labor intensive and costly than measurement of cover.

As with macroalgae, our study defined two types of thresholds for sediment %OC and %N in intertidal flats: 1) tipping points associated with aRPD approaching near zero at concentrations and 2) a reference envelope of %OC and %N. Our threshold for ecological effects (1.2% OC) is lower than in other previously published work in this field, much of which is based on empirical work in subtidal areas. Thresholds or tipping points in %OC leading to adverse effects to benthic invertebrates have been reported at: 2 to 3% (Diaz *et al.* 2008, in Boston Harbor); 2.8% (Magni *et al.* 2009, in Mediterranean lagoons); 3.5% (Hyland *et al.* 2005, in seven coastal regions of the world). These authors developed useful thresholds for screening over broad coastal areas, but did not quantify sources of variability related to the thresholds. In contrast, Pelletier *et al.* (2010) used a large data set to evaluate %OC thresholds linked to adverse effects to benthic invertebrates, and quantified variability due to sediment grain size and region. Sediment designated as “enriched” were more likely to have reduced water column dissolved oxygen and adverse effects to benthic invertebrates. This approach provides a more satisfying comparison to our dataset, because %OC varies as a function of grain size. The median grain size distribution in our study for plot level data was 16% fines, with a 90th percentile of 45% fines. For grain sizes of <45% fine, Pelletier *et al.* (2010) predicted subtidal impairment and enrichment thresholds at %OC values above 1 to 1.5%OC for the three Atlantic Coast regions, agreeing well with the

range in slope thresholds of 1.1 to 1.2 %OC found in our study. Because low oxygen is one of the primary faunal stressors associated with high %OC (Hyland *et al.* 2005) and the intertidal zone is re-oxygenated on a daily basis, we might expect macrofauna to remain healthy at higher levels of %OC than would those in subtidal habitats (Magni 2003). However, our data do not provide evidence for a difference in these thresholds for sediment organic matter along this intertidal-subtidal continuum.

Pelletier *et al.* (2010) also defined reference envelope of %OC at 0.2 to 0.9% over our range of 0 to 45% fines, values that also agree well with the 0.2 to 0.7 %OC reference transition range identified in our study. In addition to grain size, further sources of variability in empirical relationships between %OC and benthic fauna include the quality and form of organic carbon (Pusceddu *et al.* 2009) and a variety of other co-varying factors such as dissolved oxygen, toxicants and nutrients (Hyland *et al.* 2005). However, Pelletier *et al.* (2010) accounted for many of these other variables and found that grain size accounted for 65.6 – 85.5% of the variation in %OC. This suggests that many of the subtidal studies reporting higher thresholds %OC for reference (< 1% OC; Hyland *et al.* (2005)) and adverse effects of %OC may have been conducted in muddier sediments than we saw in our mostly sandy intertidal setting. Like sediment % OC, %N appeared to exhibit a strong tipping point with respect to aRPD. This is not surprising given that sediment %N was strongly correlated with %OC. Sediment molar C:N ratios, which averaged 9:1 with a range from 2 to 17:1, were reflective of algal (typically <10:1) rather than terrestrial sources of carbon (typically >20:1; Ruttenberg and Goni 1997). This is a logical result in a data set dominated by lagoonal estuaries with little freshwater input. A review of literature shows no studies that provide thresholds specifically for sediment %N; all work has focused on %OC (e.g., Hyland *et al.* 2005). In general, only in estuaries with strong terrestrial or refractory sources of organic matter would you expect a deviation of %OC and %N thresholds beyond 10:1 ratio.

It was interesting to note that thresholds associated with aRPD for both %N and %OC were tighter than for macroalgal biomass. This is likely due to the fact that aRPD is directly driven by the introduction of organic matter that increases oxygen demand and stimulates sediment diagenesis, thereby shallowing the aRPD; the effect of macroalgae

on aRPD is an indirect effect of feedback loops involving macroalgae and the biogeochemistry of sediment organic matter. Live macroalgae uptakes nitrogen from the water and sediment porewater at a high rate, while releasing large amounts of labile organic carbon and nitrogen as exudates (Valiela *et al.* 1997, Fong *et al.* 2004, Fong and Zedler 2000). However, when macroalgae decay after senescence or shading, they release large amounts of bioavailable organic nitrogen and labile carbon: thus, macroalgal blooms during growth phases draw down porewater N and during decay phase can enrich sediment %OC and %N in surficial sediments. Sediments with high organic matter content are often associated with chronic macroalgal blooms (Kamer *et al.* 2004); high macroalgal biomass was present under a range of %N, but above 0.3%N, macroalgal biomass was consistently high (>100 g dw m<sup>-2</sup>). This relationship is reflective of strong feedback between macroalgae and sediment biogeochemical processing. Plots from ES-1 and HB-2, identified and removed as outliers because of high algal biomass, high aRDP and very low %OC and %N, were sites characterized by high hydrodynamic energy that likely led to transport or rafting of macroalgal mats into the site (Rhoads and Germano 1982). This suggests an important consideration the use of macroalgal biomass as an indicator of eutrophication: high biomass in the absence of high sediment %OC or %N may indicate rafting rather than a bloom event. If so, evaluation of sediment organic matter content would be a useful line of additional evidence in diagnosing eutrophication.

Our work presents a significant step forward in quantifying ranges of reference and severe adverse effects associated with macroalgal blooms on intertidal flats, thereby increasing the confidence in use of this indicator for eutrophication assessment and establishment of nutrient-related water quality goals. The inclusion of eight estuaries (representing a range in geoform, tidal forcing, and rainfall in a Mediterranean climate) expands our understanding of uncertainty in applying thresholds from earlier work conducted in single estuaries. Further, our thresholds were selected through statistical analyses, rather than through visual interpretation of the data; confidence intervals in our estimates provide a measure of variability in response across systems. Not all sources of variability were explored in our study. For example, it is reasonable to expect that thresholds of adverse effects as well as reference transition ranges

may differ by macroalgal genus. The C:N ratio of biomass, surface area to biomass ratios, and growth form (filamentous, presence of thali, etc.) could also be expected to influence the lability of carbon loading to sediments (de los Santos *et al.* 2009). Because of the lack of sufficient range and sample size at the genus level, we aggregated the data to identify adverse effect levels. The adverse effects ranges identified are most applicable to *Ulva spp.*, the genus that dominated our data set at high biomass. Lack of information on the duration of macroalgal blooms and the longevity of mats is another source of variability important to threshold identification. For this reason, we see our study as a complement to field experiments in which biomass and duration were tightly controlled (Green *et al.* Submitted). Application of these thresholds in a management context must consider these uncertainties; confidence in their application will increase in circumstances where macroalgal blooms are documented to persist over long period of time (duration) or greater spatial extent (McLaughlin *et al.* Submitted).

Use of macroalgal indicators in regional and national assessments of estuarine eutrophication has previously been hampered by the lack of quantitative data on thresholds (McLaughlin *et al.* Submitted, Bricker *et al.* 2007). This study statistically defined a reference envelope and exhaustion thresholds for the effects of macroalgae and sediment organic matter on benthic habitat quality, providing data that will help refine the diagnostic frameworks with which these assessments are made (Bricker *et al.* 2003, Scanlan *et al.* 2007, Zaldivar *et al.* 2008, Borja *et al.* 2011).

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