Algae Biomonitoring and Assessment for Streams and Rivers of California's Central Coast

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Introduction

California has made substantial progress in the field of bioassessment over the past 20 years, since the California Department of Fish and Game published its first standardized procedure on bioassessment in 1993. Both the California Water Resources Control Board and the Department of Fish and Game have invested considerable effort and resources in the development of programs, research and expertise in the field. These efforts have been made in recognition of the economic importance of preserving the health and integrity of California's aquatic ecosystems for beneficial uses, as well as to meet the requirements of the Clean Water Act to "restore and maintain the chemical, physical and biological integrity of the nations waters." The California Department of Fish and Game (CDFG) has an Aquatic Bioassessment Laboratory dedicated to the mission of "supporting the use of biology in California's water quality management and assessment programs." The CA Department of Pesticide Regulation has used bioassessment as a tool for ascertaining reference conditions and documenting expected macro-invertebrate communities in specific areas of interest for pesticide effects, for example the San Joaquin Valley (Bacey 2007). The progress that has been made in California includes the consolidation of data management from multiple programs under the Surface Water Ambient Management Program (SWAMP), development of citizen monitoring programs, field methods courses, extensive guidance for quality assurance, protocols and tools, and investment by several of California's regions in the development of indices of biological integrity (IBI). In 2009, an external review of California's bioassessment program concluded that while the state had made great strides forward in the field of bioassessment, technical recommendations for further advancement included the addition of algal assemblages for bioassessment and the implementation of the Reference Condition Management Plan, which was published in 2009 (Yoder and Plotnicoff 2009).

Bioassessment is one of several biological monitoring tools, which include toxicity monitoring, tissue chemistry, invasive and indicator species monitoring and the development of fish habitat indices. The purpose of bioassessment is to directly characterize stream health through biological, rather than chemical of physical, indicators. Methodologies for of bioassessment rely on the identification of organism assemblages that occur in undisturbed or minimally disturbed sites and expected under natural environmental conditions. One ways to quantify the composition of these assemblages is through indices of biological integrity (IBI). Macroinvertebrates had been California's primary biological indicator and were the focus of the first regional IBIs (Herbst 2001). Multimetric indices of biological

integrity for BMI are available for the Eastern Sierras, the Northern Coast, the Central Valley and the South Coast (SWAMP 2006). Tools have been developed and published online for regional use for both the Eastern Sierras and the South & Central Coast based on these IBIs for macroinvertebrates. These tools enable users to characterization of the biological health of streams of interest based on regional research of reference sites and the development of IBIs. In addition to IBIs, predictive models of expected BMI assemblages based on natural environmental gradients have been developed. Models, such as River Invertebrate Prediction and Classification system (RIVPACS), use an observed to expected ratio to assess stream health. Application of RIVPACS to macroinvertebrate populations have been used in California streams (Hawkins et al. 2000, Ode et al. 2008), however evidence of regional application of such models was not found during our literature review. More recently Ecologstis and resource agencies have acknowledged the potential to use freshwater algae assemblages for stream and river bioassessment.

Algae production, primarily in the form of suspended chlorophyll concentration, is the most common way in which algae is applied in bioassessment in California. More limited progress has been made in California in the use of algae species composition for ecological characterization of stream health. Identification of periphyton communities for bioassessement in California began in the Lahontan region in 1996. In 2003, the Lahontan Region published a report identifying diatom and soft algae species that could serve as indicators of environmental conditions and ecosystem integrity for the Lahontan Basin (Blinn and Herbst 2003). Following their initial study, they developed a preliminary index of biotic integrity (IBI) for the Eastern Sierra Nevada region of California (Herbst and Blinn 2008). In 2007, Proposition 50 grants for the research funding this report on algae bioassessment and monitoring in the Central Coast region of California and for Southern California were signed. In 2008 a technical advisory committee (TAC) composed of researchers, scientists and regulators recommended that the California Water Resources Control Board include algae as a bioassessment tool in SWAMP, focusing first on wadeable perennial streams and later on nonperennial streams (Fetscher and McLaughlin 2008). The TAC encouraged use of diatoms and soft algae based on their responsiveness to nutrients as a stressor, because they are helpful to diagnosing other forms of impairment such as siltation or heavy metals, and as a way of meeting the USEPA recommendation to use multiple indicators as lines of evidence, i.e. used in conjunction with macroinvertebrate bioassessment. A further recommendation of this team was to form a workgroup for taxonomic harmonization for stream algae in the southwest. The Central Coast research team and the Southern California research teams working on the Proposition 50 grants have worked collaboratively

to achieve harmonization of taxonomic identification. In 2011, a meeting between the three labs involved in taxonomic identification (Portland State University, University of Colorado, and Michigan State University) and project researchers generated a harmonized taxonomy list, which included a master list with valid names and images.

Our bioassessment of periphyton on California's Central Coast has been guided by a technical advisory committee (TAC) including membership from the Southern California Coastal Water Research Project (SCCWRP), the Central Coast Regional Water Quality Control Board, the US Environmental Protection Agency, the US Fish and Wildlife Service, the US Geological Survey, California State Parks and the California Department of Fish and Game.

The goals of this study were multifold:

- to expand the number of reference sites in the Central Coast Region and characterize algae at these reference sites
- to develop an algae index of biotic integrity (IBI) to help evaluate and monitor water quality use in the Central Coast region
- to develop a tool for use in classification of stream ecological condition based on our IBI
- to harmonize the taxa for the Central Coast and Southern Coast
- quantify nutrient-algae relationships that will assist in the development of nutrient criteria protective of beneficial uses.
- to develop a predictive model, similar to River Invertebrate Prediction and Classification system (RIVPACS), for determining an observed to expected (O/E) ratio for diatom assemblages for potential use in characterizing stream health on the Central Coast.

Background

Bioassessment

The Clean Water Act was written with the objective "to restore and maintain the chemical, physical, and biological integrity of the Nation's waters." As a result of the law, States and Tribes have monitored many chemical pollutants for decades and evaluated biological integrity using laboratory toxicology assays. Researchers have made considerable progress and developed sophisticated techniques for identifying the chemical constituents of water quality and the potential sources of pollution

(Cude 2001); however, traditional monitoring of chemical water quality and toxicological data can underestimate biological degradation by failing to assess the extent of ecological damage in streams (USEPA 1996; Yagow et al. 2006). Compounding the challenge to define 'clean' water is the complex and dynamic nature of lotic systems and the range of characteristics such as biological, physical, and chemical attributes of stream environments (Vannote et al. 1980; Resh et al. 1988; Dodds et al. 1998; Allan and Castillo 2007). Sole reliance on stream chemistry monitoring is an incomplete indication of stream health; whereas, biological indicators provide a more effective tool to monitor the ecological response to physical and chemical stressors in the environment (Barbour et al. 1999; Karr 1999; Karr and Chu 2000; Yagow et al. 2006).

Water quality, measured as the concentration of toxic chemicals and reduced toxicity in bioassays, has generally improved through regulation of point-source reductions. Over time, biological monitoring—primarily of fish and aquatic invertebrates—has been incorporated by many States and Tribes and now supplements laboratory toxicology assays (Karr 2006).

These monitoring approaches have shown some effectiveness in reducing point-source pollutants; however, non-point source pollutants have been more difficult to regulate and manage (Smith et al. 1999). Agriculturally derived nutrients are often non-point source pollutants, entering waterways from diffuse locations rather than discharge pipes. Biological monitoring can be complicated, as many biological organisms respond indirectly rather than directly to nutrients, especially as their impact cascades up the food web. Perhaps even more problematic to interpreting the consequences of anthropogenically added nutrients, nitrogen and phosphorus, unlike DDT or atrazine, are naturally found in aquatic ecosystems, are necessary to the survival of living organisms, and can vary with non-anthropogenic factors such as geology and climate. Thus, in cases where nutrient enrichment may threaten the integrity of surface waters, accounting for background variation in nutrients and the effect of increases on organisms that respond directly to nutrients in biological monitoring and assessment may be important (Rollins 2005, Soranno et al. 2008).

Biological assessments and the associated biocriteria evaluate the integrity of freshwater streams. Stream taxa, such as fish, invertebrates or diatoms, have the potential to assimilate the effects from anthropogenic changes into their community structure (Karr 1981; Wright et al. 1984; Barbour et al. 1999; Stevenson and Pan 1999). Changes in assemblage composition thus can be used to quantify changes in the biological integrity of streams caused by changes in stream chemistry, physical

modifications, or introduction of non-indigenous species (Barbour et al. 1999; Bailey et al. 2004). Biological integrity, in this instance, refers to the unimpaired condition and the ability of aquatic taxa, communities and guilds to respond and recover from natural fluctuations (Angermeier and Karr 1994; Karr 1999). As part of the long-term national goals for clean water, the United States Congress incorporated a concept of biological integrity into United States water quality policy. The Federal Water Pollution Control Act Amendments of 1972 and 1987, referred to as the Clean Water Act (CWA), requires federal and state governments to restore and maintain the "biological integrity of the Nation's waters" (USEPA 2002). The CWA established the need to preserve and protect the biological integrity of aquatic resources and institute the appropriate biocriteria to assess water quality.

Aquatic bioassessments interpret the ecological condition of a waterbody by directly measuring the resident, surface-water biota (USEPA 1996). Bioassessments often utilize communities of organisms to communicate broad meaning beyond the measurement of a single organism (Karr 1981; Norris and Hawkins 2000). The inferences of indicator species can aid scientific knowledge, policy and management decisions and communicate the condition of a waterbody to a larger audience (Norris and Hawkins 2000). Biocriteria can provide the narrative guidelines or the numeric targets used to evaluate the biological integrity of a waterbody (USEPA 2000). States commonly designate the beneficial uses for a waterbody, such as important fisheries or critical habitats for species of concern. Biocriteria help evaluate and protect these aquatic life uses (USEPA 2000).

Defining "Reference Condition" for this Document

To evaluate the health of a system, researchers often compare sampled sites against an expected condition. Expected conditions are often established through the use of comparison sites that lack disturbances that are expected to affect water quality. Historically, these were sites upstream of a point source of concern. This approach is more problematic for water quality assessment when nutrient enrichment is a suspected source of impairment. First, point sources of nutrients are less common than non-point sources, making it difficult to identify a suitable upstream site. Second, statistical inference based on upstream sites can be confounded due to lack of sample independence. More recently, large field surveys that sample many sites with minimal human disturbance throughout a region are being used to help establish expected conditions at sites where impairment is suspected (Wang et al. 2005, Stevenson et al. 2008). These approaches that incorporate minimally disturbed regional sites either apply a set of reasonably independent samples (i.e., spaced sufficiently to reduce the effects of spatial autocorrelation) within a physical

region of similar sites (e.g., the bioregion approach) or are used in combination with abiotic factors such as climate, geology, and geography to model expected conditions at individual sites (e.g., the RIVPACS approach).

Commonly, these minimally disturbed sites are referred to as "reference sites," despite potential for confusion in the use of this term. Stoddard et al. (2006) suggest that the term "reference condition" should be reserved for sites that exemplify true naturalness. However, in practice, the term "reference site" is applied to sites establishing varying degrees of expected conditions. For example, in highly disturbed regions, the expectation may be that reference sites reach "best attainable conditions," because these sites likely represent the best attainable conditions for the region. In this study, we use the term "reference" to describe sites that range from what Stoddard et al. call "minimally disturbed" to "least disturbed" allowing for limited anthropogenic activities within the watershed.

Application of Reference Sites to Establish Expected Conditions

The reference condition approach (RCA) quantifies ecological conditions at sites with minimal human disturbance and applies these values as the expected conditions at test sites where water quality is being assessed. Bioassessments using a RCA can measure the deleterious effects anthropogenic stressors have on organisms by first measuring stream integrity at sites unaffected by human influence. In other words, RCA establishes benchmarks with which one can evaluate stream health by defining "healthy".

Several bioassessment methods use the reference condition approach. For example, current applications of Multi-Metric Indexes (MMIs), also called indexes of biotic integrity (IBIs), assign values (metrics) to multiple biological attributes and compare results of reference streams to streams suspected of impairment. Likewise, the river invertebrate prediction and classification system (RIVPACS), applies the reference condition approach. Several studies in California have successfully used a RCA approach to bioassess changes in invertebrate assemblages (Hawkins et al. 2000; Ode et al. 2005; Herbst and Silldorff 2006; SWRCB 2006).

Algal Ecology

Generally, algal assemblages grow in a variety of streams from mountainous, low-order streams to relatively flat, high-order rivers. Algal assemblages contain a diverse collection of plant-like organisms constituting the basis of stream food webs and are important elements in the stream ecosystems (Cushing and Allan 2001). These include diatoms, soft algae and cyanobacteria.

Diatom Characteristics

Table 1: Environmental factors that affect diatom growth (Weitzel 1979)

Availability of light
Solar incidence
Turbidity
Substrate type
Depth
Currents
Water Velocity
pH
Alkalinity
Nutrients
Dissolved metals

Diatoms (Bacillariophyceae) make up part of the micro-flora of submerged, benthic organisms, commonly referred to as periphyton (Weitzel 1979). Though microscopic, periphyton can be "seen" and felt as the greenish or brownish slippery substance covering substrate material in many streams. The unicellular eukaryotic diatoms contain photosynthetic pigmentation and silica infused cell walls (Figure 1). Multiple environmental factors affect diatom growth. A small list of these factors are provided in Table 1 (Weitzel 1979), but diversity of environmental factors affecting algae growth, in addition to the interactions between these factors is extensive (Stevenson et al. 1996).

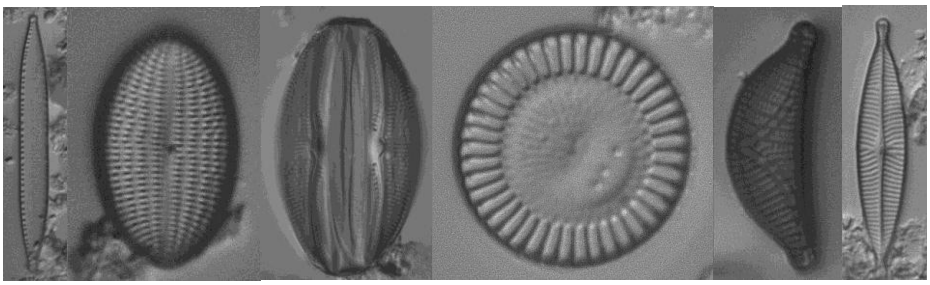


Figure 1. 7Example of diatoms from California Central Coast. From left to right: *Nitzschia palea* (Kützing) Smith, *Cocconeis placentula* var. *euglypta* (Ehrenberg) Grunow, *Amphora ovalis* (Kützing) Kützing, *Cyclotella menenghiniana* Kützing, *Epithemia sorex* Kützing, and *Navicula capitatoradiata* Germain. Diatoms not shown to relative scale. Images from the Pajaro River watershed by Dr. Nadia Gillett.

Diatoms and Nutrients

Many investigators have documented the use of algal assemblages, specifically diatoms, to characterize the effects from anthropogenic changes (Patrick 1968; Hansmann and Phinney 1973; Pan et al. 1996; McCormick and Stevenson 1998; Chessman et al. 1999; Carpenter and Wait 2000; Fore and Grafe 2002; Passy and Cao et al. 2007). Furthermore, multiple researchers have established relationships between diatom assemblages and levels of nitrogen and phosphorous (Pan et al.

1996; McCormick and Stevenson 1998; Leland et al. 2001; Munn et al. 2002; Weilhoefer and Pan 2006; Ponader et al. 2007; Lavoie et al. 2008). As indicator taxa, diatoms have multiple benefits because diatoms are short-lived organisms; diatoms rapidly assimilate stream nutrients, a relatively abundant and important component in the food web (McCormick and Stevenson 1998).

Availability of nitrogen and phosphorous limit diatom biomass and growth (Smith et al. 1999; Dodds et al. 2002). The availability of these inputs and other environmental conditions influence the abundance and composition of diatom assemblages (Sigee 2005). McCormick and Stevenson (1998) argued diatom abundance, rapid growth and early senescence allowed assemblages to quickly integrate environmental changes into their community structure.

Nutrient Enrichment on California's Central Coast

Cultural eutrophication[1] has been recognized as a water quality problem in several California Central Coast watersheds (e.g., nitrate TMDL's in the Pajaro and Salinas rivers). For several years, drinking water standards were being used as nutrient reduction targets because the effects of nutrients on other beneficial uses in surface waters of the region were not well documented. While municipal drinking water standards provide numeric nutrient reduction targets, these concentrations are far above those found naturally in most surface waters. These targets are unlikely to protect beneficial uses because aquatic organisms generally respond to nutrients at lower concentrations. Nutrient targets that are too high will not reduce the risk of exceeding water quality standards for biostimulation, dissolved oxygen (DO), and pH. Excess nutrients can also increase the probability of toxic algal blooms. As a result, some management plans drafted by the Water Board have called for further study examining the effects of primary production on beneficial uses (e.g., CCRWQCB 2011a, 2011b, 2005), including resolutions to "conduct further monitoring to investigate and obtain information to determine causes of algal blooms and dissolved oxygen conditions that may be causing impairment" (CCRWQCB 2005). More recently California Regional Water Boards are moving toward nutrient numeric endpoints (NNEs) in their water quality programs (Creager et al. 2006). Rather than using predefined limits, this approach selects nutrient response indicators that can be used to evaluate impairment. The framework to develop NNEs is founded on the concept that biological response

[1] Unfortunately, the term eutrophication is problematic and based on simplistic categories that fail to appreciate the diversity of aquatic systems. We suggest the word not be used by the regional board because the term lacks scientific specificity.

indicate risks beneficial use impairment, rather than using pre-defined nutrient limits that may or may not result in mitigation of excess primary production for a particular water body. The method re3lies on the assumption that this approach is a more robust link to actual impairment of use, rather than an approach that relies on concentration data alone. In general, the California NNE framework depends on

- Biological response indicators that provide a better direct risk-based linkage to beneficial uses than nutrient concentrations alone.
- Multiple indicators will produce NNE with greater scientific validity.
- For many instances there are no clear scientific consensus exists on a target threshold that results in impairment.

Without a clear scientific consensus on a target thresholds associated with impairment for many of the biological indicators of biostimulation, the California NNE framework can be used to classify water bodies into the Beneficial Use Risk Categories. Although these goals are beyond the scope of this project, the IBI and RIVPACs model can both be used to develop these risk categories.

Applying the IBI for Development of Effects-based Criteria

Biological attributes such as the IBI can be used to establish effects-based water quality criteria. This approach is often used for toxic chemicals. Generally, criteria for toxic chemicals are established at a level well below a threshold above which individual organisms are likely to die in laboratory tests. This individual-based dose-response approach has been criticized for its difficulty in extrapolating to higher levels of biological organization and indirect or limited ecological relevance; critics favor the use of more ecologically relevant endpoints (Cairns and Pratt 1986, Cairns 1983). Some researchers have proposed that stressor-response thresholds in more realistic systems may be useful for establishing water quality criteria (Stevenson et al. 2004, King and Richardson 2003). These thresholds can be good indicators that human activities have affected water quality in aquatic ecosystems and can be used to establish benchmarks for nutrient criteria.

Effects-based criteria are one of three approaches recommended by the USEPA for nutrient criteria development. In addition to effects-based approaches, reference-based and distribution-based approaches are noted in the criteria development guidance document for streams and rivers (USEPA 2000). The criticism of the distribution-based approach, which establishes the numeric benchmark, for example, at the 25th percentile of nutrient measurements across all sites, is that the approach can be under protective for regions that lack high-quality reference site

data or over-protective for regions that have few disturbed sites. Likewise, the reference-based approach has been criticized for its potential in being over-protective because it assumes that all sites must be minimally disturbed, assuming that systems lack assimilative capacity or valuing naturalness over other beneficial uses. Effects-based criteria, however, help establish nutrient criteria at levels that are ecologically relevant.

RIVPACS

The RIVPACS-type predictive model interprets the biological integrity of stream sites using biological assemblages. The approach compares observed biological assemblages with those expected at minimal human disturbance as predicted by models developed from reference site data. The approach was first developed for benthic macroinvertebrates, but we apply it here to diatom assemblages.

Overview of RIVPACS

Stream researchers first developed the RIVPACS method in Great Britain to establish the baseline health of streams and rivers (Wright et al. 1984; Moss et al. 1987). Researchers evaluated the process in the United States and a similar process in Australia (Norris 1996; Hawkins et al. 2000). RIVPACS compares the expected occurrence of macroinvertebrate species at reference sites with observed occurrence at test sites (Hawkins et al. 2000). The strength of the predictive models relies partly on how effectively the reference sites represent the gradient of conditions found at the test sites (Norris and Hawkins 2000). Model construction first clusters reference sites biologically, grouping like sites according to the occurrence of assemblages. Discriminant analysis associates the biological groupings with major natural environmental attributes of the reference sites (Figure 2). In an effort to isolate potential stressors, discriminant modeling only utilizes non-anthropogenic environmental attributes, for example latitude, elevation and precipitation. Lastly, an appraisal of test sites assigns each test site a probability of membership in each of the reference clusters (Moss et al. 1987; Hawkins et al. 2000).

The endpoint indices consist of observed to expected ratios (O/E) for stream test sites. Impairment is a measurement of how far the assemblage of a test site deviates from the assemblage predicted to occur if the site is in a reference state. For example, an O/E value significantly less than one ($O/E << 1$) would indicate the absence of expected taxa at the test site, thus a degraded site. A non-impaired score of an O/E equal or close to one ($O/E \approx 1$) indicates the observed occurrence of taxa at a test site is approximately equal to the expected occurrence at reference sites.

Model construction commonly excludes the occurrence of assemblages at the 95% level and 5% level (Hawkins et al. 2000). This exclusion increases the sensitivity of the models by removing taxa occurring at nearly all the reference sites, and decreases exaggerated exclusivity by eliminating rare occurrences. Thus, the O/E metric can represent a precise measurement of biological integrity. Post O/E processing, a comparison of chemical levels, such as nitrogen and phosphorous, present at the test sites and the O/E index can relate the effect changes in stream chemistry have on the resident biota. Figure 2 shows an overview of their entire RIVPACS process from reference site selection to O/E index endpoints.

Instead of invertebrates, several researchers have employed benthic diatoms (Bacillariophyceae) to assess streams using RIPACS-type predictive models, but their success has been mixed. Cao et al. (2007) found that periphyton models performed similar to macroinvertebrate models in Idaho streams and rivers. Chessman et al. found that their models did not perform as well as similar models developed for macroinvertebrates, suggesting that greater temporal variability and different responses to environmental conditions may be to blame. However, diatoms were only identified to the genus level in this study. Diatoms are more easily identified to the levels of species and variety than for aquatic macroinvertebrates. Thus, low taxonomic resolution may also be a factor. On the other hand, Mazor et al. (2006) found that periphyton provided the best bioassessment performance, but that macroinvertebrates were more sensitive to real disturbance, likely due in part to the strong classification strength of periphyton assemblages. Environmental conditions on the California Central Coast and diatom life history attributes may lend themselves to a RIVPACS diatom evaluation on the Central Coast. Conditions such as the Mediterranean climate can account for multiple annual growth cycles, and the ephemeral status of some streams can support quick growth populations and potential for stream flashiness, allowing diatoms to incorporate chemical fluctuations into their assemblage structure. However, multiple and variable growth cycles may serve to confound sampling data when comparing assemblages at various levels of growth.

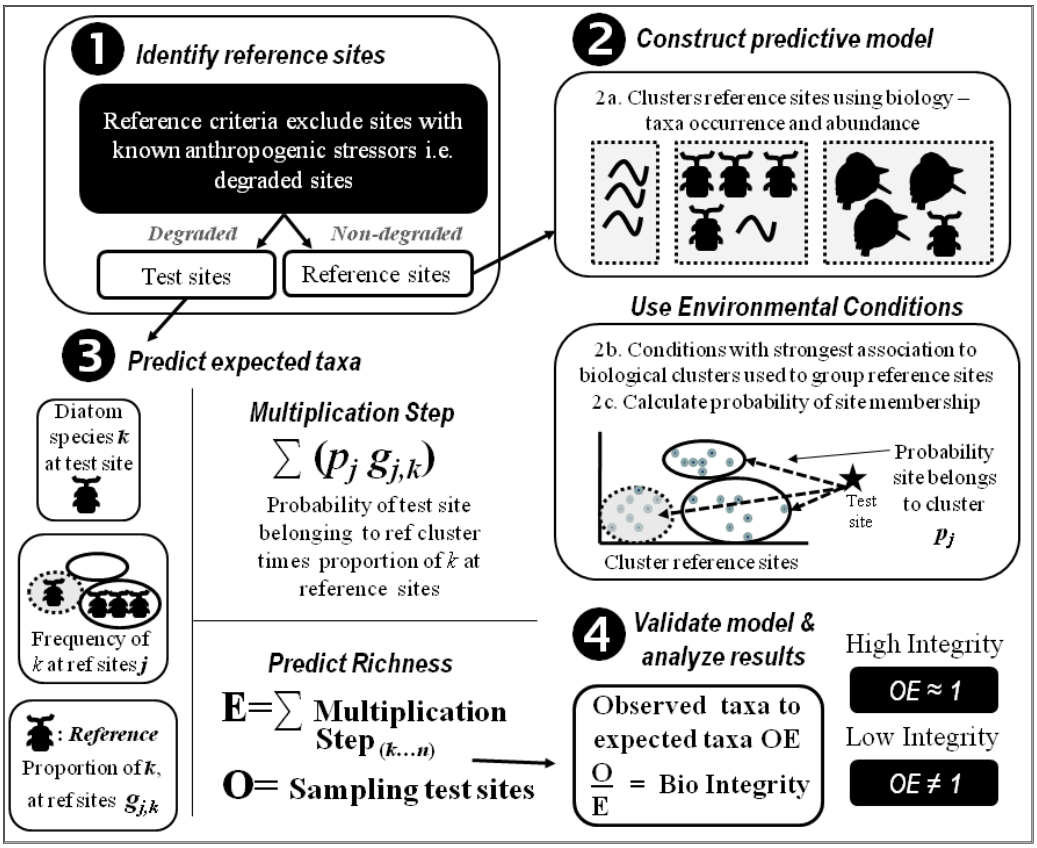


Figure 2. Process overview of RIVPACS method.

Implication of a RIVPACS application in Coastal California

Stream health on the California Central Coast affects many individuals including farmers, residents and outdoor enthusiasts. Streams in this region provide a mix of beneficial uses such as replenishment groundwater recharge, drainage, endangered species habitat (e.g. Steelhead, *Oncorhynchis mykiss*) and scenic destinations. Detection of human caused degradation, in this region, can be difficult to detect against a background of normal chemical and biological variations and the pervasive and historic anthropogenic influences.

A diatom RIVPACS investigation adds a line of evidence available for interpreting the biological integrity and impact on aquatic life uses. A suite of evaluation techniques, such as indicator assessments and water quality monitoring can help discern the overall health and status of Central Coast streams. A diatom assessment can inform resource managers on the potential effects from biological stressors due to nutrient over-enrichment. The results of this project may have a significant

bearing on the agricultural community and other land-use stakeholders. A review of numeric nutrient objectives and OE scores could have policy and economic ramifications, such as assessing CWA compliance, prioritizing monitoring and remediation efforts or measuring management effectiveness.

Report Organization

Three different types of bioassessment were prepared through this study:

1. Development of an index of biotic integrity (IBI) that determines which metrics are best associated with regional stream health based on diatom species observations.
2. Application of the IBI to recommend effects base criteria through change point analysis that applies biocriteria to water quality thresholds for trophic status, total nitrogen, total phosphorus, and nitrate.
3. A RIVPACS type predictive model that associates regional diatom communities with natural gradients to establish site-specific community structure benchmarks with which individual sites may be assessed.

Each of these analyses are described in the methods and results sections and are labeled with appropriate descriptive headings.

METHODS

Individual diatom samples (n=291) were collected from 221 wadeable stream sites along the California Central Coast region during the 2007, 2008, 2009 summer and fall sampling seasons, with the exception of a small number of samples collected in March 2008 from intermittent-type streams. The majority of sample sites were located in a State Water Resources Control Board Region 3, which is the region overseen by the Central Coast Regional Water Quality Control Board (Figure 3). This region covers 29,200 square kilometers, includes approximately 3,798 kilometers of perennial and annual streams and 378 miles of coastline (SWCRB 2002). The area encompasses portions of Santa Cruz County on the coast, inland to the counties of Santa Clara, Monterey, San Benito, San Luis Obispo and south to parts of Santa Barbara County and Ventura County. Multiple north-south trending mountain ranges populate the region, such as the Santa Cruz Mountains, Diablo Range and Santa Lucia Range. The mountains are steep but relatively low in elevation with the highest peaks less than 1800 m. Runoff events from the watersheds typically have short lag times after rainfall events and high peaks due to the relative size and steepness of the surrounding mountains (Mount 1995). Unstable rock and soil types,

such as alluvium and sandstone separate the mountains forming valleys such as the Salinas and Santa Maria river valleys. Characterized by a Mediterranean climate, the Central Coast contains several ecological regions. Ecoregions include Coast Range, California coastal sage, chaparral and oak woodland, and southern and Baja California pine-oak mountains (Omernik 1987). Climatic attributes for the region include mild wet winters, dry hot summers and mild coastal temperatures (Sugihara et al. 2006). Precipitation patterns vary greatly from 1700 mm mean annual precipitation in the Santa Cruz Mountains to 250 mm mean annual precipitation the dryer interior Salinas River valley (PRISM 2011).

Sampling Design and Sample Collection

In conjunction with California State University Monterey Bay and a state-funded project studying periphyton-based bioassessments, a team of researchers performed fieldwork and sample collection. Staff used landscape analysis with geographic information systems (GIS) to generate a random set of possible sample locations throughout the region. Sites were originally identified in part by calculating accessibility (proximity to public roads) and stream order. However, field teams were unable to utilize some of the randomized sites. Limited accessibility, logistical considerations and a multi-year drought constrained the ability of teams to sample from pre-identified locations. Field crew leaders used best professional judgment and consultation with area experts to identify the majority of sample locations. We sampled wadeable streams with varying morphological features and a range of ecological characteristics. This included headwater streams, mid-valley streams, and low-valley streams with diverse land uses in the surrounding watershed. Land uses examples such as urban areas, forests, recreation and agricultural settings were sampled. In addition to sampling impaired test sites, we sampled sites with minimal disturbance in the watershed such as state parks, reserves and undeveloped regions of the Central Coast.

Field personnel used field assessment techniques consistent with methods described in Ode (2007) and a modified algae collection method from Barbour et al. (1999) and Peck et al. (2006) to record and collect samples. Sampling consisted of 150m reaches for streams less than 10m wide and 250m for streams greater than 10m wide. Each reach was subdivided into 11 transects of 15m or 25m respectively. Crews collected benthic diatom samples, physical measurements and stream habitat observations at each transect (e.g. depth, substrate type, velocity, riparian cover, etc.). Field notes for geomorphic and riparian features included sediment deposition, stream incision, herbivory, water clarity, channel slope (%) and evidence of fire. We collected water samples prior to diatom collection, placed the samples on ice, and

processed for nutrient content at California State University Monterey Bay and University of California Santa Cruz water quality laboratories.

Diatom sampling consisted of gathering the benthic substrate at each transect location. Field crews systematically collected substrate material from the left, middle or right of the stream channel along a transect at 25%, 50% and 75% of the wetted width, according to SWAMP protocol and also followed by the Southern California team (Ode 2007). The collection technique included sampling rocks or loose substrate material at each subsection. Personnel processed diatom collection by using a circular template (12.5 cm^2) to scrape rocks with a plastic spatula and toothbrush. Crews collected fines, sand and gravel type substrates with a similarly sized circular cup (12.5 cm^2) and spatula. In rare cases, bedrock and large boulder sampling for diatoms was not performed. If needed, substrata in close proximity to these substrate types were used as a proxy. In total, 137.5 cm^2 was collected per reach. Field crews rinsed the template region or the collected loose material into a container bucket. The total liquid volume was measured (ml), transferred into a 45ml aliquot sample bottles and placed on ice. Field personnel added a solution of glutaraldehyde within a 12-hour holding time to preserve samples. Diatom samples were refrigerated and sent to Center for Water Sciences at Michigan State University or to the School of the Environment at Portland State University for identification to lowest possible taxonomic level, usually genus or species. Labs at MSU and PSU held taxonomic harmonization meetings with the taxonomic lab at University of Colorado that conducted the diatom work for a similar project in southern California. Labs agreed on a set of taxonomic names that kept the finest resolution that could reliably be identified by all three labs. Hereafter, these taxa are referred to as operational taxonomic units (OTU). Relative abundances for OTUs were established the Center for Water Sciences from a count of 600 individuals. Laboratory sample processing and identification followed methods applied in the USEPA EMAP studies and the USGS NAWQA program. Laboratory sample processing and diatom identification was conducted by labs involved in both of the former projects.

Field and Laboratory Water Chemistry Methods

Nutrient concentrations, namely total nitrogen (TN), total dissolved nitrogen (TDN), nitrate (nitrate + nitrate), ammonium, total phosphorus (TP), total dissolved phosphorus (TDP), and orthophosphate were determined using a Lachat Instruments, Inc. QuikChem 8000 Series Flow Injection Analyzer. This is a multi-channel continuous flow analyzer that uses flow injection analysis (FIA) to allow automated handling of sample and reagent solutions with strict control of reaction conditions. In FIA, a fixed volume of sample is injected into a carrier stream where it

is mixed with reagents to form a color reaction. The product is measured photometrically to determine the concentration of nutrients that reacted using the methods in Table 2.

Table 2. Methods used for chemical analysis of water samples.

Nutrient	***Method***	***Principle***
nitrate + nitrite-N	(SM 4500-NO3 F)	Cadmium reduction
Ammonia-N	(SM 4500-NH3 G)	Distillation, automated phenate
Ortho-Phosphate-P	(SM 4500-P F)	Ascorbic acid reduction
TN/TP	USGS Method # I-4650-03	Acid/ oxidant digestion
Dissolved TN/TP	USGS Method # I-2650-03	Acid/ oxidant digestion

Major anions (Cl^-, SO^{2-}_4, NO^-_3, NO^-_2, Br^-, F^-, PO^{3-}_4) were analyzed by Ion Chromatography (IC). FIA analysis is considered to be more accurate for NO_3 than IC analysis, but measurement of other anions adds some redundancy and provides more conservative reference data to which changes in nutrient concentrations can be compared.

Dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were determined via high temperature catalytic combustion using a Shimadzu TOC Analyzer (SM 5310-B). Silicate was analyzed using the Lachat QuikChem Method 10-114-27-1-A. Calcium carbonate, a measure of alkalinity, was determined by Hach Alkalinity Method 8203. Dissolved metals were determined via ICP-MS. The low detection limits (sub ug/L) achieved using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, EPA 200.8) provided for the determination of dissolved metals over a wide measuring range. ICP_MS was employed in this study to determine the concentration of the following dissolved metals: aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), nickel (Ni), and zinc (Zn). Suspended Sediment Concentrations were determined using a filtration method, modified from the Standard Methods for the Examination of Water and Wastewater 20th Ed, Method 2540 D. Residual sediments were weighed on a Sartorius precision balance to establish concentrations.

Water-column and benthic chlorophyll a samples were analyzed using the laboratory procedure specified for periphyton (EPA 445), which involves the extraction and fluorometric determination of chlorophyll a pigments. Benthic algae was also quantified by the combustion of organic biomass to determine the ash-free dry mass (AFDM).

Field measures of water quality parameters at each sampling location involved using a Hach Hydrolab with appropriate sensors for dissolved solids, dissolved oxygen, pH, salinity, specific conductivity, water temperature and turbidity.

Quality Assurance

Implementation of the project was conducted according to the Quality Assurance Project Plan (QAPP) approved in June 2007. The QAPP documents the sampling and analytical methods, procedures, and requirements, training and certification, equipment maintenance, the data quality objectives regarding precision, accuracy and completeness, and corrective actions for quality assurance problems.

Both water quality and taxonomy data were reported in SWAMP compatible format and submitted for inclusion in the publically available data on the California Environmental Exchange Network (CEDEN) website (SWAMP 2010). Once this data is uploaded by CEDEN, interested people can utilize the "data query tool" available on the CEDEN website to access the data: http://www.ceden.org/ceden_data.shtml.

Geographic Information System Methods

We delineated watersheds for each site from the US Geologic Survey (USGS) NHDPlus data sets which includes the National Elevation Dataset (NED), National Hydrography Dataset (NHD), and Watershed Boundary Dataset (WBD). The elevation data was used to determine elevation, slope, and aspect at the site. Hydrologic stability at each site was computed from stream gage data by first computing the ratio of the minimum reported flow divided by the maximum reported flow for each gage included in the NHDPlus data set. From this we developed a raster with a 4 km^2 resolution by using inverse distance squared interpolation including the closest 12 points across a radius of up to 100 km. From this raster we developed the hydrologic stability index for each site. The mean, minimum and maximum elevation of watersheds were determined using a USGS 30 m resolution digital elevation model (DEM). Dominant watershed rock type was characterized from USGS digital geology maps (http://pubs.usgs.gov/of/2005/1305/). Watershed soil permeability and depth to bedrock were computed using the State Soil Geographic Data Base (STATSGO) from

the US Department of Agriculture, Natural Resource Conservation Service (http://www.soilinfo.psu.edu/index.cgi?soil_data&conus&background). Climate data were obtained for each site from PRISM (Parameter-elevation Regressions on Independent Slopes Model) and WorldClim. Mean annual precipitation and the minimum and maximum temperatures at sites in 2007 and 2008 were obtained from PRISM (http://www.prism.oregonstate.edu). A number of biologically relevant climate variables compiled by WorldClim to portray climate extremes, seasonality and annual trends were used from available grid formats (http://www.worldclim.org/current).

Reference/Nonreference Site Determination

Department of Fish and Game determined conditions for assessing whether a site was suitable as a reference site (minimal human disturbance) (Ode and Schiff 2009, Yoder and Plotnickoff 2009). These conditions included landscape analysis, proximity to mines and dams, number of paved road crossings, and water chemistry criteria (Table 3).

We reviewed the 221 monitoring sites using criteria developed by the Department of Fish and Game (DFG) and separated them into reference or nonreference sites (Figures 4 & 5). DFG supplied the results of their GIS analysis of all sites reviewed (2400 locations in the Central Coast) locations and with the R code for determining reference sites. We applied all conditions recommended by DFG for determining site suitability as a reference site, with the exception of the water chemistry parameters (total nitrogen, total phosphorus, and conductivity) and the W1_HALL parameter, which includes specific site inspections that were not performed by us at the time of monitoring. As shown in Table 3, the conditions for reference site determination included a review of the site at three geographic scales: 1 kilometer radius, 5 kilometer radius and the watershed above the monitoring site. The conditions included maximum percent of agricultural and urban landuse, a NLCD landuse code designating urban grasses/ roadside vegetation (Code 21), road density, number of upstream paved road crossings, distance to a dam, gravel mine density in the riparian zone, no productive mines within 5 km and at the watershed scale a maximum percent of canal and pipe waterways. Although 50 monitoring sites were not included in the DFG review, other sites close to reviewed DFG sites allowed us to infer whether these missed sites were likely to meet the reference criteria or not. The final reference analysis identified a total of 63 reference sites and 158 non-reference sites (Table 4).

Quantifying the Human Disturbance Gradient

A metric based on the reference site classification criteria was developed to evaluate the response of the IBI's response to human disturbance. This metric was also used to examine the response of individual metrics used in the final IBI. The human disturbance gradient metric (HDG) was quantified using the proportion of individual reference criteria that failed for a given sample site. In theory, the metric could have ranged from 0 to 1; however, the maximum failure rate in the dataset was 73%.

Individual reference criteria were also used to classify samples as the "best" and "worst" with respect to site quality (i.e., level of human disturbance). These classes were used to evaluate the responsiveness of individual candidate criteria to human disturbance. Samples from reference sites—those that failed none of the reference criteria—were the "best" samples. Samples from sites that failed >20% of the individual reference criteria were classified as the "worst". The 20% threshold was used because it was the cutoff the produced the class size most similar to the size of samples from the reference site pool. To help ensure the selection of responsive and reproducible metrics for the IBI, diatom samples were treated as independent.

Index of Biological Integrity

Algae Metric Screening and Selection

A large set of possible metrics was evaluated for inclusion in the California Central Coast multimetric algal index of biotic integrity. The initial list of metrics was compiled based on those used in the past by others and those available as part of the Western Environmental Monitoring and Assessment Program (WEMAP). Methodologically problematic metrics, including those that we could not calculate with the available data or resources, were eliminated from the candidate metric list, (e.g. metrics based on absolute abundance). This initial screening resulted in the initial candidate metric list (Table 5).

Individual candidate metrics were screened using the approach of Stoddard et al. (2008). The aim of this approach is applicability to regional and national scales through identification of a metric set that meets 4 criteria: 1) based on a data range with sufficient variation among sites, 2) temporal stability to allow for reproducibility, 3) responsive to stressor gradients, and 4) relatively good independence between metrics of the others (Stoddard et al. 2008). Metrics were classified and evaluated for sufficient range and reproducibility. Individual metrics were evaluated for responsiveness to human disturbance, assessed for ecological redundancy and, when necessary, adjusted for correlation with natural gradients.

This iterative, formalized approach allowed us to quantitatively cull a long list of candidate metrics, reducing the size to a manageable number. Furthermore, it allowed us to maintain a compatible set of approaches with development of another multimetric index simultaneously being developed for Southern California streams and rivers by another research group. While the two research groups had slightly different goals, development of an algae-based IBI for our respective regions was a common goal.

The two teams worked together at various stages of project development in order to coordinate methods and harmonize taxonomy to a reasonable extent. Field protocols were developed together, using USEPA EMAP protocols as a starting point. Extensive effort was made to ensure compatibility with existing SWAMP protocols. Field protocols used by the Southern and Central California research teams differed primarily in the collection, identification, and use of soft-bodied algae. The research group from Southern California intended to make use of soft-bodied algae, using and budgeting for methods that had not been applied in USGS NAWQA and USEPA EMAP. In the Central California Coast, our goal was to greatly increase the number of sites for which periphyton data were available, particularly with respect to reference sites. In the Central Coast, we applied methods similar to those used by USEPA EMAP for soft-bodied algae collection and processing. We identified soft-bodied algae in several samples, but found limited utility in these data, relative to the diatom data. Diatom identifications were harmonized with the Southern California through a series of conference calls and meetings, resulting in a single taxonomic list for the two regions. Index development protocols proposed by the Southern California group were shared. Most of these protocols followed Stoddard et al. (2008) relatively closely. Thus, we applied the peer-reviewed and published methods of Stoddard et al. (2008) in our IBI development. Field sampling protocols, diatom identification, and IBI development between the two regions should be highly compatible and may even allow for the development of a single diatom-based IBI for the two regions, should it become a priority.

Metric Classification

The first step of the Stoddard et al. (2008) approach is classification of metrics, with preference for a classification scheme that relates inherent qualities of aquatic biota to important elements of biotic condition. For our study, all candidate metrics were classified into one of five ecological categories: 1) autecological preferences, 2) community structure, 3) ecological guilds, 4) tolerance and intolerance, and 5) production. All metrics derived from van Dam et al. (1994) were classified under "autecological preferences". Van Dam's autecologies include species-level

classifications for ecological preferences in pH, salinity, nitrogen uptake metabolism, oxygen requirements, saprobity, trophic state, and moisture. Metrics derived from relative individual abundance, relative species abundance, dominance, evenness, and measures of diversity were classified under "community structure". Metrics derived from motility and morphological classifications were included in "ecological guilds". Metrics pertaining to presence, dominance, and abundance were included in community structure. All metrics derived from the pollution tolerance index developed by Bahls (1993) were classified as tolerant and intolerant. Additionally, tolerance and intolerance metrics were developed from our data, specific to taxa whose abundance most effectively discriminated between sites with the least human disturbance and sites with the greatest human disturbance. Finally, metrics derived from measures of biomass such as chlorophyll, ash-free dry mass (AFDM), microalgal growth and macroalgal growth were classified as "production". Our original intent was to develop the index of biotic integrity (IBI) using one to three metrics from each of these ecological categories.

Individual Metric Range

To help reduce the size of the initial pool of candidate metrics, Stoddard et al. (2008) suggests evaluating metrics for their range across sites to insure the metric can aid in discriminating variability in conditions. Range consideration includes not only the statistical range of a given metric, but also the exclusion of metrics that exhibit a large proportion of similar values, such as zero, at many sites. It is important to note that Stoddard et al. use this filter on regional and national scale in which metrics are generally likely to exhibit sufficient range. Furthermore, they do not set specific criteria for meeting range requirements, but note that they often remove metrics with a range <4 or >1/3 samples with zero. Because we are working at a more localized scale, range criteria were much more liberal. Furthermore, we were interested not only in linear responses of metrics to stressors but also threshold effects. Assuming that some metrics exhibit nonlinear responses to stressors, it is possible to have metrics with small ranges but a strong ability to discriminate between reference and non-reference sites. Therefore, metrics for which >80% of sites were a single value, such as zero, were eliminated, as were metrics that exhibited fewer than 4 levels. Because some metrics are based on only a few taxa, these criteria allowed us to preserve highly responsive metrics, even if they only exhibited different values at little more than 20% of sites.

Reproducibility

Suitable metrics should be stable within a site, responding to environmental changes of interest with minimized variance due to sampling (Stoddard et al. 2008).

Several sites were visited at least two times and some duplicate counts were conducted on samples collected from a single site visit, allowing us to estimate the within site variation, in addition to the variance across sites. The signal-to-noise ratio (S/N) was calculated for each metric by dividing the pooled site variance (signal) by the mean within site variance (noise). All metrics with a S/N <1.5 were eliminated. This is at the more conservative end of the <1.0 S/N criteria that Stoddard et al. (2008) suggest for periphyton metrics.

Responsiveness

The ability to distinguish between most-disturbed and least-disturbed sites is the most important criterion for metric selection (Stoddard et al. 2008). Welch's two-sample *t*-test was used to screen variables for responsiveness to human disturbance. Within each metric classification, metrics with the highest absolute *t*-values were considered for inclusion in the final multimetric index. Although selecting metrics from each class may ultimately lead to a slightly less responsive multimetric index, however doing so does help ensure that the index is ecologically representative of multiple types of variables that best indicate conditions associated with biological integrity. All statistical analyses were performed using R statistical software (R Core Group 2011).

Accounting for Natural Gradients

In addition to responding to human disturbance gradients, metrics may respond to natural gradients (Stoddard et al. 2008). Because natural gradients and human disturbance gradients can be correlated, it can be important to adjust metrics to help ensure that they are responding to the human disturbance gradient rather than the natural gradient. One way to do this is to model the response of metrics to natural gradients using only reference sites (Stoddard et al. 2008). Therefore, we created linear regression models using only reference sites for metrics that passed all other screening criteria and were among the most responsive metrics. Statistically significant (α=0.05) models were then used to predict how metric values relate to natural environmental variables such as elevation and slope. Expected values based on environmental variability were subtracted from observed values, resulting in a natural gradient-corrected metric. Environmentally corrected metrics were then reevaluated for responsiveness.

Scaling, Direction-Corrections, and IBI Calculation

After individual metrics were identified, they were scaled and summed to yield an IBI score somewhere between 0 and 100. Scaling followed recommendations by Stoddard et al. (2008). Scaled values were calculated as follows:

$$\text{Scaled metric} = (x - 5^{th}\ \%\text{ile of } x)/(95^{th}\ \%\text{ile of } x - 5^{th}\ \%\text{ile of } x),$$

where x is an individual metric score at a given site.

Some metric values increase with human disturbance while others decrease. In order to produce an IBI for which higher values represent higher biological integrity, metric values that are positively correlated with human disturbance must first be reversed. Subtracting values from one changes the direction of these metrics that decrease at lower levels of human disturbance. The result is a set of 11 individual metrics that have values near 1 at high quality sites and values near 0 for low quality sites.

The final IBI was calculated by summing individual metric values and scaling to 100 as follows:

$$\text{IBI} = 100\Sigma(\text{scaled metrics}/11).$$

Count data for new samples can be entered into the spreadsheet (CaliforniaDiatomIBICalculator.xlsx) provided to calculate the IBI score for the site (see Appendix I). The spreadsheet scales individual metrics, makes necessary corrections for covarying natural gradients, corrects for the direction in which individual metrics change in response to human disturbance, and calculates the overall IBI score. Most algae bioassessment labs can readily calculate many of the individual metrics; however, the spreadsheet should allow IBI users to easily calculate scores without pre-calculated metrics.

Using the IBI to Establish Biocriteria

The IBI can be used for biomonitoring and assessment. Assessment, by its nature, places a value on levels of the multimetric (e.g., "good", "bad", "impaired", "unimpaired", "meeting standards", etc.). Several recommendations are made for IBI biocriteria based on the reference site distribution and response of the IBI to trophic status. Common distributional breakpoints, such as the median, quartiles, and minima are generally applied to establish criteria using reference distributions. Effects-based biocriteria are established using thresholds in the metric along an environmental gradient, such as trophic status or human disturbance. We quantified human disturbance as the proportion of DFG reference criteria that failed. To quantify trophic status, we used the criteria established by Dodds et al. (1998) in two ways. We used the TN, TP, mean benthic chlorophyll, and sestonic chlorophyll trophic classification boundaries (Table 6). First, sites were classified as oligotrophic, mesotrophic, or eutrophic if any one of these measures placed them

into a higher trophic state. Then we gave oligotrophic a value of one, mesotrophic a value of two, and eutrophic a value of three. The values for all four measures (TN, TP, mean benthic chlorophyll and sestonic chlorophyll) were summed to create a trophic status index (TSI). Additionally, a principle components analysis-derived trophic status index (PCA-TSI) was created by entering nutrient and algal production measures into a principle components analysis. The first principle component axis site scores were then used as a measure of trophic status.

Table 6. Suggested boundaries for stream trophic classifications by Dodds, Jones, and Welch (1998). Boundaries were used to classify streams as eutrophic or mesotrophic if one of these measures exceeded the benchmark.

Variable	Oligotrophic-mesotrophic boundary	Mesotrophic-eutrophic boundary
Mean benthic chlorophyll (mg/m^2)	20	70
Sestonic chlorophyll (μg/L)	10	30
TN (mg/L)	0.700	1.500
TP (mg/L)	0.025	0.075

Various methods have been applied to quantify thresholds, some of which have been applied to water quality criteria development. Here, we used a non-parametric changepoint analysis (Qian et al. 2003) that acknowledges uncertainty in the changepoint, allowing for a risk-based establishment of criteria. Using such an approach, the median of the changepoint distribution represents a 50% chance that the threshold has been passed. More conservative or lenient criteria may be established using other quartiles or percentiles along the changepoint distribution.

Changepoints can be difficult to conceptualize, particularly when displayed graphically because people are accustomed to viewing linear models. Soranno et al. (2008) compare the relative reduction in deviance from the changepoint analysis with R^2 values from linear regression. Each describe the proportion of variation explained by their respective model, providing for a rough comparison between threshold and linear responses.

RIVPACS

Site Classification Based on Diatom Assemblages

Fifty-five diatom samples from the pool of reference sites were classified into groups based on their diatom assemblages. This "calibration set" had one randomly selected sample from each site. Any duplicate samples or samples collected during a site revisit were placed into the "model validation set" which was later used to assess bias in the predictive model. Diatom abundances from the calibration sites were first transformed into presence-absence data. Assemblage dissimilarities were calculated using the Bray-Curtis dissimilarity index. Hierarchical agglomerative clustering was used to associate diatom assemblages using the flexible beta method (Beta=-0.6).

The dendogram resulting from the analysis was then pruned to produce site classes of similar diatom assemblages. Although pruning level is subjective, the goal is to organize sites into classes of sufficient dissimilarity and size. Lengths of branches in the dendogram are proportional to the variance in assemblage dissimilarities explained. Therefore, pruning branches that are long rather than short helps maximize dissimilarity between groups. Additionally, it is important that each class resulting from pruning should be represented by a sufficient number of sites without being so overly conservative as to create large, heterogeneous classes. Our pruning level was chosen to maximize distance between classes with a target class size between ten and thirty sites. Thus, based on the size of our calibration set, the number of classes would fall somewhere between two and five.

Predictor Variables

RIVPACS-type models use environmental variables to predict class membership, thus establishing expected conditions at a site. Because predictor variables for these models are used to infer biological assemblages if a site were experiencing minimal human disturbance, it is important that the predictor variables are influenced little by human activities at the landscape scale being assessed. Therefore, measures of climate, geologic, and geographic characteristics are ideal, whereas measures related to water chemistry or correlated with streamside human activity should be avoided. Furthermore, variables that can be derived from GIS allow expected conditions to be calculated for every stream reach within the region without collecting field data.

Approximately fifty potential predictor variables were screened for redundancy, high correlation with other variables, and sufficient range. This process resulted in

fifteen candidate variables (Table 7) that were used to construct a predictive model for assemblage class membership.

Predictive Model

Backward stepwise linear discriminant analysis was used to construct a predictive model for diatom assemblage class membership in the calibration set. This association of environmental variables with reference clusters allows the model to make future predictions for expected taxa at a given test site. The predictor variables were used to develop the OE metric by establishing a strong association to biological groups at reference sites and comparing those environmental characteristics at test sites to make expected taxa predictions. This stepwise approach resulted in the same final model as the best-subset routine used by others (e.g., Van Sickle et al. 2006). More in-depth discussions of the statistical steps for RIVPACS model construction are described elsewhere (Wright et al. 1984; Moss et al. 1987; Kaufman and Rousseeuw 1990; Wright 1995; Marchant et al. 1997; Hawkins et al. 2000; McCune and Grace 2002); however a basic outline is provided by the following steps:

Step One: Organize reference sites. One diatom sample was randomly selected from each reference site to develop the calibration set (n=55). These sites were used to develop the diatom assemblage classes used in the predictive model. All remaining duplicate samples from reference sites were used for the validation set (n=17). The validation set was used to evaluate the accuracy of the predictive model. We evaluated model performance by generating an OE score for the calibration sites, and for the validation sites and reviewed how close to one, or high biological integrity, they scored (Hawkins et al. 2000; Van Sickle et al. 2006). One measure of good model performance is O/E scores at validation sites of one or very close to one.

Step Two: Biological clustering. A first step in building the model involved biological classification of sites containing similar assemblages of diatoms at the species or variety level. Reference sites were clustered into groups containing taxonomically similar assemblages by using cluster analysis. In later steps, these reference clusters provided the basis for associating environmental variables to biological groups in order to create a predictive model. The predictive model was developed by clustering reference sites into taxonomically similar assemblages and determining natural environmental predictor variables that related group members. Use of the RIVPACS method assumes that species composition and abundance within assemblages varies and conforms along changing environmental gradients and settings (McCune and Grace 2002). We started by removing rare species (those

occurring at fewer than 5% of the reference sites) prior to the biological clustering in order to decrease the "noise" from rarely occurring species (Hawkins et al. 2000, McCune et al. 2000). After clustering, we added the previously removed taxa back into the data used for final O/E predictions. These clusters of self-similar assemblages were used to find predictor variables strongly associated with the cluster groups in order to predict assemblages along natural gradients and verify these at test sites. We accomplished this by using discriminant analysis. These strongly associated predictor variables would be used to predict expected taxa at degraded sites.

To achieve the clustering of sites into groups based on their taxonomic composition, we created a hierarchical dendrogram using an agglomerative nesting technique (AGNES). The agglomerative nesting constructed a *tree-like* dendrogram, which related biologically pairs of individual sites at one end and built upward to relate branches to a top cluster containing all sites (Kaufman and Rousseeuw 1990; McCune and Grace 2002). A flexible, unweighted, pair-group average method (UPGMA) used presence-absence data in conjunction with a Bray-Curtis dissimilarity coefficient to determine ordination distances (McCune and Grace 2002; Van Sickle et al. 2006). Calibration sites were linked with a flexible-β method (β= −0.6), where $\beta = 1\text{-}2\alpha$ (Hawkins et al. 2000; McCune and Grace 2002; Van Sickle et al. 2006). To reflect an ordination strategy similar to Ward's linkage method (Ward 1963), which minimized sum of square errors derived from Euclidean distances. Once the dendrogram was created, we "pruned" the tree to establish cluster groups. Cluster groups were formed by creating a cut-off point on the dendrogram to maximize the formation of taxonomically self-similar groups with 10-30 sites per cluster (Hawkins et al. 2000).

Step Three: Predictive modeling with environmental variables. This portion of the model construction associated environmental characteristics with previously established biological clusters. After model construction, this step enables the model to predict reference assemblages at any site based on similar environmental characteristics. In order to identify the environmental predictor variables establishing membership of a test site in one of the taxonomic groups identified in the cluster analysis above, we used discriminant analysis. Linear DA is analogous to multiple regression analysis, as it employs predictor variables to determine the best fitting classification of a sample set to a group (Williams 1983). Linear DA was used to identify predictors with the strongest association to the biological clusters to classify and group the calibration sites to match the dendrogram of biological clusters (Wright et al. 1984; Marchant et al. 1997; Hawkins et al. 2000; Van Sickle et

al. 2006). We used backwards stepwise linear discriminant analysis to select the best subset of predictor variables. This approach resulted in the same model produced by the best-subset algorithm applied by others (Van Sickle et al. 2006; Poquet et al. 2009).

Step Four: Group membership probability and taxon frequency. This step determined the probability of any site belonging to a reference group and was used to generate expected taxa. DA had a dual purpose for model development by first grouping the reference site data (Step 3 above), and second by assigning the probability of any site (test or reference) being a member of any one of the classified reference groups (P_j). DA was used to accomplish this by maximizing the separation between a fixed number of groups (previously discerned from biological clusters) along an orthogonal scale in ordination space and calculated the probabilities of each site belonging to each group (Mahalanobis distance in multidimensional space between each site and the centroid of cluster groups) (McCune and Grace 2002; Poquet et al. 2009). A frequency of occurrence for each taxon (k) was established within each cluster group (g). The average proportion of each taxon within the member-established reference cluster groups ($g_{j,k}$) was calculated (Marchant et al. 1997).

Step Five: Probability of capturing observed taxa at reference sites. Final taxa counts were established using statistical operations to generate the expected diatom assemblages. To facilitate prediction of taxa at each site, the program summed the product of $g_{j,k}$ and P_j to determine the 'probability of capture' (P_C) for each taxon. P_C uses the final set of selected predictor variables and predicts the expected taxa for all sites. For this investigation, a probability of capture (P_C) level of 0.5 was used as a starting point for predictive model use following variable selection. By applying the model constructed using the calibration set to the validation set, bias in O/E estimates can be assessed. P_C level was adjusted to a level that was sensitive but unbiased. A P_C value of 0.1 was eventually selected for the model.

Step Six: Expected prediction and OE calculation. The final model construction step calculated total expected taxa for a site and produced the observed to expected (O/E) metric. The O/E score was then used as a measure of biological integrity at each site. Observed taxa from the test sites were counted only if the species were identified at reference sites. Species observed but not part of the expected lists were not incorporated into the OE metric. The procedure calculated observed taxa (O) at all the sites by summing the total of each expected taxon observed in the actual sample data. The outcome of the predictive models was an O/E score for each site. The O/E score was used to determine degradation by establishing and upper baseline score

and lower baseline score for O/E values. O/E scores near one were identified as non-degraded. OE scores outside the upper and lower bands were identified as degraded. We deemed a site degraded based on the 0.10 and 0.90 percentiles of calibration O/E results (Van Sickle et al. 2005). While traditionally, RIVPACS models use only lower bounds, Van Sickle et al. also incorporate the upper bounds. For diatoms this may be particularly important because low nutrient regions often exhibit an increase in diatom species richness as a result of nutrient enrichment.

Results and Discussion

Taxonomical Results

Benthic samples were sent to two different labs for identification of taxa: Dr. Jan Stevenson at Michigan State University (MSU) and Dr. Yangdon Pan at Portland State University (PSU). Occasionally samples from the same site and collection date were evaluated at both labs for comparison of results. Taxonomic evaluation of samples sent to MSU were evaluated for diatoms and sometimes soft algae, whereas samples sent to PSU were evaluated solely for diatoms. All diatom counts were harmonized between MSU, PSU, and the Southern California project's taxonomic lab at the University of Colorado. A single list of California diatom taxa was created through several conference calls and meetings. Taxa that could be reliably distinguished by all three labs were retained in counts analyzed. In some cases, the same entity was given different names by labs. After discussion and analysis, labs agreed upon a single taxonomic name. In rare cases that diatom taxa were not differentiated similarly among labs, taxa were lumped into a single operational taxonomic unit (OTU) for data analysis. All taxa that were not identified to the level of genus were eliminated from the data set. Taxa identified to the level of genus, but not species, were included and counted as separate species; thus the total number of species may be slightly over represented. Taxonomic identification of samples from 212 sites, found a total of 501 different diatom species representing 85 genera were collected in Central Coast wadable streams. The number of species detected at the 56 reference sites was 347 and at the 156 nonreference sites was 453 (Table 8). From 39 sites, a total of 37 soft algae species representing 25 genera were identified in benthic samples. Of the soft algae, 13 species were found at the 8 reference sites whereas 36 species (all but 1) species were found at the 31 nonreference sites (Table 9

Metric Screening

During initial screening of the potential metric list, all potential metrics based on absolute abundance were removed. These metrics require an estimate of cell densities from the original streambed or riverbed, which was not available for much of our data. Furthermore, the method by which these estimates are made can be biased, based on the method applied to estimate diatom densities (Alverson et al. 2003).

Initial screening led to a list of 250 candidate metrics. Classification is the first step in determining an IBI (see Methods: Metric Classification). Classification of these metrics into environmentally relevant groupings resulted in 10 metrics being classified as tolerant or intolerant, 21 as ecological guild, 94 as community structure metrics, 117 as autecological, and 8 as production metrics (Table 10).

Several metrics were eliminated from candidacy for failing the range test. Metrics exhibited inadequate range if either 80% of their measures were of a single value or if there were fewer than 4 levels measured in the dataset. These metrics included the autecology variables: proportion of individuals that are van Dam's pH Class 1, proportion of individuals that are van Dam's pH Class 2, Richness of van Dam's Moisture Class 5, proportion of species belonging to van Dam's Moisture Class 5, species richness of van Dam's pH Class 1, species richness of van Dam's pH Class 2, proportion of species belonging to van Dam's pH Class 1, and proportion of species belonging to van Dam's pH Class 1. Community structure metrics with inadequate range included proportion of individuals belonging to the genus *Achnanthes*, proportion of individuals belonging to the genus *Eunotia*, proportion of individuals belonging to the genus *Frustulia*, species richness within the genus *Achnanthes*, proportion of species belonging to the genus *Achnanthes*, species richness within the genus *Eunotia*, proportion of species belonging to the genus *Eunotia*, species richness within the genus *Frustulia*, proportion of species belonging to the genus *Frustulia*, species richness within the genus *Rhoicosphenia*, proportion of individuals belonging to the genus Achnanthes divided by the sum of the proportion of individuals belonging to the genera *Achnanthes* and *Navicula*, and proportion of species belonging to the genus Achnanthes divided by the sum of the proportion of species belonging to the genera *Achnanthes* and *Navicula*,. All tolerance/intolerant and ecological guild metrics met range criteria.

A number of metrics exhibited signal-to-noise ratios below 1.5, failing the reproducibility criterion. These metrics are indicated in Table 5 by an asterisk in

front of the abbreviated name. Additionally, all production metrics failed this criterion; so all production metrics were excluded from the IBI.

Metrics that met other screening criteria were then evaluated for responsiveness to human disturbance. *T*-statistics from Welch's two-sample *t*-test were used to quantify the ability of each candidate metric to distinguish between the best and worst sites (see Quantifying the Human Disturbance Gradient above). The two or three metrics with the highest absolute value for the *t*-statistics were evaluated for significant correlations with the two environmental factors that we felt would most likely confound responsiveness to the human disturbance gradient: elevation and slope because agriculture, urbanization, and residential development tend to be more concentrated in valleys. Only two of the top metrics were significantly correlated with one of these non-anthropogenic environmental variables (Table 10). The weighted pollution tolerance metric was positively correlated with slope and the proportion of species belonging to the genus *Epithemia* was positively correlated with elevation. When weighted pollution tolerance was corrected for slope (see section "Accounting for natural gradients"), its t-statistic dropped below that of the proportion of individuals belonging to pollution tolerance class 3, which was not significantly correlated with slope or elevation. The proportion of species belonging to the genus *Epithemia* was elevation-corrected and retained for use in the IBI. Additionally, metrics that were biologically correlated (as opposed to statistically correlated) were considered redundant because they provide the same information. If two or more metrics were redundant, only the metric with the highest absolute *t*-statistic was retained. Eleven metrics (three autecological, three community structure, three tolerance/intolerance, and two ecological guild metrics) were retained for the final IBI. Each of these metrics responded significantly to human disturbance (Table 10, Figures 7A-7K).

Diatom Index of Biotic Integrity (IBI) for wadable streams and rivers of California's Central Coast

The multimetric algal index of biotic integrity contained eleven individual metrics. Boxplots of each IBI metric for the best and worst sites (see "Quantifying the Human Disturbance Gradient") are displayed in Figure 7 (A-H) and results of Welch's t-test in Table 10. These individual metrics were scaled as described in the methods and summed, resulting in a single value for each sample. While the metric could theoretically range from 0 to 100, the observed range was 17.28 to 92.57. The distribution of IBI values across all samples was unimodally distributed, with a median value of 55.04.

Worst sites had higher median values for presence of species in all three autoecological metrics indices that distinguished best and worst sites on the Central Coast: proportion of species in van Dam's trophic class 5, abundance-weighted average of van Dam's salinity score, and proportion of species in van Dam's oxygen requirement class 5.

It can be difficult to classify diatoms according to a positive or negative response to nutrients, and there may be local, regional or continental scale differences (Patapova and Charles 2005). Van Dam (1994) describes 7 trophic states, where species tolerant of high nutrient levels are categorized in the eutraphentic level (Figure 7A). Whereas US national NAWQA metrics classify the common species *Achnanthidium minutissimum* as a low-nutrient indicator, van Dam's classification from European waters places it in the trophic level indifferent to nutrients (Potapova and Charles 2005). In our study, *A. minutissimum* was present in equal percentages of reference and nonreference sites (68%). For the Central Coast, using the van Dam class 5 metric, we observed a significant difference between best and worst sites on the Central Coast (t-stat = 4.9).

A second metric from this classification was the weighted average of the van Dam salinity score, which had similar medians for best and worst sites. However there was a greater range of species in the worst sites and a significant difference between best and worst sites (t-stat = 4.3). Van Dam (1994) classifies oxygen requirements into 5 levels, with very low oxygen requirements at about 10% saturation. On the Central Coast, the proportion of Van Dam species with very low oxygen requirements were more prevalent in worst sites (Fig. 4.B) and the t-test showed strong discrimination between best and worst sites (t-stat = 4.0).

In the community structure metric class, the three metrics that passed all the tests were related to species dominance (Figures 7D, 7E and 7F). The two species richness indices were correlated with these metrics and the not included in the final IBI because the higher t-statistic was associated with the dominance metrics. The final IBI metrics were the proportion of species belonging to the genus *Epithemia* (t-stat = -6.0), the proportion of species belonging to the genus *Amphora* (t-stat = 5.4), and the proportion of individuals belonging to the genus *Achnanthes* divided by the sum of individuals belonging to the genera *Achnanthes* and *Navicula* using genera from Kramer and Lange-Bertalot (1986, 1988, 1991a,b) rather than the current taxonomically accepted genera (t-stat= -5.3). The two species richness indices were for the genera *Epithemia* and *Amphora*, with *Amphora* having greater species richness in worst sites (t-stat = -6.1) and *Epithemia* in best sites (t-stat = 5.4). The

most prevalent species on the Central Coast was *Amphora pediculus*, observed at 199 out of 236 sites (84%) and present at 72% of reference sites and 89% of nonreference sites. A total of 8 different *Amphora* species were identified and each was present at a higher percentage of nonreference sites compared with reference sites (Table 8). We observed 3 species belonging to the *Epithemia* genus and in each case, these species were, by contrast, more likely to be found at reference than at nonreference sites. The species *Epithemia adnata* was present at 47% of reference sites and only 19% of nonreference sites. *Epithemia* contain endocellular cyanobacteria, making them capable of nitrogen fixation (Floener and Bothe 1980). This may explain their presence in reference conditions with generally lower concentrations of total nitrogen and nitrate as compared with higher concentrations in nonreference sites (Fig. 13).

Two metrics from the ecological guild class were good indicators of site quality on the Central Coast: the proportion of individual with minimal motility (Fig 4.G, t-stat = -4.4) and the proportion of individuals with vertical morphology (Fig. 4.I, t-stat = 3.2). Ecological guild is a useful classification because diatom behaviors are distinct and predictable within guilds along a nutrient and disturbance gradient (Passy 2006). Differences between guilds include motility and profile (Passy 2006). A higher proportion of individuals with minimal motility was seen at best sites and a higher proportion with vertical morphology at worst sites. Motile diatoms can move on and around streams with high sedimentation because of their raphe and may have increased abundance at locations with a silty bottom (Stevenson and Bahl 1999, Wang et al. 2005). On the Central Coast, motility may be less important in best streams, i.e. those more removed from human disturbance and less likely to have associated sediment, and at higher elevations where sediment is less likely to accumulate. Diatoms with a more vertical profile are susceptible to disturbances in stream hydrology whereas prostate diatoms are less sensitive to hydraulic disturbance (Stevenson and Bahl 1999). The higher proportion of individuals with vertical morphology at more disturbed sites on the Central Coast may be related to the greater control exercised over preventing hydraulic disturbance in these areas in order to use surrounding lands for human purposes, ie. agriculture and urbanization.

From the tolerance/intolerance metric class, the three indices were indicative of site quality on the Central Coast: the proportion of individuals classified as California Central Coast most sensitive, the proportion of individuals classified as California Central Coast most tolerant, and the proportion of species classified as Bahl's pollution tolerance class 3. Sensitive species are by definition those present and

abundant in reference streams, whereas tolerant species prevail in more impaired streams. Bahl (1993) developed pollution tolerance classes for diatoms observed in Montana based on Lange-Bertalot's classes. Bahl's pollutions tolerance class included three groups: (1) most tolerant, (2) least tolerant and (3) sensitive. The species classified as most tolerant for California's Central Coast were *Amphora pediculus,* found in 89% of nonreference sites (Table 8), and *Navicula gregaria* found in 83% of nonreference sites. The species classified as least tolerant for California's Central Coast were *Encyonopsis microcephala* (9% of reference sites), *Epithemia sore* (23% of reference sites), *Gomphonema acuminatum*(9% of reference sites), *Gomphonema pumilum* (14% of reference sites), *Navicula canalis* (4% of reference sites), *Navicula cryptotenella* (52% of reference sites), *Navicula cryptotenelloides* (2% of reference sites), and *Navicula radiosa* (15% of reference sites). The criteria for inclusion in the two California Central Coast-specific metrics were a positive S/N and a statistically significant difference in abundance between the highest quality and least quality sites. These criteria are quite liberal and did lead to the inclusion of three relatively rare species in the intolerant metric. A larger set of reference sites may be necessary to determine whether these are truly intolerant species. Because of their rarity, the IBI is unlikely to be substantially affected by their removal.

Multimetric biotic indices are a recent approach used in biological monitoring of streams to aid resource managers in assessing biotic condition and identifying stressors (Hill et al. 2003, Wang et al. 2005). Karr and Chu (2000) describe multimetric biological indices as a stalwart means for diagnosing, minimizing and preventing river degradation. Wang et al. (2005) asserts that diatom biotic indices are also valuable toward aiming restoration efforts at protecting resources and restoring the base of the food chain, i.e. the primary producers in streams. Others have criticized multi-metric indices because they are a greatly simplified single number representing a complex set of relationships and because there is poor understanding of their distribution (Suter 1993, Norris 1995). The advantage of single number index is that it provides for quick assessment and may aid resource managers in focusing their efforts in managing a large scale region.

The Central Coast IBI was negatively correlated with the human disturbance gradient metric (Pearson Correlation = -0.61; Figure 8). The IBI effectively distinguished between sites with the highest human disturbance and the sites with the lowest human disturbance ($t = 9.2953$, $df = 144.201$, $p < 0.001$; Figure 9). Only 7% of sites in the "worst" human disturbance class had IBI values greater than the first quartile of the "best" human disturbance class and only 6% of sites in the "best" class had values below the third quartile of the "worst" class. Reference site IBI

values (mean = 50.96) were also significantly higher than IBI values for the full set of nonreference sites (mean = 66.51) ($t = -9.6364$, $df = 131.277$, $p < 0.001$).

Identifying Potential IBI-based Water Quality Criteria

Distribution-based water quality criteria often use quartiles or percentiles of data distributions from the set of reference sites. Following this approach, the first quartile of the reference site IBI distribution can be applied as the cutoff between sites with "good" and "fair" scores, and the minimum value of the references site IBI distribution (excluding statistical outliers) can be applied as the cutoff between "fair" and "poor" scores (Figure 10). Using these values, IBI scores >58 are "good", IBI scores ranging from 48 to 58 are "fair", and IBI scores <48 are "poor". Using these reference-based distribution-derived criteria, 29% of nonreference sites have good IBI scores, 32% have fair IBI scores, and 39% have poor IBI scores.

Examining response of the IBI to the human disturbance gradient revealed a negative linear relationship with no apparent threshold (Figure 8) and thus, a reasonable biocriterion could not be derived. The relationship the IBI to trophic status, however, revealed several potential numeric thresholds that could be used to develop biocriteria. The IBI did exhibit a threshold response to trophic status, however. Sites with lower IBI scores had a higher probability of being eutrophic; the median threshold value was 66.47 (Figure 11A). A threshold was also observed with TSI; sites below the median of 41.57 had higher TSI values than sites above the median threshold value (Figure 11B). A third measure of trophic status was also used. Principle components analysis was used to reduce the dimensionality of the chemistry matrix. The first principle component axis was highly correlated with all measures of nitrogen and chlorophyll and thus may be thought of as a measure of trophic status. Using this PCA-derived TSI, a strong threshold exists around an IBI score of 35 (Figure 12). Like the reference distribution approach, the effects-based approach suggests upper and lower breaks that provide potential boundaries between good, fair, and poor IBI scores. Using the median value, of the eutrophic threshold, good IBI scores would be those above 66.47. This would mean that many reference sites would not qualify for good IBI scores. A less stringent approach would be to use the lower quartile of the threshold distribution. This would place the boundary at 51.49, which is closer to the break suggested by the reference distribution approach. At the lower end, median thresholds based on TSI responses were 35.45 and 41.57. These are lower than the boundary suggested by the reference distribution approach. Using the upper quartile on these threshold distributions has little effect, since these thresholds were so strong. Using the

effects-based boundary would reduce the number of "poor" sites and increase the pool of "fair" classifications in the nonreference sites.

Application of the IBI to Recommend Effects-based Criteria

In addition using the IBI to establish quantitative biocriteria, we used it as a response variable to demonstrate its application in effects-based criteria for nutrients, i.e. using change point analysis to establish the thresholds. Change point analysis attempts to estimate the point in a series of observations where statistical properties (typically the mean and variance) of a response variable indicate a structural change in an ecosystem (Qian et a. 2003). Thresholds along environmental gradients are logical locations to establish water quality criteria for nutrients. These approaches are demonstrated for total nitrogen [TN (mg/L)], nitrate as nitrogen [NO3-N (mg/L)], and total phosphorus [TP (mg/L)].

The median threshold value for response of the IBI to TN was 0.584 mg/L (Figure 13A), a significantly lower threshold than those produced using the algal production endpoints suspended chlorophyll (median = 1.646 mg/L; Figure 14A) and benthic chlorophyll (median = 2.340 mg/L; Figure 14B). The algal biomass endpoint thresholds were also weaker than the threshold with IBI.

The median threshold value for response of the IBI to TP was 0.046 mg/L (Figure 13B). The median IBI threshold was roughly half the median threshold using suspended chlorophyll as the endpoint (median = 0.088 mg/L; Figure 15A). The threshold produced using benthic chlorophyll as the endpoint was weaker but had a similar value to that using the MIABI endpoint (median = 0.0391 mg/L; Figure 15B).

The nitrate-IBI threshold had a median value of 0.298 mg/L (Figure 16). This is a value much lower than the 1.0 mg/L proposed screening criterion to protect aquatic life uses (Worcester et al. 2010). At a nitrate concentration of 1.0 mg/L, there is an 86% chance that the threshold has been surpassed, leading to lower average IBI scores. Despite the appearance of a linear relationship when the x-axis is transformed, the relative reduction in deviance (0.22) for the changepoint analysis is larger than the R^2 (0.21) for the linear model using log-transformed nitrate. Histograms comparing nitrogen at reference and nonreference sites show not many nonreference sites have high total nitrogen or nitrate (>10 mg/L); however no reference sites had high total nitrogen or nitrate (Figure 17).

Qian et al. (2003) recommend that a change point may sometimes be better estimated as a small range rather than a single value by applying Bayesian or nonparametric changepoint analysis. Here, we have applied the nonparametric

approach and show the median as a solid line, along with the 5th and 95th percentiles as dashed lines (Figures 11-15). Thus values within this range may be considered for use as effects based biocriteria in the Central Coast region based on the risk that managers are willing to take in surpassing the threshold at various levels of nitrate. . The threshold represents a nitrate value above which IBI values are, on average, significantly lower than below. The mean IBI value below the median nitrate threshold (0.2980 mg/L) is significantly higher than above (mean below = 58.4, mean above = 42.7, $t = 9.2841$, $p < 0.0001$).

RIVPACS

Agglomerative hierarchical clustering followed by dendogram pruning resulted in three assemblage classes in the calibration set (Figure 18). Class 1 was the largest with twenty-one sites, class 2 contained 20 sites and class 3 contained 14 sites.

Backwards stepwise linear discriminant analysis resulted in a predictive model with four GIS-derived geological variables that resulted in the best predictions of diatom class membership. These variables were mean rock depth, percent sedimentary clastic, percent metamorphic, and mean soil permeability. The average mean rock depth for diatom classes 1-3 were 59.0 m, 67.7 m, and 57.2 m, respectively. Percent sedimentary clastic averages were 69%, 50%, and 41%. Percent metamorphic averages were 12%, 4%, and 19% (Figure 19). Mean soil permeabilities were 3.8 in/h, 3.4 in/h, and 4.4 in/h.

ANOVA using Helmert contrasts was used to test for the significance of O/E values for calibration, validation, and test sets (Figure 20). O/E values for the calibration set were not significantly different from 1.0 ($t=0.319$, $p=0.750$), with a mean of 1.010 and a standard deviation of 0.245. Validation O/E values were not significantly different from the calibration set ($t=-0.026$, $p=0.980$). Despite a relatively high variance in the calibration O/E set, test sites composed of all nonreference sites had O/E values that were significantly lower than the reference sites making up the calibration and validation sets ($t=-2.695$, $p=0.007$; ANOVA $F=4.435$, $df=2,283$, $p=0.0127$).

The 10th and 90th percentiles of the calibration set can be used as impairment benchmarks (Figure 21). For our calibration set O/E distribution, these values are 0.66 and 1.32. Sites with O/E below 0.66 may be impaired. Likewise, sites with O/E > 1.32 could be of concern. (Figure 19). Fourteen percent of nonreference site samples had O/E values less than 0.66 and 4% had O/E values above 1.32.

Conclusions

A multimetric algal index of biotic integrity (IBI) was developed for the California Central Coast. Hundreds of individual candidate metrics were classified into ecological classes and screened for adequate range, reproducibility, and responsiveness. Top metrics were selected and incorporated into the final multimetric index. The IBI responded linearly along the human disturbance gradient and reference sites had significantly higher IBI scores than nonreference sites.

The IBI reference distribution and response to trophic status provide convenient boarders between "good", "fair", and "poor" IBI scores. The reference distribution first quartile and minimum were used to establish boundaries. Scores less than 48 are "poor", 48-58 are "fair" and greater than 58 are "good". Effects-based boundaries were more extreme than those based on the reference distribution, expanding to 35 to 42 at the low end and 66 at the high end. Effects-based boundaries suggest that many reference sites are in "fair" condition and that a higher proportion of nonreference sites are in "fair" condition, rather than "poor". Thus, the reference-based boundaries seem more reasonable.

Threshold responses of the IBI to nutrients were observed. These thresholds were stronger than those observed for nutrient-biomass thresholds. Using the median of nonparametric changepoint distributions to quantify thresholds suggest a total nitrogen (TN) criterion of 0.584 mg/L and a total phosphorus (TP) criterion of 0.046 mg/L. These numbers are slightly higher, but not unlike the TN and TP criteria suggested by the USEPA for nutrient level III ecoregion 6. Using the 25th percentile of the reference site distribution for these water chemistry measures, criteria of 0.5 mg/L for TN and 0.030 mg/L for TP (USEPA 2000). The median threshold value for NO3-N was 0.298 mg/L. This would likely be considered a very low nitrate criterion in the region. The nonparametric changepoint distribution suggests that there is an 86% chance that the threshold has been surpassed at the proposed screening criterion to protect aquatic life uses. These results provide direct evidence that actual harm to aquatic life uses may be observed above the 1.0 mg/L criterion.

The RIVPACS-type model was not as precise as desired, but nonreference sites still had O/E values significantly lower than those at reference sites. O/E can be applied as a metric to assess biological integrity. Large deviations from O/E=1.0 can indicate impairment. For these data, only 18% of nonreference sites are impaired if applying the 10th and 90th percentiles of the calibration O/E as biocriteria.

Table 3. DFG criteria for determination of reference site conditions. This project used Department of Fish and Game (DFG) criteria for segregating reference and nonreference sites, excepting the W1_HALL and water chemistry criteria.

Reviewed Sites at Three Geographic Scales		
1 Kilometer	**5 Kilometer**	**Watershed**
< 3% Agriculture	< 3% Agriculture	< 10% Agriculture
< 3% Urban	< 3% Urban	< 10% Urban
Combined Ag + Urban < 5%	Combined Ag + Urban < 5%	Combined Ag + Urban < 10%
Road Density < 2 km/km^2	Road Density < 2 km/km^2	Road Density < 2 km/km^2
Code 21[*1] < 7%	Code 21 < 7%	Code 21 < 10%
Number of Paved Crossings < 5	Number of Paved Crossings < 10	Number of Paved Crossings < 50
	No productive mine	Canals and pipes < 10% in watershed
	Gravel Mine Density in Riparian < 0.1	

Reviewed Additional Conditions
Total Nitrogen < 1000 ug/L
Total Phosphorus < 500 ug/L
W1_HALL: Human reach scale unitless variable
Conductivity < 99th percentile of site-specific prediction error[*2]
Upstream dam distance > 10 km

*1 Code 21 is roadside vegetation in forested regions or urban parks, golf courses, etc. in urban regions

*2 From an in-press model of predicted conductivity by John Olson and Chuck Hawkins at Utah State University

Table 4. Site location and determination of site type. A total of 221 sites were sampled. Using DFG criteria for reference site determination, except for water chemistry and W1Hall, sites were typed into reference (sites) and nonreference (sites).

SiteCode	SiteDescription	Latitude	Longitude	Site Type
200BEABEA	Beardsly Creek @ Beardsly Rd	37.19543	-122.00260	Nonreference
200COYCOC	Coyote Creek @ Cochrane Rd.	37.16508	-121.63244	Nonreference
200COYCOI	Lower Coyote Creek at Coit Rd.	37.12618	-121.48072	Reference
200EASHEN	East Fork Coyote Creek @ Bear Mountain Road	37.19393	-121.46813	Reference
200GUAHIC	Guadalupe Creek @ Hicks Rd	37.18047	-121.87250	Nonreference
200HERALA	Herbert Creek @ Almitas Rd	37.15583	-121.84512	Reference
200LGCALD	Los Gatos Creek at Aldercraft Heights Road	37.16982	-121.98069	Nonreference
200LGCMAI	Los Gatos Creek at Main Street	37.22037	-121.98094	Nonreference
200MELSAN	McElroy Creek @ Sanborn Road	37.24897	-122.06856	Nonreference
200MIDHEN	Middle Fork Coyote Creek	37.18172	-121.50659	Reference
200STECOO	Stevens Creek @ Cooley Picnic Area	37.28437	-122.07631	Nonreference
200TODSAN	Todd Creek @ Sanborn Road	37.24231	-122.07100	Nonreference
304ALPALP	Alpine Creek @ Alpine road	37.29680	-122.25958	Nonreference
304APTMAR	Aptos Creek at Margaret Bridge	37.00215	-121.90525	Nonreference
304BATMAI	Bates Creek @ N. Main St.	36.99694	-121.95377	Nonreference
304BEAGRE	Bean Creek @ Green Valley Rd	37.05629	-122.02998	Nonreference
304BEAOLD	Bear Creek at Old Bear Road	37.13624	-122.09721	Nonreference
304BIGBIG	Big Creek @ Big Creek Road	37.07456	-122.22190	Nonreference
304BLOBCC	Bloom Creek at Blooms Creek Campground footbridge	37.16788	-122.21544	Nonreference
304BOLHW9	Boulder Creek at Rte. 9	37.12663	-122.12386	Nonreference
304BRAFOT	Branciforte Creek @ Forty Thieves Picnic Area	37.00150	-122.00144	Nonreference
304BRAMAR	Branciforte Creek @ Market St.	36.98555	-122.01362	Nonreference
304BRAOCE	Branciforte Creek @ Ocean Street	36.97319	-122.02377	Nonreference
304BURSHU	Burns Creek @ Schulties Road	37.12815	-121.96672	Nonreference
304BUTCYN	Butano Creek @ Canyon Rd.	37.22605	-122.33142	Reference
304CARCAR	Carbonera Creek at Carbonera Road	37.00220	-122.01699	Nonreference
304CARELO	Branch Carbonera Creek @ Elderberry Ct	37.06621	-121.99669	Nonreference
304ELCELC	El Corte Madera Creek @ El Corte Madera Rd	37.32055	-122.33778	Nonreference
304EMAMOO	East Majors Creek@Moore's Ranch Road	37.01738	-122.11363	Nonreference
304FALFAL	Fall Creek at Fall Creek Road	37.05322	-122.08027	Nonreference
304GAZGAZ	Gazos Creek@ Gazos Creek Road	37.18563	-122.34071	Nonreference
304HESPUL	Hester Creek at pullout on Old Soquel San Jose Rd	37.07487	-121.93823	Nonreference
304KILMIL	Kings Creek @ Miller Property	37.15972	-122.12568	Nonreference
304KINROC	Kings Creek @ Castle Rock Falls Trail	37.22003	-122.11341	Reference
304LAGSMI	Laguna Creek @ Smith Grade	37.02196	-122.13150	Nonreference
304LAHH84	La Honda Creek @ Highway 84	37.32438	-122.27271	Nonreference
304LBUOFR	Little Butano Creek at Olmo Fire Road pullout	37.20389	-122.33568	Nonreference
304LITSWA	Little Creek at Swanton Road	37.06395	-122.22697	Nonreference
304LOBLOB	Lobitos Creek @ Lobitos Creek Rd	37.39647	-122.39207	Reference
304LOCMIS	Lockheart Gulch @ Mission Spring Campground	37.06301	-122.03467	Nonreference
304LOMLOM	Lompico Creek at Lompico Creek Road	37.08838	-122.05307	Nonreference
304LOVLOV	Love Creek at Love Creek Road	37.09420	-122.08670	Nonreference
304MAJMOO	Majors Creek @ Moores Ranch Road	37.01501	-122.11340	Nonreference

Table 4. (cont'd) Site location and determination of site type.

SiteCode	SiteDescription	Latitude	Longitude	Site Type
304MANCRO	Manson Creek @ Mt. Cross	37.07239	-122.08722	Nonreference
304MILSWA	Mill Creek @ Swanton Rd	37.04271	-122.17194	Nonreference
304MORSSJ	Moores Gulch Creek off Old Soquel San Jose Road	37.03122	-121.94832	Nonreference
304NEWGLE	Newell Creek at Glen Arbor	37.08320	-122.07900	Nonreference
304OPAGAZ	Opal Creek at Gazos Creek Road	37.17040	-122.22398	Nonreference
304PESPRS	Pescadero Creek @ Portola State Park	37.25100	-122.21779	Nonreference
304PESSTA	Pescadero Creek at Stage Road	37.25489	-122.38308	Nonreference
304PETPRS	Peters Creek @ Portola State Park	37.25334	-122.21769	Nonreference
304PURHIG	Purisima Creek @ Higgins Canyon Rd	37.43663	-122.37123	Nonreference
304REGSMI	Reggiardo Creek @ Smith Grade Road	37.03421	-122.13897	Nonreference
304SANHW9	San Lorenzo River @ HWY 9	37.20618	-122.14534	Nonreference
304SANSTA	San Gregorio Creek @ Stage Rd	37.32588	-122.38753	Nonreference
304SCOSWA	Scott Creek at fish gate off Swanton Road	37.02516	-122.20721	Nonreference
304SCOSWA2	Scott Creek @ Upper Swanton Rd	37.03309	-122.20020	Nonreference
304SEM236	Sempervirens Creek at Hwy 236	37.16923	-122.21271	Nonreference
304SHIBRO	Shingle Mill Creek @ Brookside Dr	37.03903	-122.08306	Nonreference
304SLRHIG	San Lorenzo River at Highland Park	37.08099	-122.08061	Nonreference
304SOQBRI	Soquel Creek at Bridge Road	36.98904	-121.95561	Nonreference
304SOQSCR	Soquel Creek at Soquel Creek Road	37.04167	-121.94085	Nonreference
304TUNTUN	Tunitas Creek @ Tunitas Creek Rd	37.39855	-122.36292	Nonreference
304TWOTWO	Two Bar Creek @ Two Bar Rd.	37.16348	-122.10775	Nonreference
304VALVAL	Valencia Creek at Valencia Road	37.00271	-121.87140	Nonreference
304WADRED	Waddell Creek at Redwood Camp in Big Basin	37.11394	-122.26993	Reference
304WILSTP	Wilder Creek at wooden footbridge in Wilder SP	36.96661	-122.08208	Nonreference
304WSTSOQ	Soquel Creek, WestB, unnamed st before Olson Rd.	37.05904	-121.94412	Nonreference
304ZAYGRA	Zayante Creek at Graham Hill Road	37.04911	-122.06611	Nonreference
304ZAYSTO	Zayante Creek at the Store	37.08432	-122.04888	Nonreference
305AGDBEA	Beach Road Ag ditch on Beach Road	36.86865	-121.81725	Nonreference
305AGDTRA	Trafton Road Ag ditch on Trafton Road	36.87153	-121.78502	Nonreference
305BRCCAS	Baldy Ryan Creek @ Casa Loma Rd	37.14869	-121.77325	Reference
305BROHAZ	Browns Creek at Hazel Dell Road	37.02536	-121.78132	Nonreference
305BROWAT	Browns Creek at Watsonville uptake	37.01618	-121.78859	Nonreference
305CLECCA	Clear Creek in BLM Clear Creek Mngmnt Area	36.36179	-120.75908	Nonreference
305COREUR	Corralitos Creek @ Eureka Canyon Road	36.99706	-121.80380	Nonreference
305CORSCU	Corralitos Creek at Scurich Lane	36.95264	-121.79327	Nonreference
305CORWAT	Corralitos Creek, WB, Watsonville City locked site	37.00431	-121.80707	Nonreference
305GAMBRO	Gamecock Creek off Browns Valley Road	37.02613	-121.77273	Nonreference
305GREHAZ	Green Valley Creek at Hazel Dell Road	37.00152	-121.74100	Nonreference
305LLAGLE	Llagas Creek at pullout on Oak Glen Ave	37.10413	-121.67408	Nonreference
305LLAOAK	Llagas Creek @ Oak Glen Ave	37.11488	-121.68879	Nonreference
305LLAOSP	Llagas Creek@ Santa Clara Open Space Preserve	37.14750	-121.77352	Reference
305LLAWTP	Llagas Creek at Water Treatment Plant	36.99054	-121.53194	Nonreference
305MILFLR	Millers Canal at Frazier Lake Road	36.96324	-121.49232	Nonreference
305PACLOV	Pacheco Creek at Lover's Lane	36.96031	-121.43078	Nonreference
305PAJBET	Pajaro River at Betabel Road	36.91678	-121.54962	Nonreference
305PAJH25	Pajaro River at Hwy 25	36.94841	-121.51191	Nonreference

Table 4. (cont'd) Site location and determination of site type.

SiteCode	SiteDescription	Latitude	Longitude	Site Type
305LLAOSP	Llagas Creek@ Santa Clara Open Space Preserve	37.14750	-121.77352	Reference
305LLAWTP	Llagas Creek at Water Treatment Plant	36.99054	-121.53194	Nonreference
305MILFLR	Millers Canal at Frazier Lake Road	36.96324	-121.49232	Nonreference
305PACLOV	Pacheco Creek at Lover's Lane	36.96031	-121.43078	Nonreference
305PAJBET	Pajaro River at Betabel Road	36.91678	-121.54962	Nonreference
305PAJH25	Pajaro River at Hwy 25	36.94841	-121.51191	Nonreference
305PAJROG	Pajaro River at Rogge Lane	36.89447	-121.64546	Nonreference
305RAMRAM	Ramsey Creek at Ramsey Road	37.02742	-121.77718	Nonreference
305SAL129	Salsipuedes Creek at Hwy 129	36.91041	-121.74604	Nonreference
305SBRCOA	San Benito, below Hernandez Res,CoalingaRd pulloff	36.37793	-120.89848	Nonreference
305SBRH25	San Benito at Hwy 25	36.61419	-121.21091	Nonreference
305SBRPR1	San Benito at Paicines Ranch	36.73704	-121.32091	Nonreference
305SBRPR2	San Benito at Cienaga Road	36.67705	-121.28327	Nonreference
305SJCANZ	San Juan Creek at Anzar Road	36.87590	-121.56130	Nonreference
305SWACRO	Swanson Creek at Croy Road	37.08117	-121.76886	Reference
305TREMUR	Tres Pinos Creek at Murphy Road	36.74360	-121.27814	Nonreference
305UVASWA	Uvas Creek at Uvas Canyon County Park	37.08660	-121.79451	Reference
305UVAUVA	Uvas Creek at Uvas Road	37.06000	-121.67326	Nonreference
305WATLEE	Watsonville Slough at Lee Road	36.90152	-121.78103	Nonreference
307CACCAC	Cachagua Creek @ Cachagua	36.40127	-121.65927	Nonreference
307CARCAC	Carmel River @ Cachagua Park	36.39918	-121.66159	Nonreference
307CARHW1	Carmel River @ Hwy 1	36.53587	-121.91178	Nonreference
307CARLOS	Carmel River @ above Los Padres Dam	36.36934	-121.66164	Reference
307CARROS	Carmel River at Rosie's Bridge	36.47434	-121.72821	Nonreference
307CARRSC	Carmel River @ Ranch San Carlos Rd	36.53690	-121.87005	Nonreference
307FINTAS	Finch Creek @ Tassajara Rd	36.38774	-121.59228	Nonreference
307JAMTAS	James Creek @ Tassajara Rd	36.37235	-121.59094	Reference
307LASGAR	Las Garzas Creek @ Garzas Trail	36.44916	-121.81818	Reference
307LASSLP	Las Garzas Crk below Santa Lucia Lake Preserve	36.45948	-121.79587	Nonreference
307POTCHA	Potrero Creek @ Chamisel Rd	36.52472	-121.86743	Nonreference
307ROBCYN	Robinson Canyon at bridge	36.51256	-121.81218	Reference
307SALGAR	Salsiquedes Creek @ Gazas Trail	36.44463	-121.81953	Reference
307SANCLE	San Clemente Upper @ San Clemente	36.43044	-121.79780	Reference
307TRILOS	Carmel River Trib @ above Los Padres Dam	36.37118	-121.66490	Reference
307TULCAR	Tularcitos Creek @ Carmel Valley Rd	36.35868	-121.55070	Reference
308ARRHY1	Arroyo De La Cruz	35.70820	-121.30394	Nonreference
308BIGLBC	Big Creek in LBC Reserve	36.07911	-121.59505	Reference
308BIGPBS	Big Sur River at Pfeiffer Big Sur State Park	36.2474	-121.77073	Reference
308BSRHW1	Big Sur River @ Hwy1	36.26975	-121.80736	Reference
308DEVLBC	Devil Flat Creek at Redwood Camp in LBC Reserve	36.07707	-121.59159	Reference
308GARCRK	Garrapata Creek above Joshua Creek Confluence	36.41485	-121.90366	Nonreference
308JOSCRK	Joshua Creek at Ken Eukland's house	36.41646	-121.90409	Nonreference

Table 4. (cont'd) Site location and determination of site type.

SiteCode	SiteDescription	Latitude	Longitude	Site Type
305PAJROG	Pajaro River at Rogge Lane	36.89447	-121.64546	Nonreference
305RAMRAM	Ramsey Creek at Ramsey Road	37.02742	-121.77718	Nonreference
305SAL129	Salsipuedes Creek at Hwy 129	36.91041	-121.74604	Nonreference
305SBRCOA	San Benito, below Hernandez Res,CoalingaRd pulloff	36.37793	-120.89848	Nonreference
305SBRH25	San Benito at Hwy 25	36.61419	-121.21091	Nonreference
305SBRPR1	San Benito at Paicines Ranch	36.73704	-121.32091	Nonreference
305SBRPR2	San Benito at Cienaga Road	36.67705	-121.28327	Nonreference
305SJCANZ	San Juan Creek at Anzar Road	36.87590	-121.56130	Nonreference
305SWACRO	Swanson Creek at Croy Road	37.08117	-121.76886	Reference
305TREMUR	Tres Pinos Creek at Murphy Road	36.74360	-121.27814	Nonreference
305UVASWA	Uvas Creek at Uvas Canyon County Park	37.08660	-121.79451	Reference
305UVAUVA	Uvas Creek at Uvas Road	37.06000	-121.67326	Nonreference
305WATLEE	Watsonville Slough at Lee Road	36.90152	-121.78103	Nonreference
307CACCAC	Cachagua Creek @ Cachagua	36.40127	-121.65927	Nonreference
307CARCAC	Carmel River @ Cachagua Park	36.39918	-121.66159	Nonreference
307CARHW1	Carmel River @ Hwy 1	36.53587	-121.91178	Nonreference
307CARLOS	Carmel River @ above Los Padres Dam	36.36934	-121.66164	Reference
307CARROS	Carmel River at Rosie's Bridge	36.47434	-121.72821	Nonreference
307CARRSC	Carmel River @ Ranch San Carlos Rd	36.53690	-121.87005	Nonreference
307FINTAS	Finch Creek @ Tassajara Rd	36.38774	-121.59228	Nonreference
307JAMTAS	James Creek @ Tassajara Rd	36.37235	-121.59094	Reference
307LASGAR	Las Garzas Creek @ Garzas Trail	36.44916	-121.81818	Reference
307LASSLP	Las Garzas Crk below Santa Lucia Lake Preserve	36.45948	-121.79587	Nonreference
307POTCHA	Potrero Creek @ Chamisel Rd	36.52472	-121.86743	Nonreference
307ROBCYN	Robinson Canyon at bridge	36.51256	-121.81218	Reference
307SALGAR	Salsiquedes Creek @ Gazas Trail	36.44463	-121.81953	Reference
307SANCLE	San Clemente Upper @ San Clemente	36.43044	-121.79780	Reference
307TRILOS	Carmel River Trib @ above Los Padres Dam	36.37118	-121.66490	Reference
307TULCAR	Tularcitos Creek @ Carmel Valley Rd	36.35868	-121.55070	Reference
308ARRHY1	Arroyo De La Cruz	35.70820	-121.30394	Nonreference
308BIGLBC	Big Creek in LBC Reserve	36.07911	-121.59505	Reference
308BIGPBS	Big Sur River at Pfeiffer Big Sur State Park	36.2474	-121.77073	Reference
308BSRHW1	Big Sur River @ Hwy1	36.26975	-121.80736	Reference
308DEVLBC	Devil Flat Creek at Redwood Camp in LBC Reserve	36.07707	-121.59159	Reference
308GARCRK	Garrapata Creek above Joshua Creek Confluence	36.41485	-121.90366	Nonreference
308JOSCRK	Joshua Creek at Ken Eukland's house	36.41646	-121.90409	Nonreference
308LILPAL	Little Sur River @ Palo Colorado Rd	36.34094	-121.80994	Reference
308MCWJPB	McWay Creek, Julia Pfeiffer SP Canyon Trail bridge	36.16292	-121.66446	Reference
308MILHW1	Mill Creek at Hwy 1	35.98416	-121.48874	Nonreference
308POSPBS	Post Creek in Pfeiffer Big Sur campground	36.24162	-121.77336	Nonreference
308PREPVS	Prewitt Creek @ Pacific Valley Station	35.93556	-121.46769	Reference
308ROCBBQ	Rocky Creek at HW1	36.37900	-121.90080	Reference
308SALHY1	Salmon Creek @ HWY1	35.81614	-121.35765	Reference
308SANHY1	San Carporoforo Creek @ HWY 1	35.76436	-121.31902	Reference
308SANWIL	San Jose Creek @ Williams Canyon Trail	36.49764	-121.87195	Reference

Table 4. (cont'd) Site location and determination of site type.

SiteCode	SiteDescription	Latitude	Longitude	Site Type
308SOBHW1	Soberanes Creek at Hwy 1	36.45486	-121.92149	Reference
308WILHW1	Willow Creek at Hwy 1	35.89367	-121.45868	Reference
308WILWIL	Williams Canyon Creek @ Williams Canyon Trail	36.47914	-121.85640	Reference
309ARSG16	Arroyo Seco @ G16 Bridge	36.28077	-121.32271	Nonreference
309ATASGL	Astascadero Creek @ San Gabriel Rd	35.46318	-120.67525	Nonreference
309BEAPVC	Bear Gulch Creek next to Pinnacles visitor center	36.48210	-121.17994	Reference
309BGCNVS	Bear Gulch Near Visitor Center	36.47935	-121.18334	Reference
309BLADRA	Blanco Drain at Salinas River confluence	36.70666	-121.74912	Nonreference
309CHA1	Chalone Creek Site #1	36.47326	-121.15398	Nonreference
309CHA146	Chalone Creek at Hwy 146 bridge	36.48772	-121.16990	Reference
309CHA2	Chalone Creek Site #2 downstream	36.46851	-121.15519	Nonreference
309CHAPVP	Chalone near Peaks View parking	36.48508	-121.16706	Reference
309CHAWSP	Willow Spring @ North Wilderness Trail	36.50675	-121.18194	Reference
309CHLCNF	Chalone North Fork	36.51494	-121.18843	Reference
309CHLCWF	Chalone West Fork	36.50280	-121.18432	Reference
309ESTEST	Estrella River @ Estrella Rd	35.65405	-120.50696	Nonreference
309GABCSC	Gabilan Creek at Constitution Soccer Complex	36.69309	-121.62801	Nonreference
309GABOLD	Gabilan Creek at Old Stage Road	36.78062	-121.58544	Nonreference
309JACJAC	Jack Crk @ Jack Crk Rd.	35.55017	-120.79487	Nonreference
309LAS1MI	Lower Arroyo Seco	36.22855	-121.49410	Reference
309MCCMC1	McCabe Creek at Pinnacles established MC1	36.49246	-121.14973	Reference
309NACCRO	Nacimiento River in Camp Roberts	35.80948	-120.76135	Nonreference
309NACFOR	Nacimiento River @ Fort Hunter Liggett	35.85644	-121.20175	Nonreference
309NATCAS	Natividad Creek at Las Casitas Road	36.69668	-121.61281	Nonreference
309PALACP	Paloma Creek @ Paloma Creek Park (City Park)	35.45221	-120.63326	Nonreference
309PASBET	Paso Robles Creek@Bethal Rd	35.53651	-120.72808	Nonreference
309POZHMN	Pozo Creek @ High Mountain Rd	35.29556	-120.38842	Nonreference
309SAL198	Salinas River at Hwy 198	36.11593	-121.02828	Nonreference
309SALATS	Salinas River @ Atascadero	35.46232	-120.62454	Nonreference
309SALBRA	Salinas River at Bradley	35.86422	-120.80974	Nonreference
309SALCHU	Salinas River @ Chular River Rd	36.55615	-121.54782	Nonreference
309SALDAV	Salinas River at Davis Road	36.64711	-121.70264	Nonreference
309SALH58	Salinas River at Hwy 58	35.40999	-120.56855	Nonreference
309SALIVR	Salinas River @ Indian Valley	35.78879	-120.72106	Nonreference
309SALKIN	Salinas River @ King City	36.20340	-121.14279	Nonreference
309SALPIL	Salinas River at Pilitas Road	35.34912	-120.51285	Nonreference
309SALPOZ	Salinas River at High Mtn Rd	35.29359	-120.38716	Nonreference
309SALSNT	Salinas River @ Sandstone Trail	35.32176	-120.42467	Nonreference
309SANBWR	San Lorenzo Creek @ Bitter Water Road	36.26699	-121.07073	Nonreference
309SANFOR	San Antonio River @ Fort Hunter Liggett	35.91158	-121.13144	Nonreference
309SANLYN	Santa Margarita Creek @ Lynden Crossing	35.41217	-120.60586	Nonreference
309SANPIC	Sandy Creek at Pinnacles Campground	36.49069	-121.14836	Reference
309SARSAN	San Antonio River at San Antonio Lake Road	35.80870	-120.85557	Nonreference
309SC2BCG	Sandy Creek 2 below campground	36.48297	-121.15536	Reference
309TASTAS	Tassajara Creek @ Tassajara Creek Rd (misnamed 310TASTA	35.38200	-120.67006	Reference

Table 4. (cont'd) Site location and determination of site type.

SiteCode	SiteDescription	Latitude	Longitude	Site Type
309TASZEN	Tassajara Creek @ Buddist Center (misnamed 307TASZEN)	36.23339	-121.54795	Reference
309TEM183	Tembladero Slough at Hwy 183	36.75165	-121.74175	Nonreference
309TORRVR	Toro Creek @ River Rd	35.32344	-120.42166	Nonreference
309TROH58	Trout Creek at Hwy 58	35.38996	-120.58215	Nonreference
309UASIND	Upper Arroyo Seco River	36.12017	-121.46878	Reference
309WILTON	Willow Creek @ Tony Trail	36.20749	-121.55717	Reference
310AGCBID	Arroyo Grande Creek at Biddle Park	35.18082	-120.51404	Nonreference
310AGRLOP	Arroyo Grande Creek @ Lopez Road	35.18508	-120.49927	Nonreference
310BIGUPP	Big Falls Creek @ Upper Lopez Road	35.26170	-120.51249	Reference
310CHOCHO	Chorro Creek @ Chorro Creek Rd	35.35754	-120.81245	Nonreference
310ISLCAM	Islay Creek at Montana de Oro campground	35.27415	-120.88600	Nonreference
310LOPUPP	Lopez Creek @ Upper Lopez Road	35.25909	-120.51123	Reference
310MORCIA	Morro Creek @ Cerra Alta	35.42743	-120.75329	Reference
310MORCIA2	West Morro Creek @ Cerra Alta	35.43002	-120.75174	Nonreference
310OLDOLD	Old Creek @ Old Creek Rd	35.47136	-120.85651	Nonreference
310SANCAM	Santa Rosa Creek @ Cambria	35.56190	-121.08194	Nonreference
310SANRED	San Simeon Creek @ Red Mountain Rd	35.60710	-121.09112	Nonreference
310SANSAN	Santa Rosa Creek @ 6115 Santa Rosa Creek Rd.	35.58188	-121.00705	Nonreference
310SLOCUE	San Luis Obispo Creek at Cuesta Park	35.29377	-120.64280	Nonreference
310TORCAY	Toro Creek off Toro Creek Road	35.42484	-120.86016	Nonreference
312BREFR	La Brea Creek at Rancho Sisquoc	34.85685	-120.19114	Nonreference
312CUY166	Cuyama River @ Hwy 166	35.02129	-120.22794	Reference
312DAVDAV	Davy Brown Creek @ Davy Brown Camp	34.76167	-119.95338	Reference
312HUAHUT	Huasna River @ Huasna Township Rd	35.08942	-120.36918	Nonreference
312MANNIR	Manzana Creek @ Nira Camp	34.77045	-119.93703	Reference
312REYCMP	Reyes Creek @ Campground	34.67928	-119.30579	Reference
312SANSUE	Santa Maria River @ Suey Crossing	34.96881	-120.40366	Nonreference
312SISJUD	Sisquoc River Above Judell Canyon	34.73712	-119.68349	Reference
312SISRNS	Sisquoc River @ Rancho Sisquoc	34.84024	-120.16651	Nonreference
314CACHAP	Cachuma Crk @ Happy Cyn Rd.	34.69277	-119.90914	Reference
314COCGRA	Coche Creek @ Grapevine Trail	34.64308	-119.75183	Reference
314NOJNOJ	Nojoqui Creek @ County Park	34.53551	-120.17645	Reference
314OSOUPP	Los Osos Creek @ Upper Oso	34.55691	-119.77218	Reference
314SALJAL	Salsipueles Creek @ Jalama Road	34.58973	-120.40901	Nonreference
314SANCRU	Santa Cruz Creek Above Santa Cruz Camp	34.63185	-119.76145	Reference
314SANGRE	Santa Ynez River @ Greco Crossing	34.61526	-120.37322	Nonreference
314SANMIG	San Miguelito Creek @ San Miguelito Road	34.59209	-120.47099	Nonreference
314SANRED	Santa Ynez River @ Red Rock	34.54053	-119.72000	Nonreference
314SANREF	Santa Ynez River @ Refugio Road	34.58520	-120.10124	Nonreference
315GAVGAV	Gaviota Creek @ Gaviota State Park	34.47333	-120.22892	Nonreference
315HONMIG	Honda Creek @ San Miguelito Road	34.59380	-120.53049	Reference
315JALCAM	Jalama Creek @ Jalama Beach Campground	34.51280	-120.49792	Nonreference

Table 5. Candidate metric classifications, descriptions, and abbreviations. Metrics using old classification are based on genera included by Krammer and Lange-Bertalot in Süßwasserflora von Mitteleuropa (1986, 1988, 1991a,b) prior to broad application of updated genus descriptions by Round et al. (1990). Asterisks before metric abbreviations indicate that the metric failed the S/N reproducibility criterion, scoring <1.5.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Autecological Metrics	Richness of van Dam Moisture Class 5 Species (never, or only very rarely, occurring outside water bodies)	*Moisture1.richness
	Richness of van Dam Moisture Classes 1 & 2 Species	Moisture12.richness
	Richness of van Dam Moisture Class 2 Species (mainly occurring in waterbodies, sometimes on wet places)	Moisture2.richness
	Richness of van Dam Moisture Class 3 Species (mainly occurring in water bodies, also rather regularly on wet and moist places)	Moisture3.richness
	Richness of van Dam Moisture Class 4 Species (mainly occurring on wet and moist or temporarily dry places)	Moisture4.richness
	Richness of van Dam Moisture Classes 4 & 5 Species	Moisture45.richness
	Richness of van Dam Moisture Class 5 Species (nearly exclusively occur outside water bodies)	Moisture5.richness
	Richness of van Dam Nitrogen Uptake Metabolism Class 1 Species (nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen)	OrgN1.richness
	Richness of van Dam Nitrogen Uptake Metabolism Class 2 Species (nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen)	OrgN2.richness
	Richness of van Dam Nitrogen Uptake Metabolism Class 3 Species (facultatively nitrogen-heterotrophic taxa,needing periodically elevated concentrations of organically bound nitrogen)	OrgN3.richness
	Richness of van Dam Nitrogen Uptake Metabolism Classes 3 & 4 Species	OrgN34.richness
	Richness of van Dam Nitrogen Uptake Metabolism Class 4 Species (obligately nitrogen-heterotrophic taxa,needing continuous elevated concentrations of organically bound nitrogen)	OrgN4.richness
	Richness of van Dam Oxygen Requirement Class 1 Species (continuously high)	OxyReq1.richness
	Richness of van Dam Oxygen Requirement Classes 1 & 2 Species	OxyReq12.richness
	Richness of van Dam Oxygen Requirement Class 1 Species (fairly high)	OxyReq2.richness
	Richness of van Dam Oxygen Requirement Class 3 Species (moderate)	OxyReq3.richness
	Richness of van Dam Oxygen Requirement Class 4 Species (low)	OxyReq4.richness
	Richness of van Dam Oxygen Requirement Classes 4 & 5 Species	OxyReq45.richness
	Richness of van Dam Oxygen Requirement Class 5 Species (very low)	*OxyReq5.richness

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Autecological Metrics	Richness of van Dam pH Class 1 Species (acidobiontic)	pH1.richness
	Richness of van Dam pH Class 2 Species (acidophilous)	pH2.richness
	Richness of van Dam pH Class 3 Species (circumneutral)	pH3.richness
	Richness of van Dam pH Class 4 Species (alkaliphilous)	pH4.richness
	Richness of van Dam pH Class 5 Species (alkalibiontic)	pH5.richness
	Proportion of Individuals van Dam Moisture Classes 1 & 2	prop.ind.Moisture12
	Proportion of Individuals van Dam Moisture Classes 4 & 5	prop.ind.Moisture45
	Proportion of Individuals van Dam Nitrogen Uptake Metabolism Classes 3 & 4	prop.ind.OrgN34
	Proportion of Individuals van Dam Nitrogen Uptake Metabolism Class 4	*prop.ind.OrgN4
	Proportion of Individuals van Dam Oxygen Requirement Class 1	prop.ind.OxyReq1
	Proportion of Individuals van Dam Oxygen Requirement Classes 1 & 2	prop.ind.OxyReq12
	Proportion of Individuals van Dam Oxygen Requirement Classes 4 & 5	prop.ind.OxyReq45
	Proportion of Individuals van Dam Oxygen Requirement Class 5	prop.ind.OxyReq5
	Proportion of Individuals van Dam pH Class 1	prop.ind.pH1
	Proportion of Individuals van Dam pH Class 2	*prop.ind.pH2
	Proportion of Individuals van Dam pH Class 4	prop.ind.pH4
	Proportion of Individuals van Dam pH Class 5	prop.ind.pH5
	Proportion of Individuals van Dam Salinity Class 1 (fresh)	*prop.ind.Salinity1
	Proportion of Individuals van Dam Salinity Class 2 (fresh brackish)	prop.ind.Salinity2
	Proportion of Individuals van Dam Saprobic Class 1 (oligosaprobous)	prop.ind.Saprobic1
	Proportion of Individuals van Dam Saprobic Class 1 & 2 (oligosaprobous & oligo-mesosaprobous)	prop.ind.Saprobic12
	Proportion of Individuals van Dam Saprobic Classes 4 & 5 (α-meso-/polysaprobous)	prop.ind.Saprobic45
	Proportion of Individuals van Dam Saprobic Classes 5 (polysaprobous)	prop.ind.Saprobic5
	Proportion of Individuals van Dam Trophic Classes 1 & 2 (oligotraphentic & oligo-mesotraphentic)	prop.ind.Trophic12

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Autecological Metrics	Proportion of Individuals van Dam Trophic Classes 5, 6, & 7 (eutraphentic, hypereutraphentic, & oligo- to eutraphentic [hypereutraphentic])	prop.ind.Trophic567
	Proportion of Individuals van Dam Trophic Class 6 (hypereutraphentic)	prop.ind.Trophic6
	Proportion of Individuals van Dam Trophic Class 7 (oligo- to eutraphentic [hypereutraphentic])	prop.ind.Trophic7
	Proportion of Species van Dam Moisture Class 1	*prop.spp.Moisture1
	Proportion of Species van Dam Moisture Classes 1 & 2	prop.spp.Moisture12
	Proportion of Species van Dam Moisture Class 2	prop.spp.Moisture2
	Proportion of Species van Dam Moisture Class 3	*prop.spp.Moisture3
	Proportion of Species van Dam Moisture Class 4	prop.spp.Moisture4
	Proportion of Species van Dam Moisture Classes 4 & 5	prop.spp.Moisture45
	Proportion of Species van Dam Moisture Class 5	*prop.spp.Moisture5
	Proportion of Species van Dam Nitrogen Uptake Metabolism Class 1	prop.spp.OrgN1
	Proportion of Species van Dam Nitrogen Uptake Metabolism Class 2	prop.spp.OrgN2
	Proportion of Species van Dam Nitrogen Uptake Metabolism Class 3	prop.spp.OrgN3
	Proportion of Species van Dam Nitrogen Uptake Metabolism Classes 3 & 4	prop.spp.OrgN34
	Proportion of Species van Dam Nitrogen Uptake Metabolism Class 4	prop.spp.OrgN4
	Proportion of Species van Dam Oxygen Requirement Class 1	prop.spp.OxyTol1
	Proportion of Species van Dam Oxygen Requirement Classes 1 & 2	prop.spp.OxyTol12
	Proportion of Species van Dam Oxygen Requirement Class 2	prop.spp.OxyTol2
	Proportion of Species van Dam Oxygen Requirement Class 3	prop.spp.OxyTol3
	Proportion of Species van Dam Oxygen Requirement Class 4	prop.spp.OxyTol4
	Proportion of Species van Dam Oxygen Requirement Classes 4 & 5	prop.spp.OxyTol45
	Proportion of Species van Dam Oxygen Requirement Class 5	prop.spp.OxyTol5
	Proportion of Species van Dam pH Class 1	*prop.spp.pH1
	Proportion of Species van Dam pH Class 2	prop.spp.pH2
	Proportion of Species van Dam pH Class 3	prop.spp.pH3

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Autecological Metrics	Proportion of Species van Dam pH Class 4	prop.spp.pH4
	Proportion of Species van Dam pH Class 5	prop.spp.pH5
	Proportion of Species van Dam Salinity Class 1	*prop.spp.Salinity1
	Proportion of Species van Dam Salinity Class 2	prop.spp.Salinity2
	Proportion of Species van Dam Salinity Class 3	prop.spp.Salinity3
	Proportion of Species van Dam Salinity Class 4	prop.spp.Salinity4
	Proportion of Species van Dam Saprobic Class 1	prop.spp.Saprobic1
	Proportion of Species van Dam Saprobic Classes 1 & 2	prop.spp.Saprobic12
	Proportion of Species van Dam Saprobic Class 2	prop.spp.Saprobic2
	Proportion of Species van Dam Saprobic Class 3	*prop.spp.Saprobic3
	Proportion of Species van Dam Saprobic Class 4	prop.spp.Saprobic4
	Proportion of Species van Dam Saprobic Classes 4 & 5	prop.spp.Saprobic45
	Proportion of Species van Dam Saprobic Class 5	prop.spp.Saprobic5
	Proportion of Species van Dam Trophic Class 1	prop.spp.Trophic1
	Proportion of Species van Dam Trophic Classes 1 & 2	prop.spp.Trophic12
	Proportion of Species van Dam Trophic Class 2	prop.spp.Trophic2
	Proportion of Species van Dam Trophic Class 3	*prop.spp.Trophic3
	Proportion of Species van Dam Trophic Class 4	prop.spp.Trophic4
	Proportion of Species van Dam Trophic Class 5	prop.spp.Trophic5
	Proportion of Species van Dam Trophic Classes 5, 6, & 7	prop.spp.Trophic567
	Proportion of Species van Dam Trophic Class 6	prop.spp.Trophic6
	Proportion of Species van Dam Trophic Class 7	*prop.spp.Trophic7
	Richness of van Dam Salinity Class 1 Species	*Salinity1.richness
	Richness of van Dam Salinity Class 1 Species	Salinity2.richness
	Richness of van Dam Salinity Class 3 Species (brackish fresh)	Salinity3.richness
	Richness of van Dam Salinity Class 4 Species (brackish)	Salinity4.richness
	Richness of van Dam Saprobic Class 1 Species	Saprobic1.richness
	Richness of van Dam Saprobic Classes 1& 2 Species	Saprobic12.richness
	Richness of van Dam Saprobic Class 2 Species	*Saprobic2.richness
	Richness of van Dam Saprobic Class 3 Species	Saprobic3.richness
	Richness of van Dam Saprobic Class 4 Species	Saprobic4.richness
	Richness of van Dam Saprobic Classes 4 & 5 Species	Saprobic45.richness
	Richness of van Dam Saprobic Class 5 Species	*Saprobic5.richness

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Autecological Metrics	Richness of van Dam Trophic Class 1 Species	Trophic1.richness
	Richness of van Dam Trophic Classes 1& 2 Species	Trophic12.richness
	Richness of van Dam Trophic Class 2 Species	Trophic2.richness
	Richness of van Dam Trophic Class 3 Species	*Trophic3.richness
	Richness of van Dam Trophic Class 4 Species	Trophic4.richness
	Richness of van Dam Trophic Class 5 Species	Trophic5.richness
	Richness of van Dam Trophic Classes 5, 6, & 7 Species	Trophic567.richness
	Richness of van Dam Trophic Class 6 Species	Trophic6.richness
	Richness of van Dam Trophic Class 7 Species	*Trophic7.richness
	Weighted Average of van Dam Moisture Score	weighted.Moisture
	Weighted Average of van Dam Nitrogen Uptake Score	weighted.Organic.N
	Weighted Average of van Dam Oxygen Requirement Score	weighted.Oxy.Req
	Weighted Average of van Dam pH Score	weighted.pH
	Weighted Average of van Dam Salinity Score	weighted.Salinity
	Weighted Average of van Dam Saprobic Score	weighted.Saprobic
	Weighted Average of van Dam Trophic Score	weighted.Trophic
Community Structure Metrics		
	Richness of Species belonging to the genus *Achnanthes* (using old classification)	Achnanthes.old.richness
	Richness of Species belonging to the genus *Achnanthes*	*Achnanthes.richness
	Richness of Species belonging to the genus *Amphora* (using old classification)	Amphora.old.richness
	Richness of Species belonging to the genus *Amphora*	Amphora.richness
	Richness of Species belonging to the genus *Cocconeis* (using old classification)	Cocconeis.old.richness
	Richness of Species belonging to the genus *Cocconeis*	Cocconeis.richness
	Richness of Species belonging to the genus *Cyclotella* (using old classification)	Cyclotella.old.richness
	Richness of Species belonging to the genus *Cyclotella*	Cyclotella.richness
	Richness of Species belonging to the genus *Cymbella* (using old classification)	*Cymbella.old.richness
	Richness of Species belonging to the genus *Cymbella*	Cymbella.richness

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Community Structure Metrics	Richness of Species belonging to the genera *Epithemia* and *Rhopalodia* (using old classification)	EpiRho.richness
	Richness of Species belonging to the genus *Epithemia*	Epithemia.richness
	Richness of Species belonging to the genus *Eunotia*	*Eunotia.richness
	Richness of Species belonging to the genus *Fragilaria* (using old classification)	*Fragilaria.old.richness
	Richness of Species belonging to the genus *Fragilaria*	Fragilaria.richness
	Richness of Species belonging to the genus *Frustulia*	*Frustulia.richness
	Richness of Species belonging to the genus *Gomphonema*	*Gomphonema.richness
	Inverse Simpson's Diversity	invsimpson
	Richness of Species belonging to the genus *Navicula* (using old classification)	Navicula.old.richness
	Richness of Species belonging to the genus *Navicula*	Navicula.richness
	Richness of Species belonging to the genus *Nitzschia* (using old classification)	Nitzschia.old.richness
	Richness of Species belonging to the genus *Nitzschia*	Nitzschia.richness
	Pielou's Evenness	pielou.even
	Proportion of Species *Achnanthes*	*prop.Achnanthes
	Proportion of Species *Amphora*	prop.Amphora
	Proportion of Species *Cocconeis*	prop.Cocconeis
	Proportion of Species *Cyclotella*	prop.Cyclotella
	Proportion of Species *Cymbella*	*prop.Cymbella
	Proportion of Individuals belonging to the dominant species	prop.dominant
	Proportion of Individuals belonging to the dominant three species	prop.dominant3
	Proportion of Individuals belonging to the dominant five species	prop.dominant5
	Proportion of Species *Epithemia*	prop.Epithemia
	Proportion of Species *Eunotia*	prop.Eunotia
	Proportion of Species *Fragilaria*	prop.Fragilaria
	Proportion of Species *Frustulia*	prop.Frustulia
	Proportion of Species *Gomphonema*	*prop.Gomphonema
	Proportion of Individuals *Achnanthes*	*prop.ind.Achnanthes

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Metric Classification	Metric Description	Metric Abbreviation
Community Structure Metrics	Proportion of Individuals belonging to the genus *Achnanthes* (using old classification)	prop.ind.Achnanthes.old
	Proportion of Individuals belonging to the genus *Achnanthes* divided by the sum of Individuals belonging to the genera *Achnanthes* and *Navicula*	prop.ind.AchOverAchPlusNav
	Proportion of Individuals belonging to the genus *Achnanthes* divided by the sum of Individuals belonging to the genera *Achnanthes* and *Navicula* (using old classification)	*prop.ind.AchOverAchPlusNavOld
	Proportion of Individuals belonging to the genus *Amphora*	prop.ind.Amphora
	Proportion of Individuals belonging to the genus *Amphora* (using old classification)	prop.ind.Amphora.old
	Proportion of Individuals belonging to the genus *Cocconeis*	prop.ind.Cocconeis
	Proportion of Individuals belonging to the genus *Cocconeis* (using old classification)	prop.ind.Cocconeis.old
	Proportion of Individuals belonging to the genus *Cyclotella*	prop.ind.Cyclotella
	Proportion of Individuals belonging to the genus *Cyclotella* (using old classification)	prop.ind.Cyclotella.old
	Proportion of Individuals belonging to the genus *Cymbella*	prop.ind.Cymbella
	Proportion of Individuals belonging to the genus *Cymbella* (using old classification)	prop.ind.Cymbella.old
	Proportion of Individuals belonging to the genus *Cymbella* divided by the sum of Individuals belonging to the genera *Cymbella* and *Navicula*	prop.ind.CymOverCymPlusNav
	Proportion of Individuals belonging to the genus *Cymbella* divided by the sum of Individuals belonging to the genera *Cymbella* and *Navicula* (using old classification)	prop.ind.CymOverCymPlusNavOld
	Proportion of Individuals belonging to the genus *Epithemia* divided by the sum of Individuals belonging to the genera *Epithemia* and *Rhopalodia*	prop.ind.EpiOverEpiPlusRho
	Proportion of Individuals belonging to the genus *Epithemia*	prop.ind.Epithemia
	Proportion of Individuals belonging to the genus *Eunotia*	prop.ind.Eunotia
	Proportion of Individuals belonging to the genus *Fragilaria*	prop.ind.Fragilaria
	Proportion of Individuals belonging to the genus *Fragilaria* (using old classification)	prop.ind.Fragilaria.old
	Proportion of Individuals belonging to the genus *Frustulia*	prop.ind.Frustulia
	Proportion of Individuals belonging to the genus *Gomphonema*	prop.ind.Gomphonema
	Proportion of Individuals belonging to the genus *Navicula*	prop.ind.Navicula

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Community Structure Metrics	Proportion of Individuals belonging to the genus *Navicula* (using old classification)	prop.ind.Navicula.old
	Proportion of Individuals belonging to the genus *Nitzschia*	prop.ind.Nitzschia
	Proportion of Individuals belonging to the genus *Nitzschia*	prop.ind.Nitzschia.old
	Proportion of Individuals belonging to the genus *Rhoicosphenia*	prop.ind.Rhoicosphenia
	Proportion of Individuals belonging to the genus *Rhopalodia*	*prop.ind.Rhopalodia
	Proportion of Individuals belonging to the genus *Surirella*	prop.ind.Surirella
	Proportion of Individuals belonging to the genus *Synedra*	prop.ind.Synedra
	Proportion of Individuals belonging to the genus *Synedra* (using old classification)	prop.ind.Synedra.old
	Proportion of Species belonging to the genus *Navicula*	prop.Navicula
	Proportion of Species belonging to the genus *Nitzschia*	prop.Nitzschia
	Proportion of Species belonging to the genus *Rhoicosphenia*	prop.Rhoicosphenia
	Proportion of Species belonging to the genus *Rhopalodia*	prop.Rhopalodia
	Proportion of Species belonging to the genus *Achnanthes* (using old classification)	prop.spp.Achnanthes.old
	Proportion of Species belonging to the genus *Achnanthes* divided by the sum of Species belonging to the genera *Achnanthes* and *Navicula*	*prop.spp.AchOverAchPlusNav
	Proportion of Species belonging to the genus *Achnanthes* divided by the sum of Species belonging to the genera *Achnanthes* and *Navicula* (using old classification)	prop.spp.AchOverAchPlusNavOld
	Proportion of Species belonging to the genus *Cocconeis* (using old classification)	prop.spp.Cocconeis.old
	Proportion of Species belonging to the genus *Cyclotella* (using old classification)	prop.spp.Cyclotella.old
	Proportion of Species belonging to the genus *Cymbella* (using old classification)	*prop.spp.Cymbella.old
	Proportion of Species belonging to the genus *Cymbella* divided by the sum of Species belonging to the genera *Cymbella* and *Navicula*	prop.spp.CymOverCymPlusNav
	Proportion of Species belonging to the genus *Cymbella* divided by the sum of Species belonging to the genera *Cymbella* and *Navicula* (using old classification)	prop.spp.CymOverCymPlusNavOld
	Proportion of Species belonging to the genus *Epithemia* divided by the sum of Species belonging to the genera *Epithemia* and *Rhopalodia*	prop.spp.EpiOverEpiPlusRho

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Community Structure Metrics	Proportion of Species belonging to the genus *Fragilaria* (using old classification)	*prop.spp.Fragilaria.old
	Proportion of Species belonging to the genus *Navicula* (using old classification)	prop.spp.Navicula.old
	Proportion of Species belonging to the genus *Nitzschia* (using old classification)	prop.spp.Nitzschia.old
	Proportion of Species belonging to the genus *Synedra* (using old classification)	*prop.spp.Synedra.old
	Proportion of Species belonging to the genus *Surirella*	prop.Surirella
	Proportion of Species belonging to the genus *Synedra*	prop.Synedra
	Proportion of Species belonging to the genus *Rhoicosphenia*	*Rhoicosphenia.richness
	Proportion of Species belonging to the genus *Rhopalodia*	*Rhopalodia.richness
	Species (or variety) Richness	richness
	Shannon Diversity	shannon
	Simpson Diversity	simpson
	Chao's Estimated Species Pool	spp.pool.chao
	Richness of Species belonging to the genus *Surirella*	Surirella.richness
	Richness of Species belonging to the genus *Surirella* (using old classification)	*Synedra.old.richness
	Richness of Species belonging to the genus *Synedra*	Synedra.richness
Ecological Guild Metrics		
	Richness of Species with High Motility	cnt.spp.HighMotility
	Richness of Species with Moderate Motility	cnt.spp.ModMotility
	Richness of Species with Vertical Morphology	*cnt.spp.vert.morph
	Richness of Nonmotile Species	cnt.spp.Nonmotile
	Richness of Prostrate Species	cnt.spp.prostrate
	Richness of Stalked Species	cnt.spp.stalked
	Richness of Species with Minimal Motility	cnt.spp.MinMotility
	Proportion of Individuals with High Motility	prop.ind.HighMotility
	Proportion of Individuals with Moderate Motility	prop.ind.ModMotility
	Proportion of Individuals with Vertical Morphology	prop.ind.vert.morph
	Proportion of Individulas that are Nonmotile	prop.ind.Nonmotile

Table 5 (cont'd). Candidate metric classifications, descriptions, and abbreviations.

Candidate Metrics		
Metric Classification	**Metric Description**	**Metric Abbreviation**
Ecological Guild Metrics	Proportion of Individuals that are Prostrate	prop.ind.prostrate
	Proportion of Individuals that are Stalked	prop.ind.stalked
	Proportion of Individuals with Minimal Motility	prop.ind.MinMotility
	Proportion of Species with High Motility	prop.spp.HighMotility
	Proportion of Species with Moderate Motility	prop.spp.ModMotility
	Proportion of Species with Vertical Morphology	*prop.spp.vert.morph
	Proprotion of Species that are Nonmotile	prop.spp.Nonmotile
	Proportion of Species that are Prostrate	prop.spp.prostrate
	Proportion of Species that are Stalked	prop.spp.stalked
	Proportion of Species with Minimal Motility	prop.spp.MinMotility
Tolerance/Intolerance Metrics		
	Proportion of Individuals Classified as California Central Coast Most Sensitive	prop.ind.ccc.most.intol
	Proportion of Individuals Classified as California Central Coast Most Tolerant	prop.ind.ccc.most.tol
	Proportion of Individuals Classified as Bahl's Pollution Tolerance Class 1 (most tolerant)	*prop.ind.PolTol1
	Proportion of Individuals Classified as Bahl's Pollution Tolerance Classes 1 & 2 (insensitive)	prop.ind.PolTol12
	Proportion of Individuals Classified as Bahls's Pollution Tolerance Class 3 (sensitive)	*prop.ind.PolTol3
	Proportion of Species Classified as Bahls' Pollution Tolerance Class 1	prop.spp.PolTol1
	Proportion of Species Classified as Bahls' Pollution Tolerance Classes 1 & 2	prop.spp.PolTol12
	Proportion of Species Classified as Bahls' Pollution Tolerance Class 2 (less tolerant)	prop.spp.PolTol2
	Proportion of Species Classified as Bahls' Pollution Tolerance Class 3	prop.spp.PolTol3
	Weighted Average of Bahls' Pollution Tolerance Score	weighted.PolTol
Productivity Metrics		
	Benthic chlorophyll a	*ben.chla
	Suspended chlorophyll a	*susp.chla
	Benthic Ash-free dry mass	*ben.afdm
	Mean microalgae thickness class	*mean.microalg
	Mean macroalgae percent cover	*mean.macroalg
	Mean floating macroalgae percent cover	*mean.floating
	Mean macrophyte percent cover	*mean.macrophyte

Table 7. Candidate variables used in backward stepwise linear discriminant analysis to predict site assemblage classes. Variables in bold were retained as predictors in the final model.

Variable	Source
Latitude	GIS-corrected site measure using site description
Longitude	GIS-corrected site measure using site description
Minimum watershed elevation	GIS
Maximum watershed elevation	GIS
Mean watershed elevation	GIS
Site Elevation	GIS
Precipitation	GIS
Reach slope	Site-based measure
Reach aspect	GIS
Mean rock depth	GIS
Percent watershed intrinsic igneous	GIS
Percent watershed sedimentary clastic	GIS
Percent watershed metamorphic	GIS
Mean annual air temperature	GIS
Mean soil permeability	GIS

Table 8. The number of sites where diatom species were found, as well as the number of reference sites (out of 56 total reference sites) and number of nonreference sites (out of 156 total nonreference sites) where species were found.

Diatom Species	Number of Site Detections	Reference Site Detections		Nonreference Site Detections	
		(# sites)	(% sites)	(# sites)	(% sites)
Achnanthes coarctata	3	0	0.0	3	1.8
Achnanthes minutissima var jackii	3	1	1.5	2	1.2
Achnanthes oblongella	14	1	1.5	13	7.7
Achnanthes sp 1 PRW	2	0	0.0	2	1.2
Achnanthes subhudsonis var kraeuselii	3	1	1.5	2	1.2
Achnanthidium deflexum	4	2	2.9	2	1.2
Achnanthidium exiguum	94	28	41.2	66	39.3
Achnanthidium minutissimum	160	46	67.6	114	67.9
Actinocyclus	1	0	0.0	1	0.6
Actinocyclus normanii	1	0	0.0	1	0.6
Adlafia bryophila	5	5	7.4	0	0.0
Adlafia minuscula	11	4	5.9	7	4.2
Adlafia minuscula var muralis	10	6	8.8	4	2.4
Amphipleura pellucida	22	12	17.6	10	6.0
Amphora	1	0	0.0	1	0.6
Amphora coffeaeformis	2	0	0.0	2	1.2
Amphora copulata	56	6	8.8	50	29.8
Amphora holsatica	3	0	0.0	3	1.8
Amphora montana	16	4	5.9	12	7.1
Amphora ovalis	57	5	7.4	52	31.0
Amphora pediculus	199	49	72.1	150	89.3
Amphora sp 1 CAL	4	1	1.5	3	1.8
Amphora veneta	82	20	29.4	62	36.9
Anomoeoneis sphaerophora fo costata	1	1	1.5	0	0.0
Asterionella formosa	2	0	0.0	2	1.2
Aulacoseira alpigena	3	0	0.0	3	1.8
Aulacoseira ambigua	2	0	0.0	2	1.2
Aulacoseira crenulata	1	0	0.0	1	0.6
Aulacoseira distans	4	0	0.0	4	2.4
Aulacoseira granulata	6	0	0.0	6	3.6
Aulacoseira granulata var angustissima	5	1	1.5	4	2.4
Aulacoseira subartica	1	0	0.0	1	0.6
Bacillaria paradoxa	38	4	5.9	34	20.2
Biremis circumtexta	2	0	0.0	2	1.2
Brachysira exilis	4	0	0.0	4	2.4
Brachysira zellensis	1	0	0.0	1	0.6
Caloneis	1	1	1.5	0	0.0
Caloneis amphisbaena	5	0	0.0	5	3.0
Caloneis bacillum	89	32	47.1	57	33.9
Caloneis schumanniana	7	4	5.9	3	1.8
Caloneis schumanniana var biconstricta	1	0	0.0	1	0.6
Caloneis silicula	10	4	5.9	6	3.6
Caloneis thermalis	2	2	2.9	0	0.0
Campylodiscus	1	1	1.5	0	0.0
Campylodiscus clypeus	5	1	1.5	4	2.4
Campylodiscus hibernicus	2	0	0.0	2	1.2
Capartogramma crucicula	1	0	0.0	1	0.6

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections		Nonreference Site Detections	
		(# sites)	(% sites)	(# sites)	(% sites)
Chamaepinnularia bremensis	4	2	2.9	2	1.2
Chamaepinnularia mediocris	6	2	2.9	4	2.4
Chamaepinnularia soehrensis var muscicola	1	1	1.5	0	0.0
Cocconeis fluviatilis	1	1	1.5	0	0.0
Cocconeis neothumensis	3	1	1.5	2	1.2
Cocconeis pediculus	46	11	16.2	35	20.8
Cocconeis placentula	82	21	30.9	61	36.3
Cocconeis placentula var euglypta	116	30	44.1	86	51.2
Cocconeis placentula var lineata	141	35	51.5	106	63.1
Cocconeis pseudolineata	40	10	14.7	30	17.9
Cocconeis scutellum	1	0	0.0	1	0.6
Cocconeis sp 1 CAL	7	1	1.5	6	3.6
Craticula accomoda	6	2	2.9	4	2.4
Craticula buderi	3	0	0.0	3	1.8
Craticula cuspidata	6	1	1.5	5	3.0
Craticula halophila	9	2	2.9	7	4.2
Craticula halophiliodes	1	1	1.5	0	0.0
Craticula molestiformis	21	7	10.3	14	8.3
Craticula submolesta	2	1	1.5	1	0.6
Ctenophora pulchella	3	1	1.5	2	1.2
Cyclostephanos costatilimbus	2	0	0.0	2	1.2
Cyclostephanos invisitatus	4	0	0.0	4	2.4
Cyclotella atomus	10	0	0.0	10	6.0
Cyclotella bodanica var lemanica	1	0	0.0	1	0.6
Cyclotella meneghiniana	102	14	20.6	88	52.4
Cyclotella ocellata	7	0	0.0	7	4.2
Cylindrotheca gracilis	3	1	1.5	2	1.2
Cymatopleura elliptica	2	1	1.5	1	0.6
Cymatopleura solea	11	2	2.9	9	5.4
Cymbella	4	1	1.5	3	1.8
Cymbella affinis	25	7	10.3	18	10.7
Cymbella aspera	16	2	2.9	14	8.3
Cymbella cistula	2	1	1.5	1	0.6
Cymbella cymbiformis	3	2	2.9	1	0.6
Cymbella delicatula	1	0	0.0	1	0.6
Cymbella hustedtii	1	1	1.5	0	0.0
Cymbella leptoceros	1	0	0.0	1	0.6
Cymbella mexicana	8	2	2.9	6	3.6
Cymbella naviculiformis	1	1	1.5	0	0.0
Cymbella proxima	3	0	0.0	3	1.8
Cymbella pusilla	6	1	1.5	5	3.0
Cymbella sp 2 CSU	6	2	2.9	4	2.4
Cymbella sp 3 CSU	1	0	0.0	1	0.6
Cymbella sp 987 WMPNDS	3	1	1.5	2	1.2
Cymbella tumida	3	0	0.0	3	1.8
Cymbella tumidula	3	3	4.4	0	0.0
Denticula kuetzingii	23	8	11.8	15	8.9
Denticula subtilis	16	6	8.8	10	6.0
Denticula tenuis	1	0	0.0	1	0.6
Denticula valida	1	0	0.0	1	0.6
Diadesmis confervacea	5	1	1.5	4	2.4

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections		Nonreference Site Detections	
		(# sites)	(% sites)	(# sites)	(% sites)
Diadesmis contenta	14	3	4.4	11	6.5
Diadesmis gallica	1	1	1.5	0	0.0
Diadesmis perpusilla	8	5	7.4	3	1.8
Diatoma mesodon	5	2	2.9	3	1.8
Diatoma moniliformis	29	12	17.6	17	10.1
Diatoma tenuis	8	2	2.9	6	3.6
Diatoma vulgaris	47	9	13.2	38	22.6
Diploneis elliptica	2	1	1.5	1	0.6
Diploneis interrupta	2	1	1.5	1	0.6
Diploneis marginestriata	2	0	0.0	2	1.2
Diploneis modica	1	0	0.0	1	0.6
Diploneis oblongella	20	9	13.2	11	6.5
Diploneis oculata	9	5	7.4	4	2.4
Diploneis ovalis	4	1	1.5	3	1.8
Diploneis parma	2	2	2.9	0	0.0
Diploneis pseudovalis	2	0	0.0	2	1.2
Diploneis puella	38	8	11.8	30	17.9
Diploneis subovalis	2	1	1.5	1	0.6
Discostella pseudostelligera	3	1	1.5	2	1.2
Discostella stelligera	2	2	2.9	0	0.0
Encyonema auerswaldii	5	2	2.9	3	1.8
Encyonema caespitosum	7	1	1.5	6	3.6
Encyonema minutum	12	3	4.4	9	5.4
Encyonema muelleri	1	0	0.0	1	0.6
Encyonema prostratum	10	1	1.5	9	5.4
Encyonema silesiacum	19	6	8.8	13	7.7
Encyonema triangulum	1	0	0.0	1	0.6
Encyonopsis microcephala	32	17	25.0	15	8.9
Encyonopsis subminuta	2	1	1.5	1	0.6
Entomoneis alata	8	2	2.9	6	3.6
Entomoneis ornata	1	0	0.0	1	0.6
Epithemia adnata	63	32	47.1	31	18.5
Epithemia sorex	69	30	44.1	39	23.2
Epithemia turgida	12	6	8.8	6	3.6
Eunotia	9	7	10.3	2	1.2
Eunotia formica	2	0	0.0	2	1.2
Eunotia minor	4	3	4.4	1	0.6
Eunotia pectinalis var undulata	1	1	1.5	0	0.0
Eunotia soleirolii	1	1	1.5	0	0.0
Fallacia cryptolyra	1	0	0.0	1	0.6
Fallacia helensis	7	0	0.0	7	4.2
Fallacia lenzii	9	1	1.5	8	4.8
Fallacia monoculata	4	2	2.9	2	1.2
Fallacia pygmaea	18	3	4.4	15	8.9
Fallacia subhamulata	15	5	7.4	10	6.0
Fallacia tenera	57	8	11.8	49	29.2
Fragilaria	1	1	1.5	0	0.0
Fragilaria capucina	11	0	0.0	11	6.5
Fragilaria capucina var gracilis	7	2	2.9	5	3.0
Fragilaria capucina var mesolepta	9	0	0.0	9	5.4
Fragilaria cf vaucheriae	10	5	7.4	5	3.0

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections		Nonreference Site Detections	
		(# sites)	(% sites)	(# sites)	(% sites)
Fragilaria construens var exigua	1	0	0.0	1	0.6
Fragilaria crotonensis	3	0	0.0	3	1.8
Fragilaria famelica	1	1	1.5	0	0.0
Fragilaria nitzschioides	12	5	7.4	7	4.2
Fragilaria pinnata var lancettula	21	6	8.8	15	8.9
Fragilaria radians	1	0	0.0	1	0.6
Fragilaria sp 946 WMPNDS	4	2	2.9	2	1.2
Fragilaria vaucheriae	49	16	23.5	33	19.6
Fragilariforma constricta fo stricta	1	1	1.5	0	0.0
Fragilariforma virescens	5	2	2.9	3	1.8
Frustulia crassinervia	1	0	0.0	1	0.6
Frustulia saxonica	1	0	0.0	1	0.6
Frustulia vulgaris	26	7	10.3	19	11.3
Geissleria acceptata	39	10	14.7	29	17.3
Geissleria decussis	50	10	14.7	40	23.8
Geissleria paludosa	2	1	1.5	1	0.6
Geissleria punctifera	19	7	10.3	12	7.1
Gomphoneis eriense	5	2	2.9	3	1.8
Gomphoneis eriense var variabilis	1	1	1.5	0	0.0
Gomphoneis herculeana	3	0	0.0	3	1.8
Gomphoneis minuta	1	0	0.0	1	0.6
Gomphoneis olivaceoides	1	0	0.0	1	0.6
Gomphoneis olivaceum	54	15	22.1	39	23.2
Gomphonema	4	0	0.0	4	2.4
Gomphonema acuminatum	13	7	10.3	6	3.6
Gomphonema affine	12	1	1.5	11	6.5
Gomphonema angustatum	14	6	8.8	8	4.8
Gomphonema angustum	4	2	2.9	2	1.2
Gomphonema augur	1	1	1.5	0	0.0
Gomphonema bipunctatum	2	2	2.9	0	0.0
Gomphonema bohemicum	3	1	1.5	2	1.2
Gomphonema clavatum	2	1	1.5	1	0.6
Gomphonema drutelingense	2	1	1.5	1	0.6
Gomphonema gracile	8	2	2.9	6	3.6
Gomphonema insigne	4	1	1.5	3	1.8
Gomphonema intricatum	8	2	2.9	6	3.6
Gomphonema kobayasii	45	9	13.2	36	21.4
Gomphonema lagenula	9	0	0.0	9	5.4
Gomphonema mexicanum	10	0	0.0	10	6.0
Gomphonema micropus	45	18	26.5	27	16.1
Gomphonema minutum	50	19	27.9	31	18.5
Gomphonema parvulum	62	12	17.6	50	29.8
Gomphonema patrickii	1	1	1.5	0	0.0
Gomphonema productum	2	1	1.5	1	0.6
Gomphonema pseudoaugur	1	0	0.0	1	0.6
Gomphonema pseudotenellum	1	1	1.5	0	0.0
Gomphonema pumilum	38	15	22.1	23	13.7
Gomphonema rhombicum	9	5	7.4	4	2.4
Gomphonema sarcophagus	3	0	0.0	3	1.8
Gomphonema stoermeri	2	2	2.9	0	0.0
Gomphonema subclavatum	2	1	1.5	1	0.6

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections		Nonreference Site Detections	
		(# sites)	(% sites)	(# sites)	(% sites)
Gomphonema truncatum	24	8	11.8	16	9.5
Gomphonema utae	3	0	0.0	3	1.8
Gomphonema vibrio	1	0	0.0	1	0.6
Gomphosphenia	2	0	0.0	2	1.2
Gyrosigma acuminatum	29	4	5.9	25	14.9
Gyrosigma attenuatum	1	0	0.0	1	0.6
Gyrosigma macrum	1	0	0.0	1	0.6
Gyrosigma nodiferum	2	0	0.0	2	1.2
Gyrosigma obtusatum	1	0	0.0	1	0.6
Gyrosigma sp 1 CSU	3	0	0.0	3	1.8
Hantzschia amphioxys	14	8	11.8	6	3.6
Hippodonta capitata	40	7	10.3	33	19.6
Hippodonta hungarica	5	0	0.0	5	3.0
Hippodonta neglecta	4	1	1.5	3	1.8
Hippodonta subtilissima	14	1	1.5	13	7.7
Karayevia clevei	33	5	7.4	28	16.7
Karayevia laterostrata	1	0	0.0	1	0.6
Karayevia ploenensis	3	0	0.0	3	1.8
Karayevia suchlandtii	4	0	0.0	4	2.4
Kolbesia suchlandtii	5	2	2.9	3	1.8
Krasskella	5	2	2.9	3	1.8
Lemnicola hungarica	18	6	8.8	12	7.1
Luticola goeppertiana	7	0	0.0	7	4.2
Luticola mutica	2	1	1.5	1	0.6
Luticola muticopsis	3	0	0.0	3	1.8
Mastogloia elliptica	5	2	2.9	3	1.8
Mastogloia pumila	1	0	0.0	1	0.6
Mastogloia smithii	2	0	0.0	2	1.2
Mayamaea agrestis	7	0	0.0	7	4.2
Mayamaea atomus	27	6	8.8	21	12.5
Mayamaea permitis	42	9	13.2	33	19.6
Melosira varians	105	19	27.9	86	51.2
Meridion circulare	32	16	23.5	16	9.5
Meridion circulare var constrictum	2	2	2.9	0	0.0
Microcostatus krasskei	1	1	1.5	0	0.0
Navicula	13	5	7.4	8	4.8
Navicula absoluta	1	1	1.5	0	0.0
Navicula amphiceropsis	8	1	1.5	7	4.2
Navicula angusta	2	1	1.5	1	0.6
Navicula antonii	130	34	50.0	96	57.1
Navicula aquaeductae	1	0	0.0	1	0.6
Navicula arctotenelloides	1	0	0.0	1	0.6
Navicula arvensis	2	2	2.9	0	0.0
Navicula aurora	8	2	2.9	6	3.6
Navicula canalis	11	4	5.9	7	4.2
Navicula capitatoradiata	33	5	7.4	28	16.7
Navicula capitellata	1	0	0.0	1	0.6
Navicula cari	4	0	0.0	4	2.4
Navicula caterva	15	1	1.5	14	8.3
Navicula cincta	19	3	4.4	16	9.5
Navicula cryptocephala	29	10	14.7	19	11.3
Navicula cryptotenella	130	43	63.2	87	51.8

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections (# sites)	Reference Site Detections (% sites)	Nonreference Site Detections (# sites)	Nonreference Site Detections (% sites)
Navicula cryptotenelloides	9	5	7.4	4	2.4
Navicula denselineolata	2	1	1.5	1	0.6
Navicula difficillima	4	2	2.9	2	1.2
Navicula erifuga	69	7	10.3	62	36.9
Navicula escambia	5	2	2.9	3	1.8
Navicula festiva	2	1	1.5	1	0.6
Navicula germainii	4	1	1.5	3	1.8
Navicula gregaria	182	43	63.2	139	82.7
Navicula incertata	1	0	0.0	1	0.6
Navicula ingenua	1	0	0.0	1	0.6
Navicula kotschyi	3	1	1.5	2	1.2
Navicula lanceolata	57	6	8.8	51	30.4
Navicula laterostrata	50	10	14.7	40	23.8
Navicula leptostriata	1	0	0.0	1	0.6
Navicula libonensis	13	2	2.9	11	6.5
Navicula lundii	19	6	8.8	13	7.7
Navicula menisculus	45	12	17.6	33	19.6
Navicula meniscus	6	3	4.4	3	1.8
Navicula microcari	37	12	17.6	25	14.9
Navicula microdigitoradiata	1	0	0.0	1	0.6
Navicula minima	114	29	42.6	85	50.6
Navicula notha	5	4	5.9	1	0.6
Navicula novaesiberica	1	0	0.0	1	0.6
Navicula obsoleta	1	0	0.0	1	0.6
Navicula occulta	1	0	0.0	1	0.6
Navicula peregrina	8	1	1.5	7	4.2
Navicula perminuta	25	8	11.8	17	10.1
Navicula phyllepta	41	8	11.8	33	19.6
Navicula porifera var opportuna	3	0	0.0	3	1.8
Navicula pseudoventralis	1	1	1.5	0	0.0
Navicula radiosa	39	14	20.6	25	14.9
Navicula radiosafallax	1	0	0.0	1	0.6
Navicula recens	9	1	1.5	8	4.8
Navicula reichardtiana	81	21	30.9	60	35.7
Navicula reichardtiana var crassa	1	0	0.0	1	0.6
Navicula reinhardtii	8	5	7.4	3	1.8
Navicula rhynchocephala	4	1	1.5	3	1.8
Navicula rostellata	31	5	7.4	26	15.5
Navicula salinarum	4	0	0.0	4	2.4
Navicula schmassmanni	2	2	2.9	0	0.0
Navicula sp 2 CAL	2	0	0.0	2	1.2
Navicula sp 3 CAL	1	0	0.0	1	0.6
Navicula sp 5 CSU	6	0	0.0	6	3.6
Navicula sp 7 CSU	1	1	1.5	0	0.0
Navicula subminuscula	8	1	1.5	7	4.2
Navicula symmetrica	11	3	4.4	8	4.8
Navicula tenelloides	16	4	5.9	12	7.1
Navicula tripunctata	120	25	36.8	95	56.5
Navicula trivialis	9	2	2.9	7	4.2
Navicula veneta	78	19	27.9	59	35.1
Navicula vilaplanii	7	2	2.9	5	3.0
Navicula viridula	1	0	0.0	1	0.6

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections (# sites)	Reference Site Detections (% sites)	Nonreference Site Detections (# sites)	Nonreference Site Detections (% sites)
Navicula viridulacalcis	4	0	0.0	4	2.4
Navicula vitabunda	1	0	0.0	1	0.6
Navicula wildii	1	1	1.5	0	0.0
Neidium	4	1	1.5	3	1.8
Neidium affine var longiceps	1	1	1.5	0	0.0
Neidium ampliatum	3	1	1.5	2	1.2
Neidium apiculatum var constrictum	1	0	0.0	1	0.6
Neidium binodeformis	8	1	1.5	7	4.2
Neidium binodis	1	0	0.0	1	0.6
Neidium dubium	4	2	2.9	2	1.2
Nitzschia	10	3	4.4	7	4.2
Nitzschia acicularis	24	9	13.2	15	8.9
Nitzschia agnita	12	1	1.5	11	6.5
Nitzschia amphibia	61	9	13.2	52	31.0
Nitzschia angustata	4	0	0.0	4	2.4
Nitzschia angustatula	8	1	1.5	7	4.2
Nitzschia archibaldii	55	15	22.1	40	23.8
Nitzschia aurariae	5	0	0.0	5	3.0
Nitzschia bacillum	11	5	7.4	6	3.6
Nitzschia capitellata	42	5	7.4	37	22.0
Nitzschia clausii	6	0	0.0	6	3.6
Nitzschia communis	51	17	25.0	34	20.2
Nitzschia compressa var vexans	6	1	1.5	5	3.0
Nitzschia desertorum	6	0	0.0	6	3.6
Nitzschia dissipata	158	45	66.2	113	67.3
Nitzschia dissipata var media	5	1	1.5	4	2.4
Nitzschia dubia	34	4	5.9	30	17.9
Nitzschia fasciculata	1	0	0.0	1	0.6
Nitzschia filiformis	8	1	1.5	7	4.2
Nitzschia fonticola	61	13	19.1	48	28.6
Nitzschia frustulum	57	13	19.1	44	26.2
Nitzschia gracilis	21	4	5.9	17	10.1
Nitzschia hantzschiana	3	0	0.0	3	1.8
Nitzschia heufleriana	78	23	33.8	55	32.7
Nitzschia inconspicua	192	48	70.6	144	85.7
Nitzschia intermedia	3	1	1.5	2	1.2
Nitzschia lacunarum	1	0	0.0	1	0.6
Nitzschia lacuum	61	13	19.1	48	28.6
Nitzschia lanceolata	1	0	0.0	1	0.6
Nitzschia levidensis var salinarum	1	0	0.0	1	0.6
Nitzschia liebethruthii	3	0	0.0	3	1.8
Nitzschia linearis	115	37	54.4	78	46.4
Nitzschia linearis var tenuis	1	1	1.5	0	0.0
Nitzschia lorenziana	1	1	1.5	0	0.0
Nitzschia microcephala	60	11	16.2	49	29.2
Nitzschia modesta	22	6	8.8	16	9.5
Nitzschia normanii	1	0	0.0	1	0.6
Nitzschia obtusa	1	0	0.0	1	0.6
Nitzschia palea	111	24	35.3	87	51.8
Nitzschia palea var debilis	45	8	11.8	37	22.0
Nitzschia paleacea	12	3	4.4	9	5.4
Nitzschia parvula	3	0	0.0	3	1.8

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections (# sites)	Reference Site Detections (% sites)	Nonreference Site Detections (# sites)	Nonreference Site Detections (% sites)
Nitzschia perminuta	8	1	1.5	7	4.2
Nitzschia pura	8	1	1.5	7	4.2
Nitzschia pusilla	12	2	2.9	10	6.0
Nitzschia radicula	6	3	4.4	3	1.8
Nitzschia recta	53	17	25.0	36	21.4
Nitzschia reversa	17	3	4.4	14	8.3
Nitzschia rosenstockii	5	1	1.5	4	2.4
Nitzschia sigma	10	3	4.4	7	4.2
Nitzschia sigmoidea	8	3	4.4	5	3.0
Nitzschia siliqua	4	2	2.9	2	1.2
Nitzschia sinuata var delognei	6	0	0.0	6	3.6
Nitzschia sinuata var tabellaria	3	0	0.0	3	1.8
Nitzschia sociabilis	41	9	13.2	32	19.0
Nitzschia solita	56	9	13.2	47	28.0
Nitzschia sp 1 CAL	8	3	4.4	5	3.0
Nitzschia subcohaerens var scotica	3	0	0.0	3	1.8
Nitzschia sublinearis	3	0	0.0	3	1.8
Nitzschia subtilis	23	4	5.9	19	11.3
Nitzschia supralitorea	29	5	7.4	24	14.3
Nitzschia tropica	60	14	20.6	46	27.4
Nitzschia umbonata	3	0	0.0	3	1.8
Nitzschia valdecostata	35	7	10.3	28	16.7
Nitzschia valdestriata	7	2	2.9	5	3.0
Nitzschia vermicularis	9	1	1.5	8	4.8
Nitzschia wuellerstorffii	2	1	1.5	1	0.6
Parlibellus protracta	24	4	5.9	20	11.9
Pinnularia	10	5	7.4	5	3.0
Pinnularia appendiculata	4	2	2.9	2	1.2
Pinnularia borealis	4	4	5.9	0	0.0
Pinnularia divergens var media	1	1	1.5	0	0.0
Pinnularia divergentissima	1	1	1.5	0	0.0
Pinnularia erraticofossilis	1	0	0.0	1	0.6
Pinnularia gibba	3	1	1.5	2	1.2
Pinnularia ignobilis	1	0	0.0	1	0.6
Pinnularia interrupta	1	1	1.5	0	0.0
Pinnularia lundii	2	1	1.5	1	0.6
Pinnularia microstauron	9	4	5.9	5	3.0
Pinnularia pogoii	1	0	0.0	1	0.6
Pinnularia sp 1 CSU	1	1	1.5	0	0.0
Pinnularia subcapitata	2	2	2.9	0	0.0
Pinnularia substomatophora	1	1	1.5	0	0.0
Pinnularia viridis	2	1	1.5	1	0.6
Placoneis abiskoensis	1	1	1.5	0	0.0
Placoneis clementis	9	3	4.4	6	3.6
Placoneis clementoides	1	0	0.0	1	0.6
Placoneis elginensis	3	1	1.5	2	1.2
Placoneis gastrum	7	1	1.5	6	3.6
Placoneis placentula	2	1	1.5	1	0.6
Placoneis pseudanglica	1	0	0.0	1	0.6
Plagiotropis lepidoptera var proboscidea	2	0	0.0	2	1.2
Planothidium apiculatum	43	6	8.8	37	22.0
Planothidium daui	2	0	0.0	2	1.2

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections (# sites)	Reference Site Detections (% sites)	Nonreference Site Detections (# sites)	Nonreference Site Detections (% sites)
Planothidium delicatulum	20	1	1.5	19	11.3
Planothidium engelbrechtii	31	10	14.7	21	12.5
Planothidium frequentissimum	198	52	76.5	146	86.9
Planothidium granum	14	1	1.5	13	7.7
Planothidium haynaldii	19	6	8.8	13	7.7
Planothidium lanceolatum	173	51	75.0	122	72.6
Planothidium minutissimum	1	0	0.0	1	0.6
Planothidium rostratum	12	2	2.9	10	6.0
Platessa conspicua	86	18	26.5	68	40.5
Platessa hustedtii	2	0	0.0	2	1.2
Platessa rupestris	1	0	0.0	1	0.6
Pleurosigma delicatulum	1	0	0.0	1	0.6
Pleurosigma elongatum	17	2	2.9	15	8.9
Pleurosigma salinarum	13	3	4.4	10	6.0
Pleurosira laevis	7	1	1.5	6	3.6
Psammodictyon constrictum	1	0	0.0	1	0.6
Psammothidium	3	1	1.5	2	1.2
Psammothidium bioretii	4	1	1.5	3	1.8
Psammothidium grischunum fo daonensis	1	1	1.5	0	0.0
Pseudostaurosira	1	0	0.0	1	0.6
Pseudostaurosira brevistriata	39	6	8.8	33	19.6
Pseudostaurosira parasitica	42	7	10.3	35	20.8
Pseudostaurosira parasitica var subconstricta	3	0	0.0	3	1.8
Pseudostaurosira subsalina	7	1	1.5	6	3.6
Pseudostaurosira trainorii	2	0	0.0	2	1.2
Pseudostaurosiropsis connecticutensis	1	0	0.0	1	0.6
Reimeria sinuata	78	19	27.9	59	35.1
Reimeria uniseriata	77	20	29.4	57	33.9
Rhoicosphenia abbreviata	188	48	70.6	140	83.3
Rhopalodia acuminata	1	1	1.5	0	0.0
Rhopalodia brebissonii	34	9	13.2	25	14.9
Rhopalodia gibba	37	15	22.1	22	13.1
Rhopalodia rupestris	3	2	2.9	1	0.6
Sellaphora bacillum	29	8	11.8	21	12.5
Sellaphora hustedtii	13	1	1.5	12	7.1
Sellaphora laevissima	9	1	1.5	8	4.8
Sellaphora pupula	45	10	14.7	35	20.8
Sellaphora seminulum	54	19	27.9	35	20.8
Sellaphora stroemii	4	3	4.4	1	0.6
Simonsenia delognei	36	10	14.7	26	15.5
Stauroforma exiguiformis	1	0	0.0	1	0.6
Stauroneis anceps fo gracilis	1	0	0.0	1	0.6
Stauroneis kriegeri	10	4	5.9	6	3.6
Stauroneis kriegerii	4	2	2.9	2	1.2
Stauroneis obtusa	2	0	0.0	2	1.2
Stauroneis phoenicenteron	1	0	0.0	1	0.6
Stauroneis producta	1	0	0.0	1	0.6
Stauroneis smithii	30	4	5.9	26	15.5
Stauroneis tackei	1	0	0.0	1	0.6
Stauroneis thermicola	1	0	0.0	1	0.6
Staurosira construens	5	0	0.0	5	3.0
Staurosira construens var binodis	8	1	1.5	7	4.2

Table 8 (cont'd). The number of sites where diatom species were found.

Diatom Species	Number of Site Detections	Reference Site Detections (# sites)	Reference Site Detections (% sites)	Nonreference Site Detections (# sites)	Nonreference Site Detections (% sites)
Staurosira construens var venter	66	14	20.6	52	31.0
Staurosira elliptica	4	1	1.5	3	1.8
Staurosirella leptostauron	1	0	0.0	1	0.6
Staurosirella pinnata	65	6	8.8	59	35.1
Staurosirella pinnata var intercedens	1	1	1.5	0	0.0
Stenopterobia curvula	2	1	1.5	1	0.6
Stephanodiscus hantzschii	9	0	0.0	9	5.4
Stephanodiscus medius	3	0	0.0	3	1.8
Stephanodiscus minutulus	5	0	0.0	5	3.0
Surirella	2	1	1.5	1	0.6
Surirella amphioxys	38	8	11.8	30	17.9
Surirella angusta	21	8	11.8	13	7.7
Surirella brebissonii	11	2	2.9	9	5.4
Surirella brebissonii var kuetzingii	1	0	0.0	1	0.6
Surirella brightwellii	10	3	4.4	7	4.2
Surirella linearis	1	0	0.0	1	0.6
Surirella minuta	51	9	13.2	42	25.0
Surirella ovalis	15	2	2.9	13	7.7
Surirella robusta	1	0	0.0	1	0.6
Surirella splendida	7	1	1.5	6	3.6
Surirella tenera	8	1	1.5	7	4.2
Synedra acus	25	9	13.2	16	9.5
Synedra delicatissima	4	0	0.0	4	2.4
Synedra goulardi	1	0	0.0	1	0.6
Synedra mazamaensis	7	4	5.9	3	1.8
Synedra rumpens	4	2	2.9	2	1.2
Synedra ulna	122	41	60.3	81	48.2
Synedra ulna var amphirhynchus	3	3	4.4	0	0.0
Tabularia fasciculata	21	3	4.4	18	10.7
Tabularia tabulata	25	2	2.9	23	13.7
Thalassionema nitzschioides	1	0	0.0	1	0.6
Thalassiosira weissflogii	13	4	5.9	9	5.4
Tryblionella apiculata	74	11	16.2	63	37.5
Tryblionella calida	12	2	2.9	10	6.0
Tryblionella compressa	8	1	1.5	7	4.2
Tryblionella constricta	2	0	0.0	2	1.2
Tryblionella hungarica	18	0	0.0	18	10.7
Tryblionella levidensis	1	0	0.0	1	0.6
Tryblionella littoralis	19	3	4.4	16	9.5
Tryblionella scalaris	11	2	2.9	9	5.4
Tryblionella victoriae	10	1	1.5	9	5.4

Table 9. The number of sites where soft algae species were found as well as the number of reference sites (out of 8 total reference sites) and number of nonreference sites (out of 56 total nonreference sites) where they were found.

Soft Algae Species	Number of Site Detections	Reference Site		Nonreference Site	
		(# sites)	(% sites)	(# sites)	(% sites)
Anabaena	5	2	25	3	9.7
Arthrospira sp.	0	0	0	0	0.0
Arthrospira massartii	1	0	0	1	3.2
Borzia sp.	0	0	0	0	0.0
Calothrix	4	1	12.5	3	9.7
Ceratium hirudinella	1	0	0	1	3.2
Chroococcus	1	0	0	1	3.2
Chroococcus <5µm MUSSRW	1	0	0	1	3.2
Chroococcus >5µm MUSSRW	2	0	0	2	6.5
Chroococcus minimus	1	1	12.5	0	0.0
Chroococcus minutus	3	1	12.5	2	6.5
Closterium	2	1	12.5	1	3.2
Cosmarium	1	0	0	1	3.2
Euglena gracilis	1	0	0	1	3.2
Gloeocapsa	2	0	0	2	6.5
Homoeothrix	3	1	12.5	2	6.5
Homoeothrix janthina	30	7	87.5	23	74.2
Homoeothrix juliana	2	0	0	2	6.5
Leptolyngbya	1	0	0	1	3.2
Lyngbya	3	1	12.5	2	6.5
Merismopedia	1	0	0	1	3.2
Microspora	5	0	0	5	16.1
Mougeotia sp 1	4	0	0	4	12.9
Oedogonium	3	0	0	3	9.7
Oscillatoria	1	0	0	1	3.2
Pediastrum sp.	0	0	0	0	0.0
Pediastrum duplex	2	0	0	2	6.5
Pediastrum tetras	1	0	0	1	3.2
Phormidium	17	4	50	13	41.9
Pseudanabaena sp.	0	0	0	0	0.0
Rossithidium duthii	1	0	0	1	3.2
Scenedesmus sp.	0	0	0	0	0.0
Scenedesmus longus	1	0	0	1	3.2
Scenedesmus quadricauda	3	0	0	3	9.7
Spirogyra	1	0	0	1	3.2
Spirulina	3	1	12.5	2	6.5
Staurastrum	1	0	0	1	3.2

Table 10. IBI Metrics for California's Central Coast. Metrics with the highest responsiveness for each metric class, measured as the absolute value of the *t*-statistic. Metrics in bold were used in the final IBI. Production metrics are not shown because they all failed the reproducibility criterion (S/N > 1.5).

Metric	Abbreviation	*t*-Statistic	Notes
Autecological Metrics			
Proportion of species in van Dam's trophic class 5	**prop.spp.Trophic5**	4.936	
Proportion of individuals in van Dam's salinity class 1	prop.ind.Salinity1	4.6474	Doesn't meet reproducibility criterion
Richness of van Dam oxygen requirement class 5 species	OxyReq5.richness	4.4492	Doesn't meet reproducibility criterion
Proportion of species in van Dam's trophic class 5,6,&7	prop.spp.Trophic567	4.3203	Correlated with prop.spp.Trophic5
Weighted average of van Dam salinity score	**weighted.Salinity**	4.2609	
Proportion of species in van Dam's oxygen requirement class 5	**prop.spp.OxyTol5**	4.0097	
Community Structure Metrics			
Proportion of species *Epithemia*	**prop.Epithemia**	-6.0657	Positive correlation with elevation Correction based on reference site regression: Expected = 0.01636+0.00004567*Elevation; Elevation-corrected metric = Obs. – Exp. Elevation-corrected *t*=5.9543.
Richness of species belonging to the genus *Epithemia*	Epithemia.richness	-5.8267	Correlated with prop.Epithemia
Proportion of individuals belonging to the genus *Achnanthes* dividied by the sum of individulags belonging to the genera *Achnanthes* plus *Navicula*	**prop.ind.AchOverAchPlusNavOld**	-5.3165	
Proportion of species *Amphora*	**prop.Amphora**	5.4003	
Richness of species belonging to the genus *Amphora*	Amphora.richness	5.4899	Correlated with prop.Amphor
Ecological Guild Metrics			
Proportion of individuals with minimal motility	**prop.ind.MinMotility**	-4.4087	
Proportion of individuals with vertical morphology	**prop.ind.vert.morph**	3.1915	
Proportion of species with vertical morphology	prop.spp.vert.morph	3.5422	Doesn't meet reproducibility criterion
Tolerance/Intolerance Metrics			
Proportion of individuals in the California Central Coast most tolerant	**prop.ind.ccc.most.tol**	-3.9883	
Weighted average of Bahl's pollution tolerance score	weighted.PolTol	-3.7238	Positive correlation with slope. After correction, prop.ind.PolTol3 had a higher *t*-value.
Proportion of individuals classified as Bahl's pollution tolerance class 3	**prop.ind.PolTol3**	-3.2481	
Proportion of individuals in the California Central Coast most sensitive	**prop.ind.ccc.most.intol**	5.3238	

Figure 3. Central Coast region as defined by Central Coast Regional Water Quality Control Board (California Interagency Watershed Map of 1999); diatom sample-site locations including reference and degraded sites and National Land Cover Dataset (2001); shaded relief derived from USGS National Elevation Dataset.

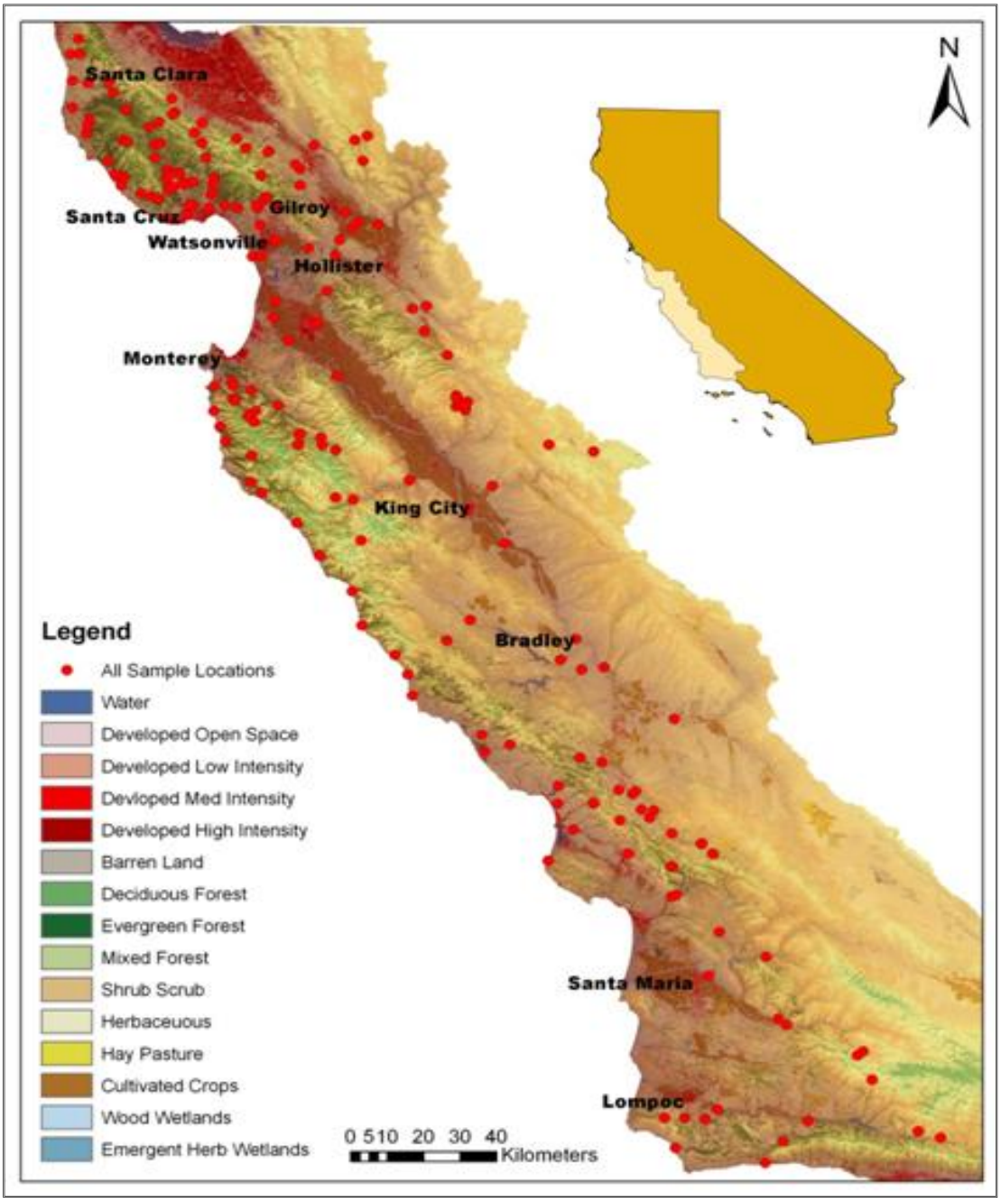


Figure 4. Reference sites (63 out of 221 sampling locations) were identified using DFG criteria. Locations and Site IDs of reference sites within hydraulic units (HUC) are displayed.

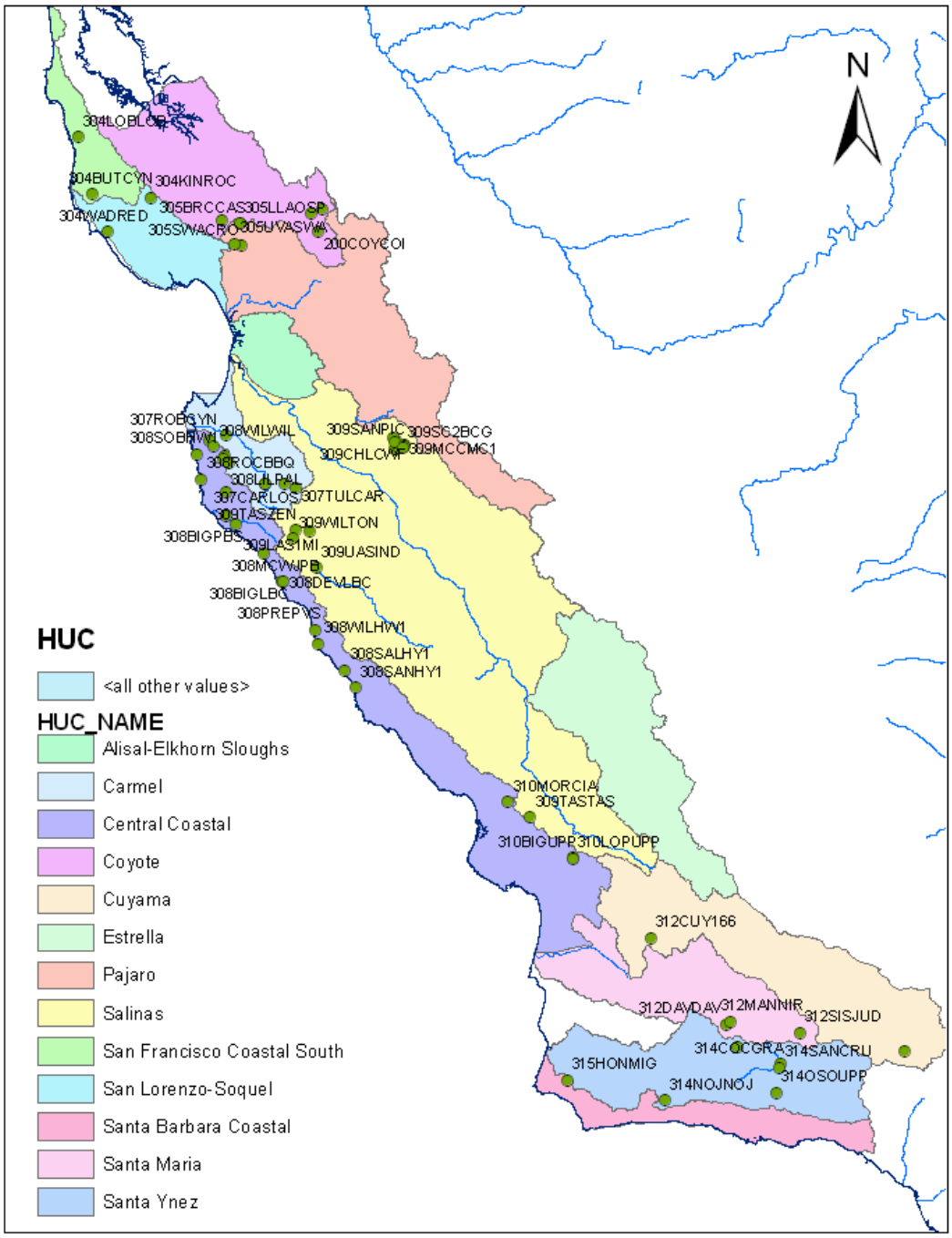


Figure 5. Nonreference sites (158 out of 221 sampling locations) were identified using DFG criteria. Locations and Site IDs of reference sites within hydraulic units (HUC) are displayed.

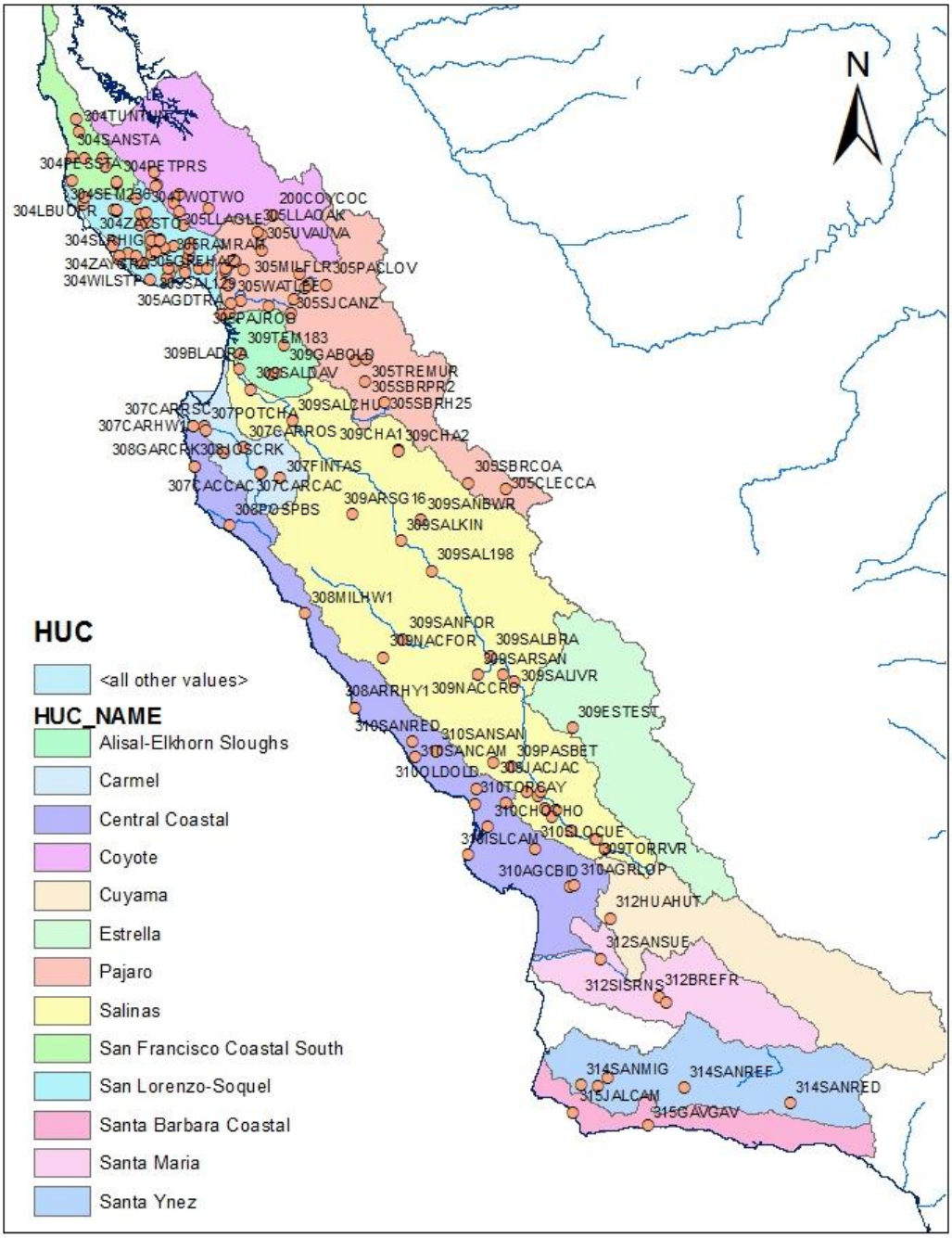


Figure 6. Histograms of log abundance for the most prevalent species found on California's Central Coast. Species are ordered from those most frequently detected (*Amphora pediculus* at 199 sites, 68% of reference and 68% of nonreference sites) to the 12th most frequently detected (*Synedra ulna* at 122 sites, 60% of reference sites and 48% of nonreference sites).

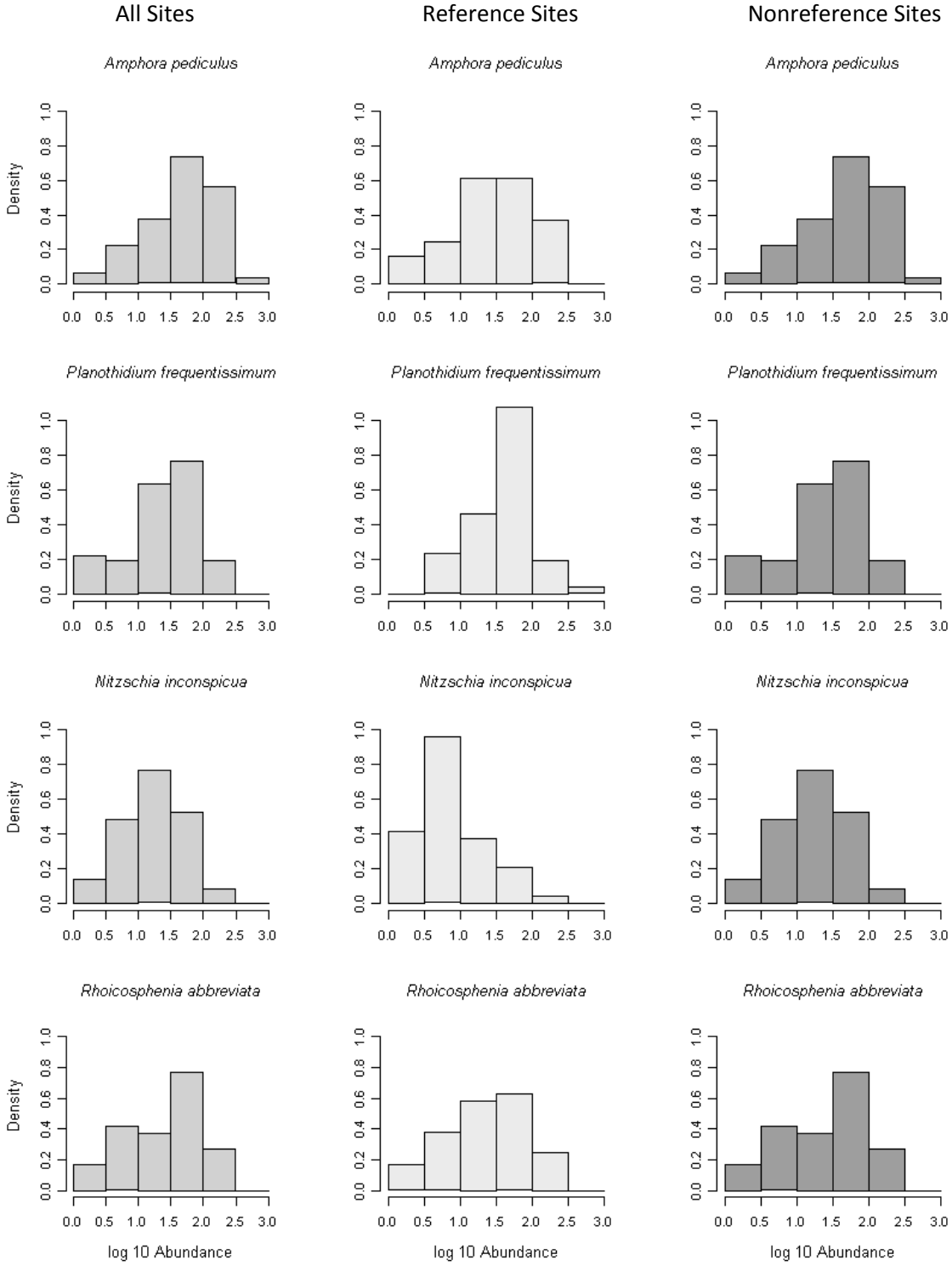


Figure 6 (cont'd). Histograms of log abundance for the most prevalent species found on California's Central Coast.

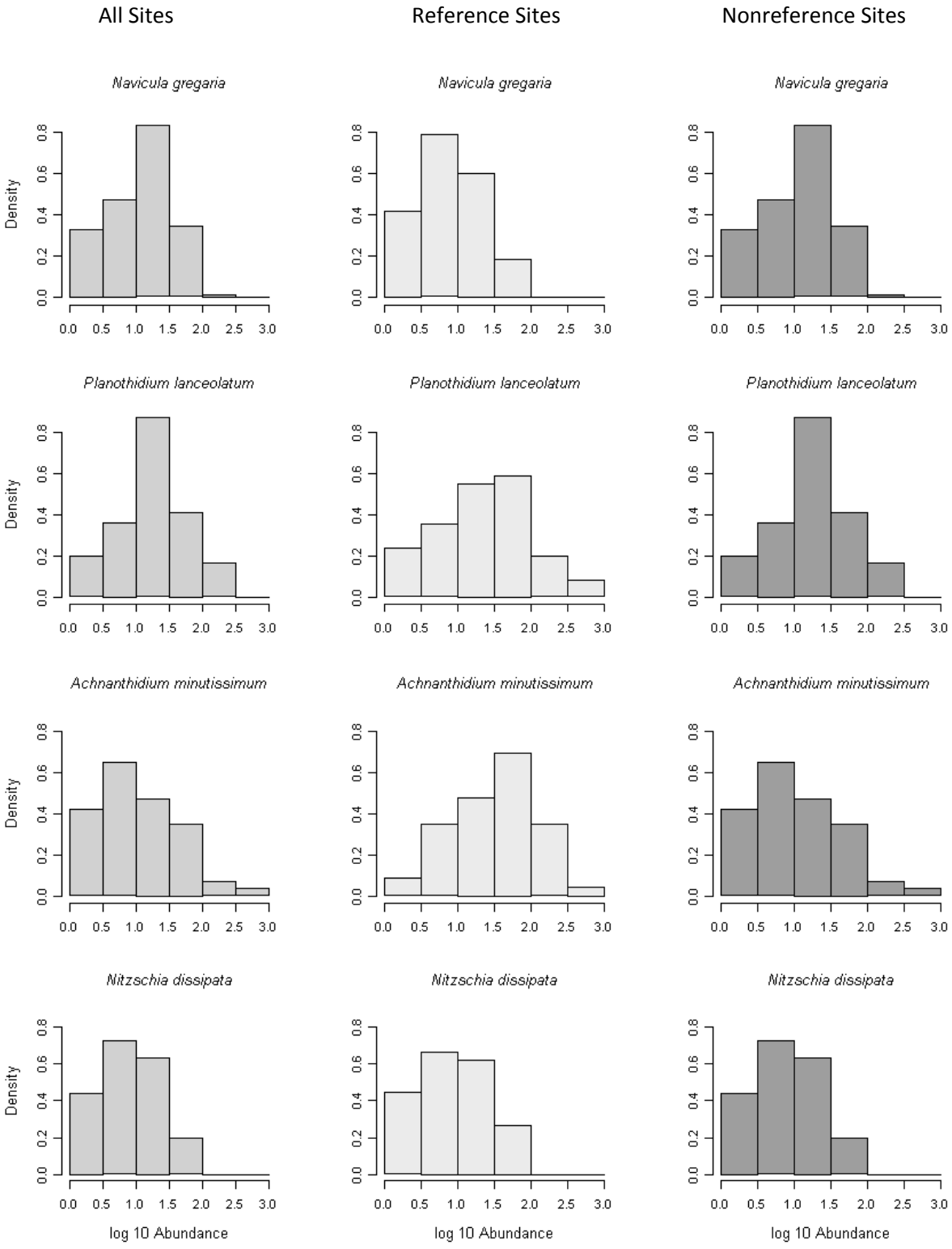


Figure 6 (cont'd). Histograms of log abundance for the most prevalent species found on California's Central Coast.

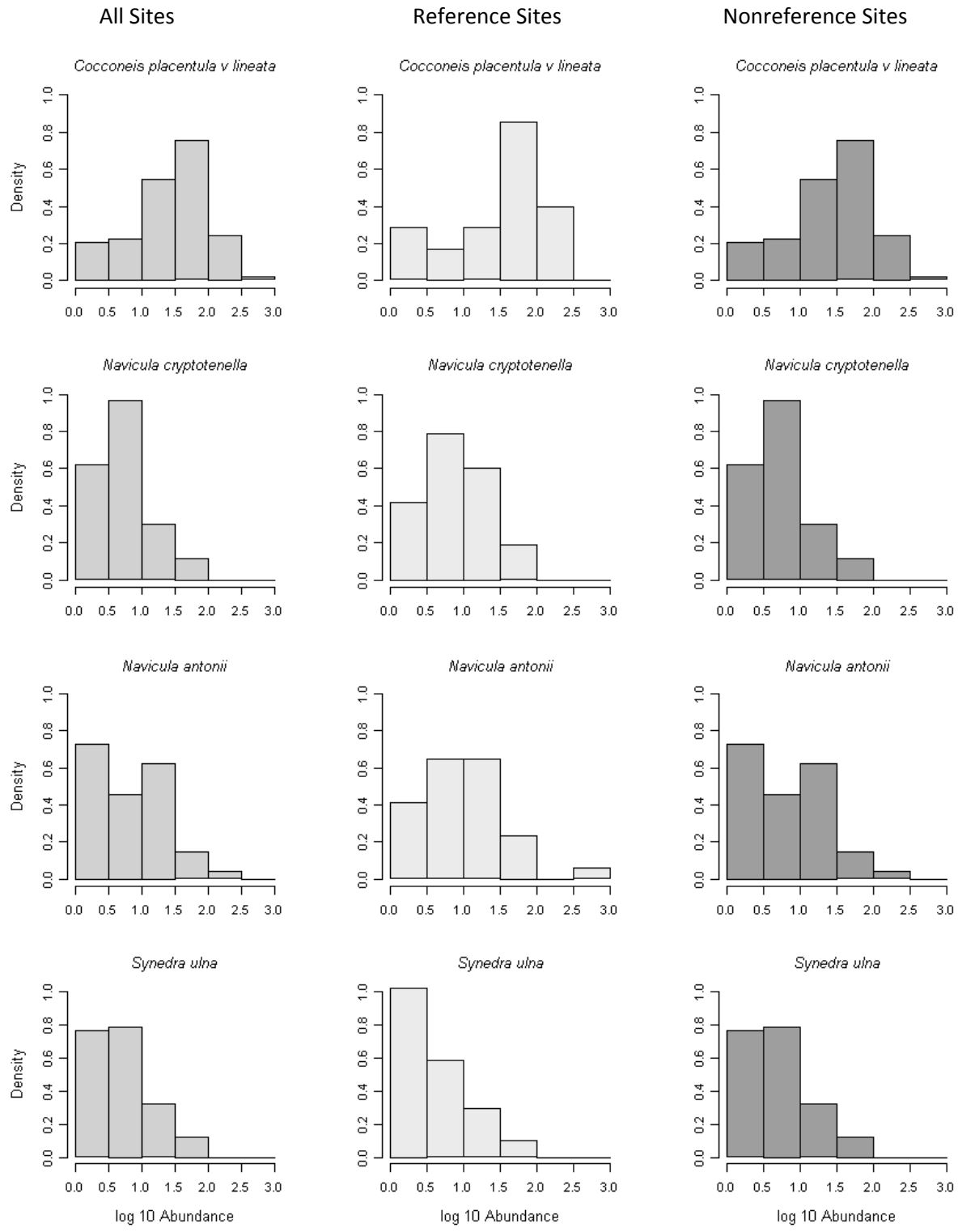


Figure 7. Boxplots of individual IBI metrics to human disturbance. "Best" diatom samples site quality were those collected from sites that met all DFG reference criteria. "Worst" site quality were those samples collected from sites that failed >20% of the DFG reference criteria. The 20% cutoff produced a "worst" class (n=75) with a diatom sample size similar to that of the "best" class (n=72).
A: Responsiveness of the proportion of species classified by van Dam as eutraphentic (nutrient tolerant) to human disturbance. B: Responsiveness of the abundance weighted-average of van Dam salinity value to human disturbance. Responsiveness of the proportion of species classified by van Dam as having very low oxygen requirements to human disturbance.

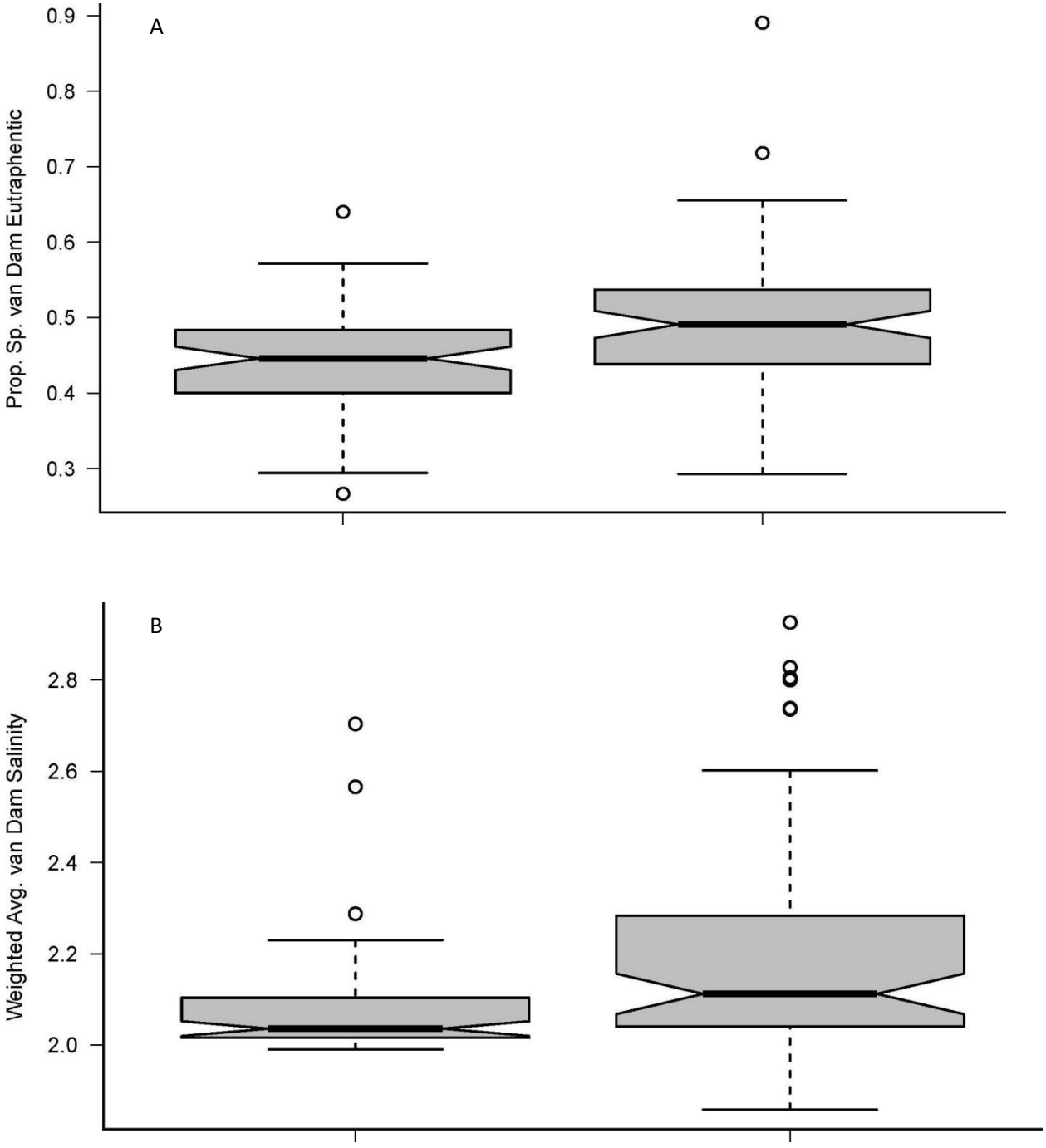


Figure 7 (cont'd). Boxplots of individual IBI metrics to human disturbance. C: Responsiveness of the proportion of species classified by van Dam as having very low oxygen requirements to human disturbance. D: Responsiveness of the proportion of species in the genus *Amphora* to human disturbance.

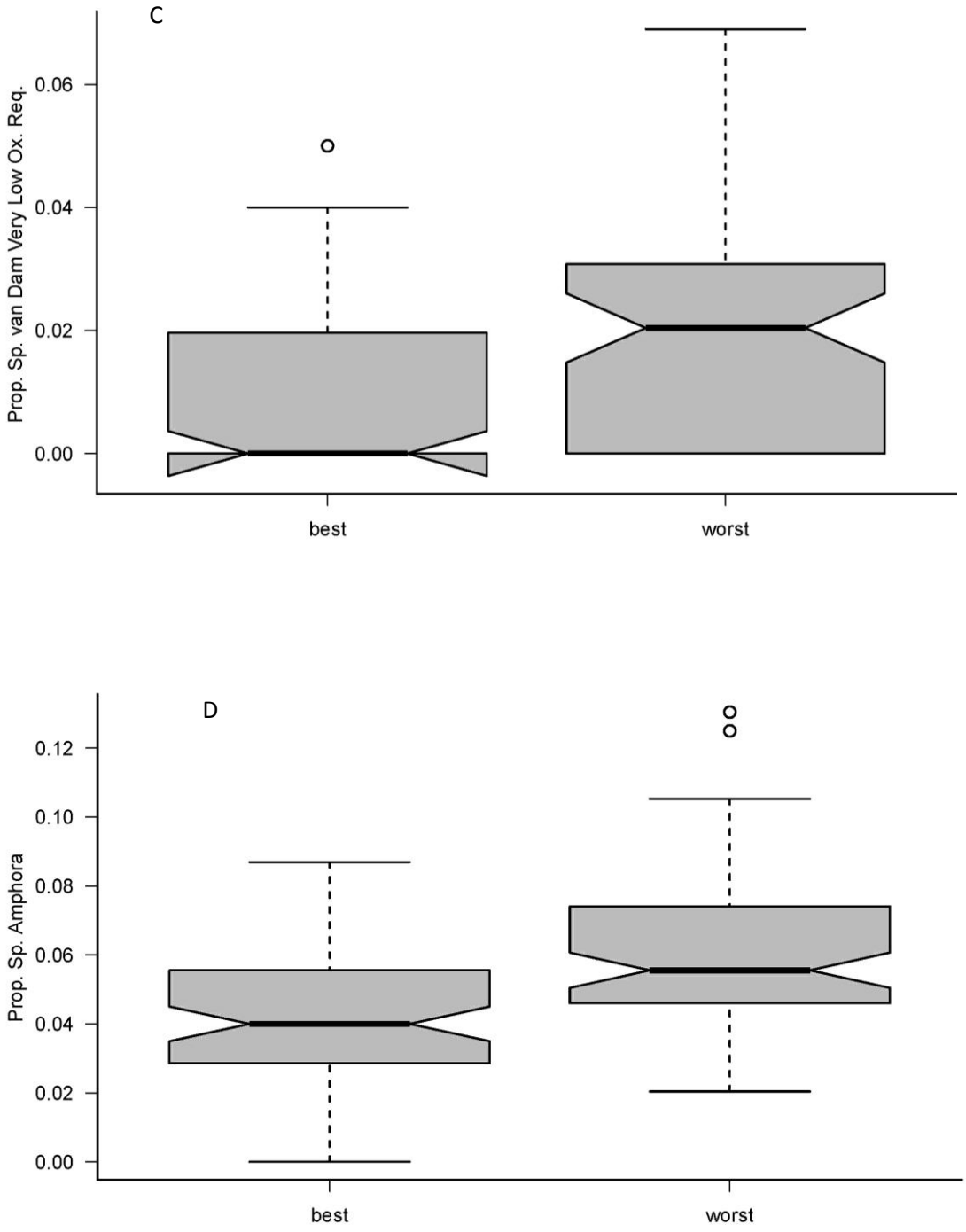


Figure 7 (cont'd). Boxplots of individual IBI metrics to human disturbance. E. Responsiveness of the proportion of species that belong to the genus *Epithemia* to human disturbance. F. Responsiveness of the ratio of the proportion of individuals that belong to the genus *Achnanthes* to the sum of individuals belonging to *Achnanthes* and *Navicula* (using the old classification) to human disturbance.

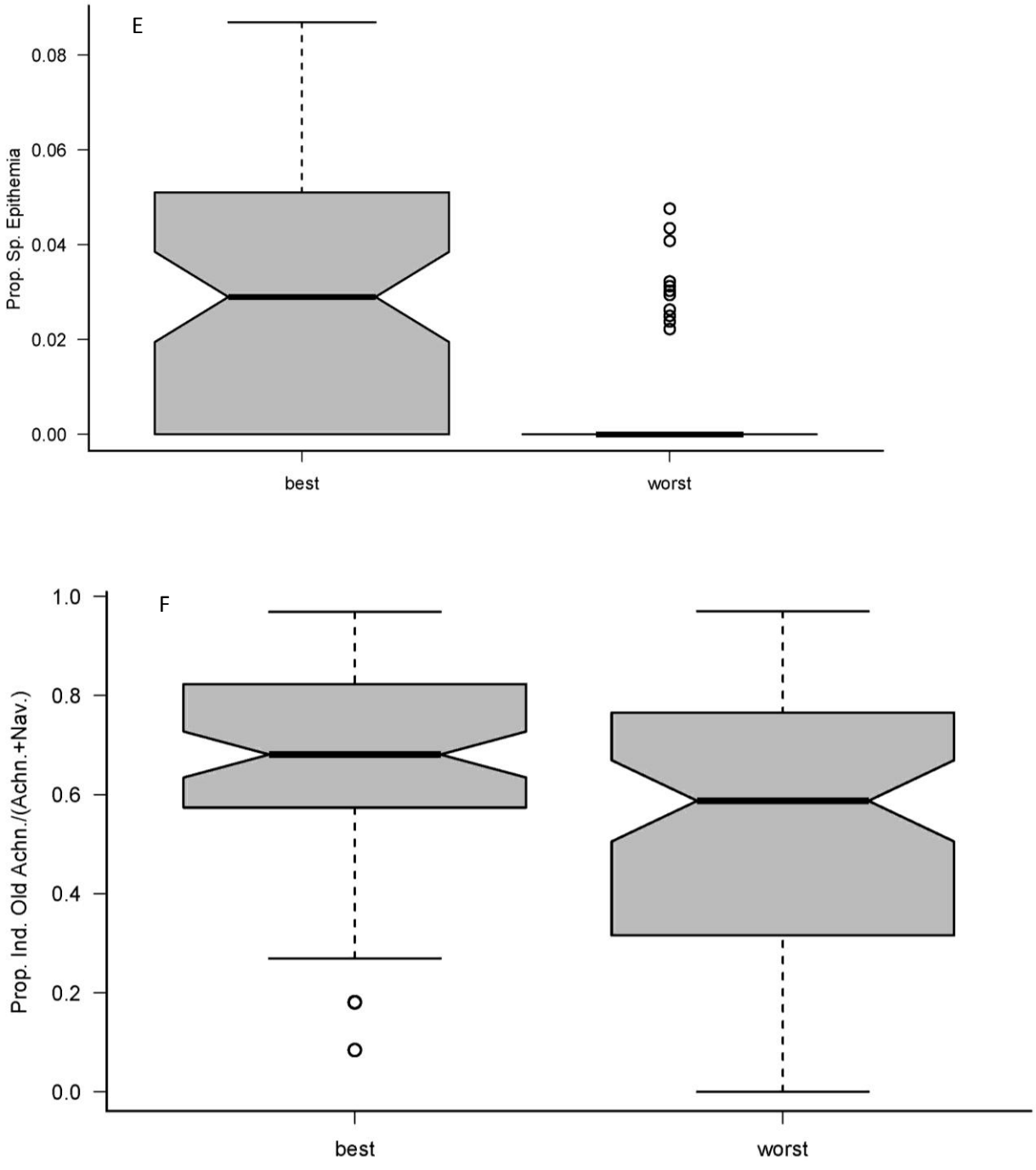


Figure 7 (cont'd). Boxplots of individual IBI metrics to human disturbance. G. Responsiveness of the proportion of individuals with minimal motility to human disturbance. H. Responsiveness of the proportion of individuals with vertical growth morphology to human disturbance.

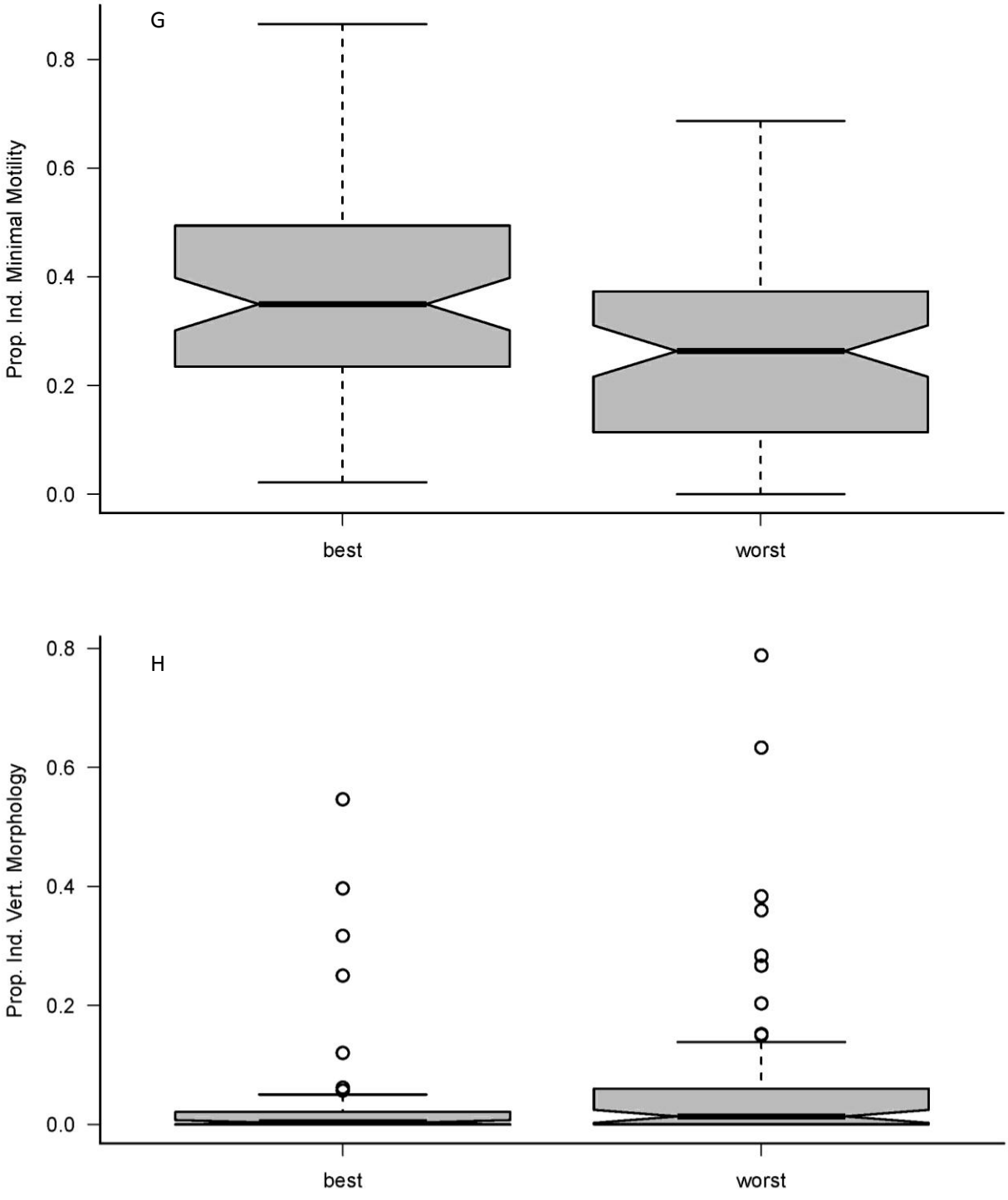


Figure 7 (cont'd). I. Responsiveness of the proportion of individuals classified as among the most tolerant species on the California Central Coast to human disturbance. J. Responsiveness of the proportion of individuals classified as among the least tolerant species on the California Central Coast to human disturbance.

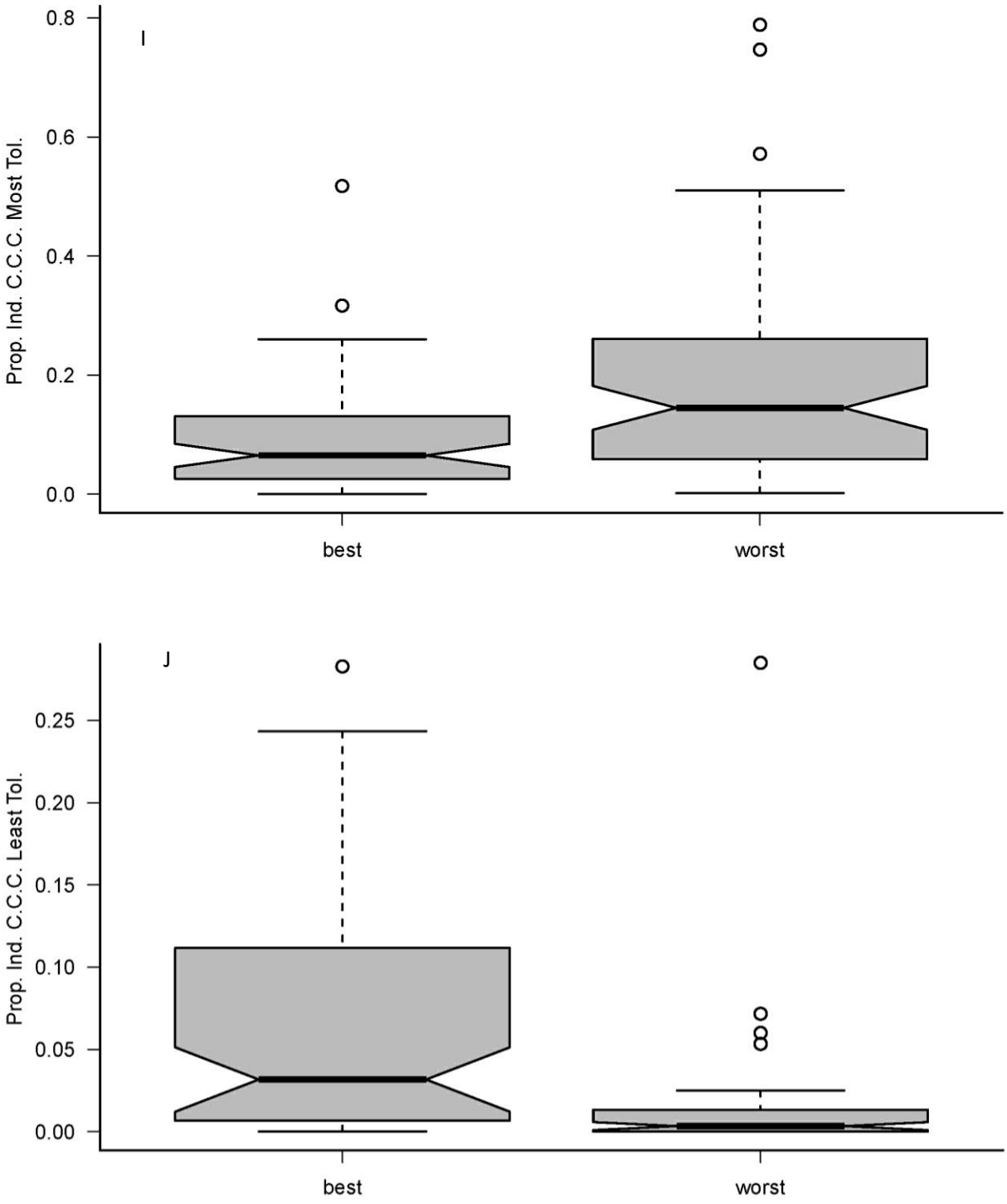


Figure 7 (cont'd). Boxplots of individual IBI metrics to human disturbance. K. Responsiveness of the proportion of individuals classified by Bahls as the most sensitive to human disturbance.

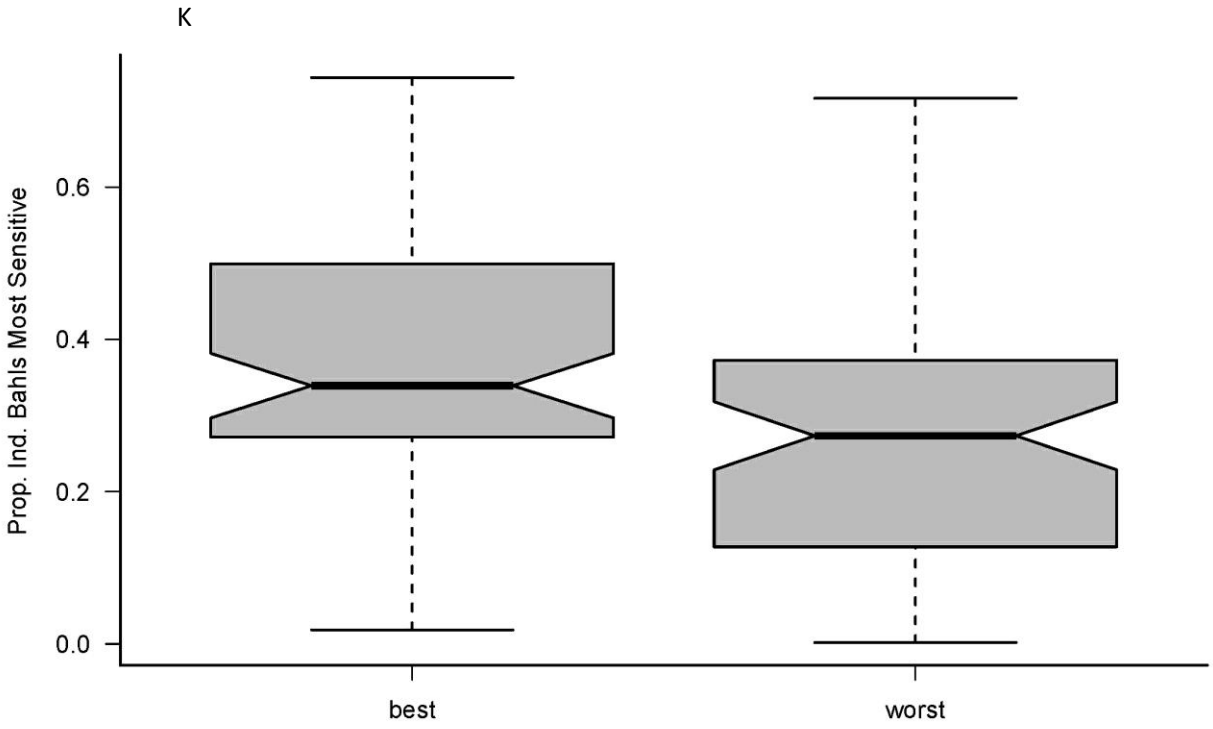


Figure 8. Response of the multimetric algal index of biotic integrity (IBI) to the human disturbance gradient (HDG). The solid line represents the least squares linear regression (IBI = 64.205-47.930(HDG), $p < 0.001$). Dashed lines represent the 95% confidence interval.

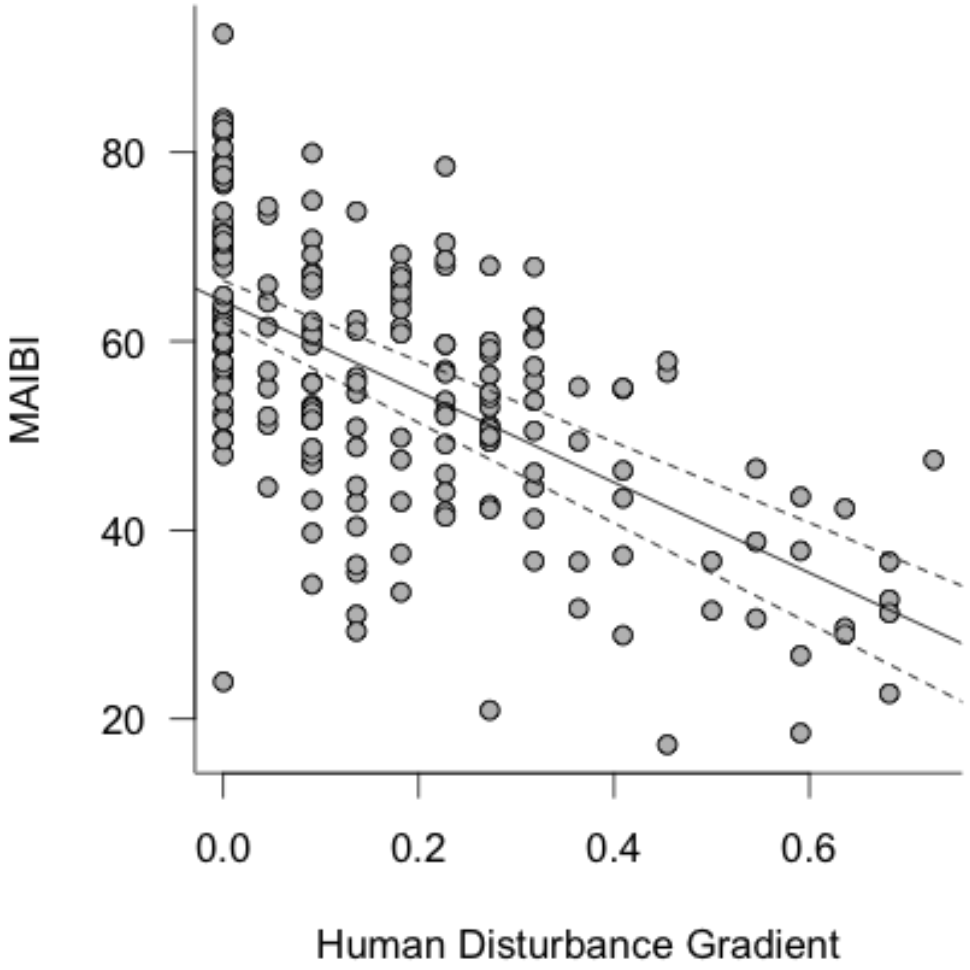


Figure 9. Boxplot highlighting multimetric algae index of biotic integrity (IBI) to human disturbance. The median IBI score for sites with the lowest human disturbance ("best" human disturbance class; n=72) was 64.48 and the median score for sites with the most human disturbance ("worst" human disturbance class; n=75) was 49.38. Best sites met all Department of Fish and Game reference criteria. Worst sites failed >20% of reference criteria. The 20% cutoff was selected to create two classes of approximately equal size.

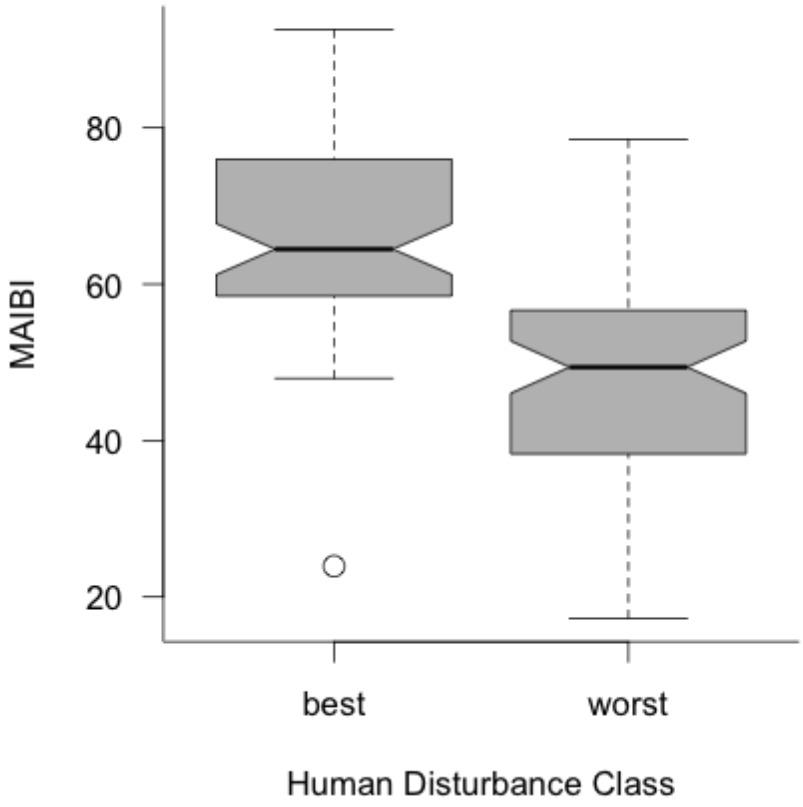


Figure 10. Boxplot highlighting the differences in the multimetric algae index of biotic integrity (IBI) for reference and nonreference sites. Horizontal dashed lines represent proposed IBI score classes. IBI values greater than the first quartile of the reference sample IBI distribution (>58) are considered in "good" condition; sites lower than the first quartile but greater than the lowest value (excluding a single outlier; 48 to 58) are in "fair" condition; sites lower than the lowest value for the reference sites (excluding a single outlier) are in poor condition.

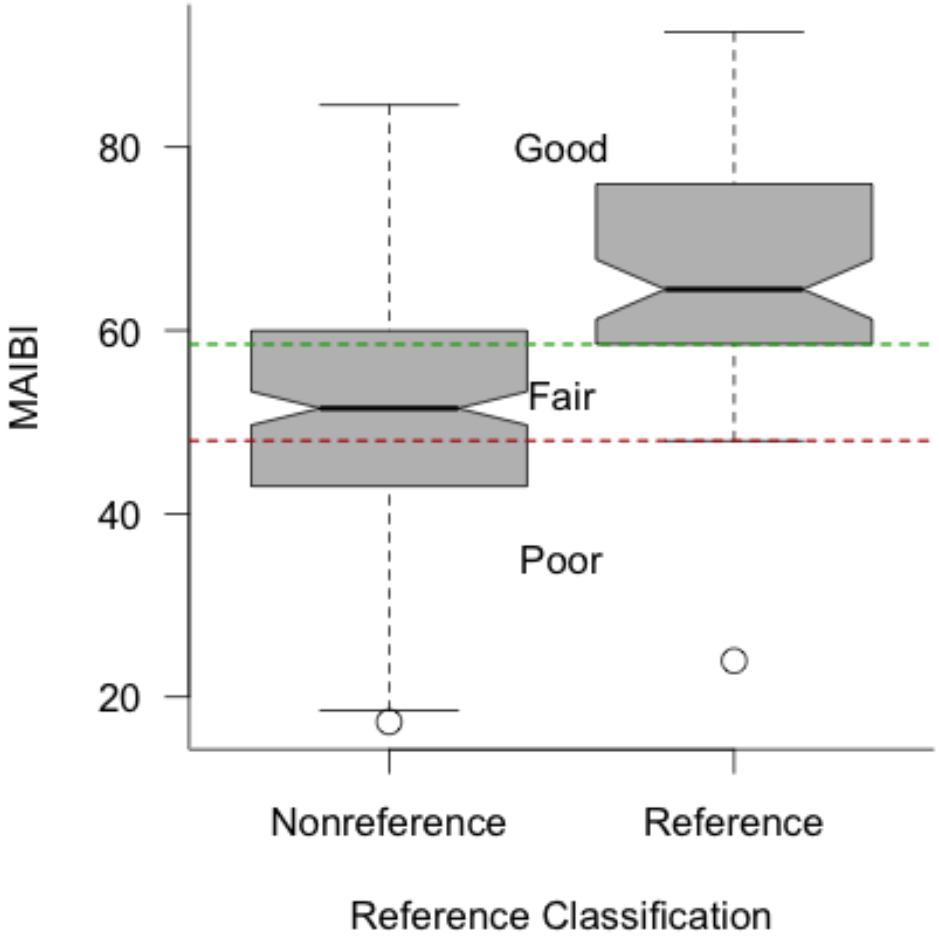
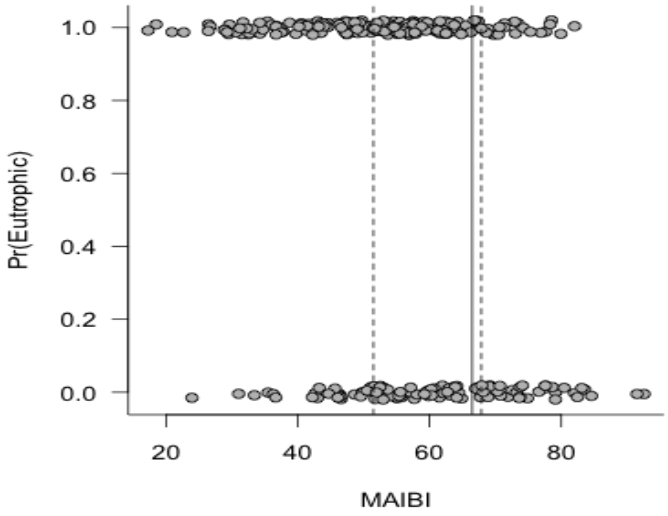


Figure 11. IBI thresholds for eutrophic and trophic status indices. A) Threshold for the IBI using the probability of eutrophic classification as the endpoint. The solid line represents the median of the nonparametric changepoint distribution and the dashed lines represent the first and third quartile. B) Threshold for the IBI using the trophic status index (TSI) as the endpoint.

A



B

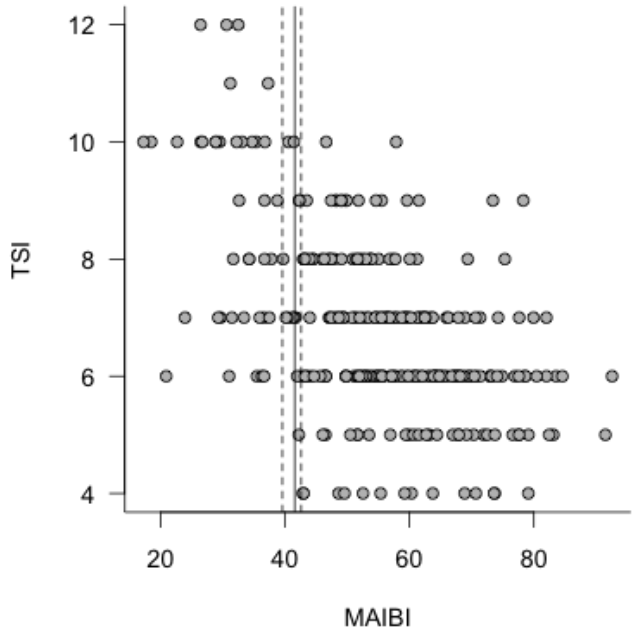


Figure 12. Threshold for the IBI using the PCA-derived trophic status index as the endpoint. The solid line represents the median of the nonparametric changepoint distribution and the dashed lines represent the first and third quartile.

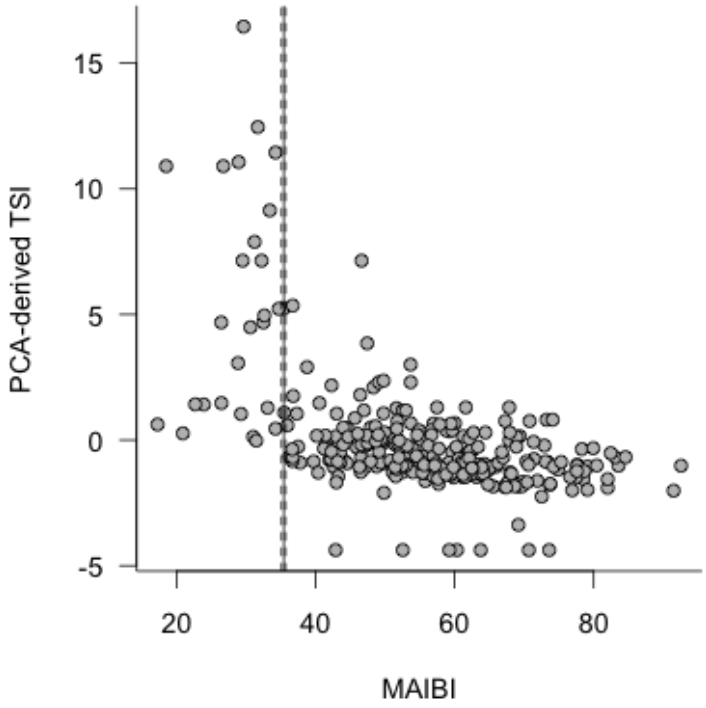


Figure 13. Change-point analysis of stressor-response variables TN and TP. A) Application of the IBI for total nitrogen (TN) criteria development. Solid line represents the median of the nonparametric changepoint. B) Application of the IBI for total phosphorus (TP) criteria development. Solid line represents the median of the nonparametric changepoint.

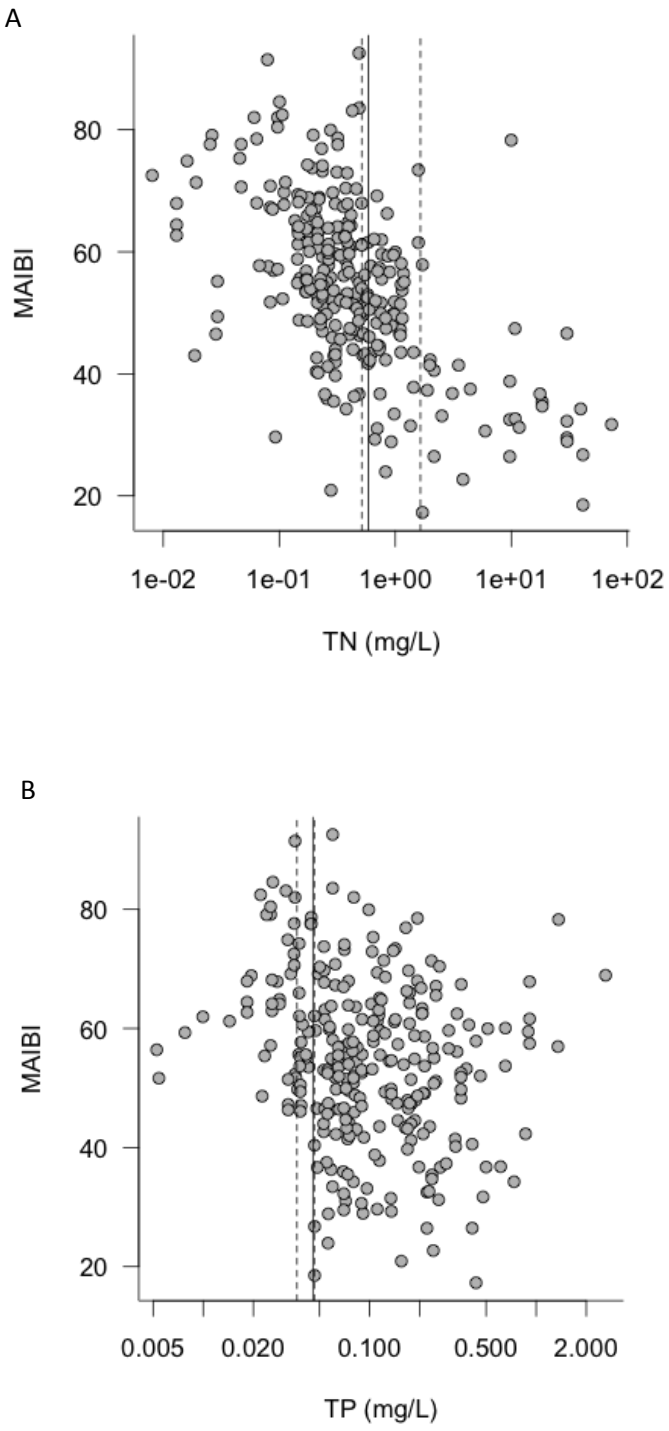
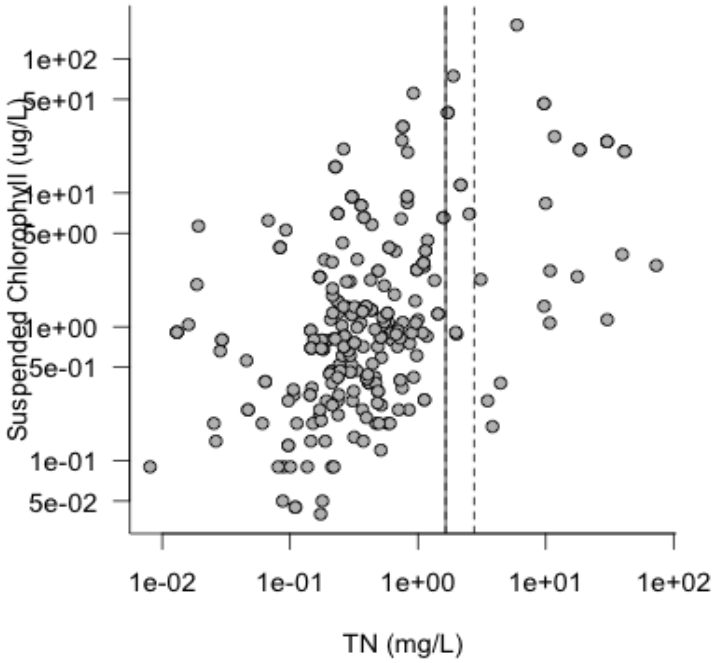


Figure 14. Nonparametric changepoint analysis applied to the TN-Suspended Chlorophyll stressor-response relationship. Solid line represents the median of the nonparametric changepoint. Nonparametric changepoint analysis applied to the TN-Benthic Chlorophyll stressor-response relationship. Solid line represents the median of the nonparametric changepoint.

A



B

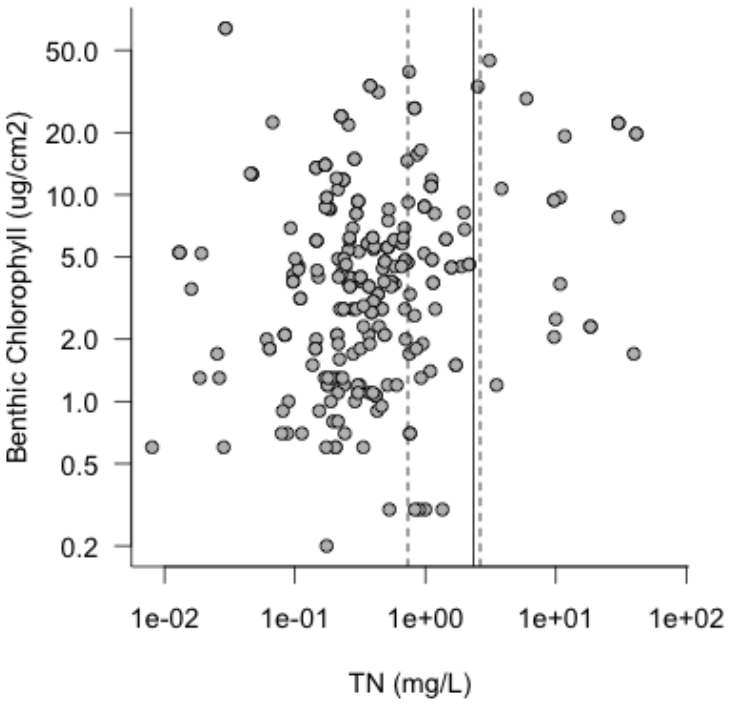


Figure 15. Nonparametric changepoint analysis applied to the TP-Suspended Chlorophyll stressor-response relationship. Solid line represents the median of the nonparametric changepoint. B) Nonparametric changepoint analysis applied to the TP-Benthic Chlorophyll stressor-response relationship. Solid line represents the median of the nonparametric changepoint.

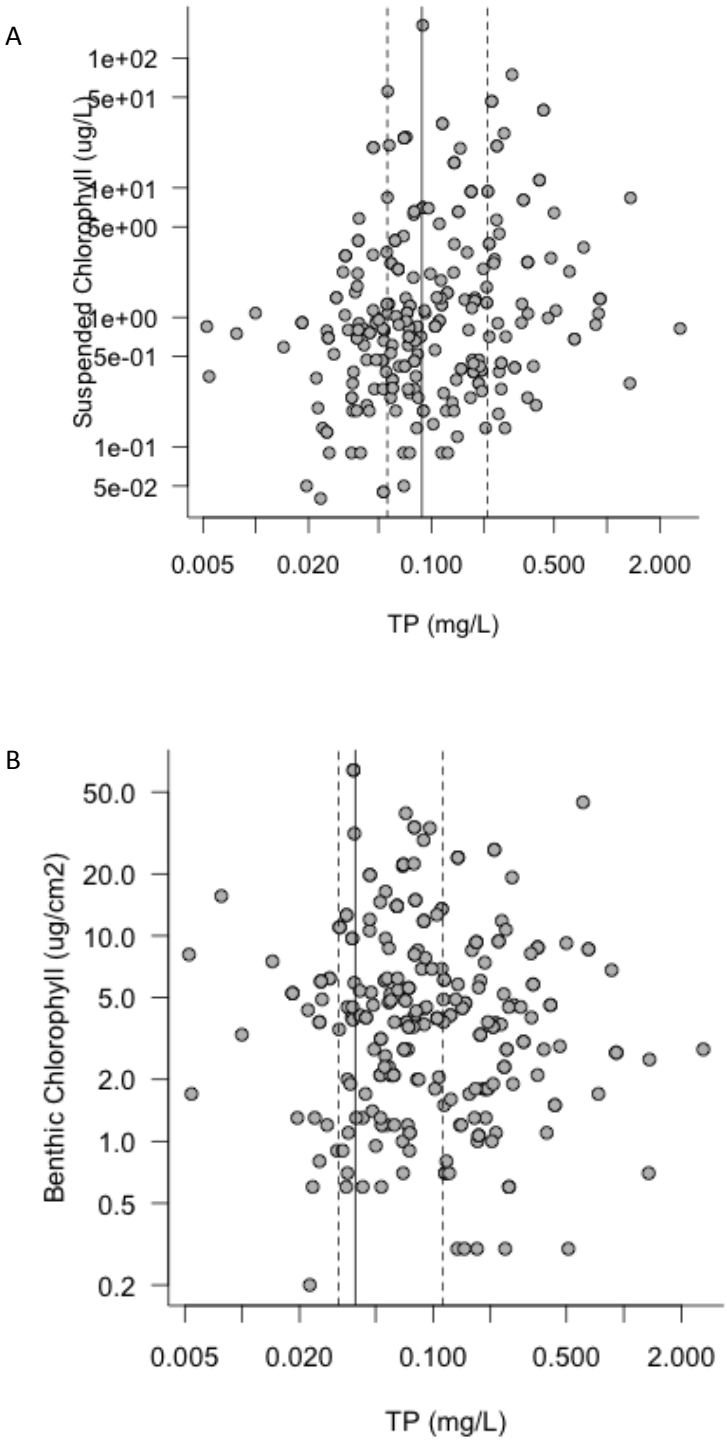


Figure 16. Nonparametric changepoint analysis applied to the nitrate-MAIBI relationship. White data points are nitrate measures that were below the detection limit. Solid black vertical line represents the median nitrate threshold of the nonparametric changepoint distribution. Black dashed lines represent the first and third quartiles of the distribution. Red dotted line represents the proposed screening criterion for nitrate to protect aquatic life uses (Worcester et al. 2010). The blue line shows the smoothed density distribution for the threshold distribution. At 1.0 mg/L, there is an 86% chance that the threshold has been surpassed.

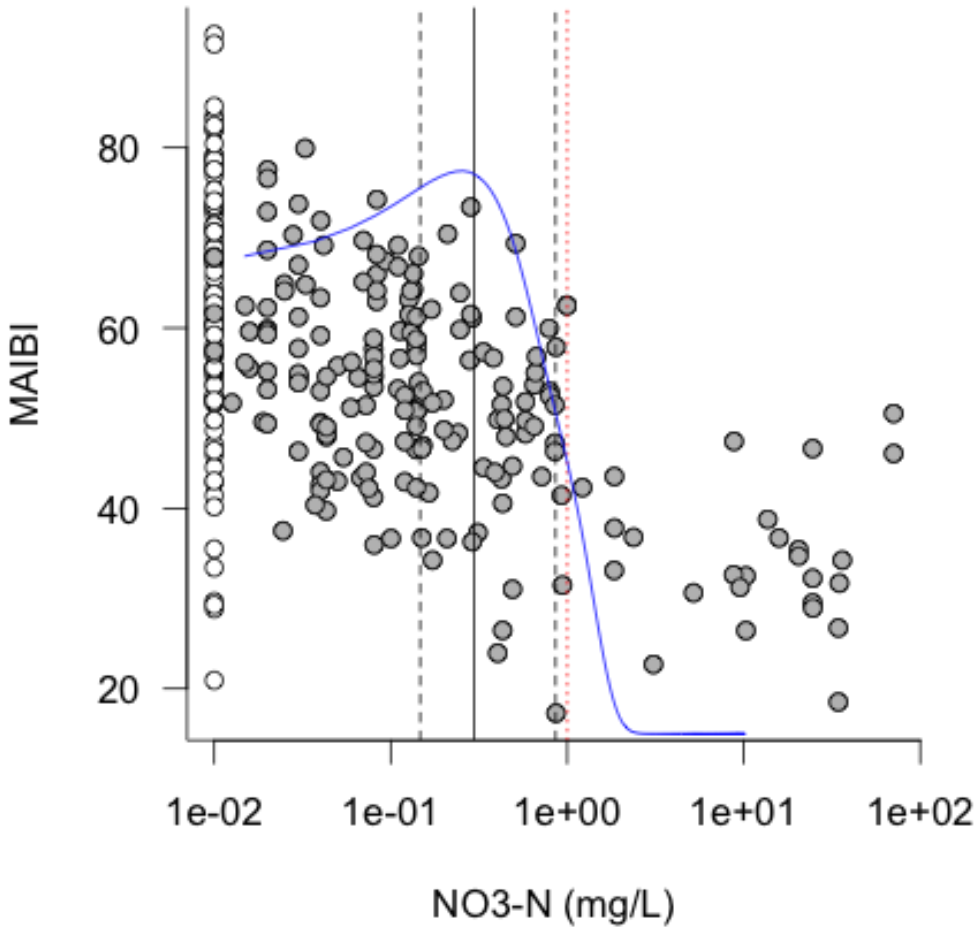


Figure 17. Histograms show that higher concentrations of nitrogenous compounds were observed in samples from nonreference than from reference sites. The mean total nitrogen concentration from nonreference sites was 2.47 mg/L and from reference sites was 0.26 mg/L. The mean nitrate concentration from nonreference sites was 2.8 mg/L and from reference sites was 0.07 mg/L. The highest nitrate concentration observed at a nonreference site was 88.7 mg/L and at a reference site was 0.9 mg/L.

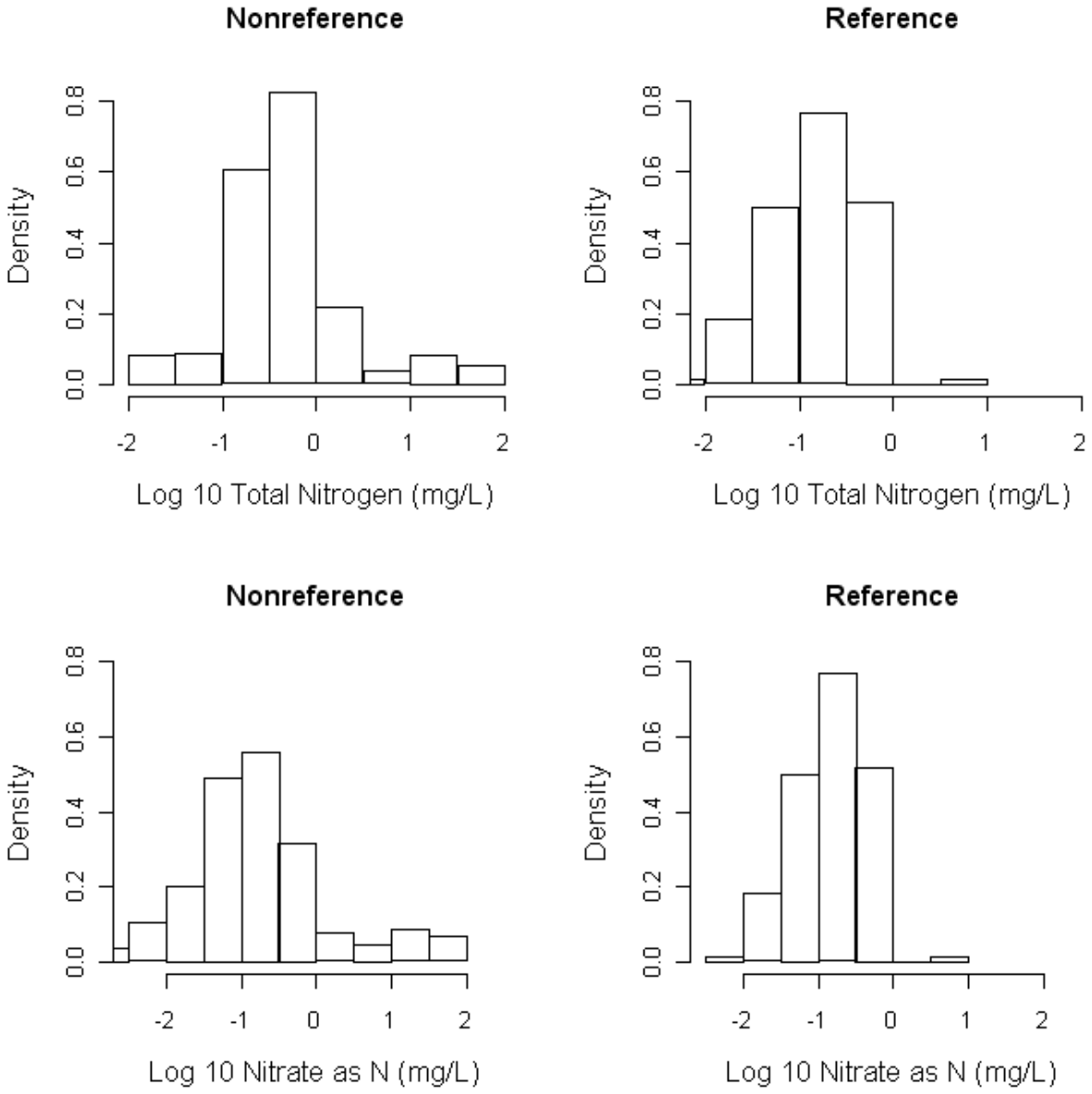


Figure 18. Site assemblage dendogram from the agglomerative hierarchical cluster analysis of calibration sites using Bray-Curtis dissimilarity and the flexible beta method (Beta=-0.6). Dashed line represents the level at which the dendogram was pruned to yield three site classes. The agglomerations coefficient was 0.91.

Class 1	Class 2
200COYCOI	304KINROC
200HERALA	304LOBLOB
200MIDHEN	305BRCCAS
304BUTCYN	307SANCLE
304WADRED	308BSRHW1
305LLAOSP	308SANHY1
305SWACRO	308WILWIL
305UVASWA	309BEAPVC
307CARLOS	309BGCNVS
307ROBCYN	309CHA146
307SALGAR	309CHAPVP
307TULCAR	309CHAWSP
308MCWJPB	309CHLCNF
308SOBHW1	309CHLCWF
310LOPUPP	309MCCMC1
310MORCIA	309SANPIC
312DAVDAV	309SC2BCG
312MANNIR	309UASIND
314CACHAP	312CUY166
314NOJNOJ	315HONMIG
314OSOUPP	

Class 3
200EASHEN
307JAMTAS
307LASGAR
307TRILOS
308BIGLBC
308DEVLBC
308LILPAL
308ROCBBQ
308SALHY1
308SANWIL
308WILHW1
309TASTAS
309TASZEN
310BIGUPP

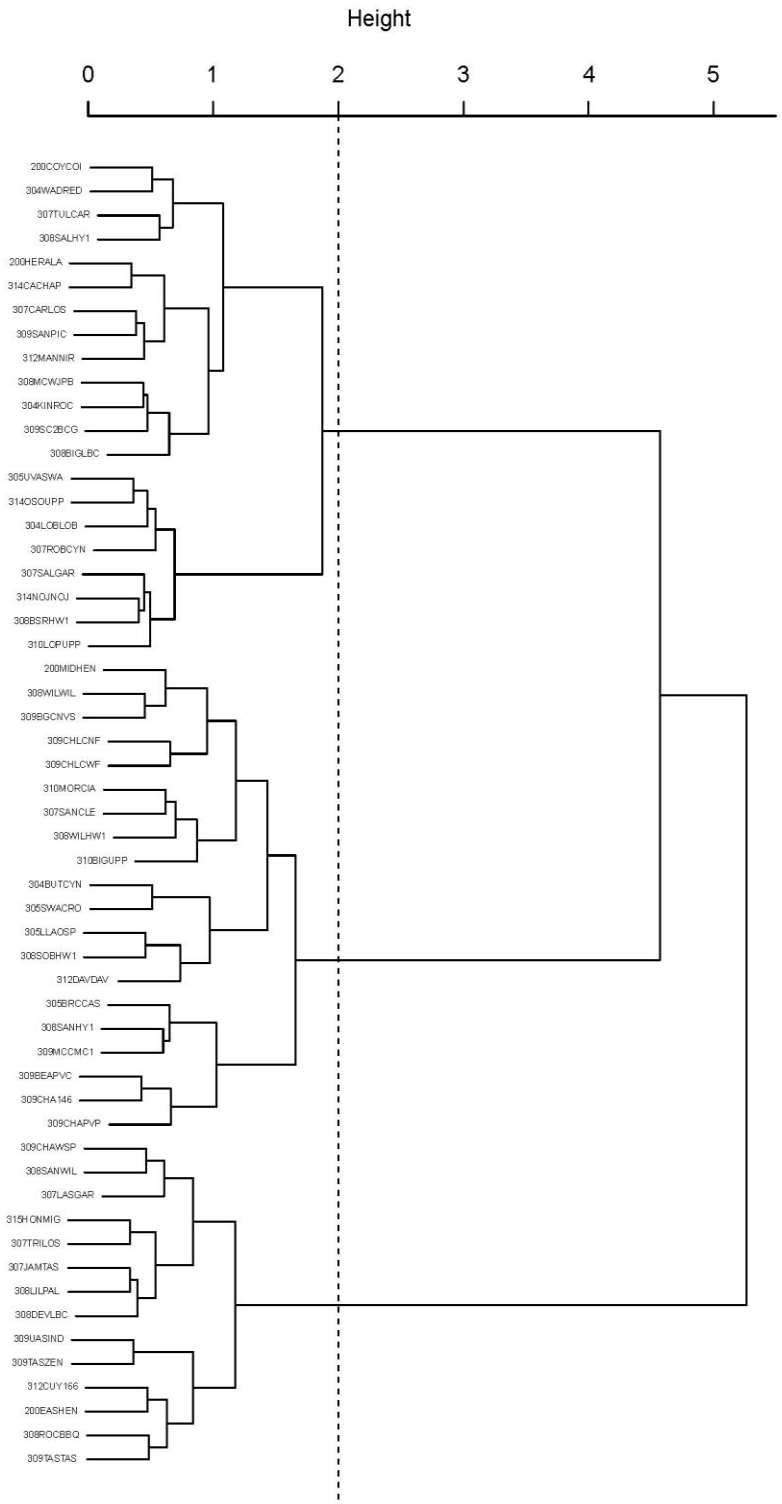


Figure 19. Observed versus expected species richness for the calibration set in the RIVPACS-type model.

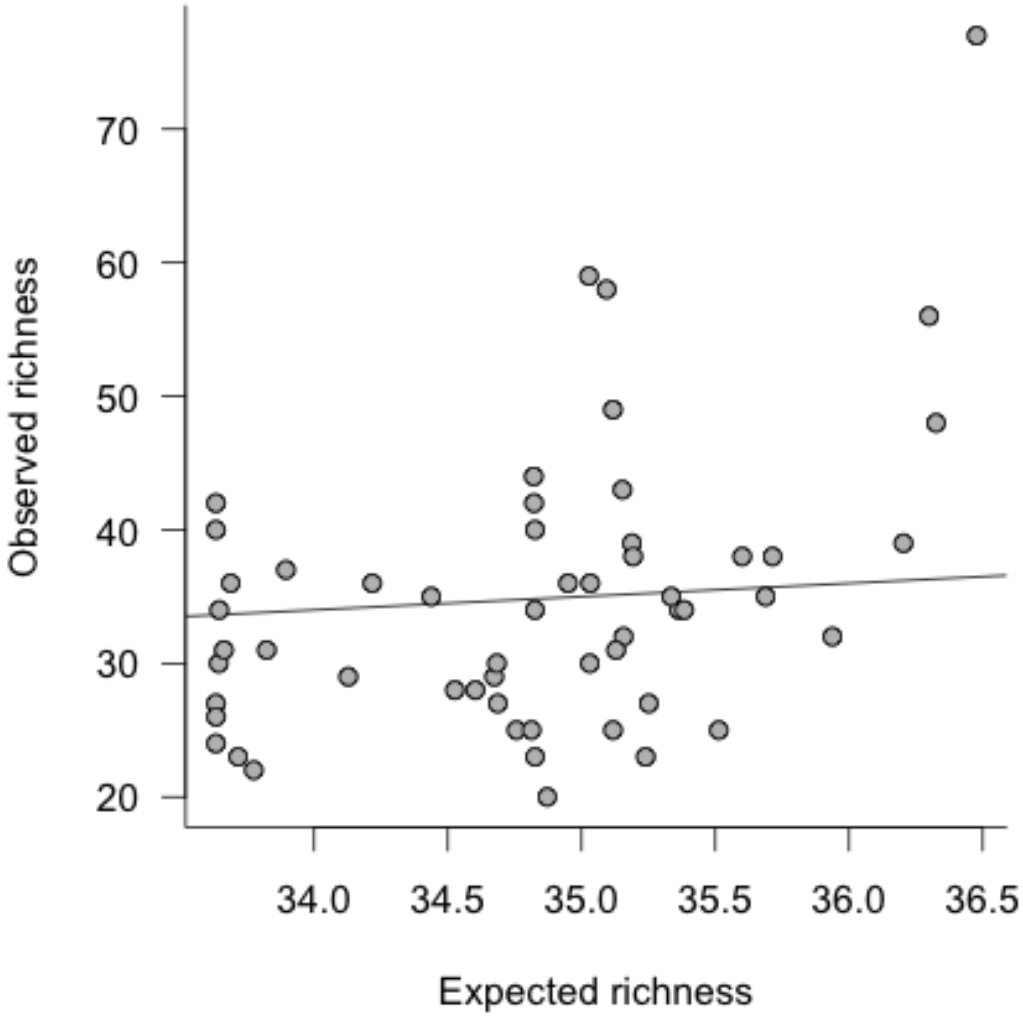


Figure 20. Boxplots of O/E values from the RIVPACS-type analysis for the calibration, validation and test sites. Analysis of variance using Helmert contrasts found the mean O/E of calibration sites was not significantly different from 1 (t=0.319, p=0.750); calibration and validation mean O/E values were not significantly different (t=-0.026, p=0.980); and the mean test site O/E values were significantly different from calibration and validation means (t=-2.695, p=0.007) (F=4.435, df=2,283, p=0.013).

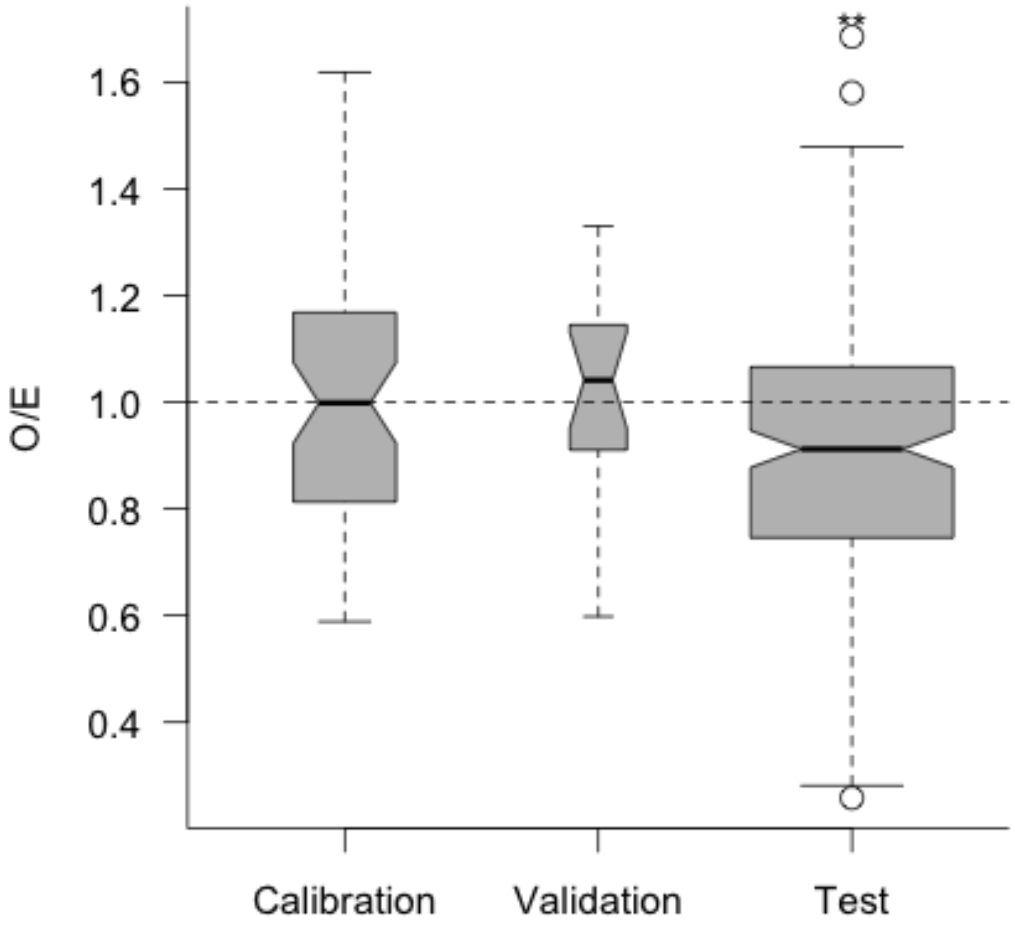


Figure 21. Distribution of O/E values for the calibration set. Vertical dashed lines represent the 10th and 90th percentiles of the distribution and represent potential criteria for assessing O/E values. Sites with O/E < 0.66 or O/E > 1.32 may be impaired.

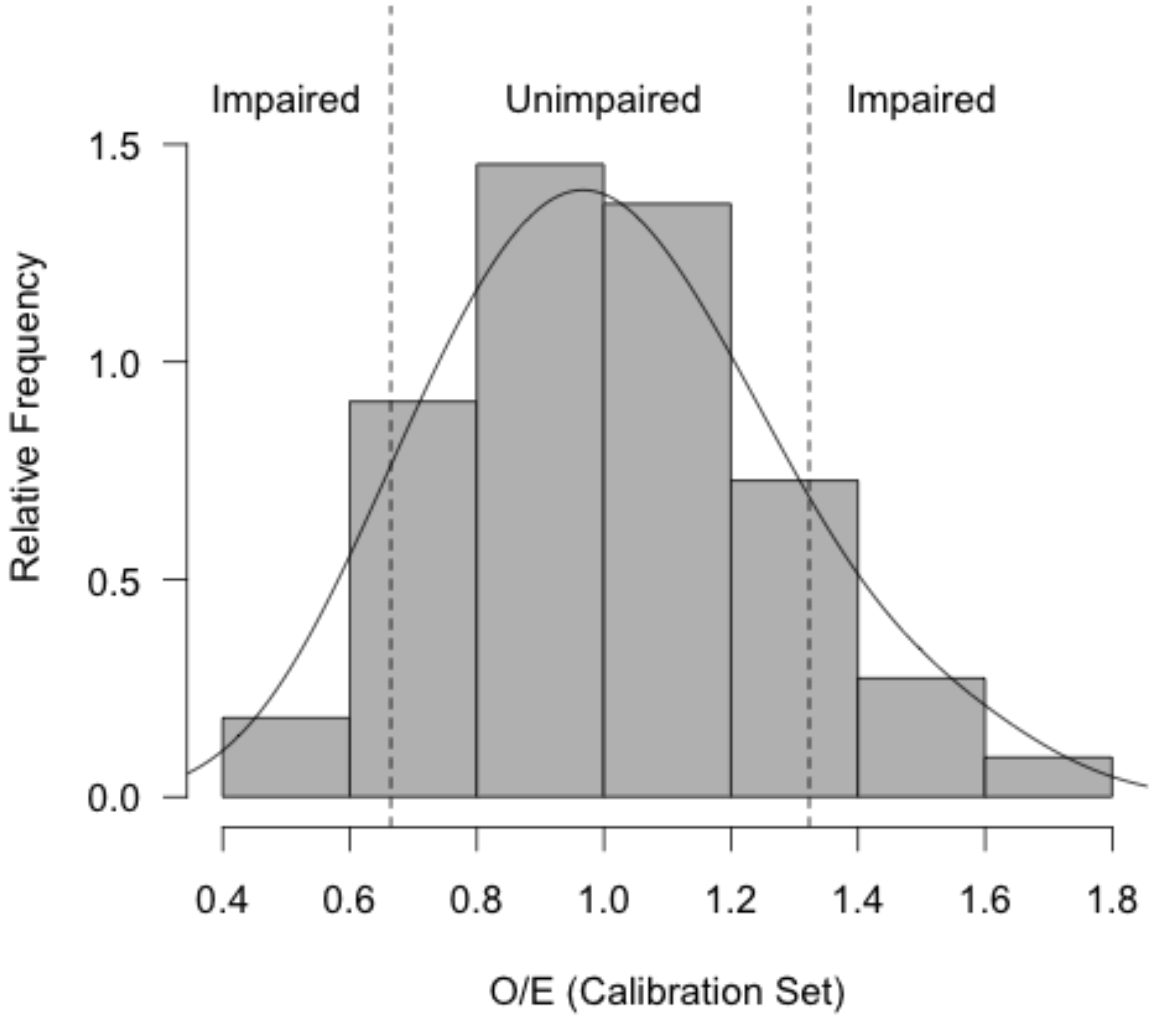


Figure 22. Proportion of metamorphic rock for watersheds were related to biological assemblages in RIVPACs. RIVPAC sites in categories 1, 2, and 3 had a mean of 12%, 4% and 19% metamorphic rock.

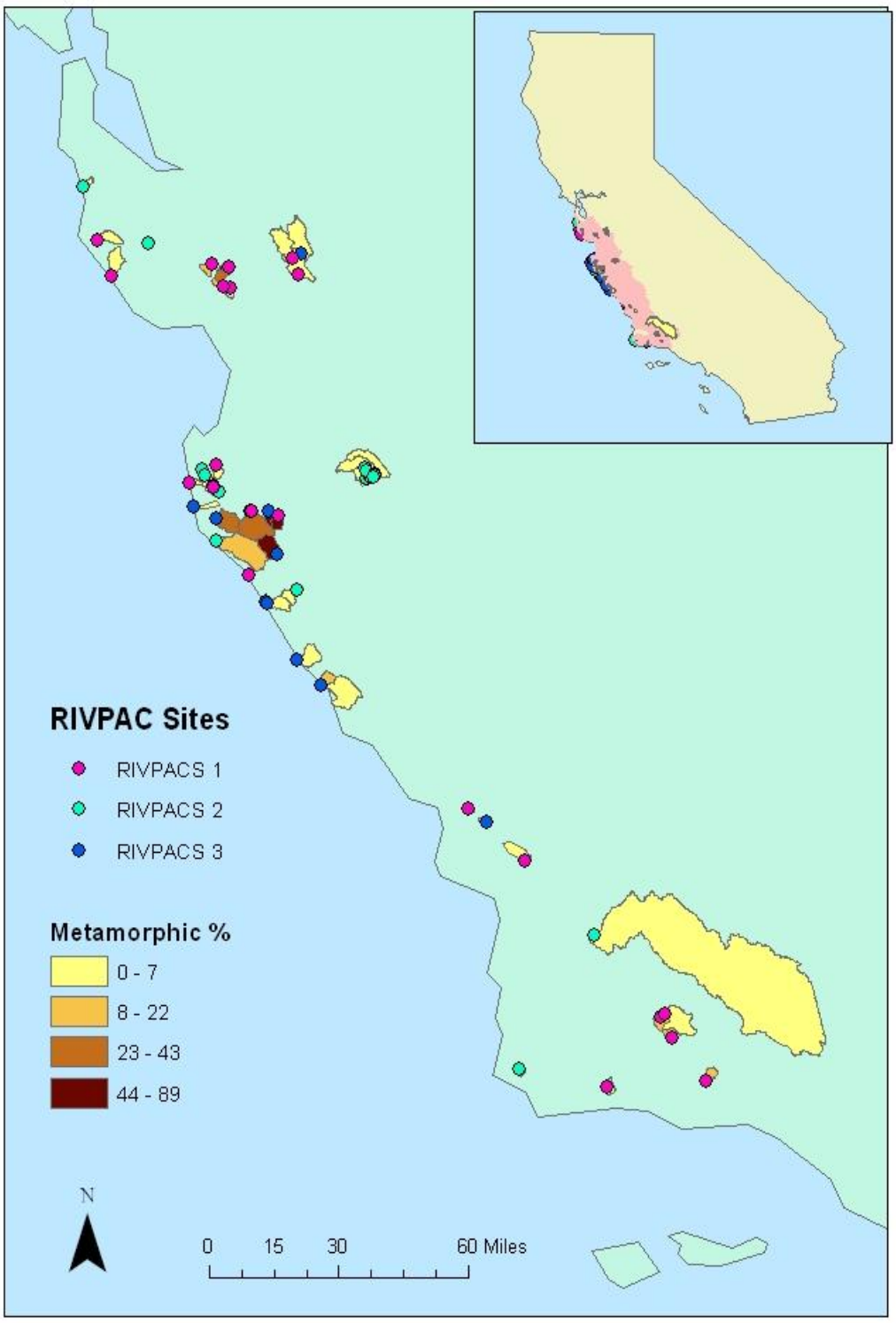
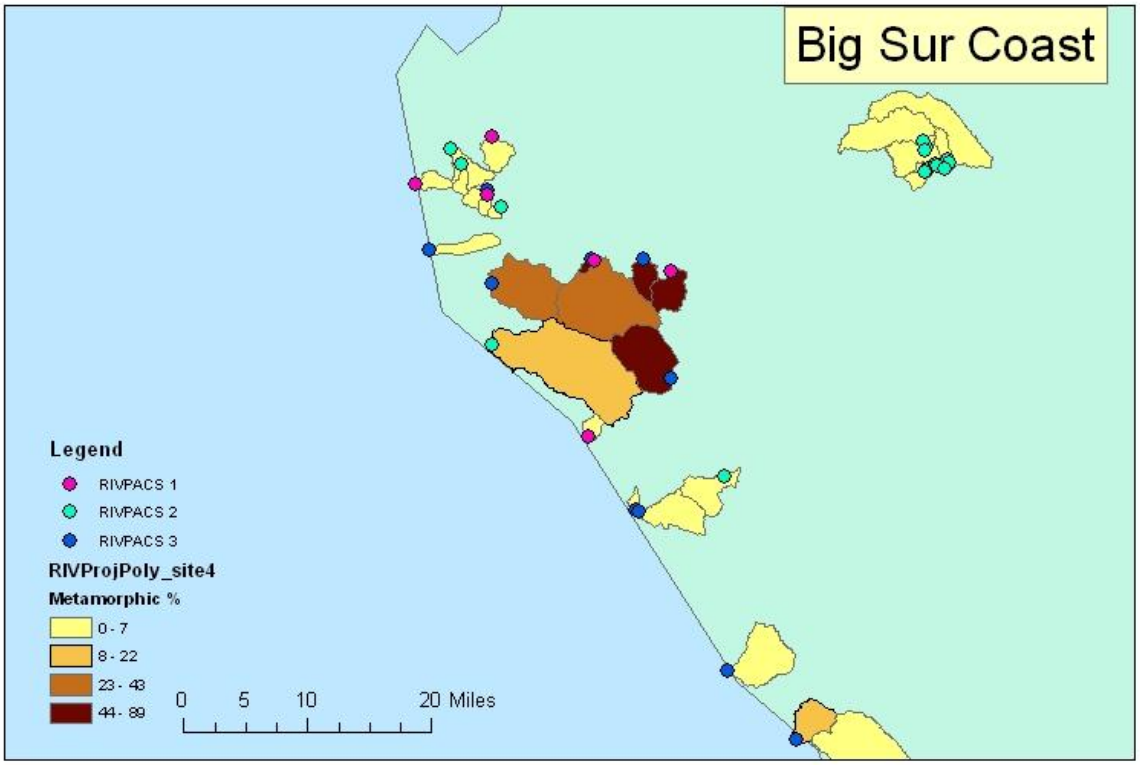


Figure 23. Metamorphic rock on the Big Sur Coast. Although much of California is sedimentary rock, the Big Sur coast is one area with a relatively high proportion of metamorphic rock.



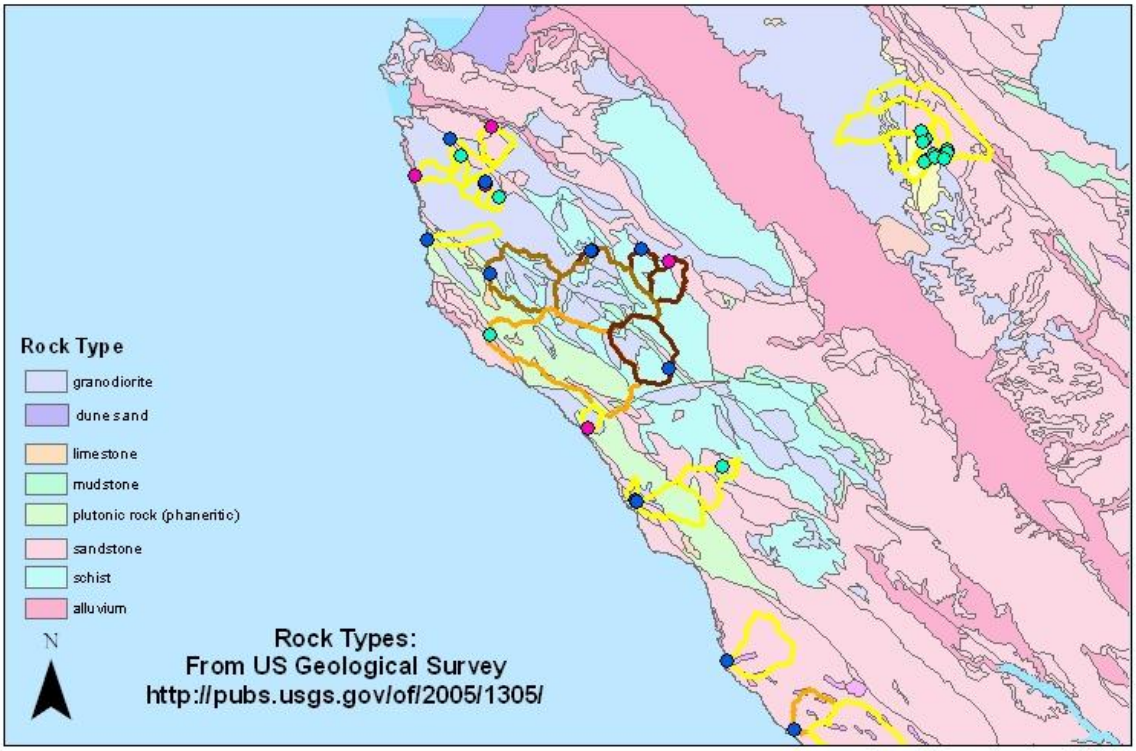
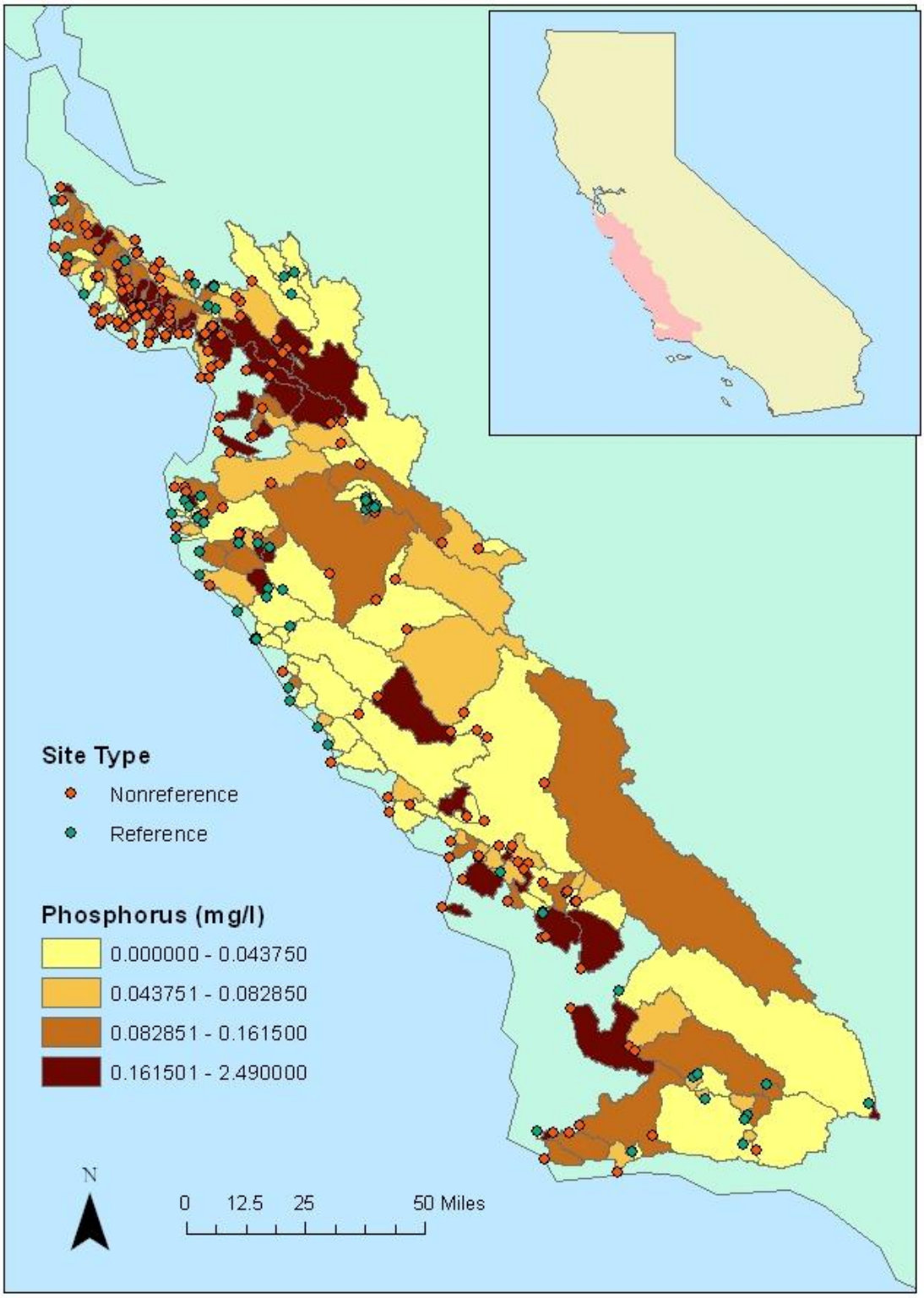


Figure 24. Phosphorus in water analyzed from sites shown in quantiles for associated watersheds. High phosphorus concentrations are found in the Pajaro watershed and near San Luis Obispo.



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Appendix I: Calculating the IBI for New Samples

An Excel spreadsheet file (CaliforniaDiatomIBICalculator.xlsx) has been created to calculate the IBI score for new sites. In order to calculate the IBI score, diatom count data and elevation from the site are needed. Diatom count data must apply the California diatom taxa list provided as a product of this research. Additionally actual count numbers, rather than diatom densities or relative abundance, should be used. The diatom data should be entered in column 'B' in the first sheet called "Site Count Data." In the second sheet, titled "IBI Calculator", GIS-derived elevation (in meters) should be entered in cell 'C6'. This elevation value will be used to detrend the 'Proportion of Species *Epithemia'* metric scores, which covary with elevation.

The spreadsheet scales all metrics and adjusts the direction, if necessary. Scaling places 90% of the data between 0 and 1. Direction-correction needs to be made because higher metric values should represent higher biological integrity. However, some metrics exhibit low values at high integrity sites and high values at low integrity sites. These must be reversed to yield higher IBI scores for sites with high biological integrity. Once metrics are scaled and direction-corrected, the sum of individual metrics is divided by 11 and multiplied by 100. This results in an IBI for which low values closer to zero are highly impaired, while sites with IBI values near 100 have high biological integrity. IBI values are output in cell 'F14'.

Appendix II: Calculating O/E for New Samples

Calculating O/E values for new sites will require some familiarity with RIVPACS-type models and experience with the statistical package R. Files necessary to calculate O/E values for new sites have been exported from R (RIVPACS.Model.Rdata). In addition, the user will need to download R script written by John Van Sickle (http://www.epa.gov/wed/pages/models/rivpacs/rivpacs.htm). Specifically, the "model.predict.v4.1.r" file will need to be sourced. This file can be found in the ZIP file at the above website. The user will also need to supply a sample-by-taxa abundance (or presence/absence) matrix and predictor data for those samples. Step 7 in the file "model.build.v4.1.r" provides instructions for calculating new O/E values and provides an example script.