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Condition of California Perennial, Wadeable Streams Based on Algal Indicators

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List of Acronyms

AFDM	ash-free dry mass
BMI	benthic macroinvertebrates
BURC	beneficial use risk classification
CDF	cumulative distribution function
EPA	Environmental Protection Agency
H20	stream algae IBI for southern California, based on diatoms and soft algae (Fetscher et al. 2013)
IBI	Index of Biotic Integrity
NNE	Nutrient Numeric Endpoints
PCT_MAP	percent macroalgal cover (attached/unattached)
PSA	Perennial Stream Assessment
SMC	Stormwater Monitoring Coalition (of Southern California)
SOP	Standard Operating Procedures
SWAMP	Surface Water Ambient Monitoring Program (of the State of California)
SWRCB	State Water Resources Control Board (of the State of California)

Executive Summary

Algae can serve as indicators of stream ecological condition in two ways. Information about algal community composition can be used for bioassessment analogous to the way in which benthic macroinvertebrates (BMIs) are employed (e.g., as described in Ode et al. 2011). When algae occur in excess (i.e., in the case of eutrophication), stress to the system can result; as such, the total amount of algae present also becomes an indicator of stream health in its own right.

To date, the state of California has invested in several initiatives to build capacity for conducting stream algal assessment. This includes support for creation of a planning document funded by State Water Resources Control Board's (SWRCB) Surface Water Ambient Monitoring Program (SWAMP) (Fetscher and McLaughlin 2008) and establishment of SWAMP Standard Operating Procedures (SOPs) for field sample collection (Fetscher et al. 2009) and a laboratory processing and enumeration SOP (currently in review) for determining algal community composition. In addition, an algae Index of Biotic Integrity (IBI) for bioassessment of southern California streams was recently completed by Fetscher et al. (2013), and a state algae laboratory, based at California State University San Marcos, has been established.

Over the past several years, the state of California's Perennial Stream Assessment (PSA) and Reference Condition Management Program (RCMP), the southern California Stormwater Monitoring Coalition (SMC), and other smaller programs have collected and processed algae samples using the SWAMP protocols. As such, a substantial amount of standardized data on stream algae and instream, vascular macrophytes (together hereafter referred to as stream "primary producer indicators") have been collected in California since 2007. However, no consolidated analysis of these data has been conducted so far. This report represents such an effort. It addresses the following assessment questions of importance to regulatory agencies, regulated communities, and the public, with the two major stream-algae assessment themes the questions encompass covered in two chapters:

Chapter 1 – Stream eutrophication:

- What are the distributions of primary producer abundance indicator values in California perennial, wadeable streams[1], and how do the statewide and regional distributions of these values relate to available endpoints[2] of concern?

- What is the distribution of these values in "Reference" sites that are subjected to minimal anthropogenic disturbance?

Chapter 2 - Stream health as assessed by algae IBI scores:

- What is the ecological health of southern California perennial, wadeable streams based on the algae IBI (Fetscher et al. 2013) developed for that ecoregion?

- How does the southern California IBI perform in other parts of the state?

[1] We used the PSA operational definition of "perennial", i.e., those stream reaches with surface flow during the index period for sampling. A "wadeable" reach was defined as that which is < 1m deep for at least 50% of its length.

[2] For the purposes of the present report, we are comparing data distributions to available endpoints in order to provide some perspective on the regional and statewide biomass values realized. The report is intended as a summary of the distribution of biomass concentrations and algae IBI score values, and is not intended as an impairment assessment.

Stream eutrophication

Excessive algal growth in response to nutrient enrichment is a major problem in California water bodies. The Nutrient Numeric Endpoints (NNE) framework (Tetra Tech 2006), proposed to be employed by the state for setting nutrient criteria, recommends the use of algal biomass in the form of benthic chlorophyll *a* as a primary indicator of nutrient impacts to beneficial uses in wadeable streams. Although the Tetra Tech (2006) report recommends a set of algal biomass endpoints[3] for various beneficial uses, to date, there has been no way to begin assessing how those endpoints relate to the actual biomass levels in streams within the state's different ecoregions, and to streams exposed to varying degrees of human disturbance. Ambient levels of stream algal biomass, as well as biomass levels at minimally disturbed "Reference" sites, are important considerations in the process of determining how realistic and meaningful the proposed endpoints are.

This report describes the estimated distributions, both regionally and statewide, of algal biomass (and other primary producer indicator) values in California perennial, wadeable streams. The results presented come from 938 stream reaches sampled between 2007 and 2011. 575 of these were sampled as part of the probability surveys conducted by the State of California Perennial Stream Assessment (PSA) and the southern California Stormwater Monitoring Coalition (SMC).

We found that California's perennial, wadeable streams, as assessed during the PSA index period[4], exhibited a skew toward the low end of the algal biomass gradient. Nearly 90% of stream kilometers had biomass values below that which represents the 95th percentile of Reference sites[5] statewide, which corresponds to 44 mg m $^{-2}$ chlorophyll *a*, 34 g m^{-2} ash-free dry mass (AFDM), and 46% macroalgal percent cover.

According to Tetra Tech (2006), values below which beneficial uses are deemed to be supported are 100 and 150 mg m^{-2} chlorophyll *a* for COLD and WARM beneficial uses, respectively. Conversely, beneficial uses are deemed to be presumptively impaired at greater than 150 and 200 mg m^{-2} chlorophyll *a* for COLD and WATER beneficial uses, respectively. It is important to note that, based on the recommendations of Dodds et al. (2002), these Tetra Tech (2006) proposed benthic chlorophyll *a* endpoints are expressed in terms of *maximum*[6] values.

Overall, more than 95% of perennial, wadeable stream kilometers in California were estimated to fall below 100 mg m^{-2} chlorophyll *a*. In certain PSA6[7] ecoregions, such as Sierra Nevada, North Coast, and Deserts-Modoc, no portion of stream kilometers was estimated to exceed that endpoint. For the other ecoregions, levels of chlorophyll *a* varied, significantly, by "site disturbance class" (a measure of anthropogenic stress to which sites are exposed, based on surrounding land use). The most highly disturbed (or "Stressed") sites supported the highest levels of chlorophyll *a*, and the least disturbed (or "Reference") sites supported the lowest. Regardless

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[3] 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.

[4] The PSA index period for stream sampling starts in May for drier parts of the state and June or July in colder/wetter parts of the state (depending upon stream flow conditions), and lasts for two to three months.

[5] In the case of the Reference sites, values are given here for all available data combined (i.e., probability plus non-probability, or "targeted", sites)

[6] In the work of Dodds et al. (2002), "maximum" appears to be intended to represent the spatially-averaged, temporal maximum algal growth potential (in response to nutrient and light availability) in the absence of temporary reductions in biomass density due to grazing, scour, and other factors. It is thus intended to be a temporal maximum, identified via multiple samples taken over the growing season.

[7] State bioassessment programs use a combination of Omernik (1995) ecoregions and Regional Water Quality Control Board boundaries to partition the state for assessment purposes. Ecoregion "PSA6" refers to the version of the classification scheme resulting in six ecoregions.

of ecoregion, no stream kilometers within the “Reference” or “Intermediate” site classes were estimated to exceed 200 mg m^{-2} chlorophyll *a*. However, because the endpoint is defined as a temporal maximum, it is difficult to draw firm conclusions regarding the frequency with which the maximum endpoint may be exceeded, because the data upon which the estimates are based constitute a one-time sample taken from each reach, generally during the late spring to mid-summer.

Stream health as assessed by algae IBI scores

We report on ambient stream condition based on southern California algae IBI scores (Fetscher et al. 2013). In addition, regional values for the IBI throughout the state are presented in terms of how they compare across sites belonging to the different site disturbance classes, in order to evaluate how well the IBI performs in ecoregions outside of southern California

Score distributions estimated for the algae IBI in the South Coast indicated that nearly half the stream kilometers in this ecoregion are indistinguishable from what would be expected among minimally disturbed “reference” sites (Fetscher et al. 2013). Score distributions varied markedly among site disturbance classes, with nearly 80% of stream kilometers in the “Stressed” class estimated to score below this boundary, compared to <40% of kilometers in the “Intermediate” class and <10% of kilometers in the “Reference” class.

With respect to IBI performance outside of the South Coast ecoregion, with the exception of the Chaparral and North Coast ecoregions, relatively poor separation in IBI scores among site disturbance classes was realized, suggesting that the southern California IBI may not be appropriate for application throughout the state. Draft algae IBIs have been developed for the Central Coast and the eastern Sierra Nevada ecoregions of the state, and are in various stages of review. For other portions of the state, it may be necessary to develop new IBIs that are specifically calibrated to local conditions.

Chapter 1 – Stream Eutrophication

Introduction

Streams and rivers provide a wide range of essential and economically valuable services ("ecosystem services") that support the health of watersheds (Paul and Meyer 2001), including clean water, opportunities for recreation, habitat for aquatic life use and protection of aquatic biodiversity, and nutrient cycling (Costanza et al. 1997). In semi-arid regions, stream ecosystems are especially vital because they provide freshwater oases on which a multitude of native wildlife species are dependent for survival (Faber and Holland 1988). In addition, wadeable streams play a critical role in denitrification, a pathway for permanent loss of nitrate by conversion to nitrogen gas (Alexander et al. 2000). Denitrification within streams can help offset the total nitrogen (N) load from runoff and groundwater to N-sensitive coastal marine environments (Howarth et al. 1996; Alexander et al. 2000).

Eutrophication is a major concern in California streams. Elevated nutrient concentrations, in concert with other site-specific factors, can result in the overabundance of algal biomass, with a suite of adverse effects. From the standpoint of Aquatic Life beneficial uses, high levels of algal biomass can negatively impact other stream organisms in several ways. High algal cover along the stream bottom can interfere with access of aquatic animals to interstitial spaces necessary for spawning, foraging, and shelter (Quinn and Hickey 1990). Large swaths of "macroalgae" (easily visible filaments or mats of algae) and macrophytes (herbaceous vascular plants within the stream's wetted channel) could block sunlight, limiting the growth of microscopic algae in benthic biofilms, a food source for primary consumers such as scraper/grazers (reviewed by Steinman 1996). Large masses of algae and macrophytes may block or slow current speeds and inflows to a reach, thus altering the hydrology (Biggs 2000, Lembi 2003, Fovet et al. 2012) in ways that could impact aquatic life. Likewise, algal respiration, as well as decomposition of material when algae and macrophytes die, can degrade water quality by creating an oxygen deficit that suffocates other resident organisms (Quinn and Gilliland, 1989), and can promote dissolution and release of metal oxides from the substratum. Dense algal mats, supported by high nutrient concentrations, have also been identified as habitat supporting polychaete worms that are hosts of myxozoan parasites that impact salmon populations in the Klamath River (Stocking and Bartholomew, 2004). In addition, some species of "blue-green algae" (cyanobacteria) can produce chemical compounds that have toxic effects on stream animals, and if these cyanobacteria achieve high biomass levels, their toxins can reach detrimental concentrations (Aboal et al. 2000). Algal blooms can also negatively impact other beneficial uses, such as Municipal and Recreational, by causing taste/odor problems, blocking filtration systems, and compromising aesthetics (Biggs 2000, Lembi 2003, Fovet et al. 2012).

Protecting stream ecosystem services from eutrophication requires a suite of tools to: 1) accurately diagnose adverse biological effects, 2) establish appropriate nutrient concentration targets, and 3) estimate the relative importance of nutrient sources to streams where an impairment has been identified. Over the past ten years, the California SWRCB, with assistance from the US Environmental Protection Agency (EPA) Region IX and Tetra Tech Inc., has developed an approach to diagnose eutrophication and address impairments from N and phosphorus (P) in freshwater streams and lakes (Tetra Tech 2006). The framework, known as the Nutrient Numeric Endpoints (NNE) framework, is intended as guidance to interpret a narrative nutrient water-quality objective.

The NNE is centered on three principal tenets:

- Assessment of waterbody condition based on regulatory endpoints of the ecological response (e.g., algal biomass, dissolved oxygen) of the waterbody to increased nutrient availability,

- Use of models or statistical "translators" to link response indicator numeric endpoints back to site-specific nutrient concentration targets, and

- Classification of waterbodies by risk of beneficial use impairment. For many of the biological indicators associated with nutrients, no clear scientific consensus exists on a target endpoint that results in impairment. To address this problem, Tetra Tech (2006) proposed to classify water bodies into the three Beneficial Use Risk Categories (BURCs) illustrated in Fig. 1.

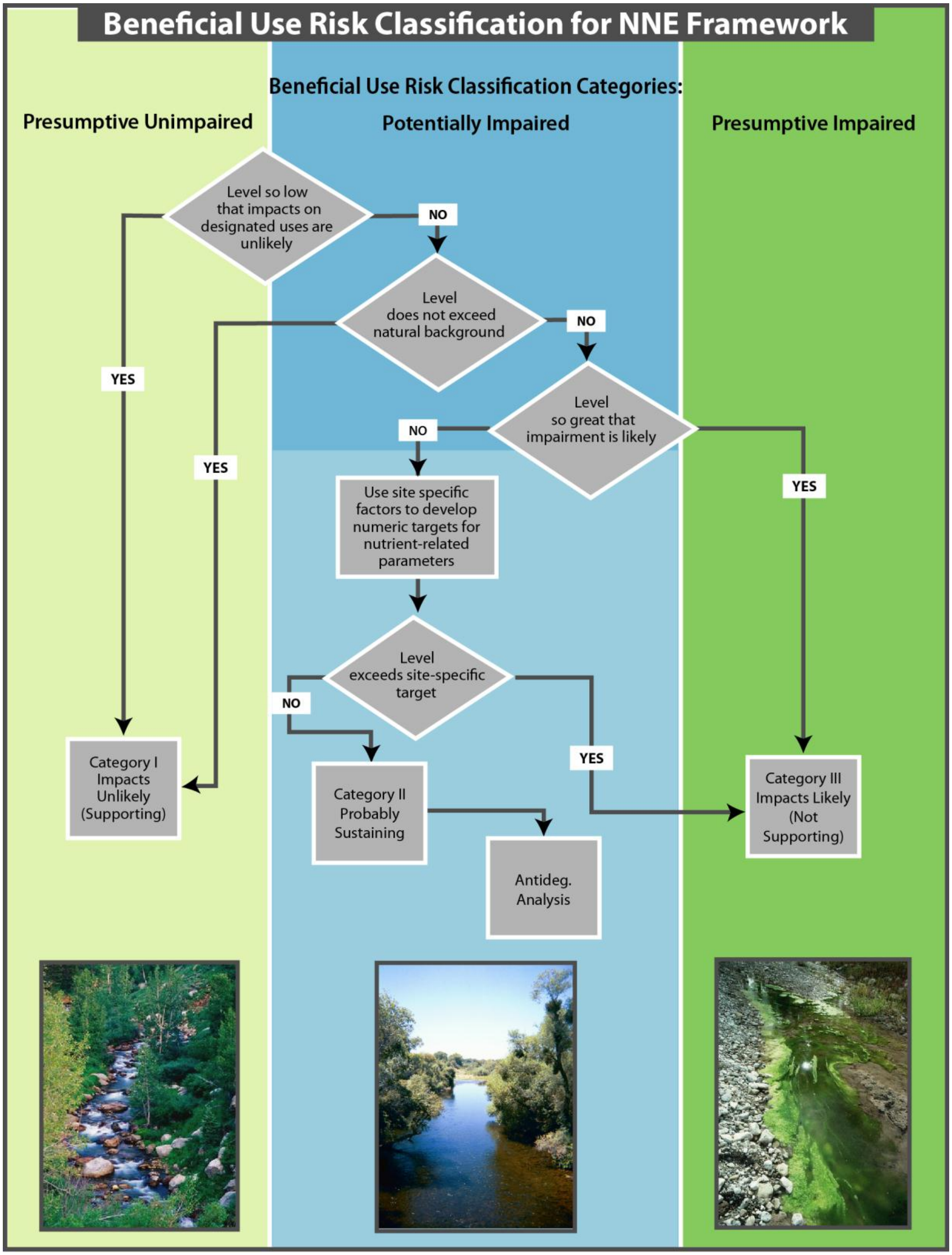

Figure 1. Conceptualization of Beneficial Use Risk Classification (BURC) categories (from Tetra Tech 2006).

BURC I water bodies are not expected to exhibit impairment due to nutrients; BURC III water bodies have a high likelihood of exhibiting impairment due to nutrients; and BURC II water bodies may require additional information and analysis. For a given beneficial use designation, the BURC I/II boundary represents a conceptual level below which there is general consensus that nutrients will not present a significant risk of impairment. This boundary should be set so that it is not less than the expected natural background. Conversely, the BURC II/III boundary represents a level that is sufficiently high that there is consensus that risk of use impairment by nutrients is probable. Within BURC II, additional water body-specific cofactors may be brought into the analysis to determine an appropriate target.

Tetra Tech (2006) proposed to use benthic algal biomass (e.g., maximum benthic chlorophyll *a*), dissolved oxygen, and pH to assess the beneficial uses status of wadeable streams. The Tetra Tech report (2006) also recommends specific values for BURC I/II and II/III regulatory endpoints, which resulted from a workshop of international experts, regulatory agencies and stakeholders (Table 1). Available documentation does not describe a quantitative basis in California data for these workshop recommendations. However, references in the main body of the Tetra Tech (2006) document cite nuisance algal impairment of REC-2 beneficial uses and adverse effects on aquatic life use associated with these levels in a variety of locations.

Table 1. Summary of recommended numeric endpoints for stream NNE indicators, by beneficial use, from Tetra Tech (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	BURC Boundary	COLD	WARM	REC-1	REC-2	MUN	SPWN	MIGR
Benthic algal biomass – max (mg chlorophyll *a* m^{-2})	I/II	100	150	C	C	100	100	B
	II/II	150	200	C	C	150	150	B
Dissolved oxygen – mean of 7 daily min. (mg L^{-1})	I/II	9.5	6.0	A	A	A	8.0	C
	II/III	5.0	4.0	A	A	A	5.0	C
pH maximum — photosynthesis-driven	I/II	9.0	9.0	A	A	A	C	C
	II/III	9.5	9.5	A	A	A	A	A

A – No direct linkage to the beneficial use
B – More research needed to quantify linkage
C – Addressed by Aquatic Life Criteria

As the SWRCB prepares to propose nutrient objectives for wadeable streams, improved data from statewide and regional ambient stream surveys can improve the scientific basis for policy decisions on NNE endpoints. In general, policy decisions on water-quality objectives are supported by information on: 1) natural background concentrations, 2) distribution of ambient concentrations across the full population of waterbodies, and 3) field surveys or experiments relating dose of the stressor (e.g., algal biomass) to adverse effects on aquatic life use. Because the NNE endpoints for algal biomass recommended by Tetra Tech (2006) represent the consensus of best professional judgment of a team of national and international stream experts, they may be reflective of the collective experience of the experts in streams with conditions atypical of stream ecosystem types found in California. At the time of the Tetra Tech (2006) report, information on the natural background concentrations of algal indicators in reference streams and the range of ambient concentrations in wadeable streams across California had not been summarized. Both of these are key considerations in the process of determining how realistic and meaningful recommended endpoints are.

The Chapter 1 component of this study seeks to address two key questions in support of SWRCB decision-making:

- What are the distributions of primary producer abundance indicator values in California perennial, wadeable streams, and how do the statewide and regional distributions of these values relate to available endpoints of concern?

- What is the distribution of these values in "Reference" sites that are subjected to minimal anthropogenic disturbance?

Methods

Data sources

The data presented here come from several wadeable stream monitoring programs in California, and largely constitute data from "probability surveys", although in some places (where noted), non-probability data (i.e., from sites subjectively selected for "targeted" sampling) are also included in analyses. In probability surveys, sites are selected in a (sometimes stratified) random manner that yields a spatially balanced distribution of sites. Because of the objective way in which sites are selected, regional/statewide estimates of stream condition, with known confidence limits, can be generated from the survey data. For more information on probability surveys, see Stevens and Olsen (2004).

The probability surveys reported on here are those of 1) the State of California Perennial Stream Assessment (PSA), and 2) the southern California Stormwater Monitoring Coalition (SMC). Results from these two programs are used to generate regional and statewide estimates of stream condition for a number of primary producer indicator data types. In addition to probability data, data from targeted sampling sites are also included in some analyses. Data from targeted sites come from the state's Reference Condition Management Program (RCMP) and a recently completely project headed by SCCWRP scientists that was geared toward developing stream algal IBIs. Taken together, the available data, sampled from 938 stream reaches, were collected from 2007 through 2011[8] and represent wadeable, perennial streams throughout the state (Fig. 2). 575 of the reaches were sampled as part of the probability surveys, and the remaining 363 were targeted. Sampling was largely conducted during a one-time site visit within the time frame spanning primarily late spring to mid-summer, with the great majority occurring in May through August.

Site selection for probability surveys

The designs for the probability surveys were based on the methods described in Stevens and Olsen (2004). The spsurvey package in R version 2.15.1 (Kincaid and Olsen 2009, The R Foundation for Statistical Computing 2010) was used in establishing the list of "probability sites" for each year's statewide (PSA) and regional (SMC) probability survey. This involved using a technique called Generalized Random Tessellation Stratified sampling site selection (GRTS; Stevens and Olsen 2004) to create spatially-balanced survey designs. As long as sites are sampled according to the order in which they appear on the site list generated for the survey,

[8] 2012 data were not available until after the data-analysis phase of report preparation.

spatial balance among sites is preserved, and the resulting dataset can be used to generate estimates of natural resource extent and condition with known confidence limits.

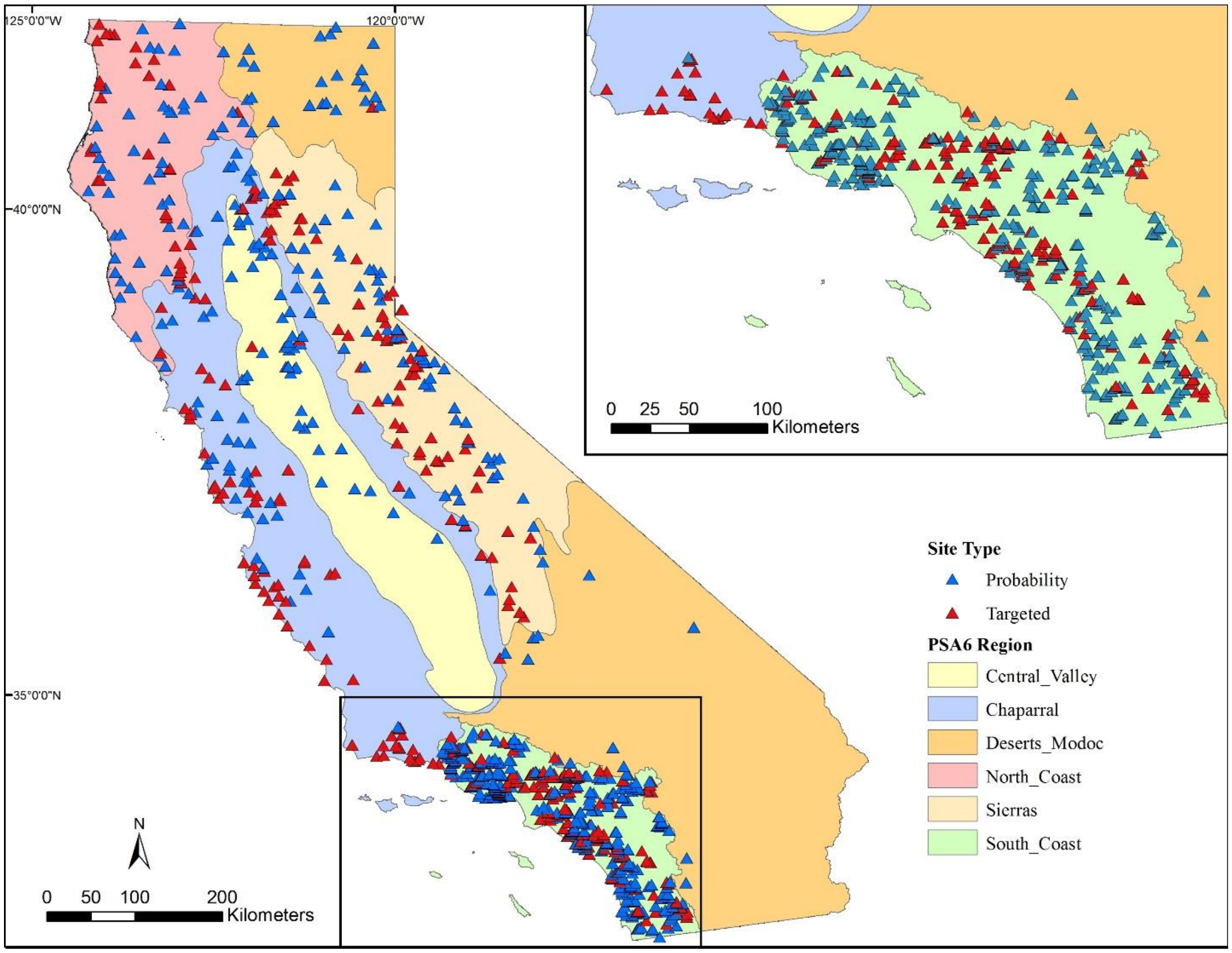

Figure 2. All algae sampling sites (probability and targeted) included in this report, shown by the Perennial Stream Assessment (PSA) ecoregion (Ode et al. 2011) in which they occur. State bioassessment programs use a combination of Omernik (1995) ecoregions and Regional Water Quality Control Board boundaries to partition the state for assessment purposes. "PSA6" refers to the version of the classification scheme that encompasses six ecoregions.

Description of Stream Algal Field Sampling and Laboratory Analysis Protocols Utilized in Compiled Wadeable Stream Survey Data

The field sampling and laboratory analysis protocols used in the compiled stream survey data provide an important context for interpretation of the findings of this study. These methods are briefly described in this section.

The samples collected and field observations recorded yielded the following data types for stream primary producer abundance indicators:

- algal biomass:
  - benthic chlorophyll *a*
  - benthic ash-free dry mass (AFDM)
- algal cover:
  - macroalgal percent cover
  - microalgal percent cover and thickness
- macrophyte percent cover

The various forms of algal/macrophyte biomass and cover data are geared toward assessing the degree to which primary production is sustained within studied streams. They provide information on the development of algal/macrophyte nuisance conditions and eutrophication. Furthermore, some of the biomass endpoints (i.e., chlorophyll *a* and AFDM) are also directly applicable for use within the current NNE framework.

A "multi-habitat" method was employed to quantitatively collect benthic algae at each sampling site. This method, SWAMP's Standard Operating Procedures (Fetscher et al. 2009), is based largely on the procedures of EPA's Environmental Monitoring and Assessment Program (EMAP; Peck et al. 2006) and is analogous to SWAMP's method for collecting BMIs (Ode 2007). It involves objectively collecting from a known surface area specimens from a variety of stream substrata, in proportions aligning with substratum type relative abundances in the stream, and combining them into a single "composite" sample for laboratory analyses. As such, a given sample may have been collected from any combination of cobbles, gravel, sand, and other substratum types. The goal is to achieve a representative sample of the benthic algae from each sampling reach, in terms of both community composition and biomass.

It is important to assess the amount of primary production supported by a stream in a number of different ways, because each method has its strengths and weaknesses (reviewed by Fetscher and McLaughlin 2008). The ability to look at a combination of measures may provide a more robust overall assessment of algal/macrophyte nuisance. Algae can occupy different "compartments" within the stream (i.e., floating on the surface, attached to cobbles/boulders, interstitially distributed within the upper layer of gravel and fine sediments—all of which are included across the sample types upon which results are reported here), so in order to arrive at a comprehensive understanding of the amount of primary production in the stream, it is best to look at various compartments.

Various measures of algal and macrophyte cover were carried out using the methods outlined in Fetscher et al. (2009). This involved recording point-intercept presence/absence of microalgae, macroalgae, and macrophytes at each of 105 points objectively positioned (in the form of a pre-determined grid) throughout each stream reach. In the case of macroalgae, that which was attached to the stream bottom, and that which was unattached and free-floating at the time of assessment, was recorded separately in order to be able to distinguish the two in the data analysis stage. In the case of microalgae, in addition to presence/absence of a biofilm on stream substrata, the thickness of the biofilm was also recorded using ordinal thickness codes.

For algal biomass, filtered isolates of quantitatively sampled algal material were analyzed for chlorophyll *a* content using EPA 445.0, and for AFDM using WRS 73A.3. Chlorophyll *a* and AFDM concentrations measured in the laboratory were transformed into mass per area of stream bottom sampled (e.g., mg m^{-2}). The quality assurance parameters for the California datasets were based on those established for the Surface Ambient Monitoring Program (SWAMP 2008).

Most algal/macrophyte field metrics were calculated as percent cover estimates in terms of the percentage of sampling points at which the type of algae/macrophyte in question was intercepted. In the case of mean microalgal thickness metrics, the midpoint values of the ranges corresponding to each thickness code recorded in the field (Fetscher et al. 2009) were averaged. A "nuisance algae" metric combining information from both macroalgae and microalgae (specifically, "thick" microalgae, meaning >1mm thick) was also calculated. A summary with descriptions of the metrics associated with algal/macrophyte cover is provided in Table 2.

Characterization of the overall level of anthropogenic stress is used throughout the analyses supporting project objectives. This was done by grouping sites into "disturbance classes". To assign sites to disturbance classes, we used the same set of screening criteria as that employed by the State of California's Biological Objectives initiative (Ode et al., under review). Under this approach, sites are classified according to the degree of anthropogenic disturbance they are exposed to, based on surrounding land uses and local riparian disturbance measures. Table 3 provides a list of the factors that were used for classifying sites into one of the three disturbance classes: "Reference", or those sites that are exposed to the lowest levels of anthropogenic disturbance based on the variables considered, "Stressed", or those sites exposed to the highest levels, and "Intermediate", or those sites falling between the "Reference" and "Stressed" groups.

Table 2. Metric descriptions and codes for stream primary producer abundance indicators.

Metric Code	Description
PCT_MAA	Percent Presence of Attached Macroalgae (defined as algal mats or filaments easily visible to the naked eye)
PCT_MAP	Percent Presence of Macroalgae (Attached and/or Unattached)
PCT_MAU	Percent Presence of Unattached Macroalgae
PCT_MIAT1	Percent Presence of Thick Microalgae (1mm+)
PCT_MIAT1P	Percent Presence of Thick Microalgae (1mm+), where Microalgae Present
PCT_MIATP	Percent Presence of Microalgae
PCT_NSA	Percent Presence of Nuisance Algae (Macroalgae + Thick Microalgae (1mm+))
XMIAT	Mean Microalgae Thickness (mm)
XMIATP	Mean Microalgae Thickness (mm,) where Microalgae Present
PCT_MCP	Percent Presence of Macrophytes

Table 3. Variables used for assigning sites to "site disturbance classes" per the state's bio-objectives process (adapted from Ode et al., under review). WS: Watershed. 5K: Watershed clipped to a 5-km buffer of the sample point. 1K: Watershed clipped to a 1-km buffer of the sample point. W1_HALL: proximity-weighted human activity index (Kaufmann et al. 1999). In order to be considered "Reference" condition, all criteria listed in the "Threshold" column for "Reference" must be met. If any of the criteria in the "Stressed" column apply, that site is considered "Stressed". Sites not falling into either of these categories default to "Intermediate". Data sources are as follows: A: National Landcover Data Set (2006, http://www.epa.gov/mrlc/nlcd-2006.html). B: Custom roads layer (P. Ode, pers. comm.). C: National Hydrography Dataset Plus (v2, http://www.horizon-systems.com/nhdplus/). D: National Inventory of Dams. E: Mineral Resource Data System (MRDS 2012). F: Field-measured variables (Fetscher et al. 2009).

Variable	Scale*	Threshold (Reference)	Threshold (Stressed)	Unit	Source
% Agriculture	1k, 5k, WS	3	50	%	A
% Urban	1k, 5k, WS	3	50	%	A
% Ag + % Urban	1k and 5k	5	50	%	A
% Code 21**	1k and 5k	7	50	%	A
	WS	10	50	%	A
Road density	1k, 5k, WS	2	5	km/km^2	B
Road crossings	1k	5	-	crossings/ km^2	B, C
	5k	10	-	crossings/ km^2	B, C
	WS	50	-	crossings/ km^2	B, C
Dam distance	WS	10	-	km	D
% canals and pipelines	WS	10	-	%	C
Instream gravel mines	5k	0.1	-	mines/km	C, E
Producer mines	5k	0	-	mines	E
W1_HALL	reach	1.5	5	NA	F

* For variables in which multiple spatial scales are used for determining site classification, in the case of the "Reference" boundary, the value indicated must apply to all spatial scales listed, whereas for the "Stressed" boundary, the indicated value need only apply for one of the listed spatial scales.
** "Code 21" encompasses a wide range of land uses primarily characterized by heavily managed vegetation (e.g., low-density residential development, parks, golf courses, highway medians)

Statewide/regional extent and magnitude of stream primary producer indicator values

To provide an overview of the values for each of the primary producer indicator data types in California perennial, wadeable streams, descriptive statistics for estimated data distributions and cumulative distribution functions (CDFs) were generated using the spsurvey package in R on the probability subset of data. CDFs depict the estimated probability distribution of values of a given indicator relative to the cumulative proportion of the geographic unit of interest (in this case, percent of stream length in the state).

Each site in the combined probability surveys for the different programs/years has an associated weight in units of stream length, which reflects how much of the state's stream network, within the stratum in which that site is found, is "represented by that site". The more sites in a given stratum, the less weight each site is assigned. Because data from multiple surveys, with different stratification schemes, were combined for this report, it was necessary to create mutually exclusive "cross-categories" corresponding to the intersection of the different strata from the various surveys. Once cross-categories were created, the weights of all sites had to be adjusted to reflect the combined numbers of sites within each new cross-category. Adjusted weights were calculated for each cross-category by dividing the total stream length within that cross-category by the number of sites evaluated during site reconnaissance (see below). Once weights were adjusted, statewide extent and magnitude estimates for the various primary producer indicator values could be computed (see below).

It is not uncommon for some of the sites in a GRTS-generated list to prove unsuitable for sampling, for a variety of reasons that include: 1) the site being found, during reconnaissance, not to be part of the survey's designated "target population", which, for the surveys reported on here, is defined as *perennial, wadeable streams in California*; or 2) the site is within the target population, but for some logistical reason, it cannot be sampled (e.g., due to landowner denial of access, or physical barriers that make the site unsafe or infeasible to reach, or sheer distance of the site from nearest roads, making it impossible to conduct bioassessment within the space of a day). Comprehensive documentation is required in order to classify sites into "evaluation categories" based on the results of site reconnaissance. If insufficient information (regarding why samples were not collected) is provided by field crews, the default classification for a site is "Unknown".

Estimates of stream condition (e.g., the percent of stream kilometers with indicator values below a certain boundary, such as the numeric endpoints discussed below) were calculated using the Horvitz-Thompson estimator (1952), which is a weighted average of sample values where weights are adjusted according to design implementation. Confidence intervals were based on local neighborhood variance estimators (Stevens and Olsen 2003), which assumes that samples located close together tend to be more alike than samples that are far apart.

For this project, we employed the numeric endpoints for benthic chlorophyll *a* proposed by Tetra Tech (2006) for COLD and WARM beneficial uses (Table 1) as endpoints for adverse effects, with the caveat that these are intended as *maximum* biomass densities. No endpoints for AFDM are specifically proposed by Tetra Tech (2006), however a ratio of 2.5 mg chlorophyll *a* : 1 g AFDM m^{-2} is utilized as a translator in the NNE "benthic biomass predictor spreadsheet tool"[9] (Tetra Tech 2006). Therefore, we use AFDM endpoints of 40, 60, and 80 g m^{-2} (i.e., benchmarked to the BURC I/II and II/III COLD and WARM benthic chlorophyll *a* endpoints) for reporting purposes here. Use of the recommended (Tetra Tech 2006) biomass endpoints in this report is for illustrative purposes only and does not imply impairment. Because stream algal survey data do not include information on designated uses, we apply these endpoints generically, and our results reflect this shortcoming.

Graphical output for all analyses in the report was generated using R (version 2.15.1, R Development Core Team (2013)) and the package "ggplot2" (Wickham 2009).

Results

Statewide/regional extent and magnitude of primary producer abundance indicator values

The proportions of sites falling into the four site "evaluation categories" (i.e., categories reflecting the outcome of site evaluations during the reconnaissance process), which were described in the previous section, are shown in Table 4. This breakdown provides perspective on the proportion of stream kilometers in the state for which condition estimates based on the probability data are generated. By far, the majority of stream kilometers in the state were estimated to fall outside of the surveys' "target population", either because they were non-perennial or non-wadeable stream reaches. The proportion of sites for which samples were collected represented about 10% of the state total stream kilometers.

[9] The "benthic biomass predictor spreadsheet tool" is provided Tetra Tech (2006) to predict biomass levels at a given site based on information about stream nutrient concentrations and other environmental co-factors.

Table 4. Extent estimates for the site-evaluation categories based on reconnaissance information across the PSA and SMC probability surveys from 2008-2011.

Site Evaluation Category	Number of Sites Sampled*	Estimated Stream Kilometers (% of State Total)	Confidence Interval (95%)
Part of survey's "target population", and sampled	572	33,499 (10)	29,101 - 37,897
Part of "target population", but not sampled	400	43,438 (13)	37,973 - 48,903
Not part of "target population"	3362	238,195 (74)	231,300 - 245,089
Unknown	174	9,510 (3)	7,270 - 11,750

* Note that each sample for the input data used in the analysis represents either a one-time sampling event, or an average (for the small subset of stream reaches for which multiple samples over time were available).

Analysis of the statewide primary producer abundance indicator data revealed that the full range (or nearly so) of possible values for each indicator type (for those indicators with a theoretical maximum, such as percent-based metrics) are represented in California. Table 5 provides a summary of the statewide estimated distributions for values of the various primary producer abundance indicators measured in the probability surveys. Appendix A provides graphical representation in the form of histograms. The algal biomass parameters, chlorophyll *a* and AFDM, exhibited a considerable degree of variability in concentrations, and broad ranges thereof, but their distributions were very highly skewed toward the low end (Appendix A).

Table 5. Statewide estimates for distributional properties of primary producer abundance indicator values in California perennial, wadeable streams. Data are from combined PSA and SMC probability surveys from 2008-2011. SE: standard error of the mean; CI: confidence interval (95%).

Indicator	Range of Measured Values (N)	Estimated Mean (SE)	Estimated Median (CI)	Estimated 90th percentile (CI)
chlorophyll *a* (mg m^{-2})	0.22 to 1504 (536)	21 (2)	8 (6-12)	47 (39-64)
AFDM (g m^{-2})	0.07 to 489 (525)	16 (2)	7 (6-8)	40 (23-50)
PCT_MAP (%)	0 to 98 (480)	16 (1)	6 (4-9)	51 (41-56)
PCT_MAA (%)	0 to 98 (480)	14 (1)	5 (3-7)	43 (36-52)
PCT_MAU (%)	0 to 87 (480)	2 (0.5)	0 (0-0)	3 (2-9)
PCT_MCP (%)	0 to 98 (480)	10 (1)	4 (2-5)	25 (20-39)
PCT_MIAT1 (%)	0 to 94 (478)	7 (1)	2 (0.5-2)	20 (13-32)
PCT_MIAT1P (%)	0 to 100 (464)	8 (1)	2 (1-3)	22 (16-41)
PCT_MIATP (%)	0 to 100 (478)	76 (2)	86 (83-93)	99 (99-100)
PCT_NSA (%)	0 to 100 (478)	20 (2)	11 (9-13)	52 (50-62)
XMIAT (mm)	0 to 6 (478)	0.5 (0.03)	0.3 (0.3-0.4)	1 (0.8-1.5)
XMIATP (mm)	0 to 20 (464)	0.6 (0.03)	0.4 (0.4-0.5)	1 (0.8-1.6)

Based on the statewide probability data, 4% of stream kilometers had benthic algal chlorophyll *a* exceeding 100 mg chla m^{-2}. However, it should be kept in mind that this 4% could include both WARM and COLD stream reaches. Under current NNE target values, the 100 mg chla m^{-2} is intended for assessing only COLD beneficial use attainment. Ecoregional estimates (Fig. 3 and Appendix B) indicated that the South Coast had the highest percentage of stream kilometers with chlorophyll *a* levels exceeding the BURC I/II and II/III Tetra Tech (2006) endpoints (e.g. 21% of stream kilometers exceeded 100 mg m^{-2} chlorophyll *a*), followed by the Central Valley (e.g. 10% of stream kilometers exceeded 100 mg m^{-2} chlorophyll *a*). The North Coast exhibited the lowest biomass levels, with an estimated nearly 90% of stream kilometers supporting <25 mg m^{-2} chlorophyll *a*. Results of chlorophyll *a* distribution estimates for “xeric” vs. “mountain” ecoregions within the South Coast are provided in Appendix C.

With respect to AFDM, 10% of stream kilometers had values exceeding 40 g m^{-2} statewide. The ranking of the ecoregions was similar to that for chlorophyll *a*. The Central Valley and South Coast ecoregions exhibited the highest levels, with approximately 25% of stream kilometers exceeding 40 g m^{-2} in each.

From the standpoint of macroalgae, every ecoregion exhibited some non-zero amount of macroalgal cover, but estimates varied greatly among ecoregions. Overall, the Central Valley and South Coast supported the highest levels of macroalgal cover, and the Sierra Nevada and North Coast the lowest. Table 6 provides a summary of estimated median values for key stream primary producer abundance indicators, statewide and by region

Table 6. Estimated median values (with 95% confidence intervals) for key stream primary producer abundance indicators statewide and by region. Data are from combined PSA and SMC probability surveys from 2008-2011.

Indicator	Statewide	Chaparral	Central Valley	Deserts-Modoc	North Coast	South Coast	Sierra Nevada
Chlorophyll *a* (mg m^{-2})	8.1 (6.2-11.5)	13 (5.6-17.4)	12.6 (7.5-21.6)	8.9 (5.8-11)	5.7 (4-11.3)	25.7 (19.2-40.7)	5.7 (2.9-12)
AFDM (g m^{-2})	6.5 (5.9-8.2)	6.6 (5.8-9.3)	13 (10.3-18.6)	10.2 (6.9-12.4)	5.5 (4.6-6.5)	17.2 (10.9-23.9)	4.8 (4.1-9.4)
Macroalgal percent cover (PCT_MAP)	6 (4-8.9)	5 (3-17.7)	16.9 (4.9-33.9)	11.9 (7-21.7)	7 (3-12.9)	20.1 (14.6-29.8)	1 (0.2-4)

In addition to evaluating distributions of primary producer abundance indicator values within the state as a whole and in individual ecoregions, a comparison was made between sites in the three disturbance classes. In terms of laboratory-measured biomass levels and percent macroalgal percent cover (PCT_MAP), Stressed and Intermediate sites supported more algae than Reference sites (Fig. 4), with the difference between site disturbance classes more pronounced for biomass than for macroalgal cover. That notwithstanding, the CDFs indicate that for AFDM and chlorophyll *a*, approximately 80 to 90% of stream kilometers statewide within the "Stressed" class are estimated to fall below the more conservative COLD BURC I/II endpoint (Tetra Tech 2006) for each biomass measure.

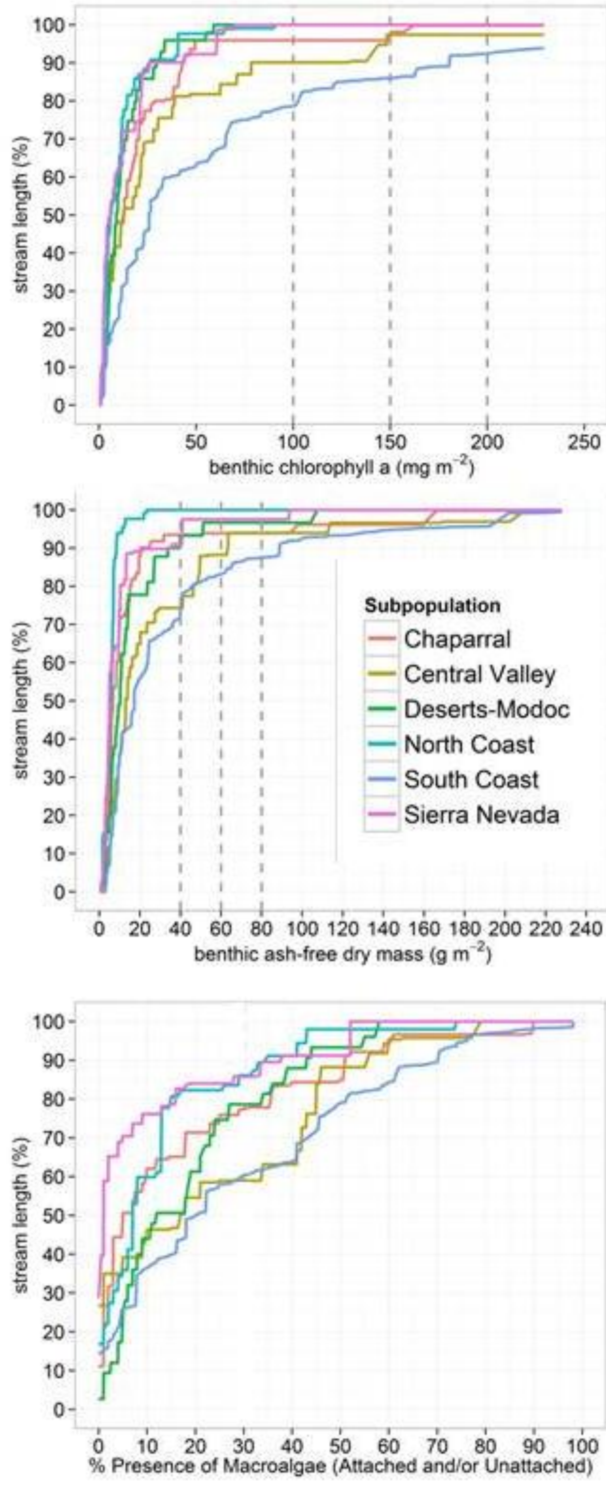

Figure 3. CDFs for biomass measures and macroalgal percent cover (attached and/or unattached combined), broken down by PSA6 ecoregion. The graphs show the estimated probability distributions of the 3 types of primary producer abundance indicators relative to the cumulative proportion of stream length. The dashed grey lines on the graphs denote the various NNE endpoints for the biomass variables (Tetra Tech 2006). Confidence intervals for each CDF can be viewed on the individual graphs for each ecoregion provided in Appendix B.

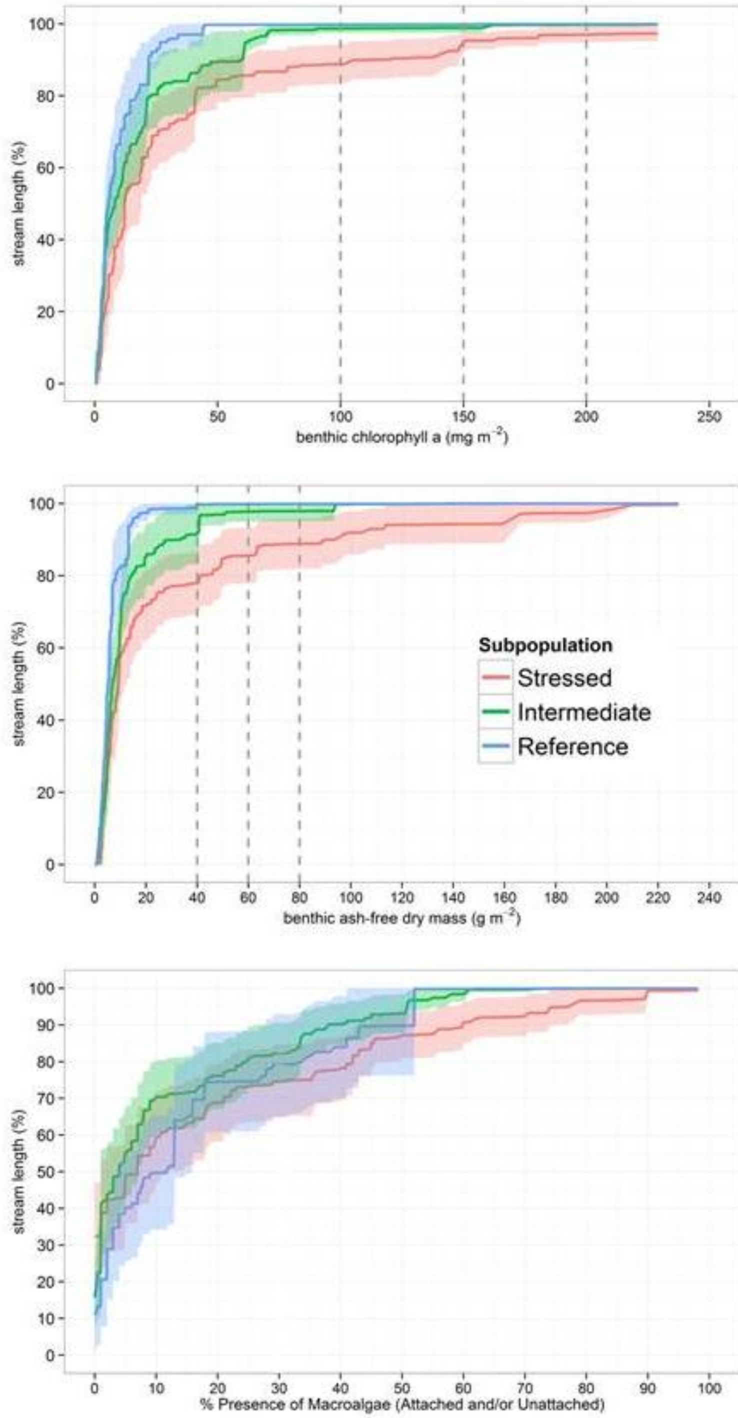

Figure 4. Statewide CDFs for biomass measures and macroalgal percent cover (attached and/or unattached combined) by site disturbance class. The graphs show the estimated probability distributions of the 3 types of primary producer abundance indicators relative to the cumulative proportion of stream length. The dashed grey lines on the graphs indicate various NNE endpoints for the biomass variables (Tetra Tech 2006). Highlighted areas delineate the 95% confidence intervals for each estimate.

Within ecoregions, "Reference" sites were most likely to fall below BURC I/II, and the "Stressed" sites were least likely. This trend was particularly pronounced in the South Coast ecoregion, where only approximately 65% of stream kilometers within the "Stressed" site class were estimated to fall below the BURC I/II COLD endpoint (100 mg m^{-2} chlorophyll *a)* (Fig. 5).

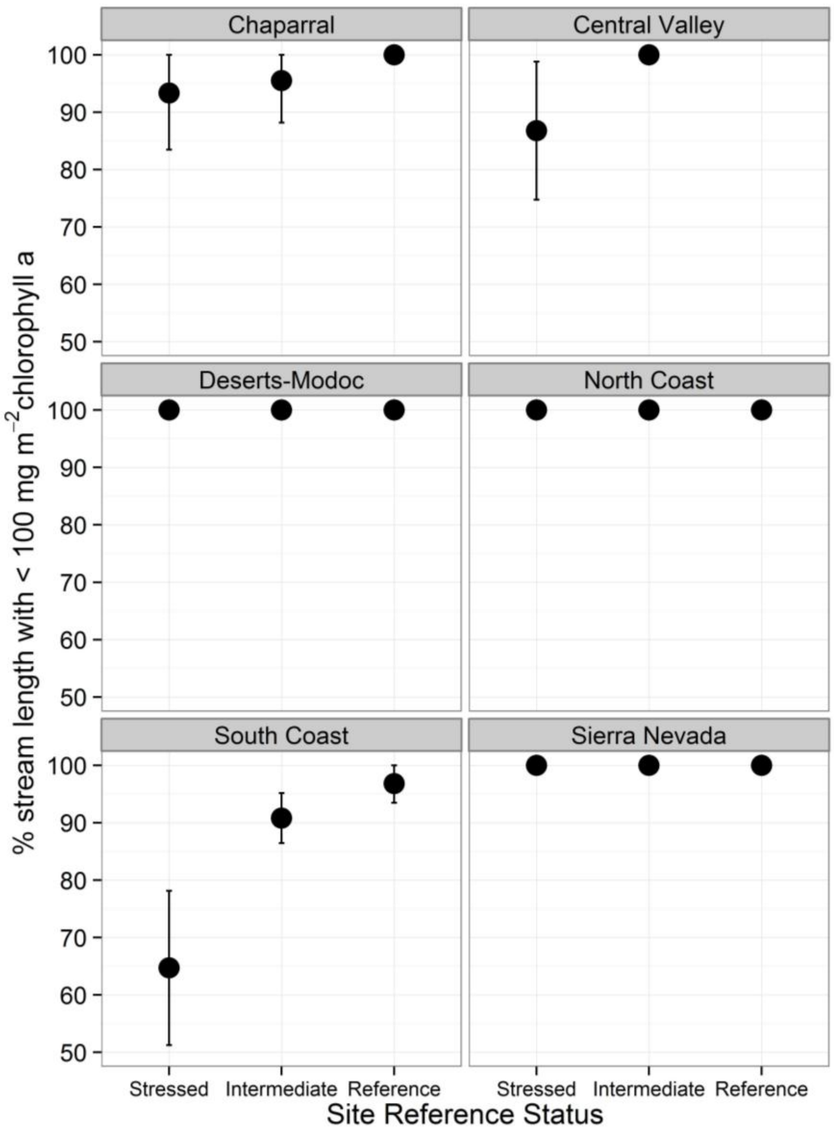

Figure 5. Within-region estimated percent of stream kilometers lower than the lowest proposed NNE endpoints for chlorophyll *a* (100 mg m^{-2}), by site disturbance class. Bars indicate 95% confidence intervals. *Note that y-axis scale begins at 50% mark*.

Statewide raw data distributions for the 3 site disturbance classes (Appendix D), which included data from both probability and targeted sites, indicated that values for benthic chlorophyll *a*, AFDM, and macroalgal percent cover (PCT_MAP) all varied with site disturbance class according to the same pattern: Stressed > Intermediate > Reference. Kruskal-Wallis tests with pairwise comparisons revealed highly significant ($p < 0.01$) differences between all site disturbance class pairs for all 3 of these indicators (Table 7). Conversely, none of the metrics based solely on measures of microalgal cover showed significant differences for all 3 pairwise comparisons, and microalgal thickness exhibited no significant differences between disturbance classes. For macrophyte percent cover (PCT_MCP), although there were highly significant differences between "Reference" sites and the other two disturbance classes, there was no difference between "Intermediate" and "Stressed" sites.

Table 7. Results of Kruskal-Wallis test with pairwise comparisons for effect of site disturbance class on a suite of stream primary producer abundance indicators, based on full dataset (probability plus targeted sites). Values are the level of significance at which the indicated pairs of site disturbance classes differed for each indicator. "NS" = "not significant". Refer to Table 2 for indicator definitions.

	Site Disturbance Class, Pairwise Comparison		
Primary Producer Abundance Indicator	Intermediate *vs* Reference	Intermediate *vs* Stressed	Reference *vs* Stressed
chlorophyll *a*	**0.01**	**0.01**	**0.01**
AFDM	**0.01**	**0.01**	**0.01**
PCT_MAP	**0.01**	**0.01**	**0.01**
PCT_NSA	**0.01**	**0.01**	**0.01**
PCT_MAA	**0.05**	**0.01**	**0.01**
PCT_MAU	**0.01**	NS	**0.01**
PCT_MCP	**0.01**	NS	**0.01**
PCT_MIAT1	**0.05**	NS	NS
PCT_MIAT1P	**0.05**	NS	NS
PCT_MIATP	NS	NS	**0.05**
XMIAT	NS	NS	NS
XMIATP	NS	NS	NS

The geographic distribution of realized chlorophyll *a* values among all sites for which data were available (i.e., data collected from sites in probability surveys as well as from targeted sampling) is shown in Fig. 6.

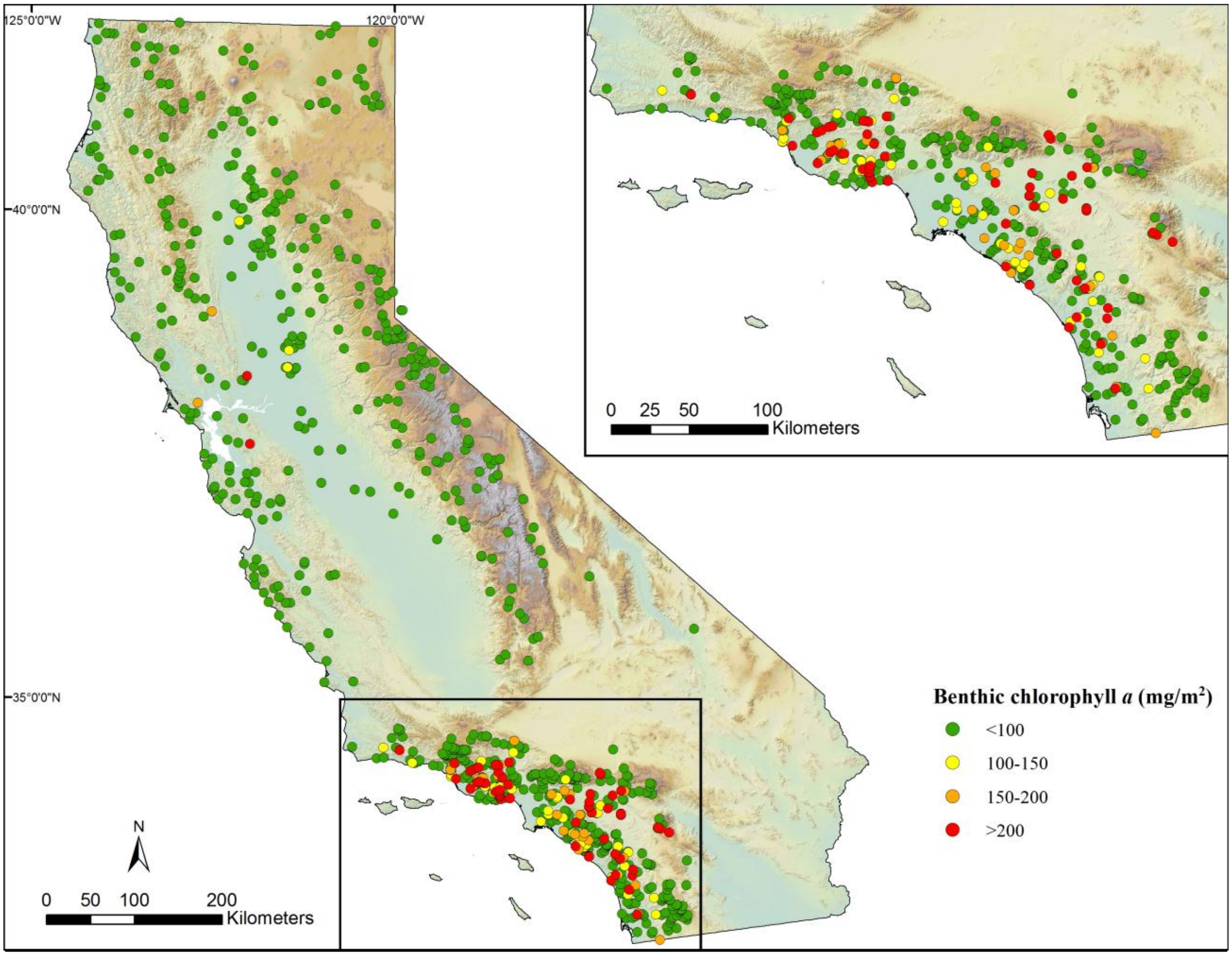

Figure 6. Geographic distribution of chlorophyll *a* values (mg m^{-2}) in California for all sites for which data were available. Inset provides zoomed-in detail for southern California. Chlorophyll *a* values are binned by NNE BURC endpoints for WARM and COLD beneficial uses (Tetra Tech 2006): green dots correspond to sites with < 100 mg m^{-2} chlorophyll *a*; yellow: 100-150; orange: 150-200; and red: > 200.

Distribution of Primary Producer Indicators at Reference Sites

As with the ambient survey data, chlorophyll *a*, AFDM, and macroalgal percent cover (PCT_MAP) exhibited a considerable degree of variability in values among Reference sites, but their distributions were highly skewed toward the low end of the biomass gradients. Table 8 provides a summary of the statewide and ecoregional median, 75th, and 95th percentiles for raw (i.e., unweighted) values for the various primary producer abundance indicators from the probability and targeted data sets, combined. At the 75th percentile, the ranges in primary producer indicator values among ecoregions, within the Reference site disturbance class, were fairly narrow (i.e., 8-27 mg m^{-2} chlorophyll *a*, 6-27 g m^{-2} AFDM, and 15-37% cover of macroalgae). At the 95th percentile, however, ranges were much broader, with values ranging up to 125 mg m^{-2} chlorophyll *a*, 131 g m^{-2} AFDM, and 60% cover in the South Coast. This 95th percentile was heavily influenced by two South Coast Reference sites that had extremely high abundances of algae (> 450 mg m^{-2} chlorophyll *a*). Removal of these two outlier sites drops the South Coast 95th percentile to 75 mg m^{-2} chlorophyll *a*.

Table 8. Median, 75^{th}, and 95^{th} percentiles of benthic chlorophyll *a*, AFDM, and macroalgal percent cover (PCT_MAP), statewide and by region, at Reference sites (both probability and targeted datasets included). SE: standard error of the mean; CI: confidence interval (95%).

Statistic by Primary Producer Indicator type		State-wide n=263	Chap-arral n=56	Central Valley n=1	Deserts-Modoc n=10	North Coast n=41	South Coast n=74	Sierra Nevada n=81
chlorophyll *a*	Median	6.9	8.9		10.7	6.2	12.5	3.1
	75^{th}	14.6	16.4	23.0[1]	26.5	9.2	24.4	7.9
	95^{th}	44.1	46.2		32.0	25.1	124.8	28.3
AFDM	Median	5.4	6.2		13.4	4.0	16.3	3.7
	75^{th}	11.9	10.0	12.9[1]	23.9	6.0	26.8	5.8
	95^{th}	34.0	19.7		36.7	14.8	130.6	12.2
macroalgal percent cover	Median	7.0	3.5		30.5	5.5	9.5	7.0
	75^{th}	22.9	15.9	41.0[1]	36.8	15.0	26.0	23.0
	95^{th}	45.7	38.9		55.9	36.5	60.0	50.3

[1] The Central Valley ecoregion only had one site in the Reference site disturbance class; values in the table represent the results of this single site.

Discussion

We found that California's perennial, wadeable streams, as assessed during the PSA index period[10], exhibited a skew toward the low end of the algal biomass gradient. Nearly 90% of stream kilometers had biomass values below that which represents the 95^{th} percentile of Reference sites[11] statewide, which corresponds to 44 mg m^{-2} chlorophyll *a*, 34 g m^{-2} AFDM, and 46% macroalgal percent cover. Similarly, 96% of stream kilometers statewide fell below the BURC I/II COLD endpoint (i.e., a *maximum* of 100 mg m^{-2} chlorophyll *a*; Tetra Tech (2006)). However, it is important to qualify that these estimates are based on one-time sampling events and time-series data necessary for identifying the seasonal maximum biomass levels at these sites were not available. In certain ecoregions, such as Sierra Nevada, North Coast, and the Desert-Modoc, no portion of stream kilometers exceeded 100 mg m^{-2} chlorophyll *a*. Conversely, ~35% of "Stressed", ~10% of "Intermediate", and ~5% of "Reference" stream kilometers in the South Coast; ~15% of "Stressed" kilometers in the Central Valley; and ~10% of "Stressed" and ~5% of "Intermediate" stream kilometers in the Chaparral ecoregions exceeded this endpoint.

With respect to the BURC II/III endpoint for WARM streams (i.e., 200 mg m^{-2} chlorophyll *a*), only in the South Coast and Central Valley ecoregions was some proportion of stream kilometers estimated to exceed this value, but this represented only small minority (<10%) of stream length, all of which fell into the "Stressed" site disturbance class. Thus, regardless of ecoregion, no portion of stream kilometers within the "Reference" or "Intermediate" site classes was estimated to exceed the highest recommended endpoint for chlorophyll *a*.

With respect to use of the BURC I/II and II/III recommended endpoints for placing California stream survey results into context, two caveats should be borne in mind. First, it is possible that the Tetra Tech (2006) BURC endpoints may not correspond to actual shifts in attainment of Aquatic

[10] The PSA index period for stream sampling starts in May for drier parts of the state and June or July in colder/wetter parts of the state (depending upon stream flow conditions), and lasts for two to three months.

[11] In the case of the Reference sites, values are given here for all available data combined (i.e., probability plus non-probability, or "targeted", sites)

Life Uses in California streams. Thus is it possible that the values reported here underestimate nutrient impacts on beneficial uses as mediated through the primary producer abundance indicators we evaluated. Research to explore linkage between algal biomass and indicators of Aquatic Life Uses in wadeable streams, which will shed additional light on this matter, is currently underway.

Second, time of year when stream algal indicators are sampled will affect the percentage of stream kilometers exceeding BURC endpoints. Tetra Tech (2006) proposed benthic chlorophyll *a* targets in terms of maximum values, based on the recommendations of Dodds et al. (2002). The definition of "maximum" has been somewhat problematic for the interpretation and application of the NNE. In the work of Dodds et al. (2002), maximum appears to be intended to represent the spatially-averaged, temporal maximum algal growth potential (in response to nutrient and light availability) in the absence of temporary reductions in biomass density due to grazing, scour, and other factors. It is thus intended to be a temporal maximum, identified via multiple samples taken over the growing season. In contrast, PSA survey data generally represent only a single time point, and the index period for bioassessment (primarily late spring through mid-summer) was established to optimize condition assessment for benthic macroinvertebrates, not stream algal biomass. This index period is not reflective of a time period when the highest stream algal biomass would be expected (i.e., at the end of the growing season—late summer to early fall). Certain streams may be experiencing algal/macrophyte blooms at certain times of year, but such phenomena might often be missed because current standard bioassessment practices call for sampling to be conducted earlier in the year than when peak algal biomass would generally be realized.

Relationships between site disturbance classes and primary producer abundance indicators

Some noteworthy trends were apparent from the comparison of primary producer abundance indicator values between site disturbance classes. Macroalgal cover tended to be dominated by attached macroalgae (as opposed to unattached), and unattached macroalgae was particularly uncommon in "Reference" sites (Appendix E), possibly because of generally higher flow regimes in such reaches. Also, whereas the algal biomass and percent macroalgal cover (PCT_MAP) variables exhibited significant differences among site disturbance classes, differences were less pronounced (and non-significant) for the other algal cover measures (i.e., in terms of microalgae). This latter discrepancy could be due in part to a greater difficulty associated with assessing microalgal presence and thickness in the field, the fact that thickness values are binned, therefore adding error to the estimates, and/or anthropogenic stressors having a more pronounced effect on the macroalgal, as opposed to microalgal, "compartments" in the stream. Understanding the reasons for the lower responsiveness of the microalgal cover variables to anthropogenic stress will be important for determining whether or how to apply them.

Chapter 2 – Stream health as assessed by algae IBI scores

Introduction

The US EPA has been encouraging states to incorporate bioassessment into waterbody monitoring programs, preferably through the use of multiple biotic assemblages because various assemblages respond differently to certain stressors and restoration activities (Yoder and Rankin 1995). Coordinated use of various assemblages yields multiple lines of evidence for assessing stream health and water quality, and can provide a broader range of perspectives on the attainment of aquatic life beneficial uses.

For over a decade, bioassessment using benthic macroinvertebrates (BMIs) has been a standard practice in several California wadeable stream monitoring programs (Ode et al. 2011). Algae can provide information complementary to that obtainable with BMIs alone in terms of types of stressors affecting them (Fore 2003, Griffith et al. 2005, Feio et al. 2007) and potentially also in terms of the levels of stress to which they are most responsive. In addition to responsiveness to stress, algal indicators are temporally complementary to BMIs (Stevenson and Smol 2003, Johnson and Hering 2004) because algae can reproduce/proliferate more rapidly than BMIs (Rott 1991, Lowe and Pan 1996, Hill et al. 2000, USEPA 2002) and communities can respond to changes in environmental conditions over shorter periods relative to other commonly used bioindicators like BMIs and fish (Stevenson and Pan 1999, Rimet et al. 2005, Lavoie et al. 2008). Another benefit of utilizing algae is the fact that they can colonize virtually any type of stream substratum, so their presence tends not to be limited by available habitat (Soininen and Könönen 2004, Feio et al. 2007). This attribute may prove particularly useful for bioassessment in highly urbanized environments, where streams may be channelized, resulting in diminished instream habitat quality (Newall et al. 2006).

Questions: Stream health as assessed by algae IBI scores

- What is the ecological health of southern California perennial, wadeable streams based on the algae IBI (Fetscher et al. 2013) developed for that ecoregion?

- How does the southern California IBI perform in other parts of the state?

An algae Index of Biotic Integrity (IBI) for bioassessment of southern California streams was recently completed by Fetscher et al. (2013), and hundreds of sites' worth of stream algae community composition data have been collected using the SWAMP field sampling SOP (Fetscher et al., 2009), particularly in southern California. The availability of probability data for application of the IBI provides an opportunity to begin evaluating the distribution of IBI scores in the South Coast ecoregion. It also provides the opportunity to begin evaluating IBI performance in other ecoregions, to determine whether the IBI might have application elsewhere.

Methods

The data sources, site selection approach, and field sampling methods for the data presented here were presented in Chapter 1.

Laboratory analyses

Laboratory analysis involved quantification of the stream benthic algal community based on the following data types:

- diatom species relative abundance (via "valve counts")
- soft algae and cyanobacteria species absolute biovolume and species tallies

Diatom samples were cleaned by the method of Van Der Werff (1955). The cleaned material was processed into permanent microscope slides, using Naphrax as the mounting medium. For each sample, 600 diatom valves were identified and enumerated using an Olympus BX-51 light microscope, under 1000x magnification using an oil immersion objective with a numeric aperture of 1.40.

For soft algae, the method of Stancheva et al. (2012) was employed for laboratory processing and taxonomic identification and quantification. Macroalgae were processed separately from the microscopical fraction of each sample. This facilitated specimen identification to the lowest possible taxonomic level due to the high-quality preservation of macroalgal vegetative and reproductive structures, as well as the even distribution of microalgal "entities", of which 300 were counted on a standard microscope slide. In addition to collecting the biovolume information for each recorded micro- and macroalgal specimen, up to 100 epiphytes were enumerated, and taxa present in a "qualitative" sample collected from the stream reach (Fetscher et al. 2009) were also recorded.

Data analyses

<u>Algae Index of Biotic Integrity</u>

Algae IBI scores were calculated for all sites for which diatom and soft algae data were available, using the IBI for southern California coastal streams that was recently developed by SCCWRP and partners (Fetscher et al., 2013)[12]. The top-performing IBI from this tool-development effort, referred to as IBI "H20" was used for this purpose.

[12] Draft IBIs have also been developed for use in the Central Coast and the eastern Sierra Nevada, but calculation of those IBIs was beyond the scope of the present report.

The algae IBI "H20" is comprised of the following 8 metrics ("d" indicates that a given metric is based on diatoms and "s" indicates soft algae; of the latter, "sp" indicates that the metric is based on relative species numbers):

- proportion N heterotrophs (d)
- proportion requiring >50% DO saturation (d)
- proportion sedimentation tolerant (highly motile) (d)
- proportion halobiontic (d)
- proportion low N indicators (d)
- proportion high Cu indicators (s, sp)
- proportion high DOC indicators (s, sp)
- proportion low TP indicators (s, sp)

The potential appropriateness of the southern California IBI for application in PSA ecoregions outside of the South Coast was evaluated within each ecoregion by comparing IBI score distributions of minimally disturbed "Reference" sites with those of sites falling in the "Intermediate" and "Stressed" site disturbance classes (described in Chapter 1). One-way Analysis of Variance (ANOVA) with Tukey-Kramer test for pairwise comparisons was used to assess the significance of difference in IBI scores between the site disturbance classes within each ecoregion. For individual, raw metrics, whose data distributions were non-normal, Kruskal-Wallis tests with pairwise comparisons were carried out to evaluate metric performance in terms of ability to distinguish among site disturbance classes.

Results

Estimated distribution of algae IBI scores within the South Coast ecoregion

IBI scores were calculated for all 331 probability sites in the South Coast for which algal community data were available. Of these, 38 were "Reference" sites, 11 of which were located within the South Coast "xeric" ecoregion, and 27 of which were in the "mountain" ecoregion (based on Omernik Level III classification scheme; 1987). Scores ranged from 1 to 98, nearly the full range possible. The mean score across the ecoregion was 53 (SE = 2), the median was 55 (95% confidence Interval (CI) = 46-65), and the 90th percentile was 78 (CI = 74-87). Figure 7 shows CDFs of IBI score estimated distributions for the South Coast region, by site disturbance class. Fetscher et al. (2013) have suggested an IBI score of 57[13] as a statistical boundary between "reference-condition" sites and "non-reference condition" sites. Using that boundary, the distribution of ambient algae IBI scores within the South Coast ecoregion indicates that nearly 80% of stream kilometers within the "Stressed" class fall below that "reference-condition" value, compared to nearly 40% in the "Intermediate" class, and <10% of stream kilometers in the "Reference" class (Fig. 7).

[13] The proposed boundary is based solely on statistical considerations, as opposed to specific knowledge that this score reflects an ecologically meaningful change point in community composition. As such, more work would be needed in order to establish the defensibility of this boundary as an ecologically-based one that could, for example, eventually be incorporated into a regulatory framework (e.g., to evaluate attainment of water body "aquatic life" goals).

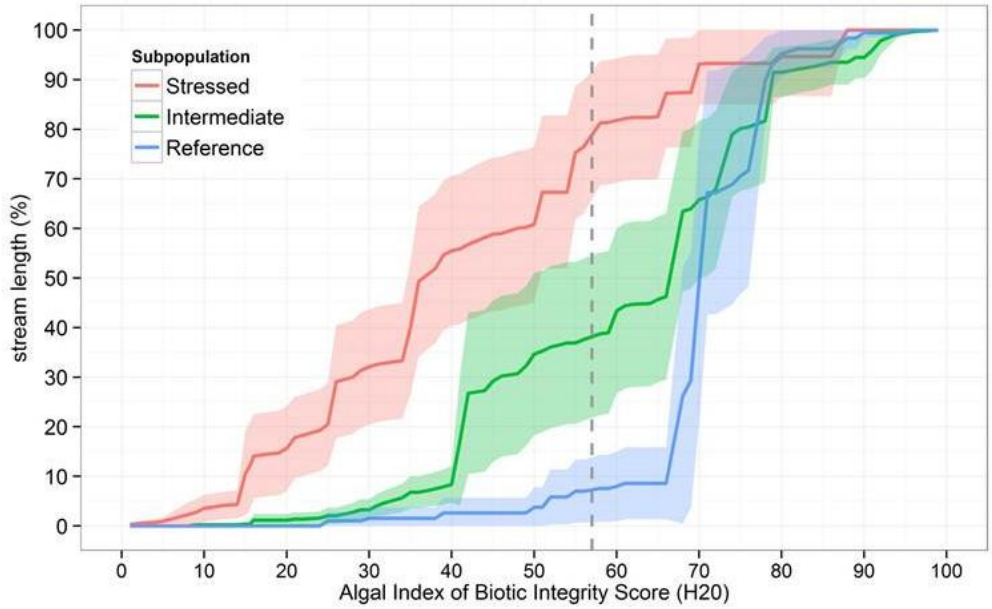

Figure 7. CDFs for algae IBI "H20" scores from probability sites in the South Coast PSA6 ecoregion. Shaded areas delineate 95% confidence intervals. The dashed grey line indicates the proposed boundary (score = 57) between reference-condition IBI scores and scores statistically distinct from reference (*per* Fetscher et al. 2013).

The geographic distribution of realized algae IBI scores among all South Coast ecoregion sites for which data were available (i.e., data collected from sites in probability surveys as well as from targeted sampling) are shown in Fig. 8.

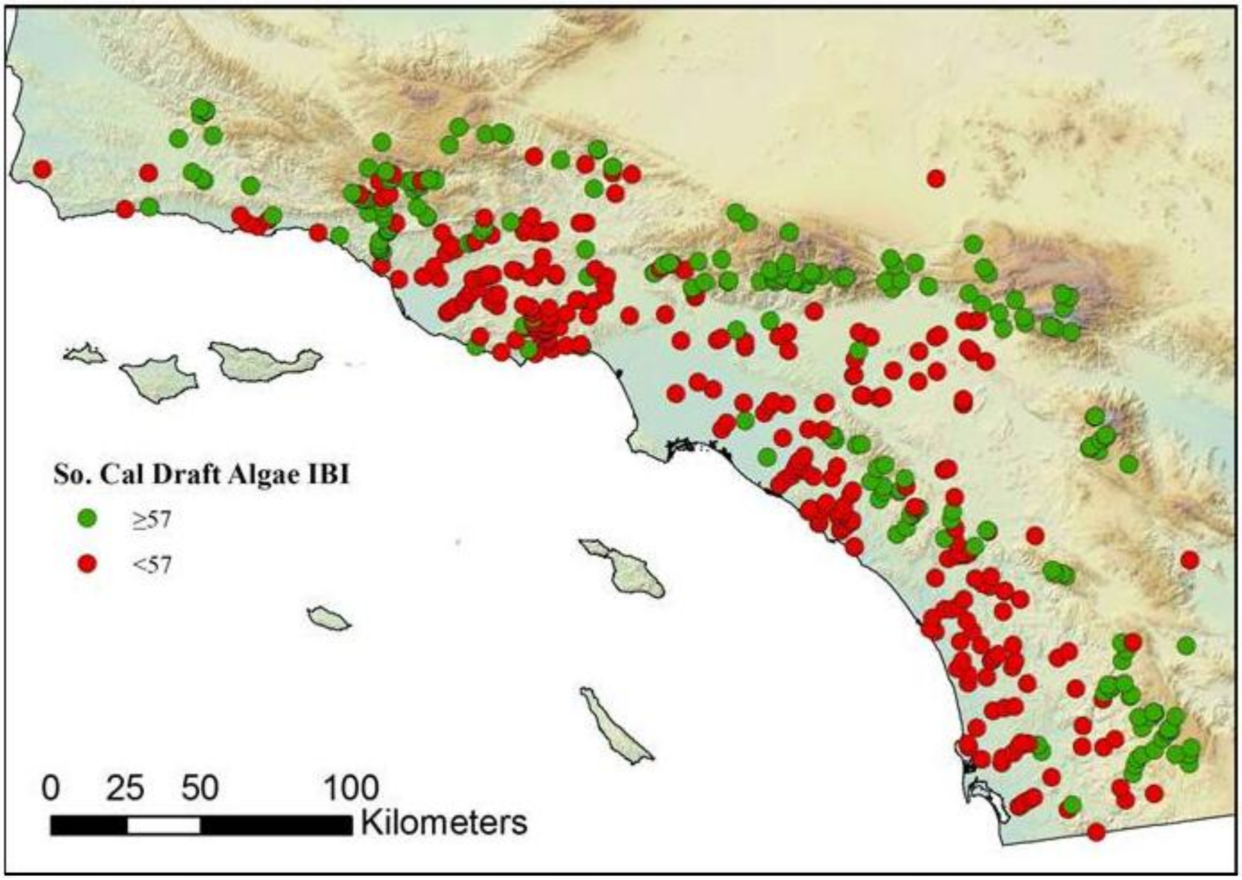

Figure 8. Geographic distribution of algae IBI scores (based on the algae IBI "H20"; Fetscher et al. 2013) in southern California for all sites for which data were available in this ecoregion. Green dots correspond to sites with IBI scores that are statistically indistinct from scores expected under "reference" site conditions; red dots correspond to sites with IBI scores that are statistically distinct from scores expected under "reference" site conditions.

Performance of southern California IBI in other ecoregions

IBI H20 scores were calculated for all California sites for which algae community data were available. This facilitated a comparison among ecoregions of how well the southern California IBI performs in parts of the state outside of the South Coast ecoregion, in terms of its ability to distinguish among sites in different disturbance classes. Figure 9 provides boxplots of IBI score distributions for each PSA ecoregion, by site disturbance class. The ecoregions with the greatest separation among site disturbance classes based on IBI scores were South Coast, which corresponds to the ecoregion for which the IBI was developed and calibrated, and Chaparral. Differences in IBI scores between site disturbance classes were highly significant ($p < 0.0001$) for all pairwise comparisons for both ecoregions. In the North Coast and Deserts-Modoc ecoregions, roughly one quarter of the variance in IBI scores was explained by site disturbance class, but in the former ecoregion, Stressed and Intermediate classes were not significantly different, and in the North Coast, discrimination between the Stressed and Reference classes was subpar, as evidenced by the overlap in their interquartile ranges. In the remaining ecoregions, only a very small portion of the variance ($R^2 \leq 0.05$) in IBI scores was explained by site disturbance class. Table 9 provides results of Kruskal-Wallis tests comparing raw metric scores among site disturbance classes for all ecoregions outside of the South Coast. All metrics comprising IBI H20 exhibited discriminatory power in at least one ecoregion besides the South Coast, and many metrics demonstrated applicability in multiple ecoregions. The ecoregion for which the most metrics exhibited discriminatory power was Chaparral. The ecoregion for which the fewest (i.e., none of them) showed applicability was Central Valley (which had a relatively low number of sampling sites, particularly in the Reference class, in the available dataset, thus compromising the ability to detect significant relationships among site disturbance classes within that ecoregion).

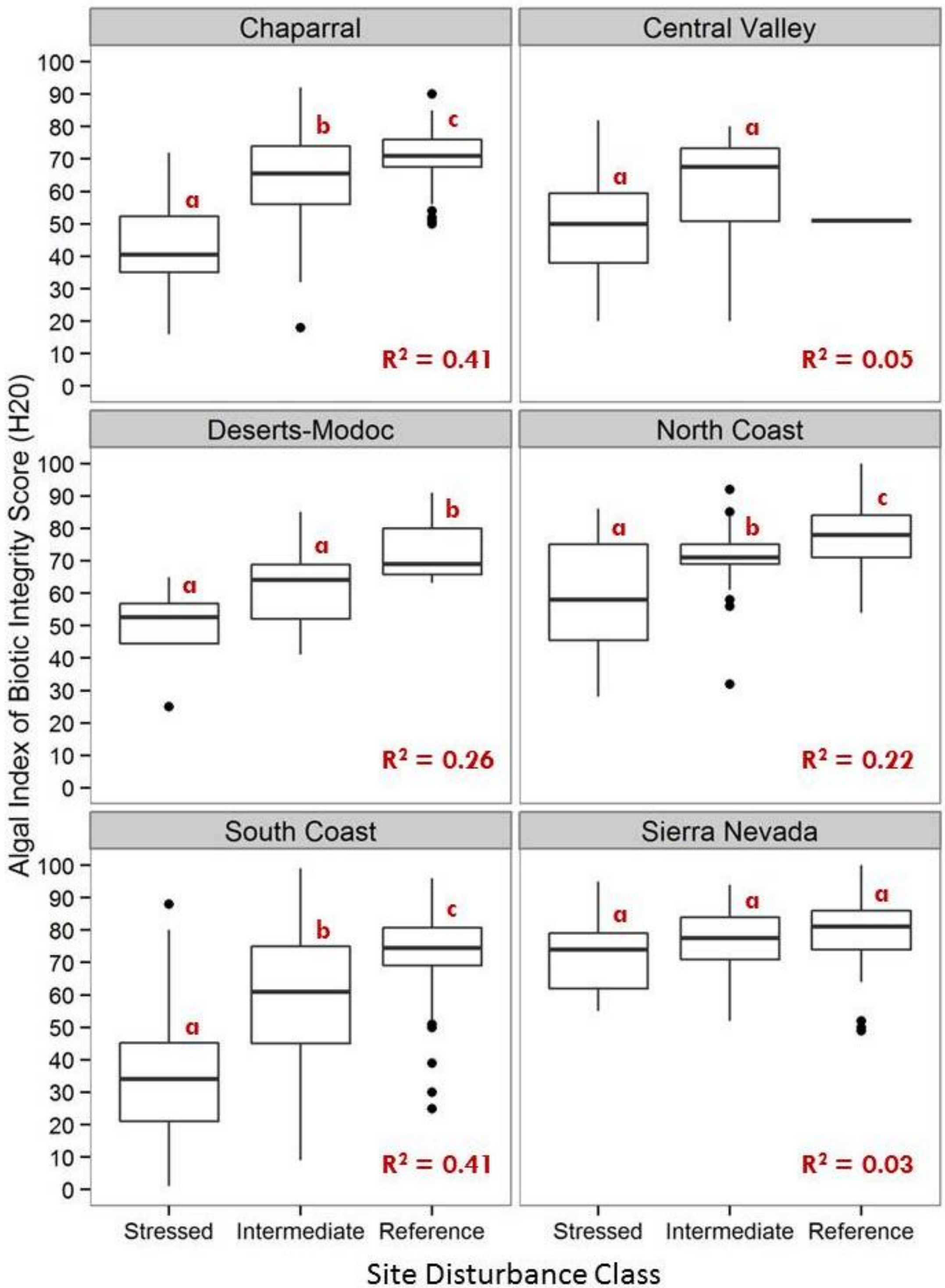

Figure 9. Discriminatory power (ability to distinguish among site disturbance classes) of IBI "H20" in the different PSA ecoregions. IBI "H20" is calibrated specifically for use in the South Coast ecoregion (see Fig. 2). R^2 values for one-way ANOVA are provided. Letters above boxplots indicate which pairwise differences are significant per the Tukey-Kramer test. Site disturbance classes that share the same letter within an ecoregion are not significantly different (α=0.05).

Table 9. Summary of results of Kruskal-Wallis tests for differences in algae raw metric scores (from Fetscher et al. 2013) among site disturbance classes, for all PSA ecoregions outside of the South Coast. "X"s indicate the pairwise comparisons for which significant ($p < 0.05$) differences were detected.

Metric	Ecoregions in which metric exhibits discriminatory power based on available data	Reference *vs.* Stressed	Intermediate *vs.* Stressed	Intermediate *vs.* Reference
proportion requiring >50% DO saturation (d)	Chaparral	X	X	
	Deserts-Modoc			X
	Sierra Nevada			X
proportion low N indicators (d)	Chaparral	X	X	
	Deserts-Modoc	X		
proportion N heterotrophs (d)	Chaparral	X	X	
	North Coast	X		X
proportion halobiontic (d)	Chaparral	X	X	
	North Coast	X		X
	Sierra Nevada			X
proportion sedimentation tolerant (highly motile) (d)	Chaparral	X	X	
	North Coast	X	X	X
proportion low TP indicators (s, sp)	Chaparral	X	X	
proportion high Cu indicators (s, sp)	Chaparral	X		
proportion high DOC indicators (s, sp)	Chaparral	X	X	

Discussion

Score distributions estimated for IBI "H20" in the South Coast indicated that nearly half the stream kilometers in this ecoregion are indistinguishable from what would be expected in minimally disturbed "reference" sites, based on the statistical boundary of 57 (Fetscher et al. 2013). Score distributions varied markedly among site disturbance classes, with nearly 80% of stream kilometers in the "Stressed" class estimated to score below 57, compared to <40% of kilometers in "Intermediate" and <10% of kilometers "Reference" classes.

Perhaps not surprisingly, the algae IBI developed for southern California coastal streams ("H20"; Fetscher et al. 2013) exhibited the best performance characteristics in the PSA South Coast ecoregion, in terms of its ability to distinguish among the 3 site-disturbance classes (Fig. 9). However, it also showed promise in the Chaparral. In the other ecoregions, it exhibited poorer performance, based on available data. However, because the dataset used for this report derives chiefly from probabilistically selected sites, the most highly disturbed sites in those ecoregions may not have been well enough represented to reveal a full gradient in IBI scores. Among the "Stressed" site class of the Sierra Nevada and North Coast that were available for analysis, IBI scores averaged higher relative to South Coast "Stressed" sites. While it is possible that the apparent relative lack of discriminatory power of IBI "H20" in the more northern ecoregions is due to a lack of tool sensitivity there, it could also be because the levels of stress in the sites sampled in these ecoregions were generally not severe enough to result in a pronounced

IBI response. It is also important to note that sample sizes within certain site disturbance classes were low for some ecoregions, thus limiting our ability to detect significant differences between them.

Draft algae IBIs have recently been developed for the Central Coast and the eastern Sierra Nevada regions of the state, and are in various stages of review. For other portions of the state, it may be necessary to recalibrate existing IBIs or develop new IBIs that are specifically calibrated to local conditions. IBIs for use in these ecoregions would ideally be developed using an adequate number of sites representing a broad disturbance gradient in order to calibrate the tool appropriately. It may turn out that existing metrics (e.g., those derived from the recent southern California (Table 9), Central Coast or eastern Sierra Nevada algae IBI development projects) can be used, but simply rescaled, once expanded datasets for the different ecoregions are available. New combinations of existing metrics might also exhibit better performing IBIs in the different ecoregions than the southern California IBI tested here.

Recommendations for next steps in State's stream algae program

Recommendations relative to eutrophication assessment

1) **Refine the biomass sampling window in terms of time of year, duration, and sampling intensity.** Relying strictly on routine bioassessment practices (i.e., as described in the current SWAMP SOP; Fetscher et al. 2009) for estimation of algal biomass may not result in documenting stream algal/macrophyte standing crop at, or even near, peak bloom. Such monitoring may occur too early in the season, before the maximal (or near maximal) amount of algae that can be supported by the stream has had time to accrue, thus yielding an underestimate. One-time assessment of a given site may fail to capture peak algal biomass, and moreover will not provide any information on the duration of algal blooms. Guidelines should be developed to ensure that sampling that is specifically geared toward assessing nutrient impacts in terms of primary producer abundance indicators is conducted during the appropriate window of time. An added benefit of refining the biomass sampling window is that it would help the State in assigning perennial status to stream reaches. A reach could be deemed perennial if it were found to "wetted at time of sampling", if sampling were conducted at the end of summer or in the early fall.
2) **Test and finalize new methods for sampling stream algal biomass in terms of how well the methods improve precision of biomass estimates.** Macroalgae often represent the bulk of algal biomass (Wehr and Sheath 2003), and tend to be highly patchily distributed, in streams (Sheath et al. 1986). This patchiness has a tendency to compromise precision of stream algal biomass estimates (AE Fetscher, unpublished data) to a degree that might jeopardize the utility of the biomass data for regulatory applications (e.g., those related to determination of nutrient impacts). New methods for sampling stream algal biomass should be evaluated in terms of how well the methods improve precision of biomass estimates. Methods to evaluate could include variations on SWAMP's existing Standard Operating Procedures (SOP) for ambient monitoring of algae for bioassessment purposes (Fetscher et al. 2009). For example, for regulatory applications, this SOP might be modified to incorporate a greater density of algae subsamples (i.e., use of more transects and/or sampling points per transect) in order to minimize sampling error. This would require a potentially non-trivial increase in field effort/expense; as such, testing should also evaluate the minimum amount of added effort required in order to achieve the desired level of precision. Other methods should also be evaluated.
3) **Explore the feasibility of incorporating "second-generation indicators", such as percent macroalgal cover, into the NNE framework.** A rapid field measure of algal cover, such as that described in Fetscher et al. (2009) and reported on here, is not subject to some of the

drawbacks of chlorophyll *a* (like the latter's tendency to degrade rapidly), and has the added advantage that it can be used to efficiently sample a higher "density" of stream area than can be achieved when collecting quantitative samples for laboratory analysis of biomass (thus helping to address the poor-precision issue discussed above). Moreover, citizen monitor groups operating on a modest budget that precludes laboratory analysis could easily conduct macroalgal field surveys, hence increasing the sources for this type of data throughout the state. Macroalgal cover may serve as a reasonable surrogate for benthic chlorophyll *a*, at least in order to identify "ballpark" chlorophyll a values, or to place a ceiling on the likely amount of chlorophyll *a* in a stream. Fig. 10 provides an example of how quantile regression might be used to establish the relationship between macroalgal percent cover and chlorophyll *a* concentration for this purpose.

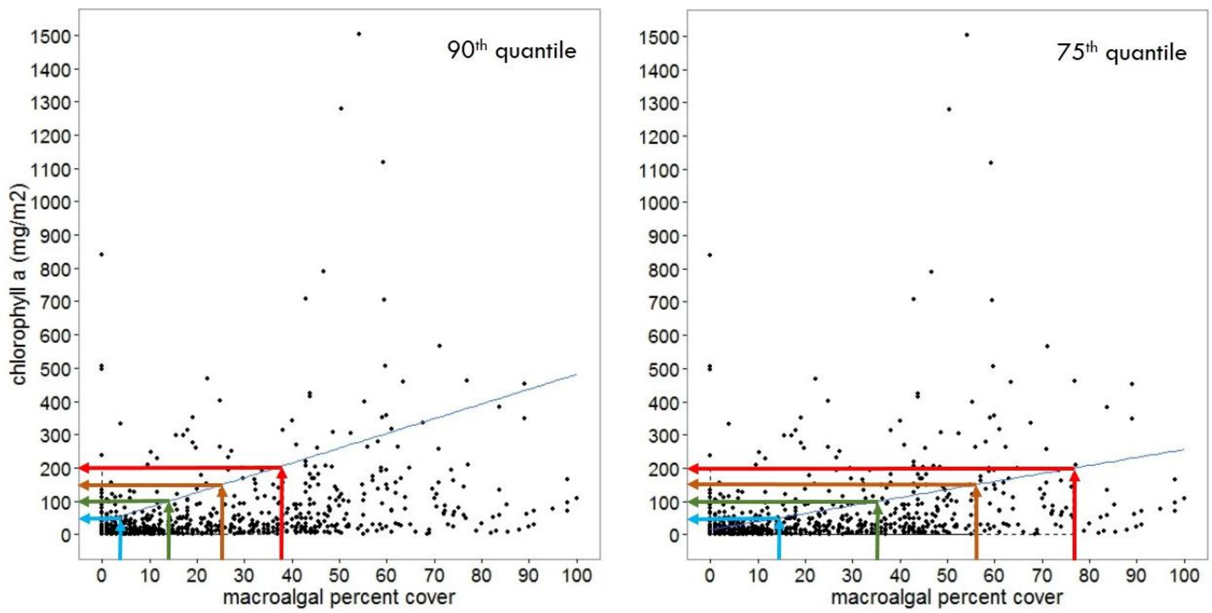

Figure 10. Quantile regressions (90th and 75th) of benthic chlorophyll *a* concentration on macroalgal percent cover. Colored lines indicate macroalgal percent cover levels associated with the three NNE BURC endpoints (100, 150, and 200 mg m^{-2} chlorophyll *a* (see Table 1) corresponding to the green, orange, and red lines, respectively), as well as for 50 mg m^{-2} (blue line), at different quantiles, based on the relationship between these two variables in the available dataset. Analysis was performed using R (version 2.15.1, R Development Core Team (2013)) and the package "quantreg" (Koenker 2013).

4) **Verify relationships between beneficial uses and proposed algal indicator endpoints for nutrient impacts.** Regulatory endpoints should ultimately be tied to beneficial uses. It is valuable to confirm the validity of proposed NNE biomass endpoints with respect to their relationship to beneficial uses in California wadeable streams, and as of the writing of this report, work has begun on this issue in coordination with the US EPA Office of Research and Development. It would also be valuable to develop scientifically valid endpoints for newer indicators such as macroalgal cover, which have implementation advantages over chlorophyll *a*, as noted above.

5) **Primary producer abundance indicators**
   a) **Determine an appropriate role for AFDM in efforts to monitor nutrient impacts.** AFDM is not as prone as chlorophyll *a* to degradation, and could be considered a more robust biomass indicator in that regard. However, AFDM has disadvantages from the standpoint that it represents not only algal material, but also other forms of organic matter in the sample (i.e., microbes, protozoa, fungi, and detritus). Worse yet is the fact that fine particulate organic matter in the sample may be partly allochthonous and thus unrelated to the stream's nutrient status. Nonetheless, AFDM could provide a good indication of the maximum amount of chlorophyll *a possible* in a given sample (i.e., the amount of chlorophyll *a* expected in the sample if it were assumed to be comprised entirely of algal material), and thus a ceiling for that estimate. Used in this way, AFDM would provide a valuable complement to chlorophyll *a* results, thus helping to mitigate the shortcomings of both biomass measures.
   b) **Determine how the various biomass and cover indicators can be used in conjunction with one another, and whether there is sufficient value in continuing to monitor microalgal thickness.** One potential advantage of continuing to look at microalgal thickness, even if it is not used as an indicator, is that it is helpful for the determination of whether there might have been a recent scour event in the sampling reach (i.e., if biofilm appears to be absent across most or all 105 sampling points). In the event of a recent scour, it would be inappropriate to rule out the potential for nutrient impacts in the stream reach in question based on a one-time low algal biomass measure (since the algal community might not have had time to develop, post-scour).

Recommendations with respect to IBIs for stream bioassessment

1) **Continue to test existing, but consider developing new, algae IBIs for parts of the state that do not currently have them. Also consider developing a statewide algae bioassessment tool.** IBIs are currently available for southern California coastal watersheds (e.g., IBI "H20" used in this report; Fetscher et al. 2013), as well as draft IBIs for Central California and the eastern Sierra Nevada. Nonetheless, regionally calibrated IBIs are unavailable for the majority of the state, and analyses presented here suggest that, with the possible exception of the Chaparral and North Coast ecoregions, IBI H20 may be inappropriate for use elsewhere. While there is a good possibility that at least some of the metrics comprising H20 could be rescaled for use elsewhere, more intensive analysis using regional datasets from sites spanning a broad disturbance gradient and a sufficient number of Reference sites would be needed for this endeavor. It would also be worthwhile to test the Central Coast and eastern Sierra Nevada IBIs in other parts of the state to evaluate their suitability. Other possibilities that merit exploration are development of models based on observed/expected (O/E) algal taxa, or a "hybrid" version of O/E with IBIs of the type described by Fetscher et al. (2013).
2) **Determine whether and how to integrate information from multiple biotic assemblages.** The state's bioassessment toolkit includes BMI-based indices as well as the recently developed algae IBIs (for southern California (used in this report), the Central Coast, and the eastern Sierra Nevada). Decisions will ultimately need to be made about if, when, and how to integrate information from BMIs and algal assemblages for different bioassessment applications.
3) **Explore approaches to utilizing algae community composition, IBIs, and/or metrics for application in causal assessment.** Identifying the cause of degraded biological condition in streams and rivers that have been classified as "impacted" by the SWRCB's new Biological Objectives (Schiff et al. 2013) has triggered a need for appropriate causal assessment tools. Algae are well suited for assessing certain stressors, such as sedimentation (Bahls 1993), as well as providing an ecologically relevant, temporally integrated assessment of the water-chemistry environment, particularly with respect to nutrients, organic pollution, salinity, and,

heavy metals (Palmer 1969; Power 1990; Cattaneo et al. 1997; Vis et al. 1998; Leland and Porter 2000; Guasch et al. 2002; Komárek et al. 2002; Sheath 2003; Douterelo et al. 2004; Porter et al. 2008; John 2011, Stancheva et al. 2013).

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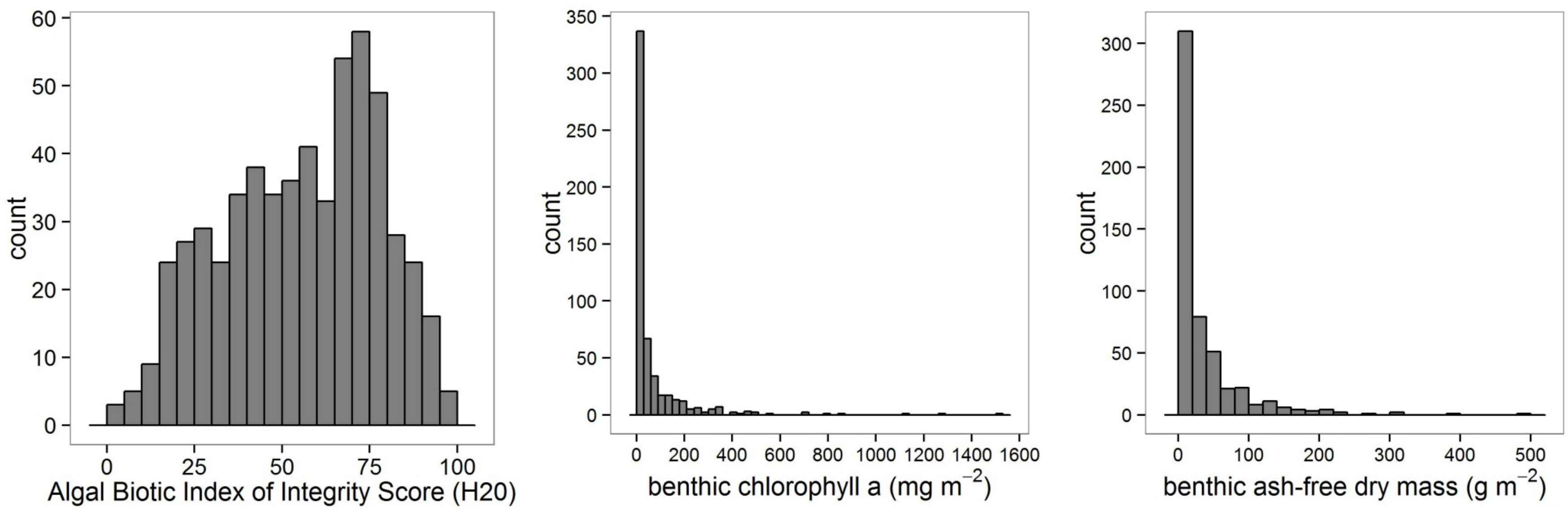
Appendices

Appendix A: Histograms of IBI, biomass, and algal/macrophyte cover data, all California probability data combined.



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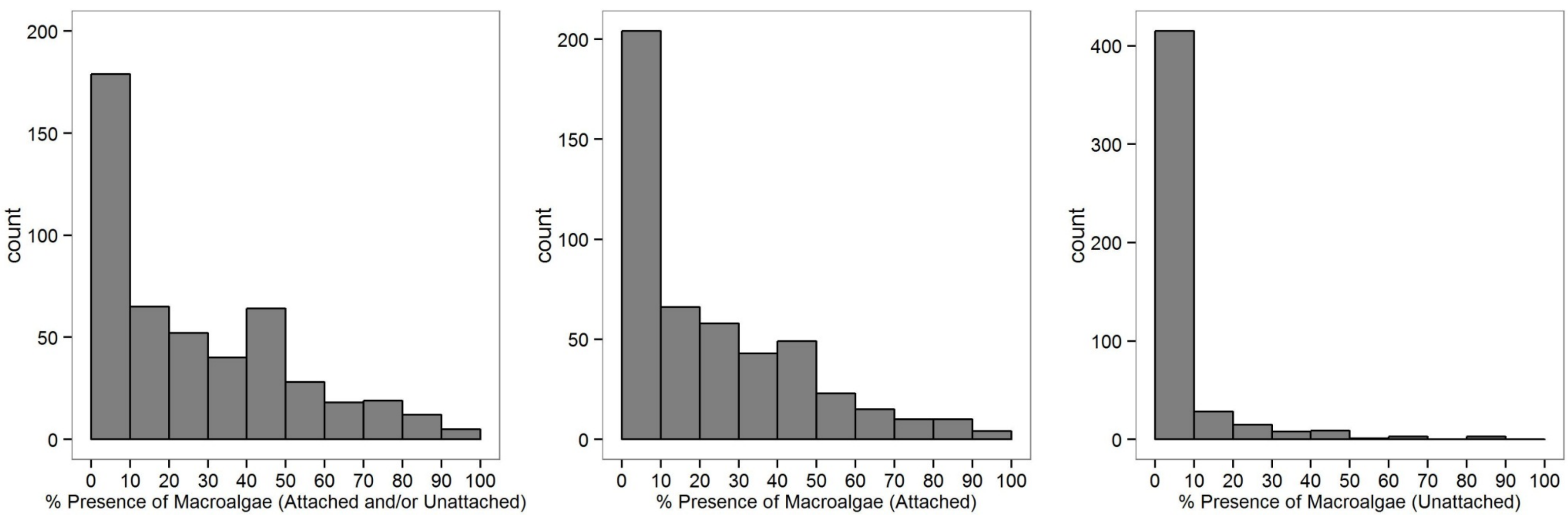
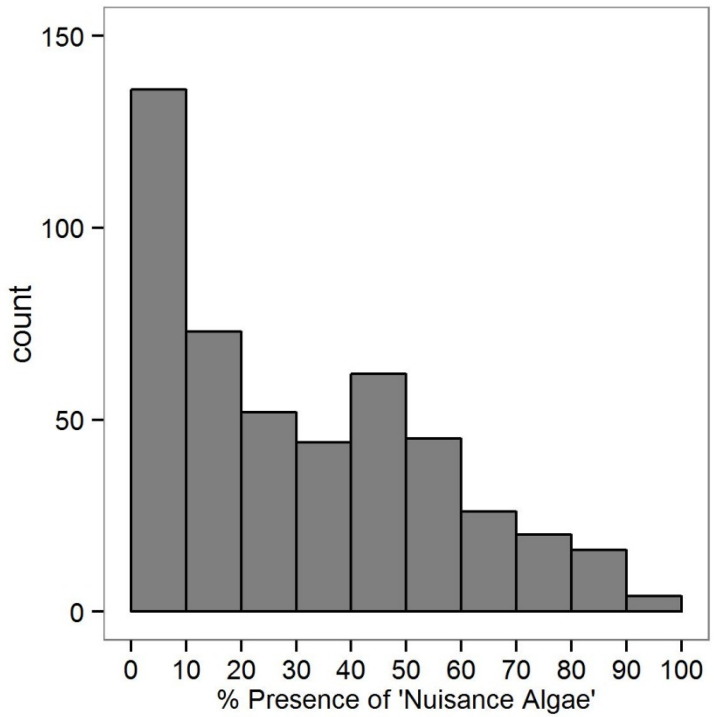
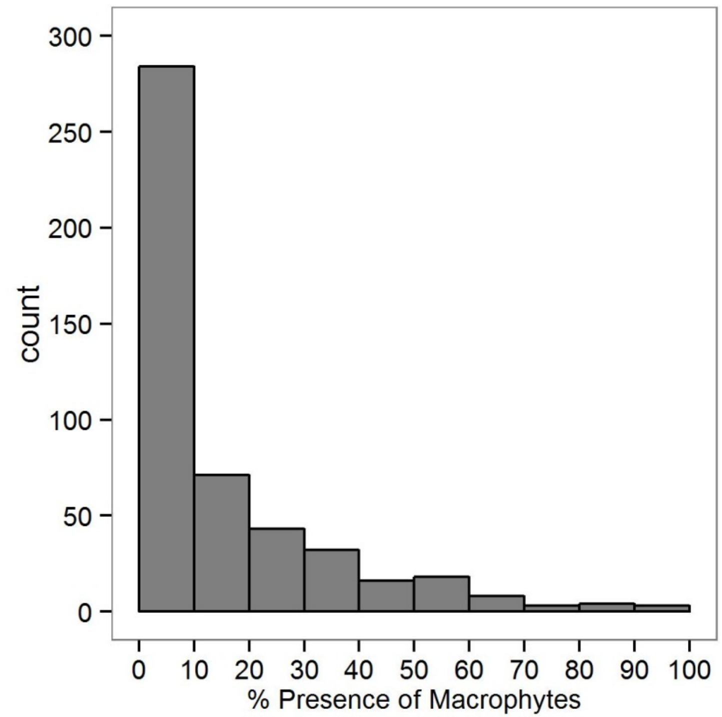
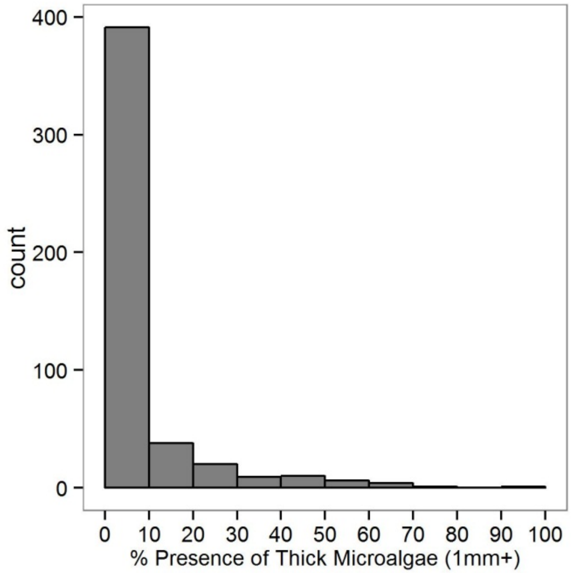


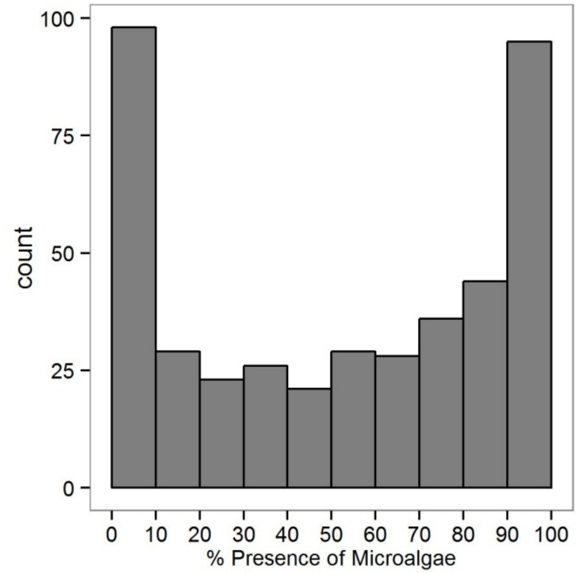
Appendix A (cont'd)

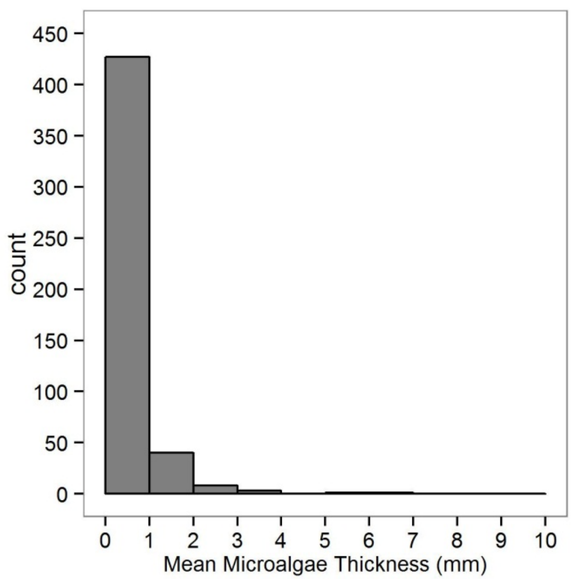


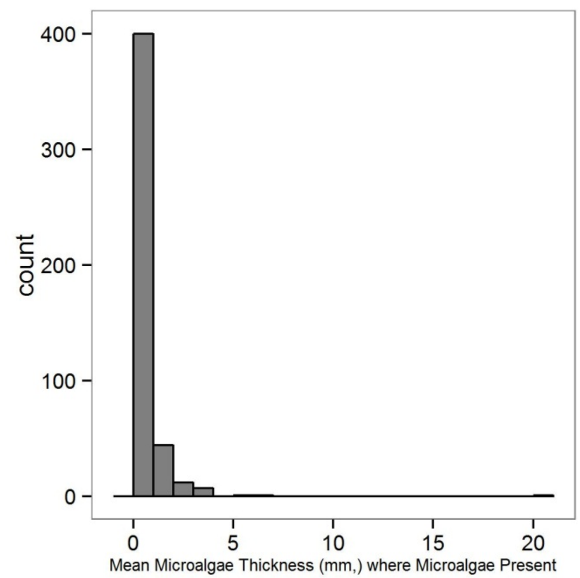


Appendix A (cont'd)

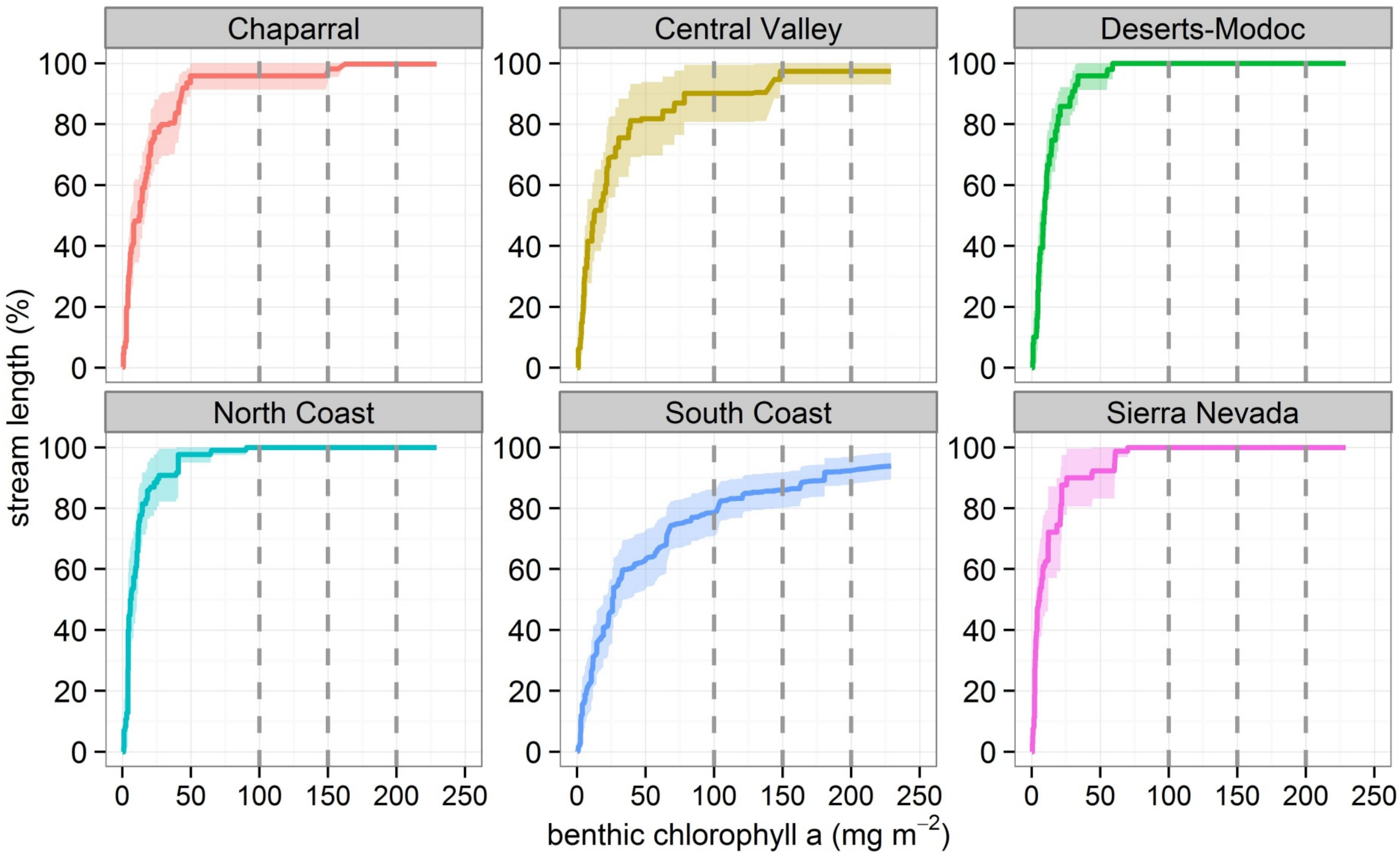








Appendix B: Cumulative distribution functions of biomass, ash-free dry mass, and macroalgal percent cover, by region, for all probability sites. Shaded areas delineate 95% confidence intervals.



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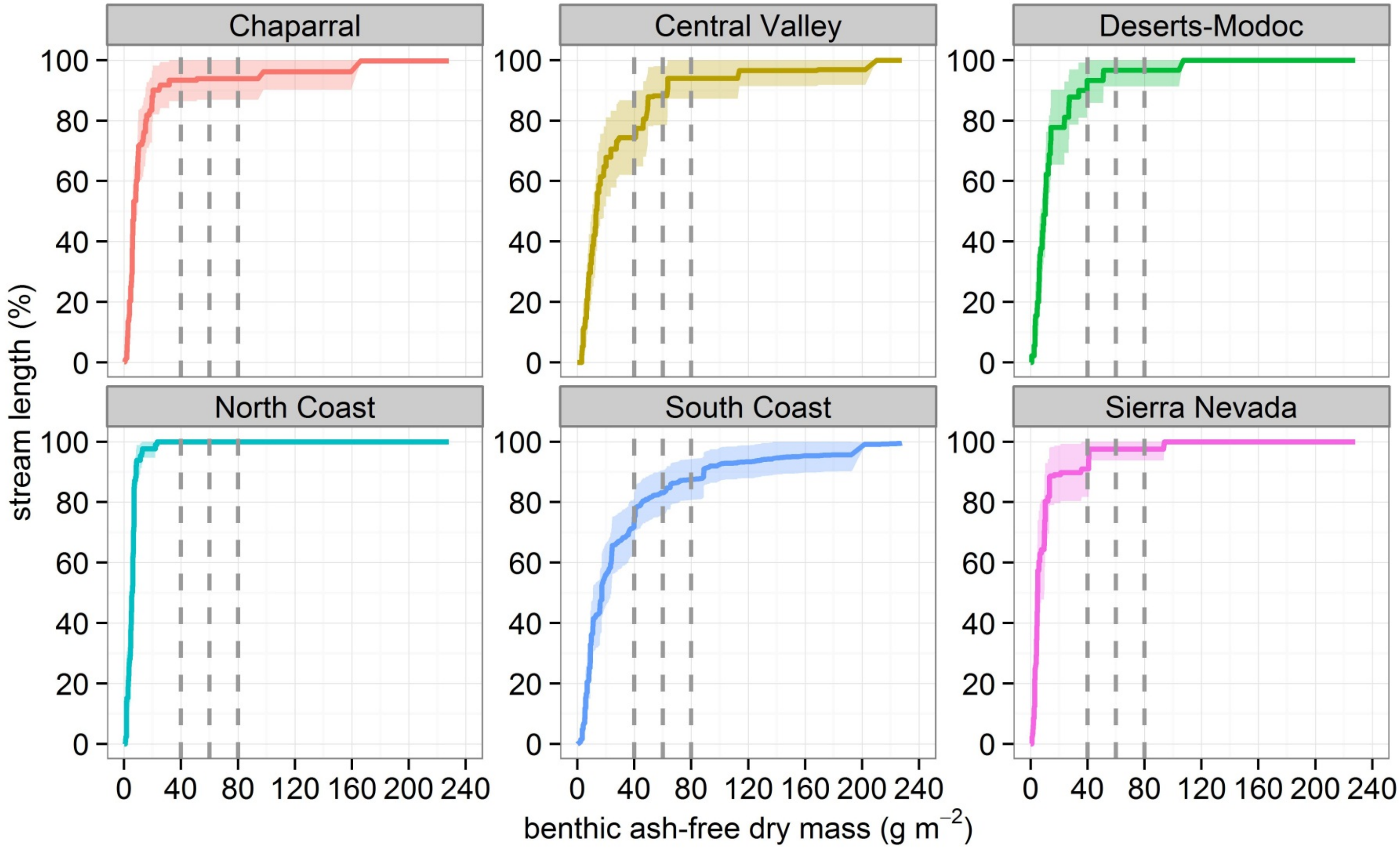

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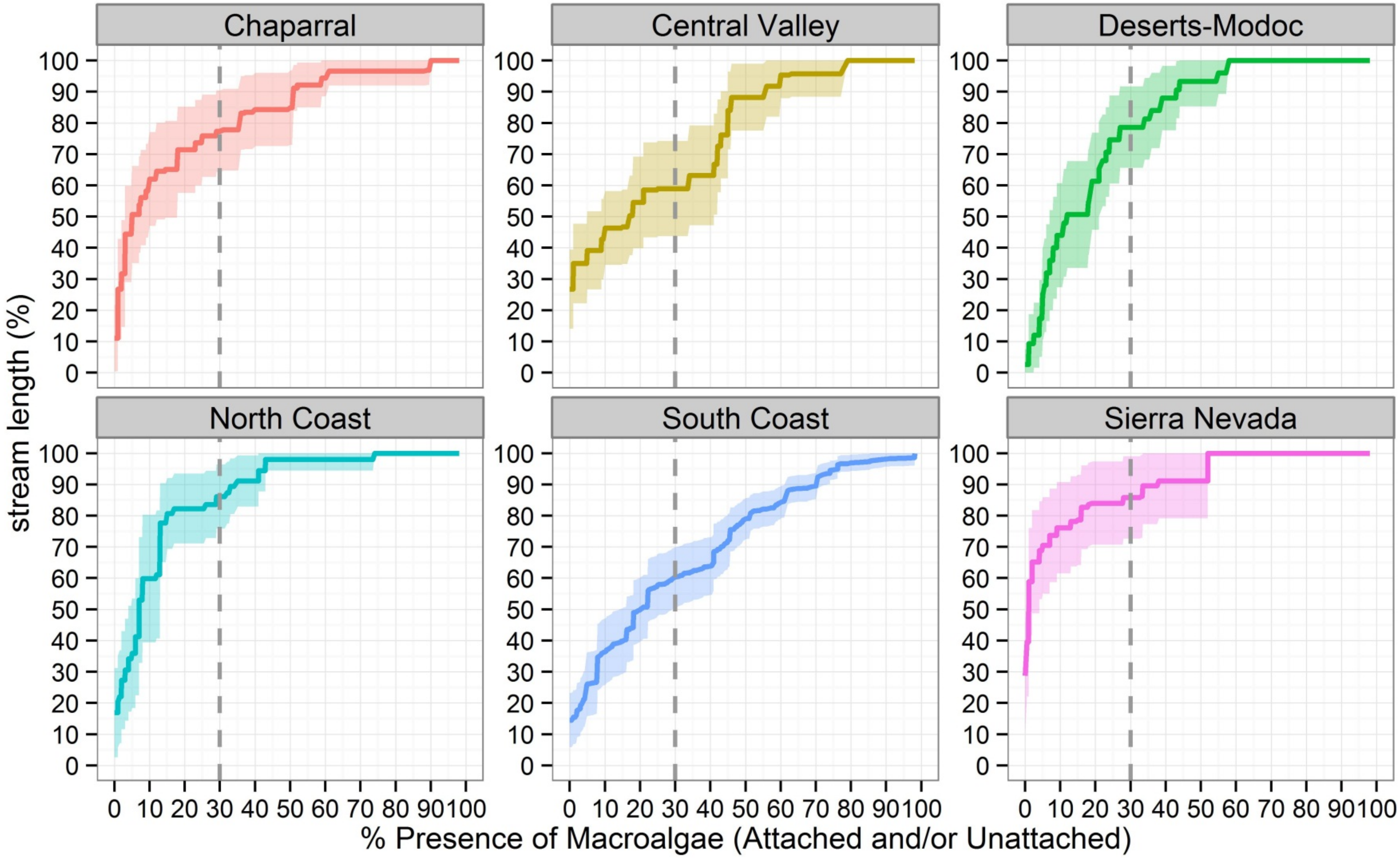


Appendix C: Chlorophyll *a* distributions within the South Coast

To investigate possible differences in chlorophyll *a* distributions within the PSA6 South Coast ecoregion, we conducted a set of analyses complementary to that which is presented in the main body of the report, in which this ecoregion was further divided into "xeric" and "mountain" zones. This subdivision was based on the Level III classification scheme of Omernik (1987). Multiple "Reference" sites were sampled for chlorophyll *a* within both regions (Table C1), however they were nearly three times as abundant in the mountain zone as in the xeric zone.

Table C1. Number of sites within each Level III ecoregion (Omernik, 1987) in the South Coast, by site disturbance class.

Ecoregion	Reference	Intermediate	Stressed
South Coast Mountain	27	33	1
South Coast Xeric	11	80	144

For each of the 3 NNE endpoints for chlorophyll *a*, higher proportions of stream length exceeded endpoints within the xeric ecoregion than in the mountain ecoregion (Figs. C1). The same tendency was observed within each site disturbance class (where data were available; Fig. C2).

Appendix C (cont'd)

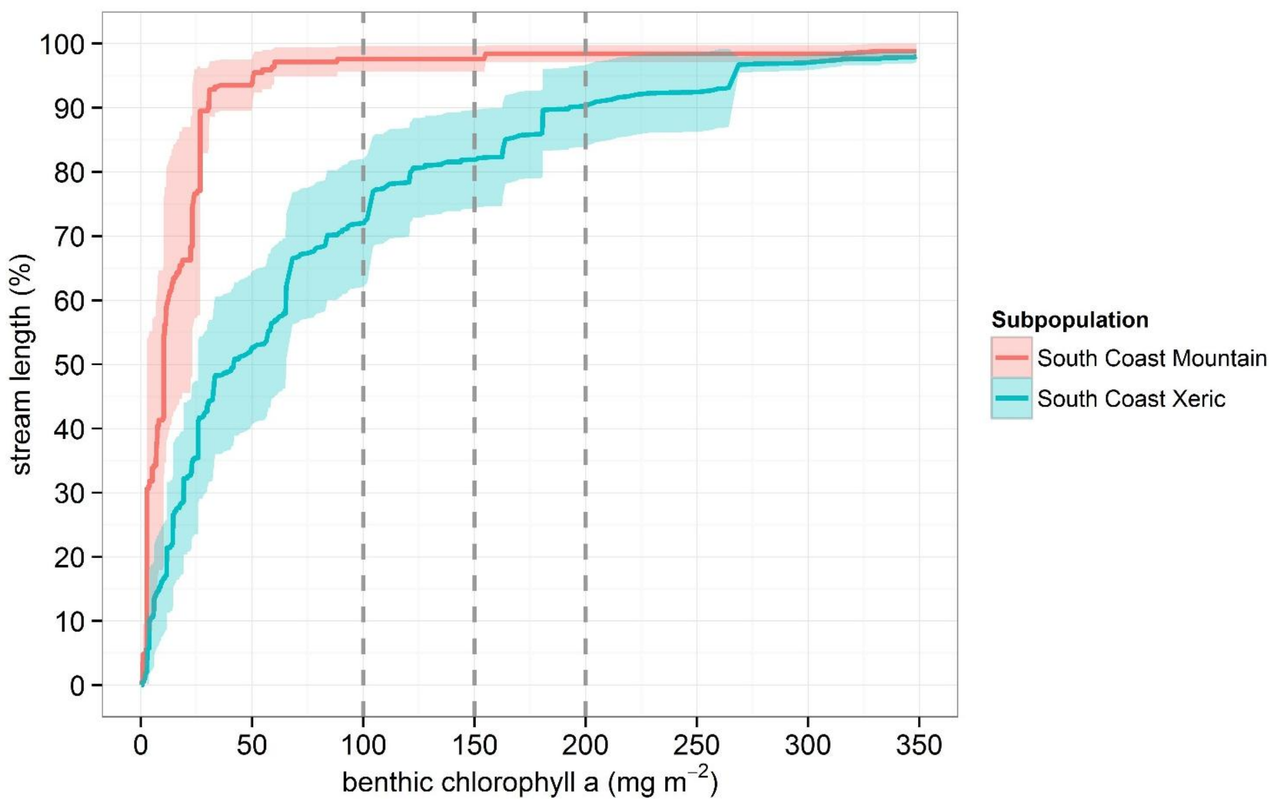


Figure C1. CDFs for benthic chlorophyll *a*, for the "xeric" and "mountain" Level III ecoregions (Omernik 1987) within the South Coast. The graphs show the estimated probability distributions of chlorophyll *a* relative to the cumulative proportion of stream length. The dashed grey lines on the graphs denote the three NNE endpoints for chlorophyll *a* (Tetra Tech 2006). Highlighted areas delineate the 95% confidence intervals for each estimate.

Appendix C (cont'd)

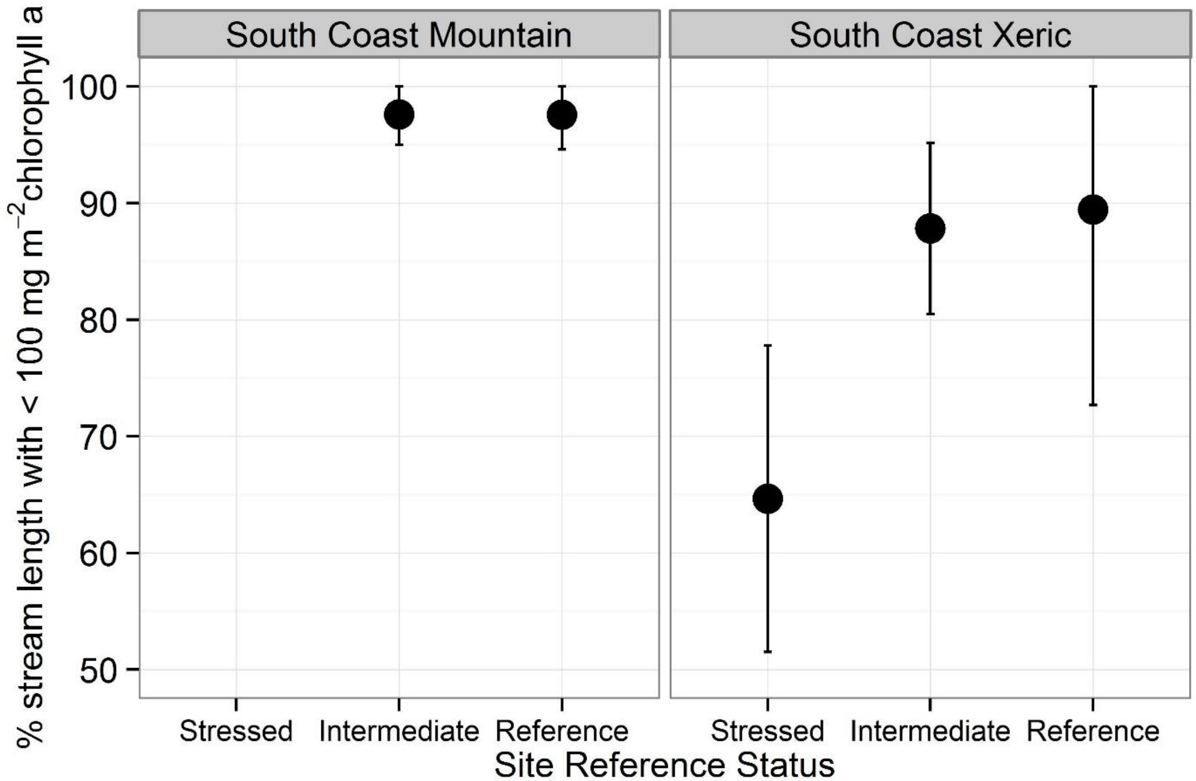
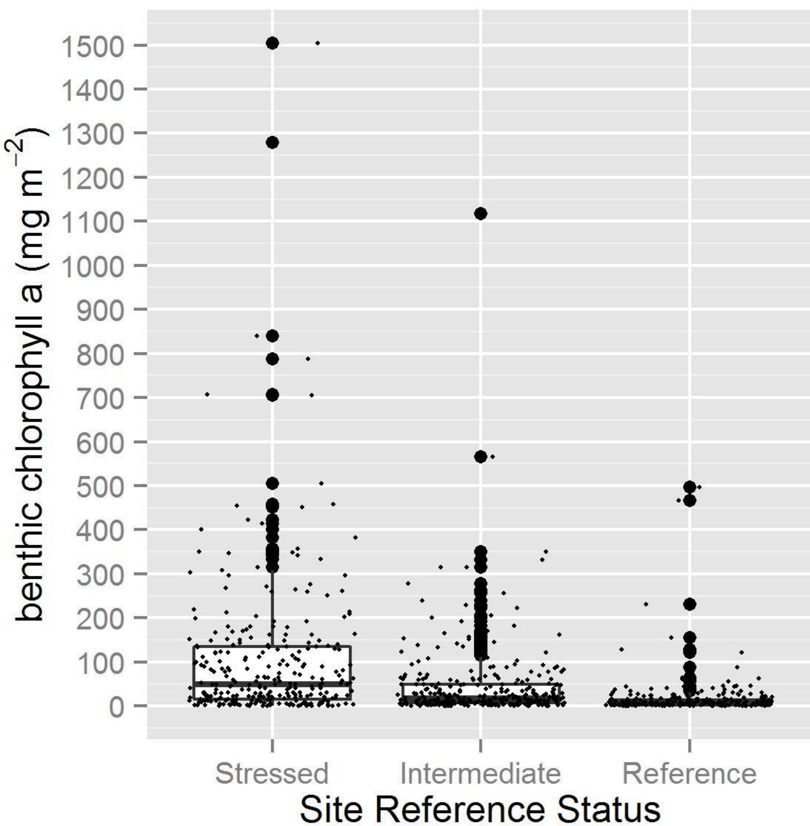
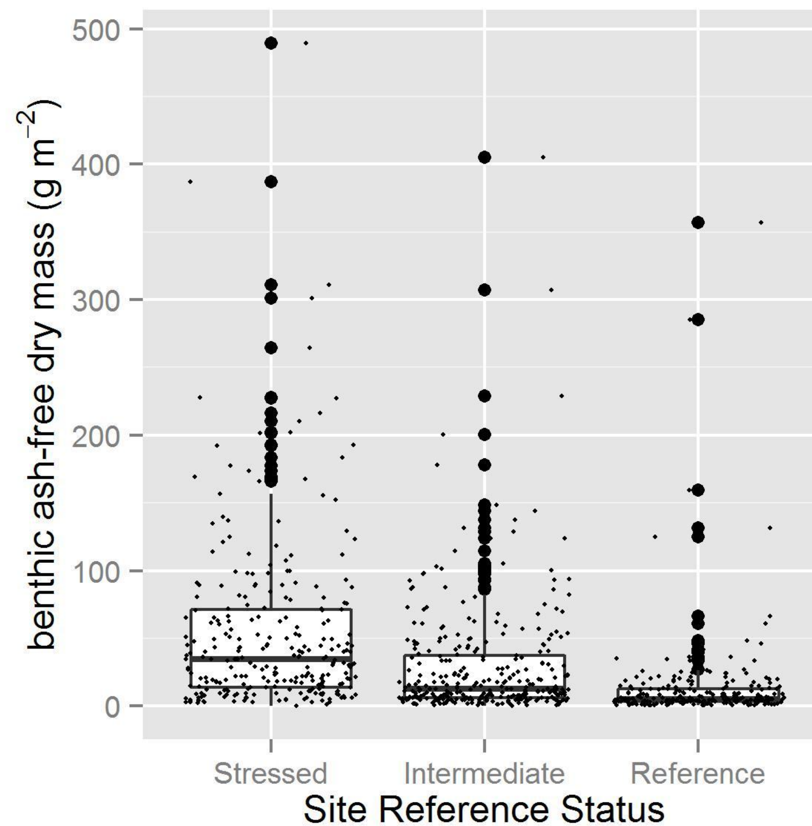


Figure C2. Within-ecoregion estimated percent of stream kilometers lower than the lowest proposed NNE endpoint for chlorophyll *a* (100 mg m^{-2}), by site disturbance class. Bars indicate 95% confidence intervals. Note that y-axis scale begins at 50% mark. Due to insufficient sample size, no estimate is available for the "Stressed" site disturbance class within the South Coast Mountain ecoregion.

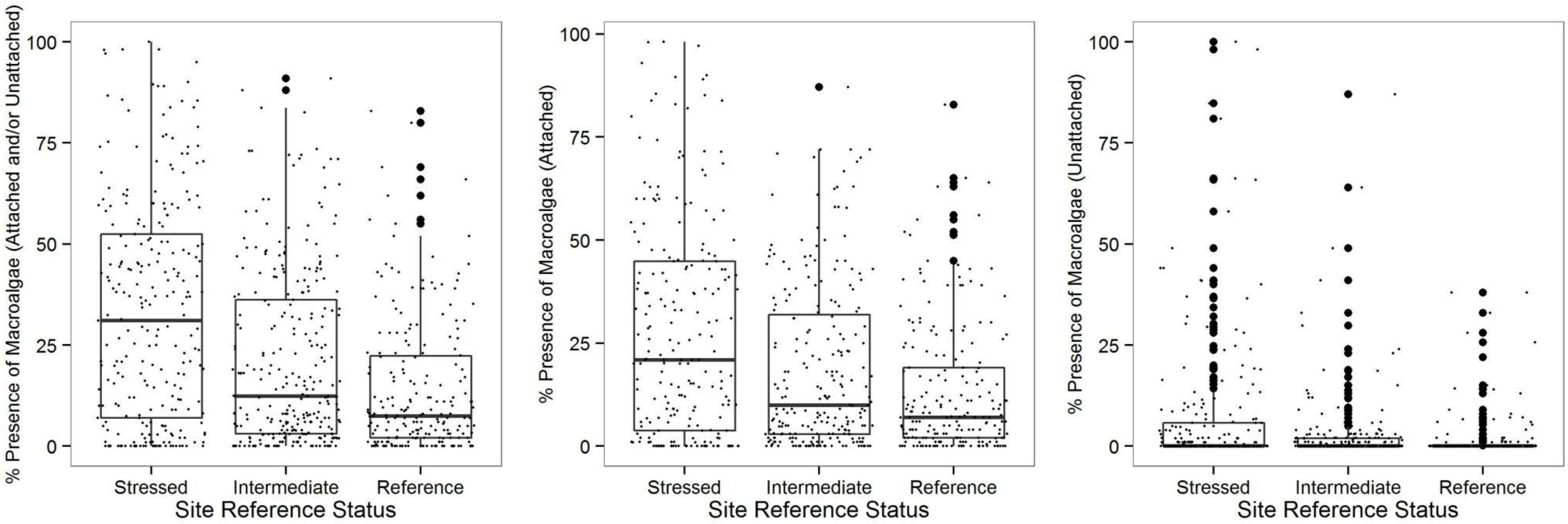
Omernik JM. 1987. Ecoregions of the conterminous United States. Map (scale 1:7,500,000). Annals of the Association of American Geographers 77:118-125.

Appendix D: Boxplots (with "jitter" data points) of biomass, ash-free dry mass, and macroalgal percent cover, for all statewide data combined (i.e., probability plus target sites).

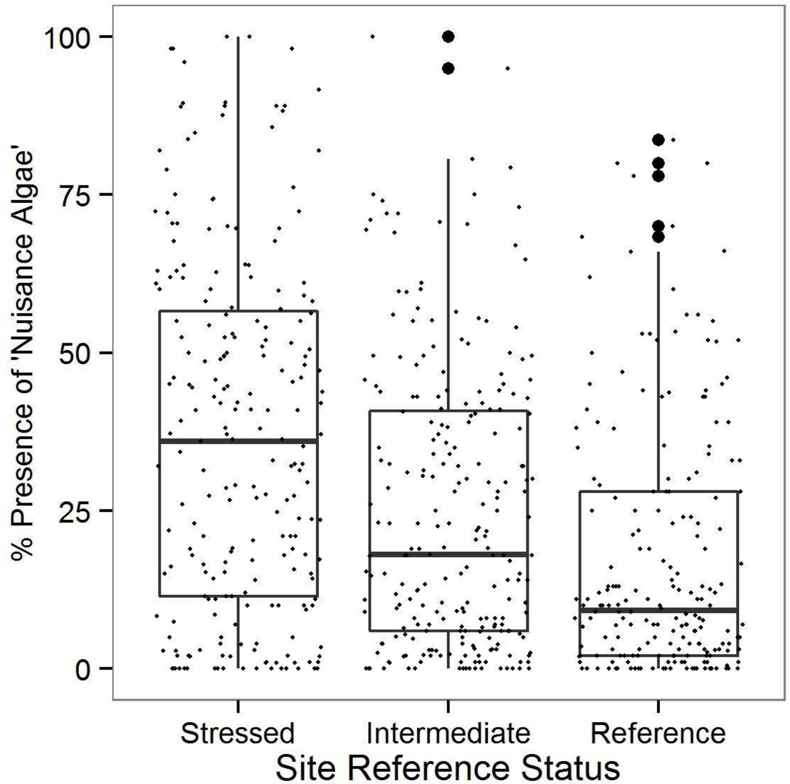


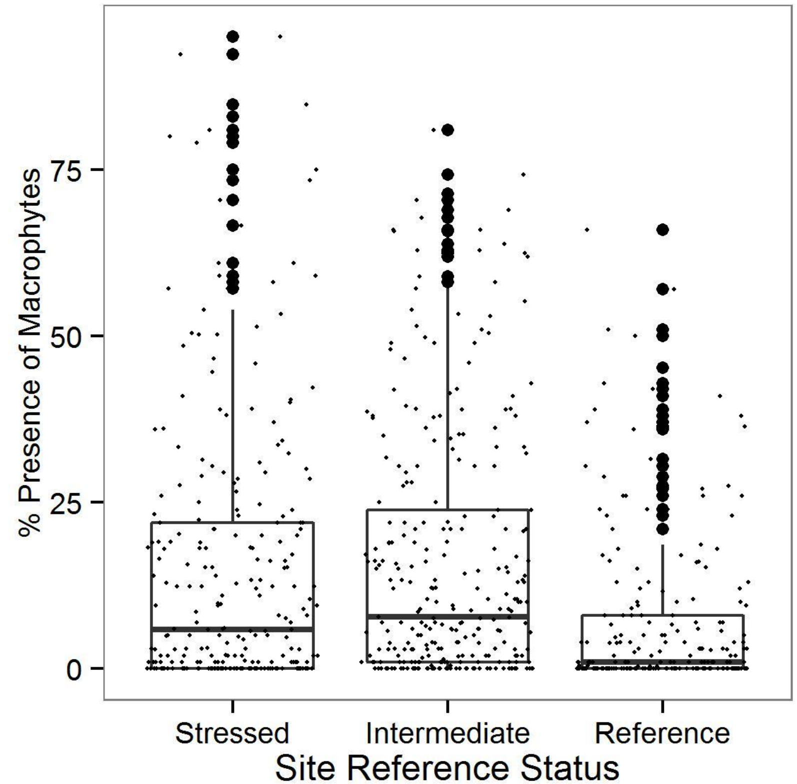


Appendix D (cont'd)



Appendix D (cont'd)





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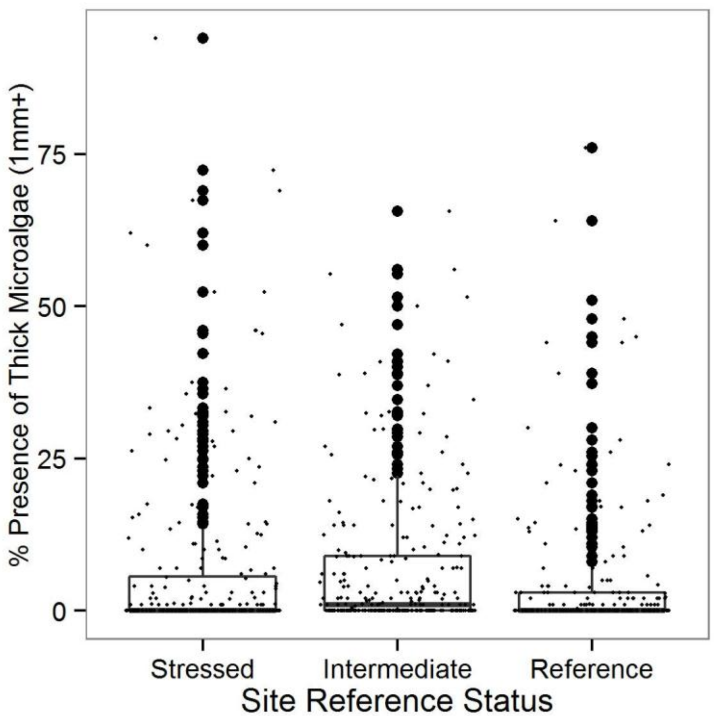
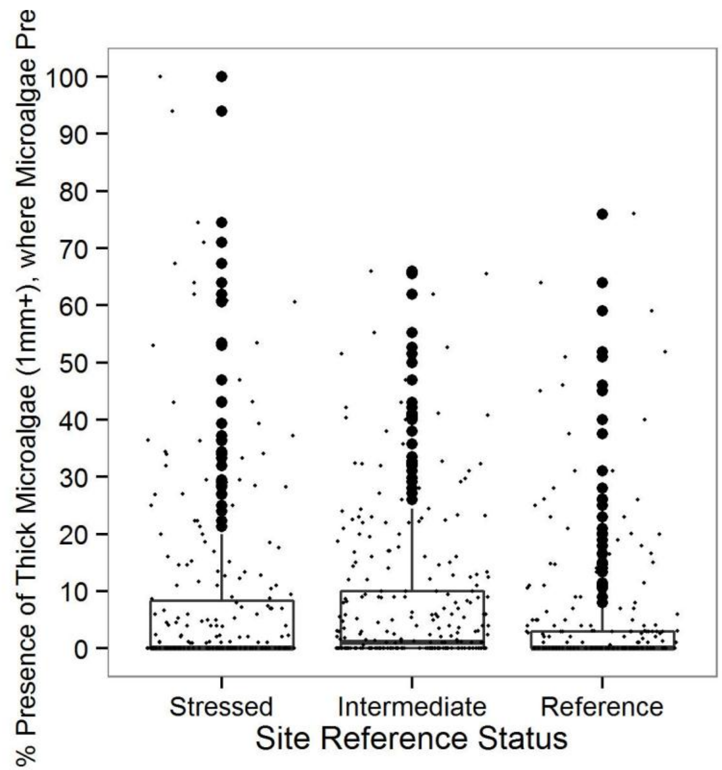
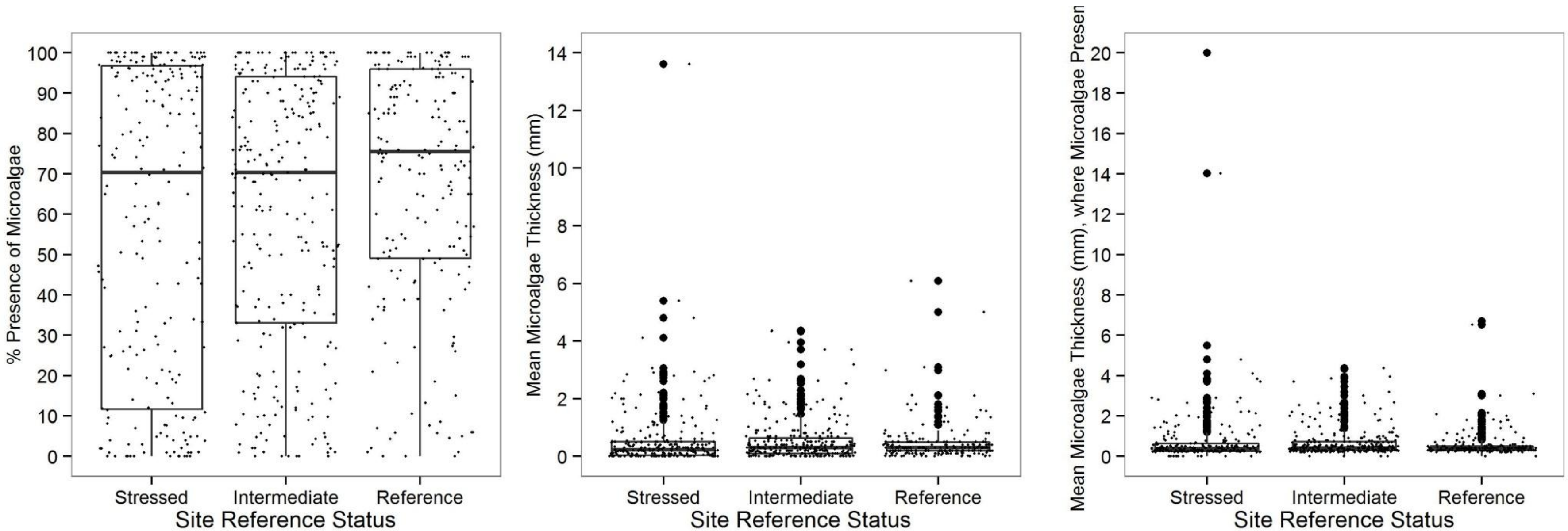


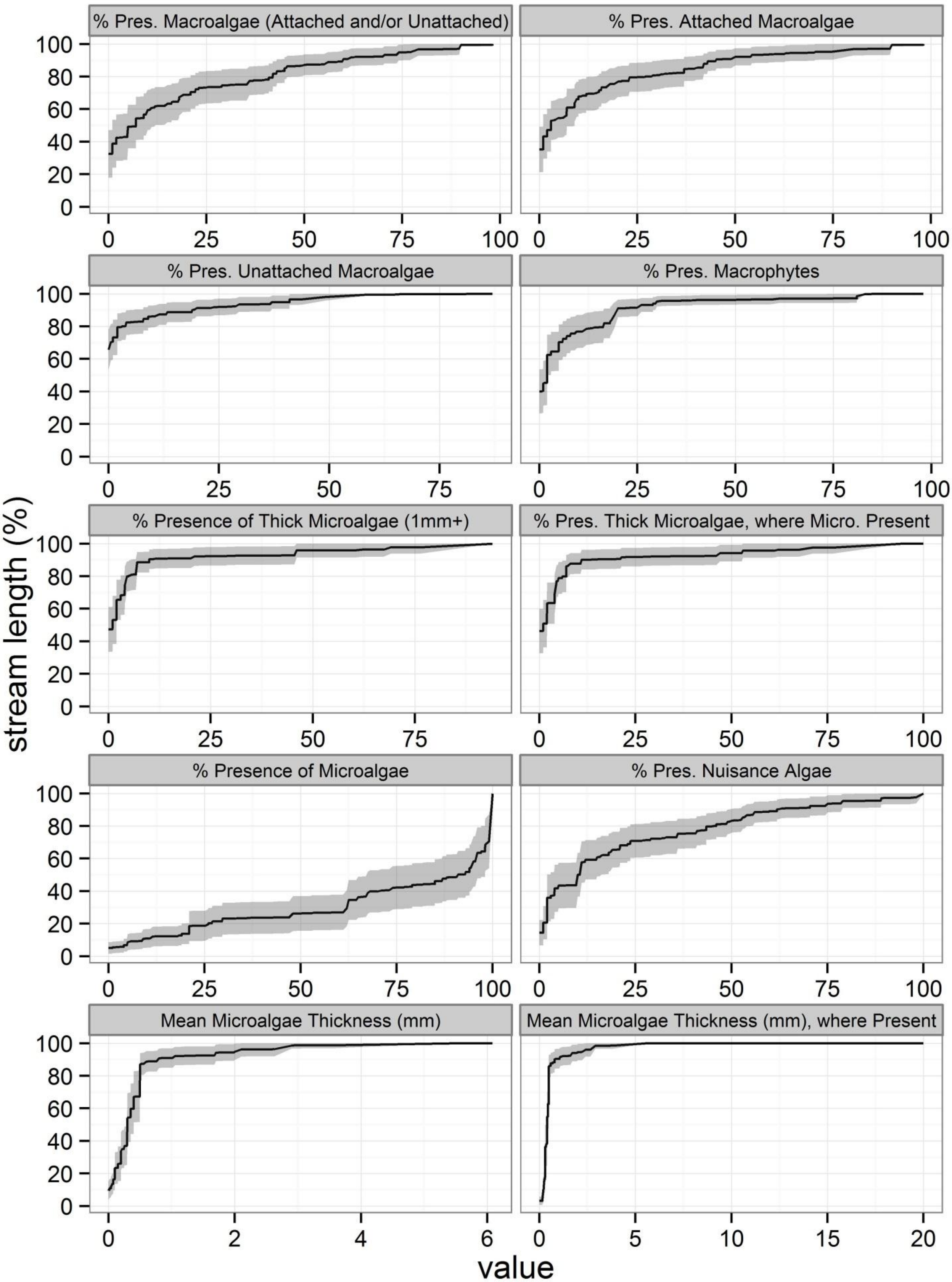


Appendix D (cont'd)

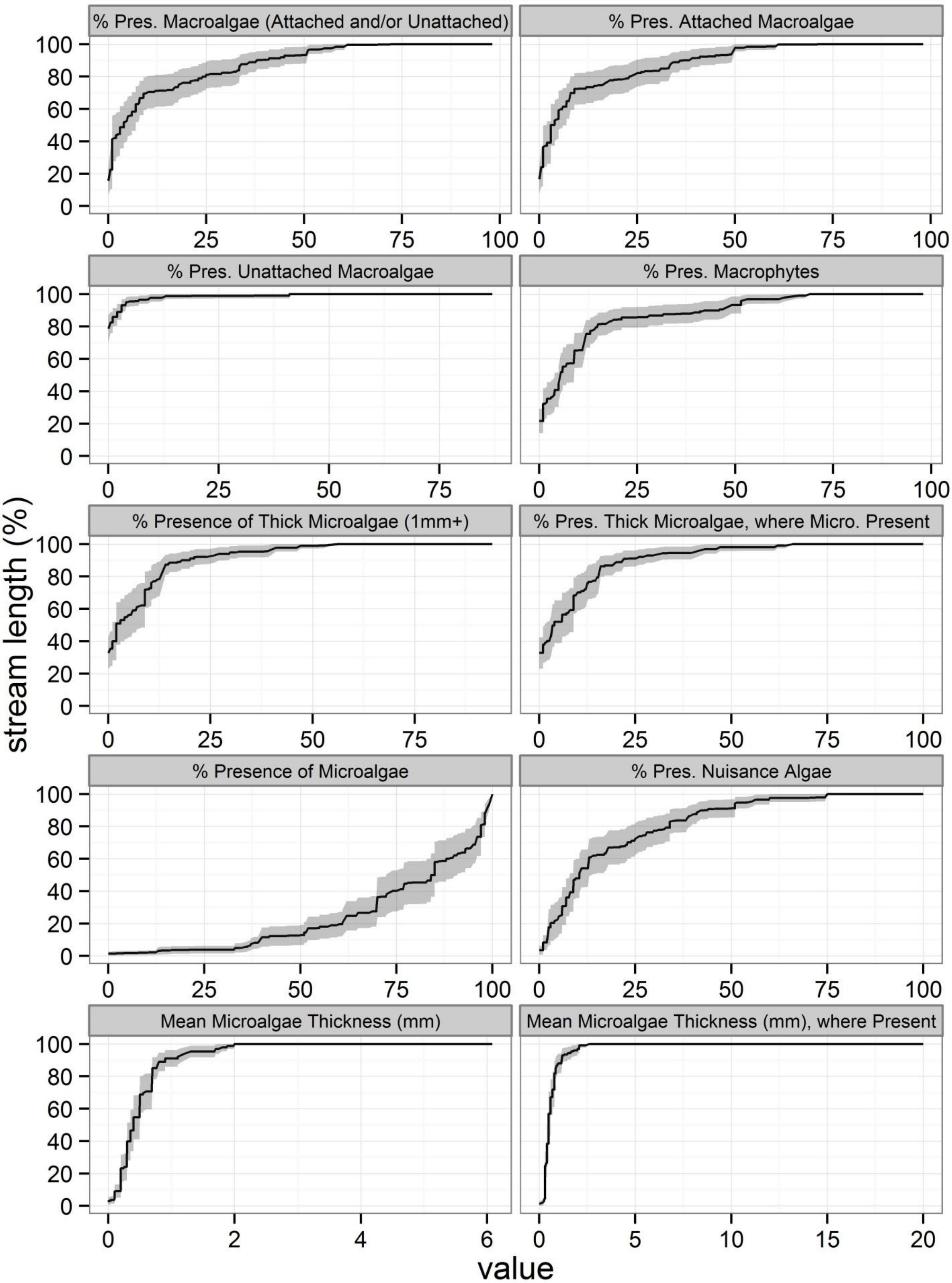


Appendix E: Cumulative distribution functions for algal/macrophyte PHab variables, by site reference category (all statewide probability data combined).

Appendix E (cont'd) : "Stressed" sites



Appendix E (cont'd) : "Intermediate" sites



Appendix E (cont'd) : "Reference" sites