A Technical Foundation for Biointegrity and Eutrophication Indicators and Thresholds for Modified Channels, Intermittent Streams, and Streams on the Central Valley Floor 2025 Update





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#### 2025 Update

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#### **EXECUTIVE SYNTHESIS**

Bioassessment tools (such as biointegrity indices) are increasingly becoming a major element of watershed management. Biological organisms have many advantages for water quality monitoring programs, such as their ability to reflect the combined impact of multiple stressors over time, and their direct relationship with aquatic life uses. Thus, indices based on the diversity and abundance of aquatic organisms are a powerful tool for establishing water quality goals, monitoring ecological conditions, as well as for evaluating the effectiveness of management programs intended to protect aquatic life.

Watershed managers in California face a number of challenges when it comes to applying bioassessment tools in certain types of streams. These challenges include uncertainty about the applicability of these tools in regions or stream types that differ from reference data sets used to calibrate tools (e.g., streams on the Central Valley floor, or intermittent streams), as well as concerns about the ability to achieve reference-based biological integrity goals in channels that have been modified for flood protection or water conveyance. Decisions on the biological integrity goals are important because they support decisions on management targets intended to control eutrophication (e.g., nutrients, algal biomass) and other stressors, which have major economic implications. These decisions have the potential to drive millions of dollars in water quality management. The first set of issues addresses questions of *natural constraints* on index interpretation (Part 1), whereas the second set addresses questions about *constraints* associated with human activity (Parts 2 and 3). Although these issues are particularly relevant in the Central Valley, they affect watershed managers in all parts of California.

This study is intended to provide these managers with a technical foundation to support the interpretation of bioassessment tools in settings where these challenges are frequently encountered:

- Streams on the Central Valley floor
- Intermittent streams
- Streams in modified channels

The first two settings represent natural constraints, whereas the third represents a constraint associated with human activity. We addressed the first challenge by comparing environmental characteristics of the Central Valley to environmental characteristics of tool calibration data sets. In addition, we evaluated the accuracy, precision, and responsiveness of bioassessment tools within this ecoregion. To address the second challenge, we compared bioassessment

index scores at intermittent reference sites to scores at perennial reference sites in arid portions of California.

For the third challenge (i.e., streams in modified channels), we developed a classification system for modified channels and evaluated ranges of biointegrity and eutrophication indicators within each class. Finally, we investigated the scientific basis for identifying biointegrity thresholds for modified channels and their linkage to eutrophication thresholds relative to those in natural (unmodified) channels. For both types of indicators, we identified thresholds using two approaches: thresholds based on indicator values at reference sites, and thresholds based on best-observed values. A reference approach can characterize ranges of indicator values associated with natural constraints (such as intermittent streams) as long as data from reference sites are available. For stream types where reference sites are lacking (e.g., streams on the Central Valley floor) or stream types defined by human activity (e.g., streams in modified channels), best-observed thresholds are an alternative way to characterize ranges of indicator values. A third approach was also evaluated for eutrophication indicators: thresholds based on statistical response models of bioassessment indices to increasing eutrophication stress. This approach allows managers to more directly evaluate the link between eutrophication stress and biointegrity. For each of these approaches, we identified thresholds under a range of scenarios to provide managers with a range of options appropriate for their needs and priorities. All analyses made use of existing, publicly available bioassessment datasets, and no new data were collected as part of this project.

For a single reach, more than one of these stream classes may apply (e.g., an intermittent hard-bottom stream on the Central Valley floor). We have created a dashboard to help managers explore all the thresholds that may be relevant for their reaches of interest and compare them to observed data: https://sccwrp.shinyapps.io/ModifiedChannelThresholds.

This study is not intended to endorse the use of specific thresholds or waterbody classifications in policy or regulatory programs. Rather, the intention is to illuminate how natural factors (like streamflow duration, and the environmental settings within the Central Valley) and channel modification can influence decisions regarding the boundaries between how poor biointegrity conditions are defined. The numeric values are presented for informational purposes, and their presentation in this report does not constitute an endorsement of their use in regulatory programs.

## Streams on the Central Valley floor: The consequences of lacking reference data

Due to the extent of modification of the landscape, nearly all stream-reaches on the Central Valley floor have been affected by human activity. Thus, this unique environmental setting remains poorly represented in reference data sets used to calibrate bioassessment indices (such as the CSCI). This lack of reference data raises concerns about using these indices (or eutrophication response models based on these



indices) in the Central Valley. However, the evidence that poor representation of the Central Valley in reference data sets affects performance or biases assessments is equivocal, with somewhat stronger evidence favoring a different approach for interpreting the CSCI than for the ASCIs.

High (reference-like) CSCI scores are uncommon in the Central Valley, and they are largely restricted to the periphery of the region in streams with less developed watersheds. This scarcity of high CSCI scores could reflect a bias in the index, or it could reflect the true condition of streams in this highly altered region. Adjusting CSCI assessment thresholds based on the best-observed scores in the Central Valley (rather than the lowest scores observed at reference sites) could account for potential bias, but lower thresholds sacrifice sensitivity. Best-observed thresholds for the CSCI are ~10% points lower than reference thresholds. In contrast, high ASCI scores were common in the Central Valley, and best-observed ASCI thresholds are considerably higher than reference thresholds (up to ~15%). Thus, best-observed thresholds are a technically defensible option for the CSCI, but not for the ASCIs.

Unlike the biointegrity indices, the eutrophication response models were developed with data that represents the Central Valley well, thanks to the fact that these models are not developed solely with reference sites. Eutrophication thresholds derived from statewide logistic regression models are appropriate for identifying levels likely to protect streams in reference conditions. If other biointegrity goals are used (e.g., those based on best-observed conditions), the updated statistical models presented in this study could be used to identify corresponding eutrophication targets that are associated with those goals.

The statewide eutrophication response models presented in Mazor et al. (2022) present an additional line based on regional reference levels of eutrophication indicators. Regional reference levels can be interpreted as natural background levels of eutrophication indicators to which stream biota are adapted. Because reference sites are unavailable for the Central Valley, best-observed thresholds provide an alternative way of identifying background levels of eutrophication indicators in the region.

#### **Intermittent streams**



Within southern California, algal and benthic macroinvertebrate biointegrity indices provide an unbiased measure of stream condition in intermittent streams. Based on provisional analyses, the same appears to be true for the ASCIs in northern California. However, the CSCI may yield lower-than-expected scores for intermittent streams in xeric portions of northern California (data are insufficient to evaluate intermittent streams in non-xeric regions, such as the Sierra Nevada

and the North Coast). Thus, the CSCI may need to be recalibrated or modified for use in these northern California streams. Until that index becomes available, we recommend two interim solutions: First, the ASCIs can serve as an unbiased measure of biointegrity in northern California intermittent streams. Second, where the CSCI is measured, alternative reference-based thresholds presented in this report will greatly reduce the likelihood of incorrectly identifying non-reference conditions (while increasing the risk of failing to detect degraded conditions). Ongoing research will provide greater clarity on the environmental factors that drive the changes in the CSCI's applicability to intermittent streams in southern versus northern California, as well as in non-xeric portions of the state. These conclusions are based on a provisional analysis of data from northern California intermittent reference streams, and they may change as these results are updated with new information. A nonperennial reference sampling program is currently underway, led by the State Water Board's Surface Water Ambient Monitoring Program.

### A better understanding of the constraints associated with channel modification

On a purely biointegrity basis, the same reference-based thresholds used to assess natural channels can accurately determine whether a modified channel supports aquatic life comparable to undisturbed streams. However, alternative thresholds based on best-observed conditions or response models may be useful for setting management goals related to aquatic life or prioritizing streams where long-term channel modifications may make the attainment of reference-based thresholds more challenging. Where resources, time, and interest allow, site-specific best-attainable thresholds may be identified through additional studies, and these thresholds are preferable to best-observed thresholds.

Channel modification by itself does not appear to constrain biointegrity, as high scores are sometimes observed, particularly for modified channels with less developed watersheds. However, channel modification typically co-occurs with substantial watershed development, and the combined impact of these two disturbances appears to limit the upper range of assessment index scores. Baseline assessments of modified channels can determine whether traditional thresholds are currently achieved.

The constraints associated with stream modification can co-occur with other constraints, such as those associated with intermittency, or with the environmental conditions of the Central Valley floor. Thus, a naturally intermittent modified channel may have a low CSCI score due to the modifications as well as to the potential bias observed in northern California intermittent streams.

#### Hard-bottom channels



The CSCI is particularly responsive to channel hardening, as high scores are rarely observed in hard-bottom channels. The loss of habitat complexity, combined with water quality conditions that commonly occur in hard-bottom channels, can be inhospitable to the benthic macroinvertebrates expected to occur in these streams, leading to poor index scores. Thus, alternative best-observed thresholds for the CSCI may be useful for setting interim management

targets. In contrast, high ASCI scores were not uncommon (>10%) in hard-bottom streams.

Therefore, best-observed thresholds were similar to or higher than traditional reference-based ASCI thresholds.

#### Soft-bottom engineered channels

Reversing the pattern that we saw in hard-bottom channels, high ASCI scores were uncommon (<10%) in soft-bottom channels, whereas CSCI scores frequently exceeded reference-based thresholds. Thus, alternative best-observed thresholds may be useful for interpreting ASCI

scores in soft-bottom channels, whereas reference-based thresholds may be useful for interpreting CSCI scores.

As an exception, soft-bottom engineered channels with 1 hardened side often scored better for both index types, compared to those with 2 hardened sides, or those with no hardening whatsoever (e.g., 40% vs. 17 – 20% for CSCI scores). Frequently, these one-sided channels occur in less developed areas with



greater ability for streams to meander and generate the habitat complexity that supports high biointegrity index scores. Less developed areas typically have less anthropogenic stress, such as lower pollutant inputs or less severe hydrologic modification.

#### Constructed channels

Although they are found in all portions of California, constructed channels (e.g., channels that have been excavated from uplands where no historic channels previously existed) have not been sampled by most bioassessment programs. This scarcity may reflect their low priority in monitoring programs, as well as their low likelihood of having suitable flow for bioassessment sampling. Thus, data are limited to a few dozen sites in the Central Valley region, plus a handful

in the Imperial Valley. Because the CSCI and ASCIs require watershed delineations in order to establish appropriate biological expectations, and because constructed channels lack traditional watersheds, the standard approach for calculating the CSCI or ASCIs does not apply, and an alternative scoring approach was developed for this study (wherein 5-km circular buffers were substituted for traditional watersheds).



Scores calculated with this alternative approach are not directly comparable to traditionally calculated scores, and they should not be evaluated with thresholds derived from traditionally calculated scores.

CSCI scores calculated with the alternative approach were lower at constructed channels than at any other channel type in the study, and thus, alternative best-observed thresholds are substantially lower than other thresholds. In fact, they were often lower than the range that could be predicted from eutrophication response models. Due to a lack of data, we could not assess ASCI scores in constructed channels.

# Evaluating eutrophication stress within modified channels: The challenge of interpreting indicator data when stressors co-occur



Eutrophication stress and stress from channel modification can co-occur with each other, and with other unrelated stressors (e.g., toxic contamination, invasive species, etc.). The eutrophication response models presented in this study evaluate each eutrophication indicator independently. Previous statewide analyses showed that indicators used in combination

have a much stronger relationship with biointegrity conditions than when they are used independently (Mazor et al. 2022), and that this study shows the same pattern within the highly altered Central Valley, where many streams are modified and affected by multiple stressors at once. Thus, the likelihood of misidentifying a stream impacted by eutrophication can be reduced by evaluating multiple indicators in assessments. Even greater confidence could result from complementary confirmatory assessment of biointegrity indicators or other response indicators (e.g., diel dissolved oxygen, algal toxin production). The highest level of confidence can be achieved by following this confirmation with causal analyses. This greater confidence comes at the cost of greater data requirements, as well as more time for data analysis. Tools under development (e.g., rapid screening causal assessment tools; Gillett et al. 2023) can facilitate data analysis. In cases where numerous stressors are found to be likely causes of poor

biointegrity, interventions that tackle multiple factors are likely to have more success than those that address one at a time.

It is important to note that excess nitrogen and phosphorus are not the only stressors that potentially affect stream biointegrity. Streams are also affected by pesticides, channel management, and lack of riparian corridors. These factors are not directly addressed in this study. Additionally, while the study focuses on nitrogen and phosphorus as drivers of eutrophication, other factors, such as hydrology, stream temperature, and sun exposure, can affect eutrophication processes.

#### Limitations of an observational study

This study leveraged several extensive biomonitoring data sets collected in California under a number of different programs that had diverse purposes. That is, the data were not specifically collected to investigate the major questions of the study. We combined data collected under probabilistic study designs, as well as data collected at sites targeted for specific purposes (e.g., reference sites, or sites below discharges). Therefore, the extent estimates of healthy streams in this report may not match their true extent in California. Furthermore, because the study was based on observational data, the relationships between stress and biointegrity are correlational; controlled experiments may establish causal relationships.

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#### STATEMENT OF DATA AVAILABILITY

The data and code used to generate all tables and figures of this study are openly available on GitHub at <a href="https://github.com/SCCWRP/BiointegrityEutrophicationIndicators">https://github.com/SCCWRP/BiointegrityEutrophicationIndicators</a>.

A spreadsheet providing basic information about sites used in each analysis, along with a data dictionary, may be downloaded here:

https://github.com/SCCWRP/ModifiedChannelThresholds/raw/main/inst/extdata/Data%20sets%20used%20in%20each%20analysis final.xlsx.

A dashboard to facilitate the evaluation of thresholds identified in this report is available here: <a href="https://sccwrp.shinyapps.io/ModifiedChannelThresholds/">https://sccwrp.shinyapps.io/ModifiedChannelThresholds/</a>.

#### HOW TO CITE THIS DOCUMENT

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streams, and streams on the Central Valley floor: 2025 Update. Technical Report #1434. Southern California Coastal Water Research Project. Costa Mesa, CA.

#### **PREVIOUS VERSIONS OF THE REPORT**

An earlier version of the report was released in 2024 as SCCWRP Technical Report #1367, and is available from the State Water Resources Control Board here:

https://www.waterboards.ca.gov/water\_issues/programs/biostimulatory\_substances\_biointegrity/docs/biointegrity-indicators.pdf.

That version should be cited as:

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#### **INTRODUCTION**

Watershed managers in California often encounter questions when considering the use of bioassessment tools (such biointegrity indices and eutrophication response models) in monitoring programs. Broadly, these questions relate to the impacts of natural and anthropogenic factors on the ability to use or interpret indicators of biological condition or eutrophication. Managers may ask: Can I use these tools in regions that lack reference sites? How does flow intermittency affect my ability to measure or interpret conditions? How can I use these tools in modified channels? Providing insight into these questions is an essential step for the integration of bioassessment tools into monitoring and management programs.

The goal of this report is to synthesize studies that provide managers with a technical foundation to address these questions through the analysis of pre-existing bioassessment data sets. Based on these analyses, we present recommendations for how bioassessment tools may be used in different natural settings within California. We also provide a range of thresholds to help interpret bioassessment data in modified channels; however, the choice of whether to use these thresholds and manage such channels differently from natural ones is a policy matter and cannot be decided by technical analysis alone.

In <u>Part 1</u> of this report, we address questions related to natural factors that affect the applicability of bioassessment tools. We focus on environmental settings that are poorly represented in the reference site data sets used to develop the tools (such as the Central Valley floor), as well as on streams with naturally intermittent flows. In <u>Part 1a</u>, we evaluate applicability of biointegrity indices in the Central Valley. First, we evaluate the representativeness of tool development data sets to see how well they compare to environmental conditions in the region where bioassessment sampling has occurred. Then, we evaluate accuracy, precision, and responsiveness of the tools. To evaluate the applicability of eutrophication response models in the Central Valley, we examine relationships between high levels of eutrophication indicators and poor biointegrity index scores within the region. In <u>Part 1b</u>, we evaluate the applicability of biointegrity indices to naturally intermittent streams by comparing scores at reference sites in known flow duration. This portion of the study applies to arid regions throughout California (and not just to the Central Valley). In addition, we conduct a brief review of the role of intermittency in eutrophication processes, and we provide guidance on evaluating bioassessment samples to see if they have been influenced by drying.

In <u>Part 2</u>, we shift focus from natural factors to the challenges associated with monitoring biointegrity and eutrophication within modified channels. First, we present a classification system for modified channels based primarily on bed and bank material. We provide a

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conceptual model for how channel modification and associated activities can affect biointegrity and eutrophication processes. We then develop a framework for selecting appropriate biointegrity and eutrophication indicators in modified channels.

In <u>Part 3</u>, we identify candidate thresholds for evaluating biointegrity and eutrophication indicators in several of the stream types discussed in previous sections (i.e., natural streams in the Central Valley, intermittent streams in arid portions of California, and different types of modified channels). For biointegrity indicators, we evaluate two approaches for identifying thresholds: reference distributions and best-observed scores for stream types lacking reference sites (e.g., modified channels). For eutrophication indicators, we evaluate these same two approaches, as well as a third approach based on eutrophication response models. We examine each approach under several scenarios to provide managers with a range of options reflecting their desired confidence in their assessments. We provide a dashboard to help managers select thresholds and apply them to sample data.

For most analyses in this report, data sets were generated by statewide and regional bioassessment programs between 1994 and 2022, and were aggregated from two public databases (specifically, the California Environmental Data Exchange Network, or CEDEN, and the data portal for the stream survey of the southern California Stormwater Monitoring Coalition, SMC). No new data collection occurred as part of this project, although a small number of site-visits were conducted to inform the classification of modified channels in Part 2.

Although the focus of this report is on the Central Valley region, these questions are relevant to most parts of California, and these studies can help managers evaluate bioassessment tools in all parts of the state. Except where specifically noted, the conclusions of these studies should be considered applicable throughout California. Priority questions for this study were identified in collaboration with staff at the Central Valley Regional Water Quality Control Board, with input from Central Valley permittees (specifically, the Irrigated Lands Working Group and the Sacramento County Stormwater Program), members of the Southern California Stormwater Monitoring Coalition, and the Bioassessment Workgroup of the State Water Resources Control Board's Surface Water Ambient Monitoring Program.

# PART 1: EVALUATING THE INFLUENCE OF NATURAL FACTORS ON BIOASSESSMENT TOOLS

#### Introduction

This study focuses on questions about applicability of bioassessment tools (specifically, bioassessment indices and eutrophication response models) to natural conditions in the Central Valley and Modoc Plateau, as well as in naturally intermittent streams (which occur in all regions of California). The Central Valley and Modoc Plateau regions lack high numbers of reference sites, which are used to calibrate the models underpinning bioassessment tools. For example, the California Stream Condition Index ([CSCI], Mazor et al. 2016), a biointegrity index based on benthic macroinvertebrates, was developed using data from nearly 600 reference sites across California, only one of which was located within the Central Valley ecoregion, and only 17 in the Modoc Plateau. For the Central Valley, the scarcity of reference sites is due to the pervasive alteration of the landscape; in contrast, the scarcity of sites within the Modoc Plateau is due to the fact that this region has only recently been the focus of bioassessment sampling efforts (Ode et al. 2011, Rehn 2021). Thus, questions about the CSCI's applicability affect both natural and modified channels in these regions.

Streamflow intermittency is known to be a major driver of the biological composition of streams (Sabater et al. 2017, Stubbington et al. 2017), which has the potential to complicate the interpretation of stream condition indices based on the structure of biotic assemblages (Mazor et al. 2014, Acuña et al. 2017, Crabot et al. 2021). Stream drying can eliminate organisms that are intolerant of high temperatures, require high dissolved oxygen concentrations, or have long aquatic life-stages, and thus has a similar impact on community composition as disturbance from pollution (Stubbington et al. 2018). If bioassessment indices are not appropriately calibrated for the types of organisms that live in intermittent streams, such streams could be mischaracterized as degraded purely due to their natural flow regimes. Investigations within the San Diego region have shown that many of the reference sites used to calibrate the CSCI are naturally intermittent (Mazor et al. 2012, 2014, Giraldo et al. 2019, Loflen 2020, Rehn 2021), suggesting that the CSCI is appropriately calibrated within southern California. However, reference sites in other parts of California have not been as thoroughly investigated.

We investigated these two natural factors to evaluate their impact on the interpretation of bioassessment tools. This understanding should give watershed managers greater confidence in

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the strengths and limitations of these tools in these settings. The objectives of this portion of this study were to answer the following questions:

- 1. How well do calibration data sets of bioassessment tools represent environmental conditions in the Central Valley and Modoc Plateau?
- 2. How do bioassessment indices perform in the Central Valley?
  - a. Are indices accurate?
    - i. Can indices attain scores similar to reference sites?
    - ii. Do less-disturbed sites in the Central Valley score similar to reference sites?
  - b. Are indices precise? Do scores at less-disturbed sites vary to the same extent as they vary at reference sites?
  - c. Are indices responsive? Do scores vary along gradients of human activity, or gradients of field measurements of water or habitat quality?
- 3. How well do eutrophication response models perform in the Central Valley?
  - a. Do models correctly predict the likelihood of good biological conditions within the Central Valley?
  - b. Are statewide thresholds for eutrophication indicators associated with significant risks to biointegrity?
- 4. How well do bioassessment indices work in intermittent streams in arid portions of California? Do intermittent reference sites have similar scores to intermittent reference sites?

#### **Ecoregional definitions**

The Central Valley ecoregion is defined by US Environmental Protection Agency (US EPA) as flat, intensively farmed plains with hot dry summers and mild winters (Omernik 1987, Griffith et al. 2016). The northern valley is dominated by the Sacramento River, whereas the southern valley is dominated by the San Joaquin river. In 1987, the US EPA issued a map defining this ecoregion, and in 2008, these boundaries were incorporated into the regional definitions used by California's State Water Resources Control Board for the statewide Perennial Streams Assessment monitoring program. In 2016, the US EPA issued a new map with updated boundaries that more precisely reflected topography and landuse patterns than the 1987 map did (Griffith et al. 2016). Although the two versions overlap considerably, the 2016 refinements resulted in expansion of the boundaries in some areas (e.g., parts of Solano County or

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northwestern Kern County are now included within the Central Valley), and retraction in other areas (e.g., western Amador and El Dorado counties are now almost completely excluded; Figure 1). Thus, sites close to the margin may be considered part of the Central Valley by one set of boundaries may be considered part of an adjacent region (e.g., the interior chaparral) by the other set. Except where noted, the 1987 boundaries were used in all analyses in study.

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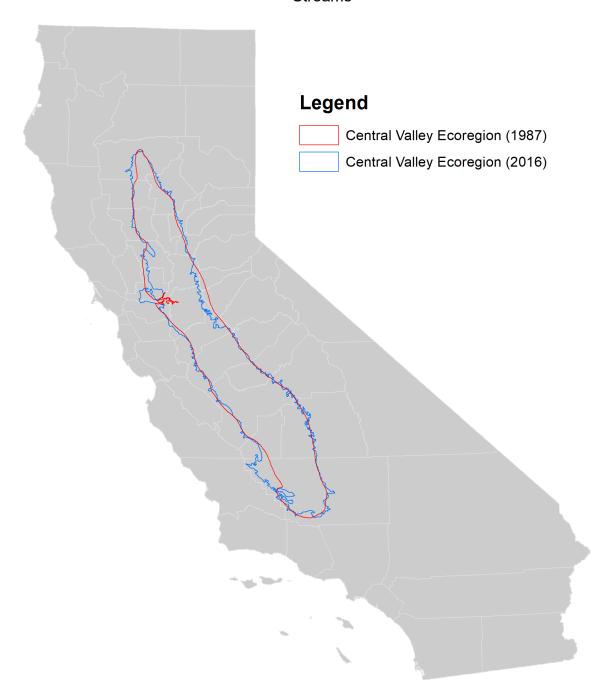


Figure 1. Ecoregional boundaries of the Central Valley. The 1987 boundary was established by Omernik (1987), and was refined in 2016 by Griffith et al. (2016). The 1987 boundaries are used to define the Central Valley region in the statewide Perennial Streams Assessment.

# How well do calibration data sets represent conditions in the Central Valley and Modoc Plateau ecoregions?

We will define "conditions in the Central Valley and Modoc Plateau ecoregions" as conditions represented sites in the aforementioned test data sets. This definition assumes all sampleable conditions have been sampled, and that unsampled locations are either a) unsampleable (e.g., lack sufficient flow for bioassessment), or b) sampleable, but similar to sampled locations.

#### Characterization of environmental conditions

We identified several environmental gradients to characterize environmental conditions at each site (Table 1). These gradients are known to influence in-stream biological communities, and are required to calculate CSCI and ASCI scores (Mazor et al. 2016, Boyle et al. 2020, Theroux et al. 2020). Abbreviations on this list refer to variable codes in Boyle et al. (2020).

Table 1. Environmental gradients used to assess the representativeness of calibration data sets.

Туре	Variable	Description
Watershed area	AREA_SQKM	Watershed area in km <sup>2</sup>
Watershed area	Logwsa	Log-transformed watershed area
Location	Latitude	Coordinates in decimal degrees
Location	Longitude	Coordinates in decimal degrees
Precipitation	PPT_00_09	Average precipitation (2000 to 2009) at the sample point, in hundredths of millimeters
Precipitation	MAXWD_WS	Catchment mean of 1961-1990 annual max number of wet-days
Precipitation	MeanP_WS	Catchment mean of mean 1971-2000 annual precipitation
Precipitation	MinP_WS	Catchment mean of mean 1971-2000 minimum monthly precipitation
Precipitation	SumAve_P	Mean June to September 1971 to 2000 monthly precipitation, averaged across the entire catchment
Temperature	TEMP_00_09	9 Average temperature (2000 to 2009) at the sample point, in hundredths of degrees Celsius
Temperature	TMAX_WS	Catchment mean of mean 1971-2000 max temperature
Atmospheric deposition	AtmCa	Catchment mean of mean 1994-2006 annual ppt-weighted mean Ca concentration
Atmospheric deposition	AtmMg	Catchment mean of mean 1994-2006 annual ppt-weighted mean Mg concentration

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Type	Variable	Description			
Atmospheric	AtmSO4	Catchment mean of mean 1994-2006 annual			
deposition		ppt-weighted mean SO4 concentration			
Soil	BDH_AVE	Average soil bulk density			
Soil	KFCT_AVE	Average soil erodibility factor			
Soil	LPREM_mean	Catchment mean log geometric mean hydraulic conductivity			
Soil	PRMH_AVE	Catchment mean soil permeability			
Soil	UCS_Mean	Catchment mean unconfined Compressive Strength			
Geology	CaO_Mean	Average calcium oxide (quicklime) in the catchment geology			
Geology	MgO_Mean	Average magnesium oxide (magnesia) in the catchment geology			
Geology	S_Mean	Catchment mean whole rock S			
Geology	P MEAN	Catchment mean whole rock P			
Filed-measured variables	% fines	Percent of fine particles (median axis < 0.06 mm) in the streamed			
Field-measured variables	slope	Reach slope (%)			

## Assessing the representativeness of the calibration data sets

#### Data sets

#### Calibration data sets

We evaluated calibration data sets for these tools:

- California Stream Condition Index (CSCI; Mazor et al. 2016)
- Algal Stream Condition Index (ASCI; Theroux et al. 2020)
- Eutrophication response models (ERM; Mazor et al. 2022)

Each of these data sets includes both reference and non-reference calibration data. For the statistical models underpinning the CSCI and ASCI, reference sites are used to predict biological expectations for different environmental settings, while non-reference sites are used to interpret and score deviations from these expectations. Thus, representativeness of the reference subset of calibration sites is particularly important.

In contrast, both reference and non-reference sites are used in the ERM models. Reference status has no influence on how sites are used in the models. Therefore, representativeness of

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the whole calibration data set, rather than the reference or non-reference subset, is most important.

#### Test data sets

We aggregated several data sets to address questions of model fitness. First, we assembled a test data set of bioassessment sampling locations collected between 7/19/1994 and 6/28/2022 to represent the range of conditions found within the study areas (the majority of samples were collected in 2008 or later). We excluded known boatable (i.e., non-wadeable) sites because bioassessment tools were developed for use in wadeable streams. After excluding these sites, these data sets included 311 unique sites in the Central Valley and 126 sites in the Modoc Plateau (437 total); however, it is possible some boatable sites that were not properly documented in bioassessment databases remain in the test data set. All test data came from the CEDEN database. All data were collected following standard SWAMP protocols (Ode et al. 2016a) or comparable methods.

Bioassessment activities have been fairly extensive within the Central Valley floor for more than two decades, and sampling within the Modoc Plateau has intensified since ~2010. Therefore, we have confidence that sampled streams within these regions are representative of typical conditions, and that unsampled reaches are either environmentally similar to sampled reaches, or are unsampleable (e.g., ephemeral) and therefore already known to be inappropriate for assessment with existing bioassessment tools.

Maps of the test data sets are provided in <u>Supplement S1-1</u>.

#### Site inventory

For each calibration data set, we tallied the number of reference and non-reference sites in each study area (Table 2, Figure 2). In general, there were many Central Valley sites in calibration data sets, but only one met the criteria for a reference site. In contrast, more numerous sites in the Modoc Plateau were reference, reflecting the recent efforts of the Reference Condition Monitoring Program targeting this region. As noted above, the 1987 ecoregion boundaries were used to define the Central Valley floor (Omernik 1987).

CSCI calibration data was collected between 1999 and 2010, and ASCI calibration data was collected between 2008 and 2014. Data to calibrate ERMs was collected between 2000 and 2015. Test data was collected between 1994 and 2020; however, over 95% of the data was collected after 2000.

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Table 2. Inventory of unique reference and non-reference sites in each data set and study area.

Data set	Study Area	Reference sites	Non-reference sites	Total
Test	Central Valley	2	309	311*
Test	Modoc Plateau	55	71	126*
CSCI	Central Valley	1	69	70
CSCI	Modoc Plateau	17	37	54
ASCI	Central Valley	1	76	77
ASCI	Modoc Plateau	17	33	50
ERM	Central Valley	1	238	239
ERM	Modoc Plateau	19	39	58

<sup>\*</sup>Of the 311 test sites in the Central Valley ecoregion, 306 are within Region 5, and 5 are within Region 2. Of the 126 test sites in the Modoc Plateau ecoregion, 104 are within Region 5, 2 are within Region 6, and 20 are within Region 1

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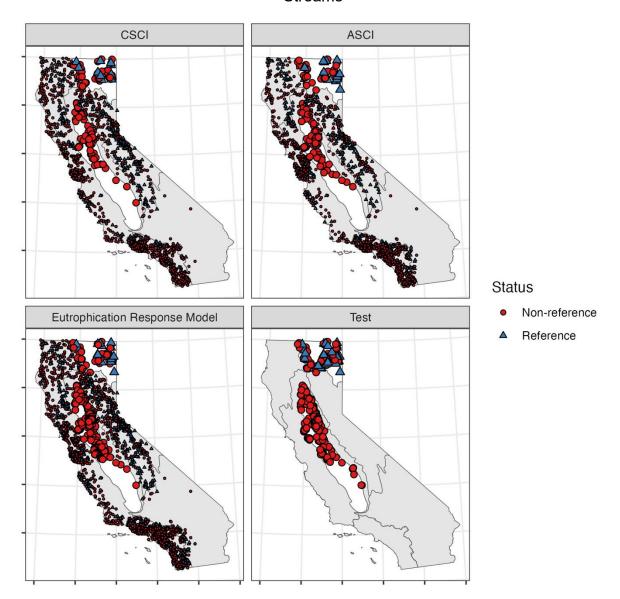


Figure 2. Locations of sites used to assess model fitness. Sites inside the study areas are plotted with larger symbols than sites outside the study areas.

#### **Evaluation of sparsely sampled areas**

For areas with very few sampled locations (e.g., the Tulare Basin in the southern Central Valley), we assume that there are very few sampleable locations due to the region's aridity. The standard SWAMP bioassessment protocol requires sufficient flow to collect benthic organisms (Ode et al. 2016a); reaches with discontinuous flow over 150 m, or reaches that are entirely stagnant are usually rejected from sampling. Analysis of sites evaluated for sampling between 2000 and 2022 as part of probabilistic bioassessment programs (such as the Perennial Streams Assessment, PSA) supports this conclusion: The vast majority of potential sampling locations in that area were identified as non-perennial (and therefore not sampled), with only a small

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number of perennial sites having been identified in the Tulare Basin. In contrast, perennial streams are more common in the northern and middle Central Valley, though still outnumbered by non-perennial streams. Only in the western portion of the Modoc Plateau do perennial streams have a higher density than non-perennial streams (Figure 3). However, this analysis also demonstrates that perennial reaches do occur within highly arid regions, and flow conditions should be assessed at each reach before determining which bioassessment tools may be applicable, such as the streamflow duration assessment methods for the arid west and western mountains developed by the US EPA (Mazor et al. 2021b, 2021d).

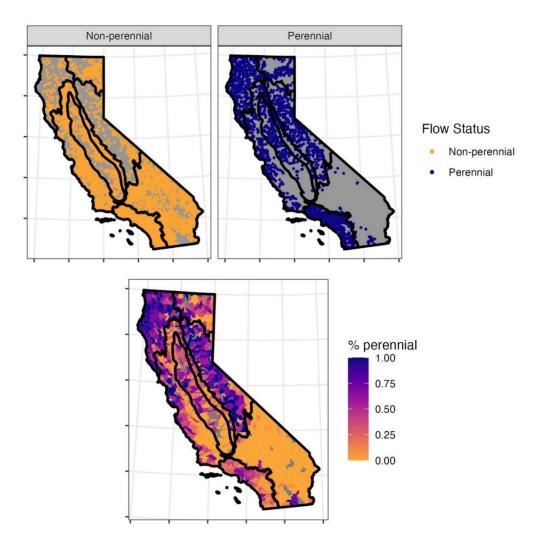


Figure 3. Locations of sites within the Central Valley, Desert, and Modoc regions evaluated for bioassessment sampling under probabilistic programs. The top row shows locations of sites that were identified as having non-perennial (left) or perennial (right) flow. The bottom row shows a map depicting the percent of perennial reaches within each HUC10-scale catchment. Sites with undetermined flow status or non-wadeable rivers were excluded from this analysis.

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### Univariate analyses

In order to compare environmental gradients represented in the calibration data sets with gradients in the study areas, we plotted values of each environmental gradient to compare ranges of values at test sites and calibration sites; reference and non-reference sites were plotted separately. Plots were made for each of the three calibration data sets (i.e., the CSCI, the ASCI, and the ERM calibration data sets) separately.

In general, sites in the Modoc Plateau were well within the values observed at calibration data sets, even when only the reference sites within the calibration data sets were considered (Figure 4). However, sites within the Modoc Plateau had larger watershed areas than sites in the calibration data sets.

In contrast, differences between the Central Valley test sites and the calibration data sets were more notable. Several sites in the Central Valley were larger, and greater ranges in elevation than at reference sites. In addition, several Central Valley sites had soil conditions that were somewhat different from reference sites, having more extreme values of soil erodibility (kfact\_ave) and compressive strength (ucs\_mean) than at reference sites (Figure 4). As noted above, the 1987 ecoregion boundaries were used to define the Central Valley floor (Omernik 1987).

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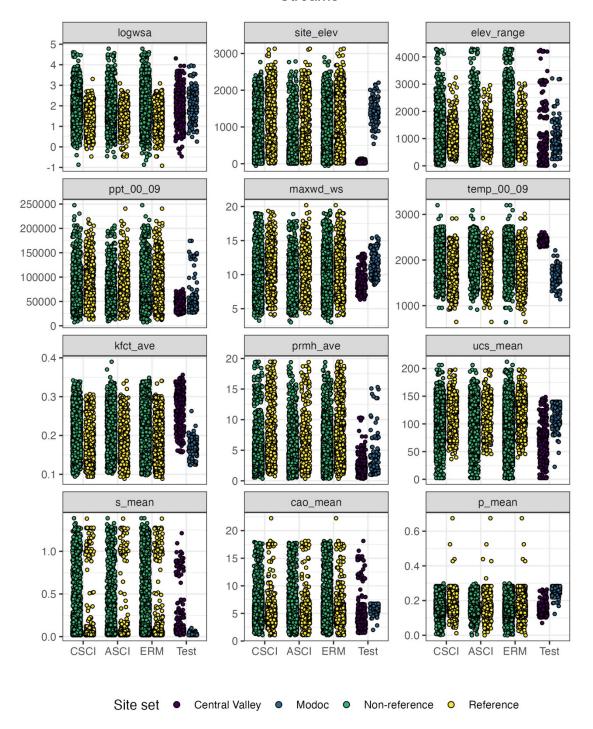


Figure 4. Distributions of selected environmental gradients within three calibration data sets (CSCI: California Stream Condition Index; ASCI: Algal Stream Condition Indices; ERM: Eutrophication Response Models) compared to test sites in the Central Valley and Modoc Plateau ecoregions.

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In addition to investigating landscape-scale characteristics of the environmental settings in the data sets, we also investigated selected reach-scale habitat measurements: slope and percent fines. Valley floor streams typically have low gradients and may naturally have a high proportion of fine particles (i.e., medial axis < 0.06 mm) in the streambed. However, many Central Valley streams may also have elevated levels of fine particles due to human activities, and discerning anthropogenic versus natural sources of sediment can be challenging.

As expected, conditions in the two study areas differed from the calibration data sets with respect to these two gradients (Figure 5). Fines were typically under 50% at ASCI calibration reference sites (max: 56%), and below 25% at CSCI calibration reference sites (max 29%). A wider range of values was observed at test sites in both study areas, with maximum values of 100%.

Likewise, low-gradient conditions that were typical of the Central Valley were not well represented in the reference data sets. For example, the median slope of Central Valley sites was 0.3, and the maximum value was 2.3. In contrast, the lowest gradient reference site in the CSCI data set was 0.9, and 0.5 in the ASCI data set. Overall, 79% of Central Valley sites and 36% of sites in the Modoc Plateau had slopes lower than the lowest CSCI reference sites.

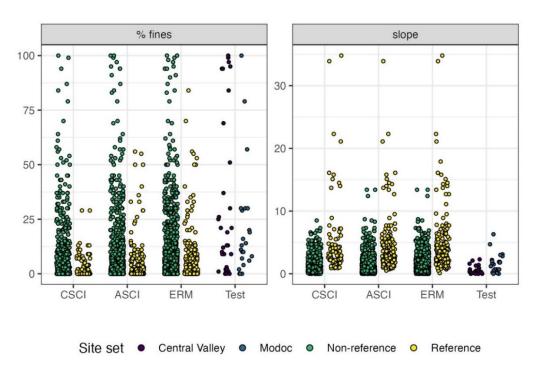


Figure 5. Distributions of slope and % fines in the streambed within three calibration data sets (CSCI: California Stream Condition Index; ASCI: Algal Stream Condition Indices; ERM: Eutrophication Response Models) compared to test sites in the Central Valley and Modoc Plateau ecoregions.

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### Multivariate analyses

The previous analysis examines individual gradients one at a time. However, data gaps may only be evident when multiple gradients are examined at the same time. For example, the calibration data set may contain sites ranging from cold to warm, and from low to high elevation, but it may lack sites that are both cold and low elevation. Multivariate analysis allows us to explore numerous gradients at once.

We examined gradients mentioned above, except for latitude and longitude (to minimize confounding of geographic location with environmental setting). We then conducted non-metric multidimensional scaling (NMDS) with a dimension of three using Gower distance on a combined data set of test and calibration data to summarize the variation in the full environmental matrix (3 axes were sufficient to reduce stress to below 0.1 in all three cases). The resulting NMDS axes were rotated with principal component analysis so that the axes are ordered according to descending proportions of total variation. Convex hulls were drawn around the reference and non-reference calibration data. Sites in the study areas that plot within or near the convex hulls are well represented by the calibration data, whereas those that plot far from the hull boundaries are not well represented. Loadings of each environmental gradient are plotted as vectors to illustrate gradients that distinguish poorly represented sites from those that are well represented.

Multivariate analyses are consistent with these results (Figure 6). The axes are plotted in order of the amount of variance they represent (axis 1: 67 to 69%; axis 2: 20 to 22%; axis 3: 11 to 12%). In all plots, sites in the Modoc Plateau were well within the cloud of points representing reference and non-reference sites in all calibration sets, whereas several Central Valley sites fell outside the cloud of reference points; this separation is most evident along axis three (as shown in the right panels of Figure 6). As indicated by variables with high NMDS scores (positive or negative) driving this discrepancy, several Central Valley sites were hotter and drier, had more erodible soils, and drained larger watersheds than the reference sites. However, when considering the non-reference sites (as is appropriate for the eutrophication response models), Central Valley sites were well within the range of environmental gradients exhibited by the calibration data sets. As noted above, the 1987 ecoregion boundaries were used to define the Central Valley floor (Omernik 1987).

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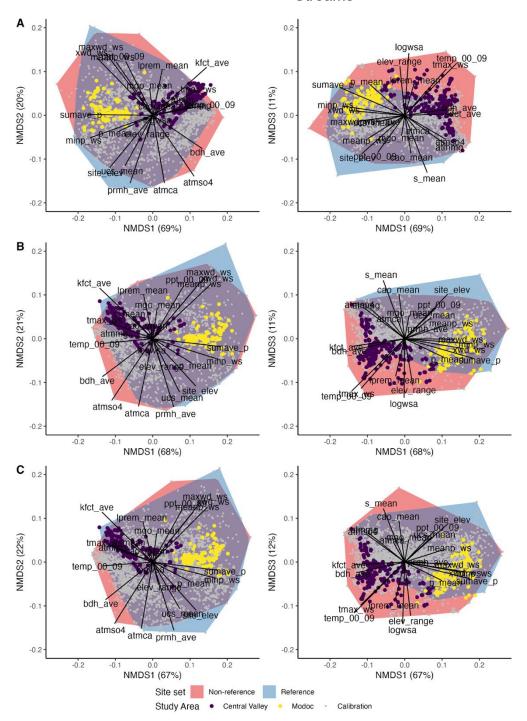


Figure 6. Non-metric multidimensional scaling of calibration and test site data sets (left panels show the first vs. second axes, while the right panels show the first vs. third axes). Vectors represent scores of environmental gradients. Convex hulls show the location of reference and non-reference sites in the calibration data sets. Percentages represent the percentage of total variation of the 3 NMDS axes after principal component analysis rotation. A: CSCI development data. B: ASCI development data. C: Eutrophication response models development data.

# Conclusions about the representativeness of calibration data sets

The development data that were used to develop the CSCI and ASCI are sufficiently representative of environmental conditions in the Modoc Plateau, and these tools may be applied there without major concern. Although there may be unique settings in this and other parts of California for which these tools are unsuitable (e.g., streams with a strong geothermal influence), the majority of stream conditions, as reflected in the test data site of bioassessment sampling locations, are not distinct from these calibration data sets.

In contrast, the Central Valley floor is characterized by conditions that are not well represented in the development data sets. These sites tend to be found in lower elevation and drain larger watersheds than reference sites used to calibrate the CSCI or ASCI. Soil conditions differ somewhat as well, having higher erodibility and lower compressive strength. Therefore, further investigation of the applicability of these indices in the Central Valley is warranted (see <a href="Part 2">Part 2</a>). As noted above, the 1987 ecoregion boundaries were used to define the Central Valley floor (Omernik 1987).

Because the eutrophication response models are calibrated with both reference and non-reference sites, their development data sets are representative of environmental conditions in both the Modoc Plateau and Central Valley ecoregions.

The dearth of sampleable locations in parts of the Central Valley (e.g., Tulare Lake Basin) suggests that there are many reaches where existing bioassessment tools are not applicable. Assessment tools for dry intermittent and ephemeral reaches are currently available (e.g., California Wetlands Monitoring Workgroup 2013, 2020) or in development (Mazor et al. 2021a), but at present, dry streams represent a potentially large knowledge gap of the status of aquatic life uses in these reaches or in adjacent waterbodies.

# Part 1a: How do the models perform at sites in the Central Valley ecoregion?

# How do CSCI and ASCI models perform?

We calculated model performance measures for the CSCI and ASCI. Standard model performance measures are presented in (Mazor et al. 2016, Theroux et al. 2020). These measures focus on accuracy, precision, and responsiveness.

Accuracy relates to the ability of an index to provide a score that correctly reflects the condition of the site, regardless of its natural environmental setting; bias is the opposite of accuracy, and

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it arises when an index provides incorrect scores in certain settings (e.g., a biased index would provide lower scores in naturally intermittent streams than in perennial streams). Accuracy may be improved by adequately representing diverse natural settings in the index calibration data set.

Precision relates to the ability of an index to provide consistent scores at sites in the same condition. A precise index will provide similar scores for multiple samples from the same site, or from multiple sites known to experience the same level of stress (e.g., reference sites). Precision may be improved by reducing sampling variability, as well as by adequately capturing sources of variability in the statistical models that underpin the index.

Responsiveness relates to the ability of an index to change in response to stress. A highly responsive index will show a greater decline in scores at stressed sites than will a less responsive index. Responsiveness can be improved by improving precision

We calculated these measures for each aspect of index performance:

- Accuracy measures:
  - o Do scores at reference sites have an average value close to 1?
  - Do natural gradients explain a negligible proportion (i.e., close to zero) of variation in index scores at reference sites?
- Precision measures:
  - O How much do scores vary among reference sites?
  - o How much do scores vary within resampled reference sites?
- Responsiveness measures:
  - o How much variation in scores do stressor gradients explain across all sites?
  - o How different are mean scores at reference vs. non-reference sites?

Several of these measures require the use of reference sites, where human impacts are minimal (i.e., minimally disturbed sites in Stoddard et al. 2006). These measures are not suitable for evaluating regions like the Central Valley, where reference sites are scarce. Therefore, we evaluated alternative measures of performance:

- Accuracy measures:
  - How many sites within the study areas are able to attain scores indicative of reference conditions (e.g., scores above the 1<sup>st</sup>, 10<sup>th</sup>, or 30<sup>th</sup> percentile of reference)?
  - O How close to one are mean scores at less-disturbed sites?
  - How much variation do natural gradients explain at less-disturbed sites?
- Precision:
  - O How much do scores vary among less-disturbed sites?
  - o How much do scores vary within resampled sites?

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#### Responsiveness:

Do index scores decline as stress levels increase?

Because the previous analysis showed that the development data sets may not be representative of conditions in the Central Valley, these analyses were restricted to that region unless otherwise noted. These measures are summarized in Table 3.

Several of these measures use a "less disturbed" definition of reference, rather than minimally disturbed (Stoddard et al. 2006). "Less-disturbed sites" are those with higher levels of human activity than allowed for a site to be considered reference following the criteria in Ode et al. (2016b) and likely reflect some impacts of disturbance. The process of identifying less-disturbed sites in the Central Valley is described in Rehn et al. (2008).

Table 3. Measures of bioassessment index performance in the Central Valley

Performance aspect	Measure	Evidence of applicability
Accuracy	Number of sites with scores above thresholds to identify reference conditions (1st, 10th, or 30th percentile of reference).	At least 1 site with index scores above referencebased thresholds.
Accuracy	Mean index score at less-disturbed sites.	Mean scores close to 1.
Accuracy	Correlation coefficients between index scores and natural gradients at less-disturbed sites	Correlation coefficients close to 0.
Precision	Standard deviation of mean scores at less-disturbed sites.	Standard deviations close to or lower than numbers reported in original studies (Mazor et al. 2016, Theroux et al. 2020)
Precision	Mean standard deviation at sites with replicate samples or repeat visits.	Standard deviations close to or lower than numbers reported in original studies (Mazor et al. 2016, Theroux et al. 2020)

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Performance aspect	Measure	Evidence of applicability
Responsiveness	Visual inspection of scatterplots of index scores vs disturbance gradients.	Scores are lower where disturbance gradients are highest.
Responsiveness	Variance in index scores represented by disturbance gradients.	High variance represented (as pseudo-R²) in a random forest model to predict index scores from disturbance gradients.

#### Are indices accurate?

#### Can sites attain high CSCI or ASCI scores?

We evaluated the distribution of high-scoring sites in the Central Valley and Modoc Plateau within the test data set described in Table 2. For sites with scores from replicate samples or samples collected on different dates, we evaluated the maximum score for each index to estimate the highest scores attainable in each study area. High-scoring sites were defined as those with maximum scores above the  $10^{th}$  percentile of reference (i.e.,  $\geq 0.79$  for the CSCI and 0.86 for the two ASCIs).

Central Valley sites with high CSCI scores were rare, and (with a few exceptions) were located close to the boundary of the adjacent Interior Chaparral ecoregion (Figure 7); all but one of these sites were considered to be within the Central Valley ecoregion by both the 1987 and 2016 boundaries (Supplement 2). In contrast, sites with high ASCI scores were more numerous and more widely distributed (Figure 7). Table 4 and Figure 8 show the number of sites with bioassessment index scores above various reference-based thresholds in the Central Valley and Modoc Plateau.

Note that because the data used in this study was collected under a number of different monitoring programs with diverse purposes, the frequency of high-scoring sites in the data set may not reflect the frequency of high-scoring reaches in the Central Valley. Such an estimate would require weighting sites according to a statistically appropriate design, and these calculations were outside the scope of the present study.

Table 4. Non-cumulative number of sites with scores in each condition category for benthic macroinvertebrate and algal indices in the two study areas.

Study Area	Index	≥30 <sup>th</sup>	<30 <sup>th</sup> and ≥10 <sup>th</sup>	<10 <sup>th</sup> and ≥1 <sup>st</sup>	<1 <sup>st</sup>
Central Valley	ASCI_D	24	10	18	37
Central Valley	ASCI_H	12	10	11	53
Central Valley	CSCI	3	7	47	244
Modoc	ASCI_D	45	15	8	4
Modoc	ASCI_H	38	13	14	7
Modoc	CSCI	64	19	16	25

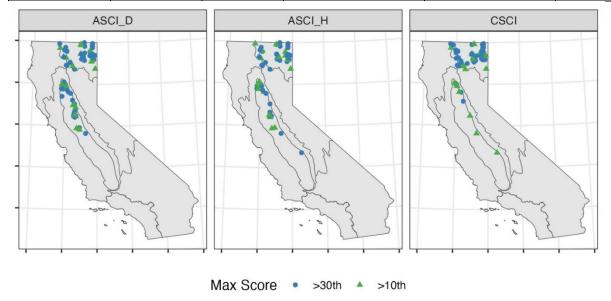


Figure 7. Locations of Central Valley and Modoc Plateau sites scoring above the 10<sup>th</sup> or 30<sup>th</sup> percentile of reference.

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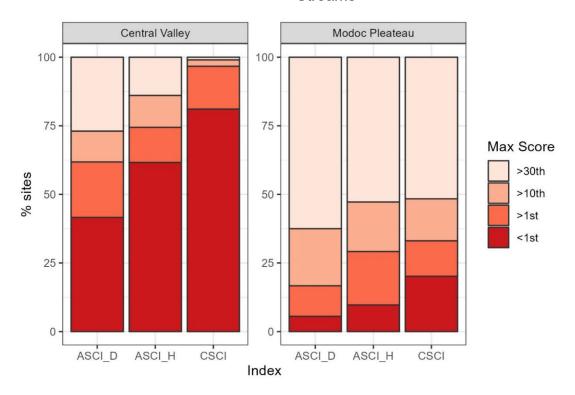


Figure 8. Number of sites with maximum scores in each condition class defined by reference-based thresholds in the Central Valley and Modoc Plateau.

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Most Central Valley sites with high CSCI scores (i.e., scores > 10<sup>th</sup> percentile of reference) drained watersheds with low levels of human activity and were located in reaches with relatively intact riparian zones. For example, two sites on Deer Creek (Tehama County) had CSCI scores above 0.9. The sites were located less than 1 km from the boundary of the Sierra Nevada ecoregion boundary, and thus may not represent the typical conditions on the Central Valley floor. The upper watersheds were mostly forested, although there was some evidence of timber harvest (Figure 9). Aerial images for all high-scoring sites (and field photos, where available) are presented in <u>Supplement S2</u>.



Figure 9. Aerial imagery (top row) and field photos (bottom row) of Deer Creek, reach on the Central Valley floor site with CSCI scores above 0.9.

#### Do less-disturbed sites score similar to reference sites?

Sites that meet the standard definition of reference used to calibrate the CSCI are nearly absent from the Central Valley ecoregion (Mazor et al. 2016, Ode et al. 2016b). However, scores at relatively less-disturbed sites that do not meet all reference criteria provide an alternative way to evaluate index accuracy. If these sites score similar to reference, then the index is not biased.

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However, if scores are lower at less-disturbed sites, then the index could be biased, or it could reflect the impacts associated with the lower levels of disturbance in this data set.

We use three sources to identify less-disturbed sites (Figure 10):

- One site within the Central Valley ecoregion meet all reference criteria in Ode et al. (2016b): "Deer Creek 2" in Tehama County (CEDEN station code 504PS0227). This site was sampled in 2009, and data from this sampling event was used to calibrate the CSCI (Mazor et al. 2016).
- A set of less-disturbed sites designated as "reference" was identified for the development of the Central Valley Index of Biotic Integrity (CV-IBI, Rehn et al. 2008). In general, these sites have relatively good habitat quality and lower levels of non-natural land use at the local scale, but extensive agricultural or urban land use in the watershed. Although all of these sites were used to calibrate the CV-IBI because they represent less-disturbed conditions within the Central Valley, about half of them were located in areas considered to part of the adjacent Chaparral ecoregion by the 2016 updated boundaries (Griffith et al. 2016).
- A site on Orestimba Creek in Stanislaus County is located near a USGS stream gage (ID 11274500) and has been designated by the USGS as a hydrologic reference site (Falcone 2011). This site nearly meets all reference criteria in Ode et al. (2016b). Unlike the other sites identified as less-disturbed reference, Orestimba Creek is intermittent.

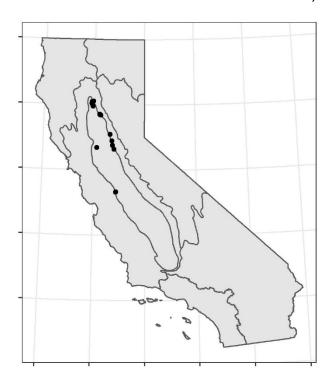


Figure 10. Locations of less-disturbed sites in the Central Valley

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Scores at less-disturbed sites were lower than would be expected at traditional perennial reference sites (Table 5; Figure 11). For example, the mean CSCI score at these sites was 0.77, which is below the 10<sup>th</sup> percentile of scores at traditional reference sites (i.e., 0.79). Scores were higher at the seven chaparral sites used in the CV-IBI than at the sites within the Central Valley ecoregion. Only four of these sites were sampled for algae, but ASCI scores were nonetheless highly variable among sites.

Table 5. Mean index scores at less-disturbed sites in the Central Valley. Mean within-site scores were used for calculations at sites with multiple visits. Sites came from three sources: Central Valley IBI reference sites (CV-IBI; Rehn et al. 2008), CSCI reference sites (CSCI; Mazor et al. 2016), and the USGS reference Gages II database (USGS; Falcone 2011). Seven reference sites used to calibrate the CV-IBI were located just outside the Central Valley border in the adjacent Chaparral ecoregion if the 2016 ecoregion boundaries are used (Griffith et al. 2016). Numbers in parentheses reflect the number of sites with ASCI scores and CSCI scores, respectively.

Subset of sites	ASCI_D	ASCI_H	CSCI
All sites (4/17)	0.90	0.85	0.77
By 2016 ecoregion	-	-	-
Chaparral (3/7 sites)	1.09	1.08	0.89
Central Valley (1/10 sites)	0.84	0.77	0.68
By source			
CV-IBI (2/14 sites)	0.85	0.76	0.75
CSCI (2/2 site)	0.95	0.94	0.94
USGS (0/1 site)	No data	No data	0.64

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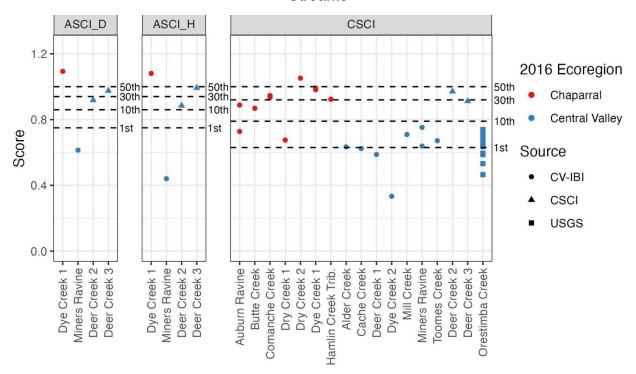


Figure 11. Bioassessment index scores at less-disturbed Central Valley sites. Sites came from three sources: Central Valley IBI reference sites (CV-IBI; Rehn et al. 2008), CSCI reference sites (CSCI; Mazor et al. 2016), and the USGS reference Gages II database (USGS; Falcone 2011). Seven reference sites used to calibrate the CV-IBI were located just outside the Central Valley border in the adjacent Chaparral ecoregion if the 2016 ecoregion boundaries are used (Griffith et al. 2016). Horizontal dashed lines indicate the 50<sup>th</sup>, 30<sup>th</sup>, 10<sup>th</sup>, and 1<sup>st</sup> percentiles of scores at reference sites for each index (Mazor et al. 2016, Theroux et al. 2020).

Based on this small data set, CSCI scores at less-disturbed sites had weak relationships with most natural gradients, indicating low levels of bias (Table 6, Figure 12). However, relationships were moderately strong (r² between 0.2 and 0.5) for 5 variables. Specifically, higher scores were associated with higher elevations (site\_elev), higher precipitation (ppt\_00\_09), lower air temperature (temp\_00\_09), higher phosphorus content in the watershed's underlying geology (p\_mean), and less erodible soils (kfact\_ave) in the watershed.

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Table 6. Pearson's correlation coefficient between environmental gradients and CSCI scores at less-disturbed sites in the Central Valley.

Variable	Pearson's r
bdh_ave	-0.18
cao_mean	0.14
elev_range	-0.13
kfct_ave	-0.41
logwsa	-0.38
lprem_mean	-0.04
maxwd_ws	0.25
mgo_mean	0.06
minp_ws	0.05
new_lat	0.27
new_long	-0.04
p_mean	0.53
ppt_00_09	0.68
prmh_ave	0.37
s_mean	-0.30
site_elev	0.63
sumave_p	0.27
temp_00_09	-0.69
tmax_ws	-0.17
ucs_mean	-0.01

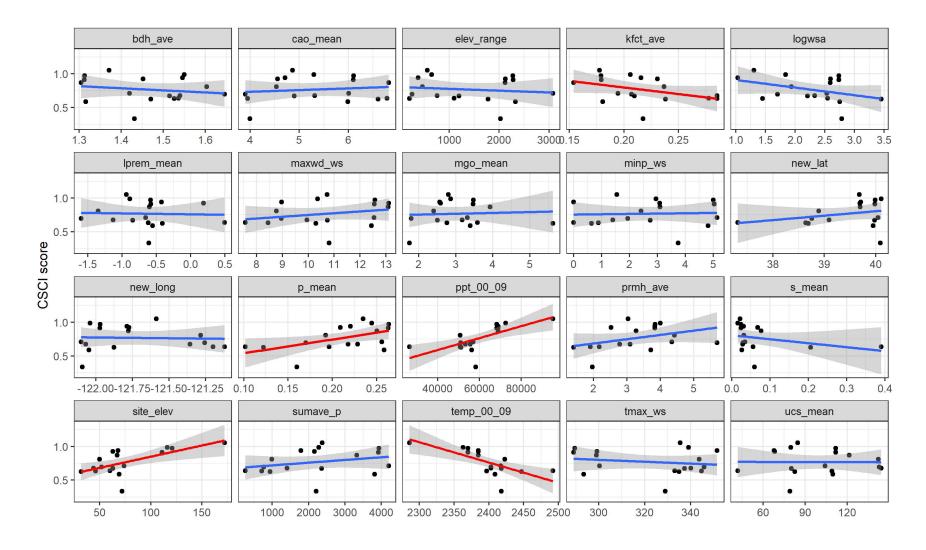


Figure 12. Relationships between natural gradients and CSCI scores at less-disturbed sites in the Central Valley. The top 5 strongest relationships are highlighted in red.

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### Are indices precise?

Precision was measured as variability in CSCI scores among and within less-disturbed Central Valley sites. Variability among less-disturbed sites was higher than at traditional reference sites, potentially indicating worse precision (Table 7). However, this analysis is limited by several factors:

- Few sites were available to assess variability among less-disturbed sites, especially for ASCI scores.
- The amount of stress experienced by less-disturbed sites likely varies among sites substantially. In contrast, all traditional reference sites likely experience consistently low levels of stress.

Table 7. Standard deviation of index scores among sites at Central Valley less-disturbed reference sites and traditional reference sites (values for traditional reference sites reported in Mazor et al. 2016 and Theroux et al. 2020)

Index	Central valley less- disturbed reference site	Traditional reference site
CSCI	0.19	0.16
ASCI_D	0.20	0.11
ASCI_H	0.28	0.11

Mean within-site variability of the CSCI at four less-disturbed sites with replicate samples was 0.06, which was lower than the mean within-site variability reported at traditional reference sites (i.e., 0.11; Mazor et al. 2016). However, this value could be an underestimate, due to the low number of less-disturbed sites with replicate data.

## Are indices responsive to stress?

#### Relationships with human activity gradients

Scatterplots of index scores versus human activity gradients generally revealed wedge-shaped relationships in both study areas (Figure 13). These wedge-shaped relationships demonstrate that the indices are responsive to human activity gradients, and that at low levels of an individual gradient, high scores are more common than at high levels of disturbance. Low scores at low levels of a human activity gradient are likely due to the influence of other stressors.

Overall, responsiveness was stronger in the Central Valley than in the Modoc Plateau. This difference is largely due to the relatively poor representation of human activity gradients within

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the Modoc Plateau, as few sites in that region had high values for these measures of human activity. A small number of sites with high scores and relatively high levels of landscape-scale alteration had outsize leverage on the Modoc Plateau models, leading to spurious increases in some of the relationships (e.g., between ASCIs and % code 21, a landcover type associated with roadsides, golf courses and other types of highly managed vegetation). The weak relationship between riparian disturbance and the algal indices is consistent with studies showing generally weaker relationships between algal communities and physical habitat degradation (e.g., Mazor et al. 2022).

A major challenge in evaluating the responsiveness of an index is ensuring that data sets include broad gradients in both stress levels and biological conditions. Thus, analyses of data at the statewide scale are usually well suited to evaluating index responsiveness, while those focused on certain ecoregions (e.g., the Central Valley floor) or stream types (e.g., modified channels) may underestimate or mischaracterize how well the index performs. In regions like the Central Valley, these gradients are particularly poorly represented — most sites experience relatively high levels of human activity, and high CSCI scores are rare. Thus, statistical tests conducted solely on data collected from the Central Valley can give the false impression that the CSCI is unresponsive to stress in this region. Well-controlled studies that make use of field manipulations (e.g., creating stress gradients within a small watershed) may be more effective in evaluating the responsiveness of the CSCI to stress within the Central Valley.

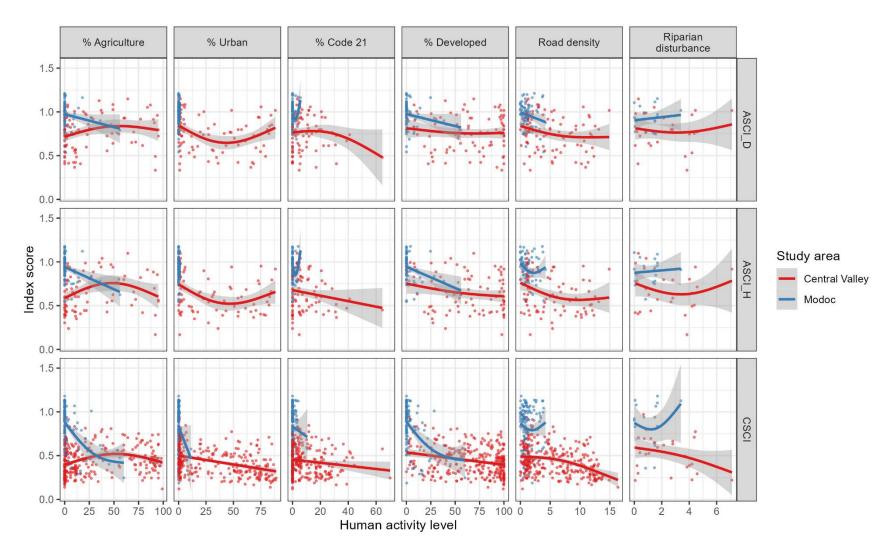


Figure 13. Bioassessment index score responses to disturbance at sites. Each line shows a general additive model calibrated for each study area. All landscape metrics were calculated at the 5-km scale.

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Random forest models calibrated to predict index scores from landcover metrics (specifically, road density, urban, agricultural, and code 21 in the watershed, within 5 km, and within 1 km of the sampling location) explained 61% of variation in CSCI scores, 46% of variation in ASCI\_H scores, and 46% of variation in ASCI\_D scores. The importance of different disturbance metrics was similar across indices (Figure 14).

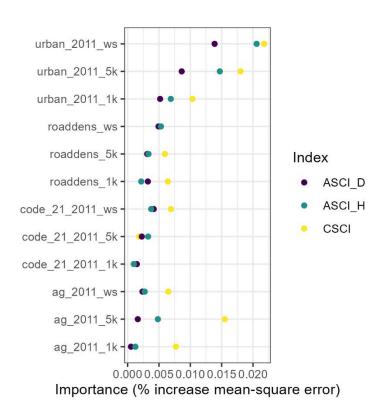


Figure 14. Importance of disturbance metrics (measured as increased mean-square error) in random forest models calibrated to predict bioassessment index scores. Metrics ending in "\_ws" were calculated at the entire watershed scale; metrics ending in "\_5k" were calculated within 5 km clips of the watershed; and metrics ending in "1k" were calculated within 1 km clips of the watershed. "Urban\_2011" metrics are urban landcover from the 2011 version of the National Landcover Dataset (NLCD, Jon Dewitz 2024); "ag\_2011" metrics are agricultural landcover from the NLCD; and "code\_21\_2011" metrics are developed open space (e.g., lawns, golf courses, road sides) from the NLCD. "Roaddens" is the

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density of paved roads and railroads. See Ode et al. (2016b) and Boyle et al. (2020) for more information about these metrics and how they are calculated.

#### Relationships with field-measured water quality and physical habitat stressors

We evaluated relationships between index scores and field-measured water quality variables and physical habitat variables (Table 8). Spearman rank correlation coefficients were generally lower for field-measured stressors than for landscape-scale disturbance gradients (Table 9, Figure 15, Figure 16). The CSCI was most strongly correlated with % fast-water habitat (a measure of in-stream habitat diversity), specific conductivity, and water temperature (followed closely by TN). In contrast, the ASCIs were more strongly correlated with % sands and fines on the streambed, turbidity, and total nitrogen concentrations. Note although high levels of % sands and fines are typically associated with disturbance, hard bottom channels that undergo regular maintenance typically have very low levels of sands and fines.

Table 8. Water quality and physical habitat stressors used to evaluate index responsiveness.

Туре	Stressor	Unit	Abbreviation
Water quality	Dissolved oxygen	mg/L	DO
Water quality	pH	Unitless	рН
Water quality	Specific conductance	μS/cm	Sp. Cond
Water quality	Water temperature	°C	Temp
Water quality	Turbidity	NTU	Turb
Water quality	Total nitrogen	mg/L	TN
Water quality	Total phosphorus	mg/L	TP
Physical habitat	Percent sands and fines	%	PCT_SAFN
Physical habitat	Riparian vegetation cover	%	XCMG
Physical habitat	In-stream natural habitat complexity	%	XFC_NAT
Physical habitat	Percent fast-water habitats	%	PCT_FAST

Table 9. Spearman rank correlation coefficients between stress gradients and index scores within the study areas.

Stressor type	Stressor	CSCI	ASCI_D	ASCI_H
Landscape	% Agriculture	-0.21	-0.25	-0.35
Landscape	% Urban	-0.62	-0.51	-0.63
Landscape	% Code 21	-0.26	-0.23	-0.35
Landscape	% Developed	-0.60	-0.46	-0.62

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Stressor type	Stressor	CSCI	ASCI_D	ASCI_H
Landscape	Road density	-0.47	-0.41	-0.51
Landscape	Riparian disturbance	-0.23	-0.10	-0.19
Habitat	PCT_SAFN	-0.51	-0.46	-0.52
Habitat	XCMG	0.23	-0.20	-0.19
Habitat	XFC_NAT	0.46	0.13	0.21
Habitat	PCT_FAST	0.67	0.25	0.36
Water quality	Dissolved oxygen	0.21	0.18	0.20
Water quality	pН	0.11	0.30	0.33
Water quality	Specific conductivity	-0.58	-0.24	-0.33
Water quality	Temperature	-0.56	-0.19	-0.29
Water quality	Turbidity	-0.52	-0.34	-0.46
Water quality	TN	-0.56	-0.35	-0.42
Water quality	TP	-0.38	-0.28	-0.38

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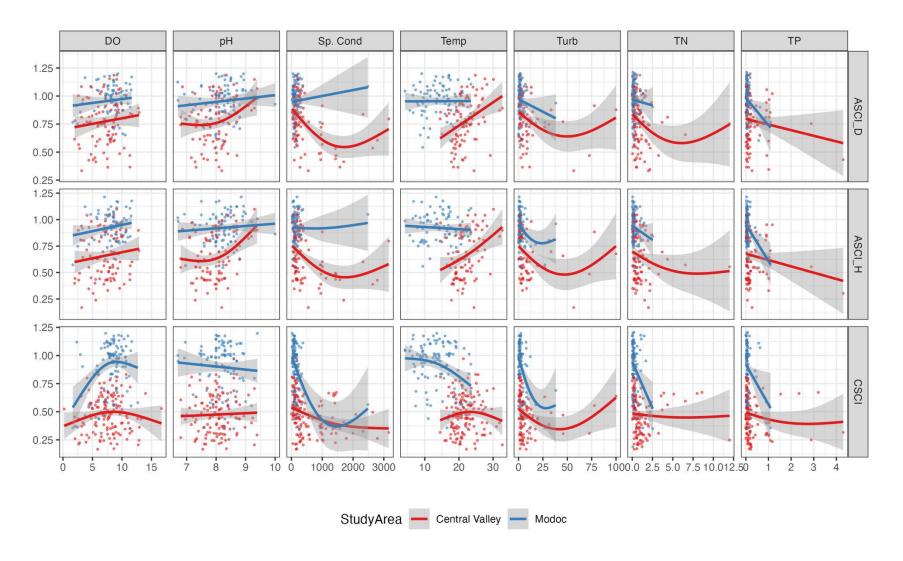


Figure 15. Index score responses to field-measured water quality stressor gradients in the Central Valley and Modoc Plateau. Each line shows a general additive model calibrated for each study area. DO: Dissolved oxygen (mg/L). Sp. Cond: Specific conductivity ( $\mu$ S/cm). Temp: Water temperature (°C). Turb: Turbidity (NTU). TN: Total nitrogen (mg/L). TP: Total phosphorus (mg/L).

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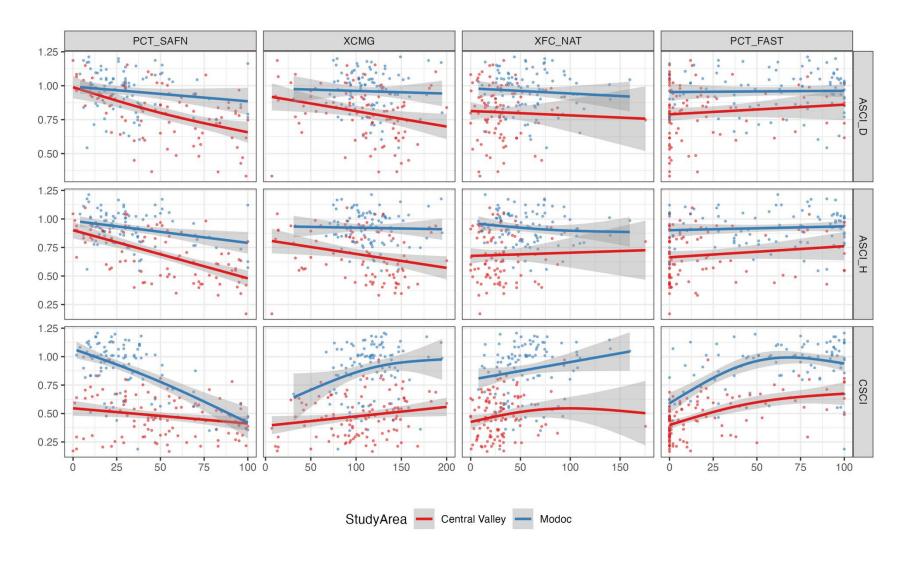


Figure 16. Index score responses to field-measured physical habitat stressor gradients in the Central Valley and Modoc Plateau. Each line shows a general additive model calibrated for each study area. PCT\_SAFN: % sands and fines. XCMG: Mean riparian vegetation cover in the upper canopy, mid-canopy, and groundcover layers. XFC\_NAT: Mean natural fish cover on the streambed. PCT\_FAST: Percent fast-water habitats.

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# How do eutrophication response models perform?

Do response models correctly predict the likelihood of good biological conditions in the Central Valley and Modoc Plateau?

We calculated probabilities of good biointegrity conditions calculated by statewide logistic regression models (Mazor et al. 2022), and compared probabilities against index scores at sites in the Central Valley and Modoc Plateau. Higher probabilities at high-scoring sites than at low-scoring sites is evidence that the models are applicable to these regions.

As expected, higher index scores occurred at levels of eutrophication indicators where models predicted a high probability of high scores (Figure 17). Strong, step-shaped relationships were evident for the CSCI and nutrients and chlorophyll a. High scores were rarely observed when the modeled probability was below 75%. Relationships between ASCIs and nutrients were considerably noisier. The probabilities predicted from the percent algal cover models were poorly correlated with ASCI scores, but had a stronger association with CSCI scores.

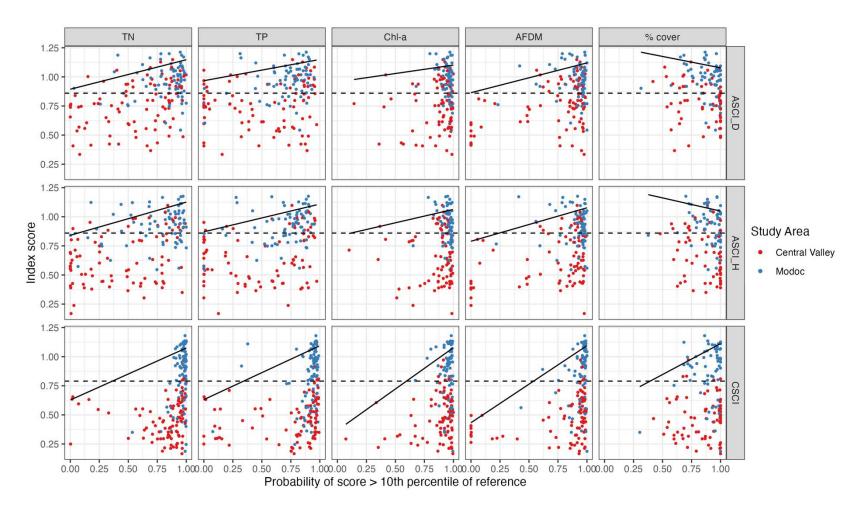


Figure 17. Index scores versus eutrophication response model predictions of the likelihood of attaining scores above the 10<sup>th</sup> percentile of scores at reference. Horizontal dashed lines represent the 10<sup>th</sup> percentile threshold). Solid lines represent regressions of the 90<sup>th</sup> quantile of index scores. TN: Total nitrogen. TP: Total phosphorus. Chl-a: Benthic chlorophyll-a. AFDM: Benthic ash-free dry mass. % cover: Percent macroalgal cover on the streambed.

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We also evaluated the applicability of the thresholds derived from those models. We repeated several analyses from Mazor et al. (2022) on the subset of sites in the Central Valley and Modoc. First, we compared the number of eutrophication targets met with the number of biointegrity indices passed. In a statewide analysis presented in Mazor et al. (2022), the proportion of sites meeting biointegrity thresholds for multiple indices increases as the number of eutrophication targets are met, demonstrating the effectiveness of using multiple indicators of eutrophication (Figure 18). In the Central Valley, the pattern was considerably weaker, in that the increase in the proportion of sites meeting biointegrity indices only increased once four of the five eutrophication thresholds were met (Figure 19). Within the Modoc Plateau, the patterns were more similar to those observed in the statewide analysis. In both regions, lack of data representing certain conditions made patterns somewhat more difficult to discern (e.g., there were no sites in the Modoc Plateau that exceeded all five eutrophication targets, and only one site in the Central Valley met all biointegrity goals).

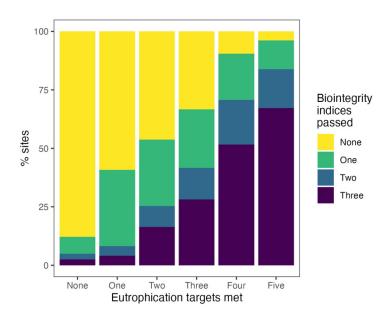


Figure 18. Percent of sites meeting thresholds for the CSCI, ASCI\_D, and ASCI\_H when different numbers of eutrophication targets are met, based on a statewide analysis presented in Mazor et al. (2022).

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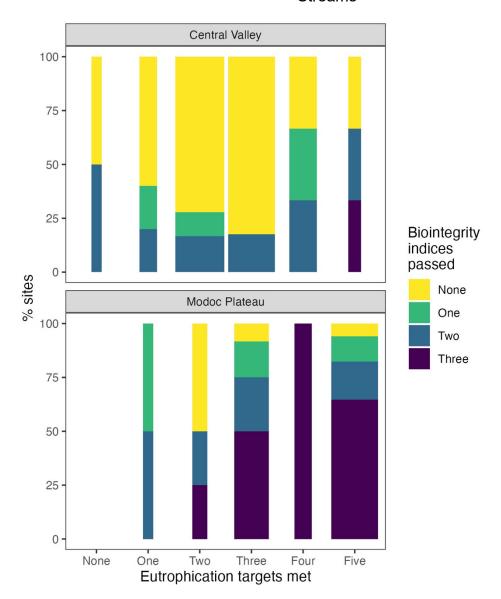


Figure 19. Percent of sites meeting thresholds for bioassessment indices when different numbers of eutrophication targets are met. Width of bars is proportional to the number of sites in each category.

Are statewide eutrophication thresholds associated with significant risks to biointegrity in the Central Valley and Modoc Plateau?

Relative risk analysis was used to validate thresholds in Mazor et al. (2022). Relative risks are calculated as the frequency of sites with a low index score among those that exceed a eutrophication threshold, divided by the frequency of low-scoring sites among the general population of sites. Relative risks greater than 1 indicate that exceeding a eutrophication target is associated with a risk of poor biology.

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We calculated relative risks on subsets of sites within the Central Valley and Modoc Plateau ecoregions (Table 10, Figure 20). Data were rarely sufficient to determine if the risks were significantly greater than 1. However, risks were considerably higher for nutrients than they were for measures of algal biomass. The % cover thresholds were below 1 for all indices in both regions. The chlorophyll-a threshold could not be evaluated in the Modoc Plateau due to the lack of sites exceeding the threshold.

Table 10. Relative risks for eutrophication thresholds presented in Mazor et al. (2022). Pass both: Number of sites that met both the biointegrity goal and eutrophication target. Fail both: Number of sites that failed both the biointegrity goal and eutrophication target. Fail BI: Number of sites that failed the biointegrity goal but met the eutrophication target. Fail ET: Number of sites that failed the eutrophication target but met the biointegrity goal. RR: Relative risk. L95: Lower bound of the 95% confidence interval around the relative risk estimate. U95: Upper bound of the 95% confidence interval around the relative risk estimate. NA: Relative risk or its confidence interval could not be calculated due to lack of data. CV: Central Valley. MP: Modoc Plateau

Indicator	Index	Area	Pass both	Fail both	Fail BI	Fail ET	RR	L95	U95
Total N	ASCI_D	CV	7	29	10	10	1.3	8.0	1.9
Total N	ASCI_D	MP	20	5	6	12	1.3	0.6	2.9
Total N	ASCI_H	CV	7	35	7	7	1.7	1.0	2.7
Total N	ASCI_H	MP	20	7	3	13	2.7	0.9	7.9
Total N	CSCI	CV	5	11	40	0	1.1	1.0	1.2
Total N	CSCI	MP	31	1	11	0	3.8	2.5	5.9
Total P	ASCI_D	CV	9	29	10	8	1.5	1.0	2.2
Total P	ASCI_D	MP	23	5	6	9	1.7	8.0	3.8
Total P	ASCI_H	CV	8	31	11	6	1.4	1.0	2.1
Total P	ASCI_H	MP	26	7	3	7	4.8	1.7	13.5
Total P	CSCI	CV	5	22	29	0	1.2	1.1	1.3
Total P	CSCI	MP	31	0	12	0	NA	NA	NA
Chl-a	ASCI_D	CV	15	5	34	2	1.0	0.7	1.6
Chl-a	ASCI_D	MP	31	0	11	1	NA	NA	NA
Chl-a	ASCI_H	CV	13	6	36	1	1.2	0.9	1.6
Chl-a	ASCI_H	MP	32	0	10	1	NA	NA	NA
Chl-a	CSCI	CV	5	6	45	0	1.1	1.0	1.2
Chl-a	CSCI	MP	30	0	12	1	NA	NA	NA

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Indicator	Index	Area	Pass both	Fail both	Fail BI	Fail ET	RR	L95	U95
AFDM	ASCI_D	CV	10	15	24	7	1.0	0.7	1.3
AFDM	ASCI_D	MP	27	2	9	5	1.1	0.4	3.0
AFDM	ASCI_H	CV	9	17	25	5	1.1	8.0	1.4
AFDM	ASCI_H	MP	29	3	7	4	2.2	0.9	5.5
AFDM	CSCI	CV	5	13	38	0	1.1	1.0	1.2
AFDM	CSCI	MP	30	2	10	1	2.7	1.1	6.5
% cover	ASCI_D	CV	6	11	27	10	0.6	0.4	0.9
% cover	ASCI_D	MP	22	0	10	8	NA	NA	NA
% cover	ASCI_H	CV	5	13	28	8	0.7	0.5	1.0
% cover	ASCI_H	MP	24	1	9	6	0.5	0.1	2.9
% cover	CSCI	CV	2	19	31	2	1.0	8.0	1.1
% cover	CSCI	MP	24	1	9	6	0.5	0.1	2.9

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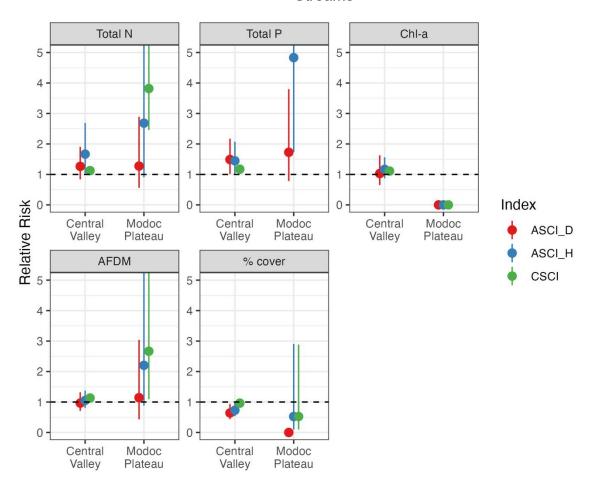


Figure 20. Relative risks of eutrophication targets derived for each biointegrity index. Thresholds were taken from Mazor et al. (2022). Vertical lines indicate the 95% confidence interval.

### Part 1a Conclusions

Although there is sufficient evidence that reference-like scores for the CSCI and ASCIs are attainable within the Central Valley, and even at sites in agricultural settings, we cannot be certain whether the high frequency of low index scores in this region are correctly reflecting the impacts of disturbance, or if they are reflecting a bias in the indices due to poor representation of valley floor conditions, or a combination. The responsiveness of the indices to stress gradients should provide confidence that the index scores may be used to measure relative conditions (e.g., differences between sites or changes in a site over time). There is less confidence, however, in the appropriateness of using reference-based thresholds to identify degraded biological conditions in the Central Valley.

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This question will not likely be resolved with additional data collection because there are perhaps no reference sites in the Central Valley left to sample. But the concern could be rendered moot if alternative thresholds based on a "best attainable" or "best observed" definition are identified, rather than one based on minimally disturbed conditions (Stoddard et al. 2006; see Part 3 for more discussion of approaches to identifying thresholds). These thresholds would already account for potential biases in the Central Valley. Best-observed thresholds are based on the best conditions observed today (e.g., the 90<sup>th</sup> percentile of CSCI scores at sites in the Central Valley). In contrast, best-attainable thresholds would be based on evidence from restorations, models, or manipulative experiments to predict likely improvements, which may exceed present-day best-observed scores.

The performance of eutrophication response models was good in the two study areas, but data limitations prevented robust evaluation of thresholds derived from statewide eutrophication response models.

#### Recommendations:

- The CSCI and the ASCIs may be used to assess biological integrity in the Central Valley, although alternative interpretations may be helpful for the CSCI.
- Explore ways to use science produced in on-going studies of modified channels to identify best-available thresholds. In addition, take advantage of any planned restoration or water quality improvement plans to identify potential best-attainable thresholds for site-specific applications.
- Fill major data gaps. Identify additional less-disturbed sites (both perennial and intermittent) in the Central Valley for further data collection. Few of these sites have been sampled recently, and thus few have been sampled for benthic algae or eutrophication indicators using up-to-date protocols.

# Part 1b: How does streamflow duration and intermittency affect model performance?

# How does intermittency affect stream biota and measures of biointegrity?

Streamflow duration is known to have a large impact on the ecological functions and biological composition of stream communities (e.g., Timoner et al. 2012, Bogan et al. 2013, Stubbington et al. 2017, von Schiller et al. 2017). Although streamflow duration varies on a continuum, reaches are often categorized into just a few categories. For example, the EPA classifies streamreaches as follows (Fritz et al. 2020):

- Perennial reaches, which experience surface flow year-round.
- Intermittent reaches, which experience surface flow for part of the year, and the local groundwater table is above the streambed for at least part of the year.
- Ephemeral reaches, which experience surface flow in direct response to rain events, and the local groundwater table is always below the bottom of the streambed.

These definitions are based on natural flow regimes, and highly modified systems may defy easy categorization. Within California bioassessment programs (e.g., Mazor 2024), intermittent streams are typically described as having at least 4 weeks of continuous flow, and at least 7 continuous days without flow in typical years. Waiting at least 4 weeks after the most recent major storm event is provided as a guideline for proper implementation of California's bioassessment protocols (Ode et al. 2016a), meaning that the protocols can be applied to intermittent streams.

Bioassessment with benthic macroinvertebrates or benthic algae is suitable for perennial reaches and intermittent reaches with long duration flows. Ephemeral streams typically do not support aquatic life, and cannot be assessed with traditional bioassessment tools, such as the CSCI or the ASCIs. For the purposes of this report, we focus on how bioassessment practices and tool applicability is affected by intermittency in reaches with long duration flows.

Desiccation resistance and resilience are some of the primary life history adaptations to life in intermittent streams (Sabater et al. 2017, Stubbington et al. 2017). Resistance traits include diapause stages or behavioral adaptations, like burrowing in refugia, which allow species to survive the dry period (Cover et al. 2015). For benthic algae, resistance traits include the ability to form protective mucilaginous structures or crusts, and cellular changes that protect photosynthetic pigments from ultraviolet radiation (Sabater et al. 2017). Resilience traits include aerial dispersal, ability to drift, and rapid reproduction, allowing taxa to recolonize a reach after it has rewetted (Robson et al. 2008, Schriever et al. 2015).

The dry-down phase can have varied impacts on stream biota, depending on a reach's geological setting. In reaches overlying coarse sand and alluvium, the dry-down can be abrupt. More confined reaches with bedrock outcrops may sustain long-lasting or perennial pools or saturated hyporheic zones, which can provide refuge for aquatic species throughout the dry period. In the latter, physicochemical conditions can vary greatly, with oxygen levels, water temperature, and chemical concentrations changing rapidly (Gómez et al. 2017). Adaptations to survive hypoxic conditions include physiological traits, such as red pigments, and behavioral adaptions, such as aerial respiration (Stubbington et al. 2017, Merritt et al. 2019). Because many of these adaptations also help biota survive pollution, it can be difficult to distinguish bioassessment samples collected from a polluted stream with those collected from an

intermittent stream late in the dry-down process (Mazor et al. 2014, Loflen 2019). In addition to the increased abiotic stresses from physicochemical changes, organisms in intermittent streams experience increased biotic stress during the dry-down phase. The decreased volume of available aquatic habitat concentrates organisms, leading to more intense competition, predation, and disease (Gasith and Resh 1999, Stubbington et al. 2017).

## How does intermittency affect eutrophication in streams?

In a review of nutrient and organic matter dynamics in intermittent streams, von Schiller et al. (2017) describe intermittent rivers and ephemeral streams as the "biogeochemical heartbeat" of watersheds in arid regions, due to their high temporal and spatial variation.

Von Schiller et al. (2017) describe four hydrologic phases of intermittent and ephemeral streams relevant to the processing of nutrients and organic matter (Figure 21):

- Expansion, when discharge rates increase, leading to an increase in aquatic habitat.
- Contraction, when discharge rates decline, leading to a reduction in aquatic habitat.
- Fragmentation, when flow ceases in some areas, leading to isolated pools and other disconnected aquatic habitats.
- Desiccation, when surface water is absent, and the substrate may dry out.

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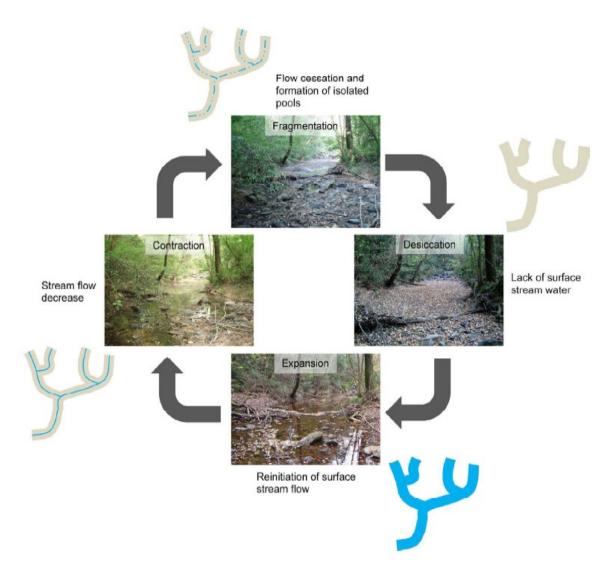


Figure 21. Typical hydrologic phases that drive temporal and spatial variation in nutrient and organic matter dynamics in intermittent rivers and ephemeral streams, reproduced from von Schiller et al. (2017). Pictures are from a Mediterranean-climate intermittent stream in Catalonia, Spain, along with schematic diagrams of a river network showing surface-water presence as blue lines.

They note that reaches vary considerably in the way they progress through these phases. For example, a stream underlain by coarse sand and alluvium may not experience fragmentation at all, and differences in weather patterns across years means that a same site might exhibit different patterns each year.

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In general, contraction increases the importance of in-stream sources of nutrients and organic matter, relative to terrestrial sources, and downstream exports may be reduced (Dahm et al. 2003).

During contraction and fragmentation, several drivers of eutrophication are likely to change, leading to an increased likelihood of eutrophication (Gasith and Resh 1999, von Schiller et al. 2017). First, during contraction periods, impacts from any point-source discharges of nutrients are typically greatest, because if any effluent discharges occur they can constitutes a larger percentage (sometimes 100%) of baseflow, with increased pollutant concentration (Oberholster et al. 2019, Ewart-Smith 2022). The effects of nutrient loading on eutrophication may be further exacerbated by seasonal increases in water temperatures, increased hydrologic residence time, reduction of scouring flows, all of which promote the growth of algae and cyanobacteria (Stevenson 1997, Atkinson and Cooper 2016). Higher algal biomass, higher water temperature and lower flow lead to greater diurnal swings in dissolved oxygen and pH. These trends are further exacerbated by shallower water depths and reduce shade, which can increase light penetration (Sturt et al. 2011, Warren et al. 2017). Increased residence time allows for rapid microbial turnover and utilization of available nutrient pools, particularly in remnant pools, maintaining high productivity of chronic algal blooms (Ardón et al. 2021).

During desiccation, organic matter deposited on the streambed (e.g., dead algal mats) is typically processed by invertebrate and microbial "clean-up crews" (Timoner et al. 2012, Sabater et al. 2016, 2017), leading to a transfer of nutrients and carbon to the nearby terrestrial environment and decreasing loads to downstream waters. However, terrestrial organic matter may accumulate in the dry streambed, leading to a large pulse of material delivered upon first flush, especially in highly seasonal climates like Mediterranean California (Gasith and Resh 1999, Datry et al. 2011, 2018). During the expansion phase, production of organic matter is typically low due to cold temperatures, and accumulation may be limited by scouring flows.

# Do indices work in intermittent streams in arid portions of California?

Several studies have been conducted throughout arid portions California (defined as the Chaparral, South Coast, Desert/Modoc, and Central Valley ecoregions; Figure 22) on the application of bioassessment indices to intermittent streams (Loflen 2020). These studies primarily focus on reference sites, which may be why none have yet been conducted in the Central Valley ecoregion. To date, there has been little bioassessment sampling from intermittent streams in non-arid portions of California. Here we summarize findings from these studies.

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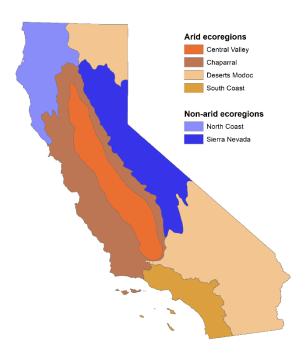


Figure 22. A map of arid and non-arid ecoregions. Ecoregion boundaries are based on Omernik 1987 and refined in 2016 by Griffith et al. (2016).

A study of intermittent streams in the San Diego region found that the Southern California Index of Biotic Integrity (Ode et al. 2005) could be used to correctly assess the condition of reference streams during baseflow conditions, but scores declined if samples were collected from reaches just before they were about to dry (Mazor et al. 2014). However, this study was limited to just a handful of sites and was conducted before the CSCI was available.

Staff of the San Diego Regional Water Quality Control Board followed up on this study by collecting samples from additional intermittent reference sites, and repeated analyses with the CSCI and ASCIs (Loflen 2020). That study corroborated the findings of Mazor et al. (2014) and determined that the lack of continuous surface flow in a reach at the time of sampling was the key factor in determining if CSCI scores in intermittent sites were comparable to reference sites. Scores from sites in "regularly flowing" intermittent streams (which flow for several months in most years) were higher than those in "seldomly flowing" intermittent streams (which may be dry or exhibit ephemeral flows in most years, but flow for several months in wet years). This study also demonstrated that the majority of reference sites in the San Diego region used to calibrate the CSCI were, in fact, intermittent. The San Diego study showed that the ASCI-H, but not the ASCI-D, tended to score intermittent streams lower than comparable perennial streams (C. Loflen, personal communication).

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Concurrent with this study, other studies were going on in other regions of the state. Targeted reference sampling in Colorado Basin, the Central Coast, the Sierra Nevada, and the Interior Chaparral included a small number of intermittent sites. In a multi-year effort in the Bay Area, Regional Board staff identified and sampled an extensive number of intermittent reference streams. Data sets in all regions were reviewed in order to exclude samples that may have been collected under drying conditions when the standard bioassessment protocol cannot be applied as required for index calculation (e.g., more than 3 dry transect). Thus, we have a dataset of intermittent reference sites in several portions of the state, which may be used to assess index applicability in intermittent streams (Figure 22). Because these data sets are continuing to grow (due to data collection at previously unsampled intermittent reference sites, and review of existing reference sites to classify them as intermittent or perennial), these analyses will be periodically updated.

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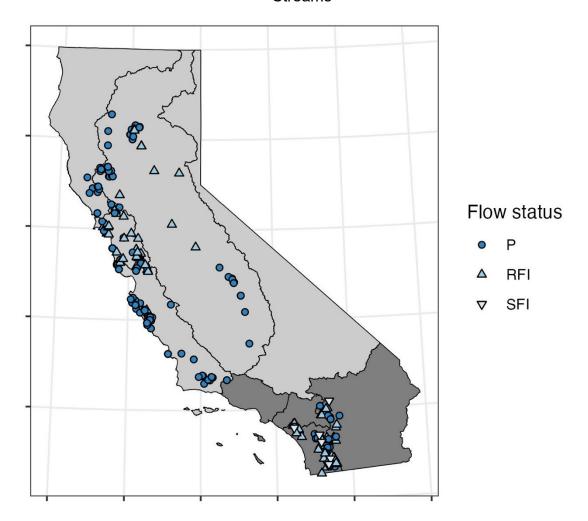


Figure 23. Location of intermittent and perennial reference sites in Southern California (dark gray) and Northern California chaparral ecoregions (light gray). Reference sites in Regions 4, 6, and 8 and non-Chaparral portions of regions 1 and 5 are not shown. P: Perennial. RFI: Regularly flowing intermittent. SFI: Seldomly flowing intermittent.

Reference sites in xeric portions of northern and southern California were classified as perennial or intermittent (Figure 23). Intermittent sites were further sub-categorized as regularly flowing intermittent (RFI), meaning that they typically flow for several months in most years, or seldomly flowing intermittent (SFI), meaning that they may flow for a month or longer in wet years, but in dry years may only exhibit ephemeral flows after storm events. Due to their short and sporadic flows, SFI streams are rarely sampled by bioassessment programs and require close attention to time sampling appropriately.

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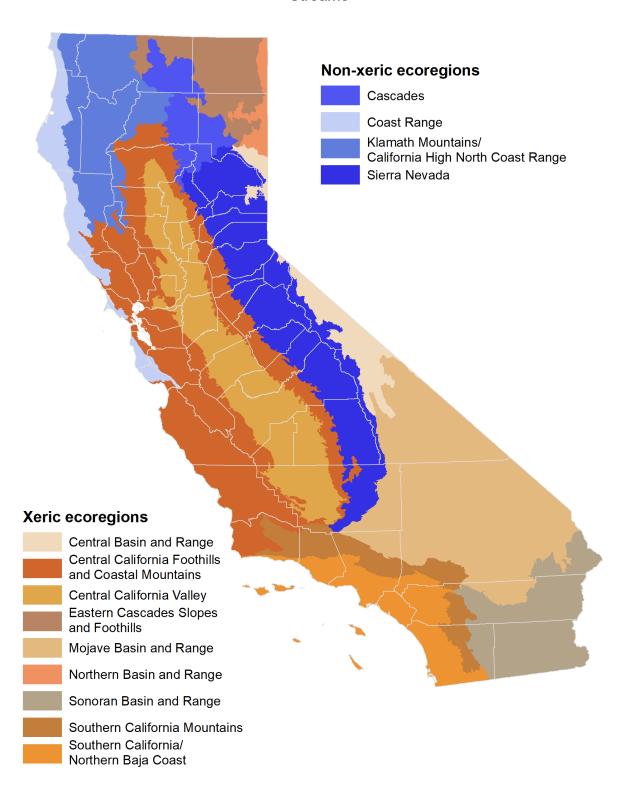


Figure 24. Xeric and non-xeric ecoregions of California. Ecoregional boundaries are from Griffith et al. (2016).

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Bioassessment data from these sites show that within southern California, CSCI scores from RFI reference sites tend to be similar to those in perennial reference sites. However, scores in intermittent reference sites in other regions (and SFI reference sites in southern California) tend to be lower (Figure 24). An ANOVA showed that streamflow duration and region had a significant (p < 0.05) influence on CSCI scores but not ASCI scores (Table 11). A post-hoc Tukey test of significant differences found that RFI reference streams had significantly lower (p < 0.05) CSCI scores than perennial streams in Northern but not Southern California (Table 12). ASCI\_D scores were slightly higher in Northern than in Southern California, but this difference was small (i.e., 0.04).

Maps of the test data sets are provided in <u>Supplement S1-2</u>.

Table 11. Analyses of variance of the effects of flow status, region, and their interaction (Flow status : Region) on index scores. DF: Degrees of freedom. SS: Sum of squares. MS: Mean squares. F: F-statistic. p: p-value. Terms with significant (p < 0.05) values are highlighted in bold.

Index	Term	DF	SS	MS	F	р
CSCI	Flow status	1	0.259	0.259	11.3	0.001
CSCI	Region	1	0.268	0.2678	11.7	0.001
CSCI	Flow status : Region	1	0.105	0.105	4.6	0.033
CSCI	Residuals	249	5.692	0.0229	4.0	0.000
ASCI_D	Flow status	1	0.013	0.0128	0.8	0.362
ASCI_D	Region	1	0.113	0.1128	7.4	0.007
ASCI_D	Flow status : Region	1	0.002	0.0015	0.1	0.751
ASCI_D	Residuals	158	2.423	0.0153		
ASCI_H	Flow status	1	0.014	0.0137	1	0.33
ASCI_H	Region	1	0.022	0.0219	1.5	0.218
ASCI_H	Flow status : Region	1	0.007	0.0068	0.5	0.492
ASCI H	Residuals	157	2.25	0.0143		

Table 12. Tukey test of significant differences. L95: Lower 95% confidence limit of the estimated difference. U95: Upper 95% confidence limit of the estimated difference. Contrasts with p-values < 0.05 are highlighted in bold. Results for regions in Northern California should be considered provisional while analyses are undergoing updates.

Index	Contrast	Difference	L95	U95	p -value
CSCI	RFI vs. P	-0.07	-0.11	-0.03	p < 0.01
CSCI	Southern vs. Northern	0.06	0.02	0.1	p < 0.01
CSCI	Northern: RFI vs. P	-0.14	-0.21	-0.06	p < 0.01
CSCI	Perennial: Northern vs. Southern	0.03	-0.06	0.11	0.83
CSCI	RFI: Northern vs. Southern	0.13	0.04	0.21	p < 0.01
CSCI	Southern: RFI vs. P	-0.04	-0.13	0.06	0.73
ASCI_D	RFI vs. P	0.02	-0.02	0.06	0.36
ASCI_D	Southern vs. Northern	-0.04	-0.08	0	0.03
ASCI_D	Northern: RFI vs. P	0.05	-0.04	0.14	0.46
ASCI_D	Perennial: Northern vs. Southern	-0.07	-0.16	0.01	0.13
ASCI_D	RFI: Northern vs. Southern	-0.06	-0.15	0.04	0.37
ASCI_D	Southern: RFI vs. P	0.07	-0.03	0.16	0.27
ASCI_H	RFI vs. P	0.02	-0.02	0.06	0.33
ASCI_H	Southern vs. Northern	-0.02	-0.06	0.02	0.32
ASCI_H	Northern: RFI vs. P	0.02	-0.06	0.11	0.91
ASCI_H	Perennial: Northern vs. Southern	-0.05	-0.13	0.04	0.52
ASCI_H	Southern: RFI vs. P	-0.01	-0.1	0.08	0.98
ASCI_H	RFI: Northern vs. Southern	0.05	-0.04	0.15	0.42

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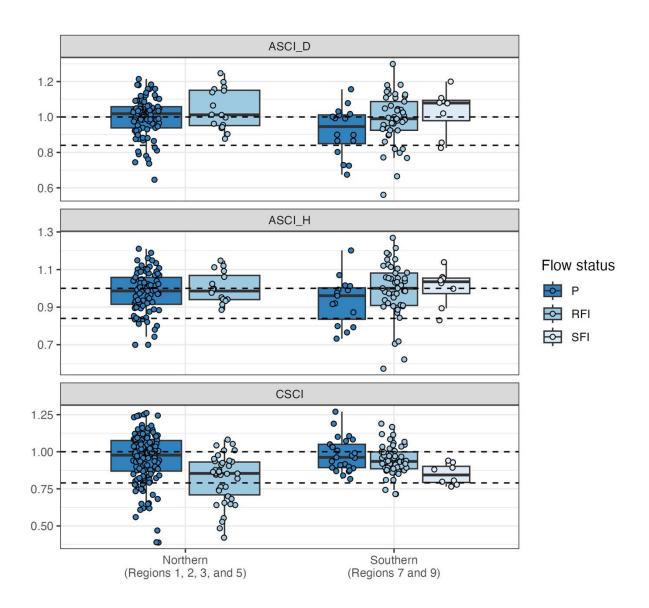


Figure 25. Comparison of index scores at perennial and non-perennial reference in Southern California (i.e., Regions 7 and 9) and the Chaparral ecoregion in Northern California (i.e., Regions 1, 2, 3, and 5). P: Perennial. RFI: Regularly flowing intermittent reference sites. SFI: Seldomly flowing intermittent reference sites. For sites with multiple samples, average scores are shown. Dashed lines are at the means and the 10<sup>th</sup> percentiles of scores at perennial reference sites (Mazor et al. 2016, Theroux et al. 2020). Results for regions in Northern California should be considered provisional while analyses are undergoing updates.

Table 13 shows statistical summaries of CSCI scores at reference intermittent sites. For regions where the means and quantiles at intermittent reference sites are substantially lower than at perennial reference sites, these summary statistics could be used to identify alternative approaches to identifying assessment thresholds. For example, the 10<sup>th</sup> percentile of CSCI scores at intermittent reference sites in northern California is 0.61 and is therefore provisionally equivalent to the perennial threshold of 0.79 in terms of risk tolerance. Table 13 shows both empirical estimates of the 30<sup>th</sup>, 10<sup>th</sup>, and 1<sup>st</sup> percentiles, as well as estimates assuming normal distributions. Normal estimates have previously been used to calculate thresholds from reference distributions to reduce the influence of extreme low-scoring outliers (Mazor et al. 2016, Theroux et al. 2020). Although very little data are available for comparison, the 10<sup>th</sup> percentile for SFI streams was similar to the 10<sup>th</sup> percentile for RFI streams (0.76 vs 0.79, Table 13).

Table 13. Summary statistics of index scores at regularly flowing (RFI) and seldomly flowing (SFI) intermittent reference streams. N: Number of unique sites. SD: Standard deviation of index scores. Q30, q10, and q01: 30<sup>th</sup>, 10<sup>th</sup>, and 1<sup>st</sup> percentiles of scores at reference sites (empirical estimates and estimates assuming normal distributions are both provided). Results for regions in Northern California should be considered provisional while analyses are undergoing updates.

Region	Flow status	Index	n	Mean	SD	q30 (empirical)	q10 (empirical)	q01 (empirical)	q30 (normal)	q10 (normal)	q01 (normal)
Regional Board 2	RFI	ASCI_D	6	0.96	0.07	0.92	0.89	0.88	0.92	0.87	0.80
Regional Board 2	RFI	ASCI_H	6	0.95	0.04	0.94	0.91	0.89	0.93	0.90	0.85
Regional Board 2	RFI	CSCI	27	0.89	0.11	0.85	0.71	0.64	0.83	0.74	0.62
Regional Board 5	RFI	ASCI_D	9	1.11	0.10	1.06	0.99	0.95	1.06	0.98	0.87
Regional Board 5	RFI	ASCI_H	9	1.04	0.09	1.00	0.93	0.92	1.00	0.93	0.84
Regional Board 5	RFI	CSCI	10	0.63	0.13	0.55	0.48	0.43	0.56	0.47	0.33
Regional Board 7	RFI	ASCI_D	10	1.04	0.07	1.00	0.95	0.93	1.00	0.95	0.88
Regional Board 7	RFI	ASCI_H	10	1.02	0.06	0.99	0.97	0.91	0.98	0.94	0.87
Regional Board 7	RFI	CSCI	10	0.88	0.09	0.87	0.74	0.72	0.83	0.77	0.67
Regional Board 9	RFI	ASCI_D	35	0.97	0.15	0.92	0.78	0.60	0.89	0.77	0.61
Regional Board 9	RFI	ASCI_H	35	0.98	0.16	0.91	0.77	0.59	0.90	0.77	0.61

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Region	Flow status	Index	n	Mean	SD	q30 (empirical)	q10 (empirical)	q01 (empirical)	q30 (normal)	q10 (normal)	q01 (normal)
Regional Board 9	RFI	CSCI	43	0.96	0.10	0.91	0.86	0.75	0.91	0.83	0.73
Regional Board 9	SFI	ASCI_D	7	1.02	0.14	0.99	0.84	0.83	0.95	0.85	0.71
Regional Board 9	SFI	ASCI_H	7	1.00	0.10	0.98	0.87	0.83	0.94	0.86	0.76
Regional Board 9	SFI	CSCI	7	0.86	0.07	0.80	0.77	0.77	0.82	0.76	0.69
Northern regions (1, 2, 3, 5)	RFI	ASCI D	16	1.05	0.12	0.96	0.92	0.88	0.98	0.90	0.78
Northern regions (1, 2, 3, 5)	RFI	ASCI_H	16	1.00	0.08	0.94	0.92	0.89	0.96	0.90	0.81
Northern regions (1, 2, 3, 5)	RFI	CSCI	38	0.82	0.16	0.74	0.61	0.44	0.73	0.61	0.44
Southern regions (7, 9)	RFI	ASCI D	45	0.99	0.14	0.94	0.81	0.61	0.91	0.80	0.66
Southern regions (7, 9)	RFI	ASCI H	45	0.99	0.15	0.92	0.85	0.59	0.91	0.80	0.65
Southern regions (7, 9)	RFI	CSCI	53	0.94	0.10	0.89	0.85	0.71	0.89	0.82	0.71

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Region	Flow status	Index	n	Mean	SD	q30 (empirical)	q10 (empirical)	q01 (empirical)	q30 (normal)	q10 (normal)	q01 (normal)
Southern regions	SFI	ASCI D	8	1.03	0.13	1.03	0.85	0.83	0.96	0.87	0.73
(7, 9) Southern regions	SFI	A3CI_D	0	1.03	0.13	1.03	0.65	0.63	0.96	0.67	0.73
(7, 9)	SFI	ASCI_H	8	1.01	0.10	1.00	0.87	0.83	0.95	0.88	0.78
Southern regions (7, 9)	SFI	CSCI	8	0.85	0.07	0.80	0.77	0.77	0.81	0.76	0.69
All regions	RFI	ASCI D	61	1.00	0.14	0.95	0.82	0.62	0.93	0.83	0.68
All regions	RFI	ASCI_H	61	0.99	0.13	0.94	0.89	0.60	0.92	0.82	0.69
All regions	RFI	CSCI	91	0.89	0.14	0.86	0.70	0.48	0.82	0.71	0.56
All regions	SFI	ASCI_D	8	1.03	0.13	1.03	0.85	0.83	0.96	0.87	0.73
All regions	SFI	ASCI_H	8	1.01	0.10	1.00	0.87	0.83	0.95	0.88	0.78
All regions	SFI	CSCI	8	0.85	0.07	0.80	0.77	0.77	0.81	0.76	0.69

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### Ongoing research on intermittency and biointegrity

At this time, the Surface Water Ambient Monitoring Program's (SWAMP) Bioassessment Program is undertaking additional research to further build capacity to assess non-perennial streams in California, and the numbers reflected in this study (e.g., in Table 13) will be updated as these projects continue. These research efforts are summarized below.

#### Generate additional data at intermittent reference sites

The results presented in Table 13 were developed from a data set of reference sites with known flow durations in arid regions of California. Two types of projects can expand this data set. An expanded data set will improve estimates of reference score distributions presented in Table 13 and expand their scope to non-arid regions. In addition, a more substantial data set might allow recalibration of bioassessment indices for use in intermittent streams.

#### Hydrologic assessments at reference sites

Many reference sites with bioassessment data were excluded from these analyses because their flow durations have not been confirmed. Within portions of the state flow duration status of these reference sites will be confirmed through appropriately timed site-visits, deployment of water presence data loggers, or evaluation of other hydrologic data. This project is best suited for regions where numerous reference sites have been sampled, the flow duration status is unknown, but many are suspected of being intermittent (e.g., the South and Central Coast). Current efforts focus on the Los Angeles and Santa Ana regions.

#### Data collection at previously unsampled intermittent reference sites

In some regions of California (particularly in non-arid regions), most reference sites with bioassessment data are known to be perennial, and new sites need to be sampled to expand representation of intermittent reference sites in these regions. Candidate sites under thorough screening and reconnaissance to confirm their status as intermittent reference sites. Current efforts focus on the Sierra Nevada, the Interior Chaparral, and the North Coast.

#### Development of indicators for dry intermittent and ephemeral streams

The CSCI and ASCI can only be used when flow conditions are suitable for sample collection, as described in the protocol (Ode et al. 2016a). Ongoing efforts (primarily in southern California) are developing biological indicators for use in intermittent and ephemeral streams when they are dry (Mazor et al. 2021a). Protocols for the collection of terrestrial arthropods and bryophytes have been developed (Robinson et al. 2018), as have taxonomic data standards for these assemblages (Mazor 2023, Mazor et al. 2023). Current efforts focus on expanding reference data collection in the South Coast to support the development of indices comparable to the CSCI.

# Guidance on considering intermittency when collecting or interpreting bioassessment data

When evaluating CSCI or ASCI scores from a site, it is important to consider whether the sampled reach was intermittent, and if the sampling event was influenced by drying or drought. A recent SCCWRP publication provides guidance on evaluating the appropriate of benthic macroinvertebrate samples for calculating CSCI scores, including guidance on interpreting the impacts of drought and intermittency (Beck and Mazor 2020), and portions of that guidance are summarized below.

Note that for streams that have undergone a conversion from perennial to intermittent (or vice versa) due to human activity, it is a policy decision whether to assess the stream based on its historic or its current flow duration status.

## Was the sample influenced by drying?

#### Why is this a problem?

Drying routinely occurs in intermittent streams, but it also occurs in perennial streams during severe drought, diversions, or changes in discharges. Drying events results in the death or aestivation of most aquatic organism, so bioassessment samples collected shortly after a drying event look very different from those collected before drying. This is particularly true in stream reaches lacking aquatic refugia (such as concrete channels), as sensitive organisms may take time to recolonize the reach.

Samples collected from a reach that is partially dry (e.g., surface water is discontinuous and/or stagnant) or shortly after a short-term drying event are known to yield low CSCI scores at reference sites. Therefore, CSCI scores from samples affected by drying are not typically considered valid. However, if the drying is related to human activity (e.g., groundwater pumping, diversions), then the low CSCI scores correctly reflect the impact of this activity.

Note that intermittency alone may not invalidate a CSCI score. Studies from Southern California reference streams have shown that the CSCI and other indices performs in these streams (Mazor et al. 2014, Rehn 2017, Loflen 2020), but the CSCI may require a different threshold to assess whether a stream is in reference condition in other parts of California (e.g., Table 13).

#### Where do you find an answer?

Normal practices defined under the stream sampling SOP (Ode et al. 2016a) require that sampling be conducted under baseflow conditions. Field notes should be consulted to determine if flow was abnormally low, possibly as a result of drought or diversions. For example, sampling transects may have been skipped if stream flow was discontinuous on the

sampling reach. The field notes should indicate if any deviation occurred from the normal protocol. Sites where the normal sampling protocol was altered may not produce accurate CSCI scores.

Short-term drying events are not always easy to detect. In streams overlying coarse alluvial substrates, streams may dry and re-wet on a diurnal cycle associated with changes in evapotranspiration by riparian vegetation; typically, flows increase overnight, when respiration is lowest, and diminish or cease by mid-afternoon, when respiration rates are highest. Field crews may note evidence of a recent rewetting, such as dried yet submerged algal mats. However, continuous data loggers or data from nearby stream gages (see section on accessing gage data) may provide more conclusive evidence that a stream was dry prior to sampling, or if the stream dries regularly.

#### How do you evaluate the answer?

Field notes may indicate if the sampling protocol was modified to accommodate drying. These modifications may invalidate a CSCI score:

- The entire sampling reach length was at least 100 m
- There were **no dry transects** within the reach
- There was some **evidence of flow** at one or more transect (that is, the entire reach wasn't stagnating).

For example, a sampling event in the SWAMP database is accompanied by this comment:

"Dry transects at C, G and GH. The entire reach was small pools with heavy tule growth.
Could not measure flow. This site was entered on a field laptop."

Based on this comment, the CSCI score for this sampling event should be considered invalid.

If data from a stream gage or a logger indicates that a drying event occurred within **1 month** of sample collection, and the drying is entirely due to natural causes (e.g., no diversions), then the CSCI score from the sample should not be considered valid. Similarly, field notes indicating that a stream has recently resumed flow (e.g., observations of submerged dead algal mats) should also be considered as potentially invalidating a CSCI score.

Note that these issues are likely to depress CSCI scores, and the observation of high scores could be taken as evidence that the sample wasn't greatly affected by low flows related to drought.

#### What can you do about it?

Additional samples may need to be collected if a sample was affected by drying.

### Was the sample influenced by drought?

#### Why is this a problem?

Drought conditions (and the management response to droughts, such as diversions or groundwater extraction) can stress stream communities in several ways, primarily by reducing baseflow conditions. In extreme cases, flow may cease altogether (see section on drying). Flow reduction can alter the physical and chemical conditions in the stream, which can adversely impact biological communities (Herbst et al. 2019). For example, reduced flow may lead to lower dissolve oxygen, increased stream temperatures, encroachment of riparian vegetation, concentration of pollutants, and saltwater intrusion in coastal streams. Natural streams have some resilience to drought, particularly those in semi-arid climates such as southern California.

The influence of drought does not invalidate a CSCI score. As described above, drought may exacerbate changes in physical habitat quality or the impacts of anthropogenic stressors. However, determining whether drought has influenced a CSCI score can be useful for assessing overall stream condition.

#### Where do you find an answer?

There are numerous ways to measure the severity of a drought. Although it is not yet clear which of these indices are most relevant to stream ecology, the Palmer Drought Severity Index (PDSI, <a href="http://www.droughtmanagement.info/palmer-drought-severity-index-pdsi/">http://www.droughtmanagement.info/palmer-drought-severity-index-pdsi/</a>) is one of the more widely used indices. Calculating the index for a given site or sampling date requires a substantial effort, but weekly drought maps (<a href="https://www.climate.gov/maps-data/dataset/weekly-drought-map">https://www.climate.gov/maps-data/dataset/weekly-drought-map</a>) may provide sufficient information on conditions at the general time and location of sampling.

If sites are visited under multiple years, comparing field notes, site sketches, photos, wetted width measurements, or water quality parameters may indicate if a stream is responding to drought.

#### How do you evaluate the answer?

Drought conditions should be evaluated relative to the magnitude (how dry) and duration (how long). Drought conditions that are more severe and that persist for longer will have a larger effect on stream health. Negative PDSI scores indicate drought conditions, whereas positive values indicate moist conditions. PDSI scores below -3 indicate severe drought, and PDSI scores below -4 indicate extreme drought. At this time, we cannot identify a threshold minimum PDSI score to determine if drought has influenced a CSCI score.

#### What can you do about it?

Where practical, revisit the site and collect new samples during years with more typical climatic conditions.

### How can you determine if a reach is intermittent?

#### **USGS Stream gages**

The best source of information on streamflow duration typically comes from stream gages that continuously measure discharge. Several reaches in California have stream gages maintained by the US Geological Survey (USGS) that provide long-term, high-resolution (i.e., daily or better) hydrologic records. These gages may be identified by consulting the USGS National Water Information System (NWIS, <a href="https://waterdata.usgs.gov/nwis/rt">https://waterdata.usgs.gov/nwis/rt</a>). When attempting to identify a gage located near a site of interest (e.g., a bioassessment sampling location), make sure that the gage is located on the same stream segment, with no intervening discharges, confluences, or diversions located between the site and the gage.

Data from the gage may be accessed through the NWIS website, or using the dataRetrival package in R (De Cicco et al. 2018). In the example shown in Figure 25, dry periods at Orestimba Creek are highlighted with red dots, consistent with an intermittent designation.

```
Library(dataRetrieval)
library(tidyverse)
# Edit the numbers in quotation marks as needed
siteNumber <- "11274500" # Gage ID for Orestimba Creek</pre>
parameterCd <- "00060" # Parameter code for discharge</pre>
startDate <- "2015-10-01"
endDate <- "2019-09-30"
# Retrieve the discharge data and site info
# Do not edit this code
discharge <- readNWISdv(siteNumber, parameterCd, startDate, endDate)</pre>
site info<- readNWISsite(siteNumber)</pre>
ggplot(data=discharge, aes(x=Date, y=X_00060_00003))+
 geom_path()+
 geom point(data=. %>% filter(X 00060 00003==0),
        aes(color="red"),
        size=1)+
 scale color manual(values="red",
        name="",
        label="Zero flow recorded")+
 ylab("Discharge (ft3/sec)")+
 ggtitle(paste("Discharge at gage ",
        site_info$site_no," ",site_info$station_nm))
```

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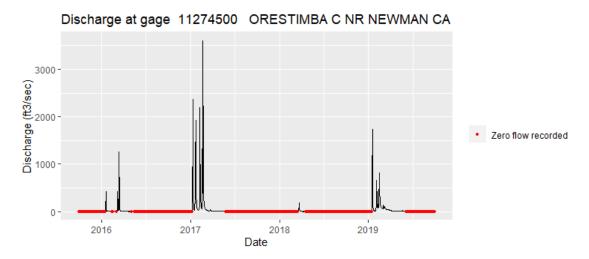


Figure 26. Example code and output graphics demonstrating use of the dataRetrieval package to investigate streamflow duration.

#### Other sources of information

Unfortunately, most reaches in California lack gages, and several gages have been discontinued or lack sufficient information to determine streamflow duration. Thus, other sources of information may be necessary.

#### Short-term deployment of water sensors

Loggers that continuously record the presence or absence of waters (e.g., Stream Temperature, Intermittency and Conductance [STIC] loggers, Chapin et al. 2014) or pressure transducers that, when corrected for barometric pressure, record water depth (e.g., HOBO U20 loggers) may be used to provide high-quality streamflow duration data at sites of interest. However, these sensors may require deployment of at least a year before a tentative classification can be made. Longer deployments may be necessary if the first year of deployment receives atypical amounts of rainfall.

Wildlife cameras that take images several times per day have also been used to interpret streamflow duration (P. Spindler, Arizona Department of Environmental Quality, personal communication). Although wildlife cameras provide excellent data, photo interpretation is necessary, which may be time-consuming.

#### Streamflow duration assessment methods (SDAMs)

The EPA has recently released two beta SDAMs covering portions of California: one for the Arid West (Mazor et al. 2021b, 2021c), and one for the Western Mountains (Mazor et al. 2021d). The Central Valley may be assessed with the SDAM for the Arid West and the eastern portion of the Modoc Plateau, whereas the SDAM for the Western Mountains is needed in the western portion of the Modoc Plateau.

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SDAMs require the measurement of streamflow duration indicators in the field. Most of these indicators are biological, although geomorphological and climatic indicators are also used in the Western Mountains method. An SDAM assessment with either method typically requires 2 hours and requires a trained crew familiar with identifying wetland plants (Arid West method only) and aquatic invertebrate taxa (both methods). Lab identifications of aquatic invertebrates may be preferable, depending on the skills and capacity of field crews.

SDAMs can classify a reach as ephemeral, intermittent, perennial, or "at least intermittent" (i.e., not ephemeral). These methods typically have the greatest accuracy in distinguishing ephemeral reaches from perennial or intermittent reaches, but they struggle to distinguish between intermittent and perennial reaches. Thus, they may not be suitable for assessing the applicability of bioassessment indices. Nonetheless, they are the best available tools for empirically determining streamflow duration at sites that lack hydrologic data.

#### **Review aerial imagery**

In many parts of California, sequences of aerial imagery can provide information about streamflow duration. Google Earth's time slider offers a convenient method of reviewing historical imagery, particularly for desert systems with little riparian vegetation that could obscure the channel (however, note that the Google Earth time slider may not have accurate image dates), as does the USGS Earth Explorer (<a href="https://earthexplorer.usgs.gov/">https://earthexplorer.usgs.gov/</a>). If surface water is observed in all interpretable images across multiple years (especially during dry seasons), this may provide evidence that the reach is likely perennial. Suppose surface water is never observed, even when other nearby intermittent streams show water. In this case, the consistent absence of surface water may provide evidence that the reach is likely ephemeral (particularly if images are captured during the wet season or after major storm events). If surface water is present in some images and dry in others, the stream may be intermittent. This evidence is strong if the images with surface water occur in the dry season, and do not coincide with storm events.

Any time that discrete observations of flow or no flow are used to inform a determination of flow duration class, it is recommended that such observations be evaluated in the context of relatively normal climatic conditions. Doing so ensures that flow duration class is not determined based on observations of flow or no flow during abnormally wet or abnormally dry periods. A useful tool to determine the antecedent precipitation conditions for any particular site and date is the Antecedent Precipitation Tool (APT), developed by the U.S. Army Corps of Engineers (<a href="https://www.epa.gov/nwpr/antecedent-precipitation-tool-apt">https://www.epa.gov/nwpr/antecedent-precipitation-tool-apt</a>). However, aerial images may not have high enough temporal resolution to confidently classify streams as ephemeral or perennial without additional data. See examples in Figure 26.

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### Perennial site: Jemez River near Zia Pueblo, NM



Intermittent site: Hassayampa River near Morristown, AZ



Ephemeral site near Las Vegas, NV



Figure 27. Examples of using aerial imagery to support streamflow duration classification. Images were taken from Google Earth using the time slider.

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#### **Commercial satellite imagery**

Commercial satellites can provide information about streamflow duration with great spatial and temporal resolution in certain conditions. CubeSat and Planet satellites have been used to determine daily surface water presence based on near-infrared imagery at 3-m cell sizes in the Hassayampa River in Arizona (Wang and Vivoni 2022), resulting in "Hovmöller" diagrams that display the spatially and temporally dynamic nature of streamflow in a desert river. An example from a pilot study in San Diego is presented in Figure 27.

There are a few limitations to this method. Because the imagery is gathered at 3-m cell resolution, small streams cannot be assessed this way. Streams in deep canyons or obscured by structures or thick vegetation also cannot be assessed. The Central Valley's famed Tule Fog will also interfere with data collection, particularly during winter months. In comparison with pilot studies in Arizona and San Diego, the Central Valley will likely have more success with this method than San Diego, but less than Arizona. Other portions of Region 5, such as the Sierra Nevada, are less likely to be successful due to their more complex topography and denser vegetation.

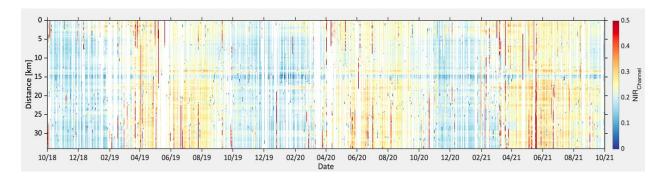


Figure 28. A Hövmoller diagram showing surface water presence in a 35-km reach of the San Luis Rey River in San Diego County. Position on the y-axis indicates distance upstream, and position on the x-axis indicates day of year over a 4-year period. Blue cells indicate water presence, whereas red and yellow cells indicate dry conditions. White cells indicate cells with no data. Blue horizontal bands indicate perennial reaches, whereas horizontal bands that are almost completely red and yellow are ephemeral. Plot provided by E. Vivoni and Z. Wang, Arizona State University.

#### **Properly timed site-visits**

A single well-timed site visit may provide sufficient hydrologic evidence about streamflow duration. For example, streams flowing at the end of the dry season (i.e., late Fall) in Mediterranean California are likely perennial, and streams that are dry a week after large rain events are likely ephemeral, assuming typical climate patterns. As with observations from aerial

imagery, any time onsite observations of flow or absence of flow are used to inform a determination of flow duration class, it is recommended that such observations be evaluated in the context of normal climatic conditions. Doing so ensures that flow duration class is not determined based on hydrologic observations of flow that occurred during abnormally wet or abnormally dry periods. The previously mentioned APT can provide this information.

### Part 1b Conclusions and recommendations

Investigate temporal dynamics of eutrophication in reference and non-reference streams, both perennial and intermittent.

- The CSCI can assess impacts at perennial streams, as well as at historically perennial streams that have become intermittent due to human activity. With the caveats noted above, the CSCI can be used to assess conditions in perennial wadeable streams in most parts of California. In historically perennial streams with artificially reduced flows, the CSCI can be used to assess the impacts of these flow reductions.
- The CSCI can assess impacts in regularly flowing intermittent streams in Southern California. In southern California (i.e., regions 7 and 9), the CSCI can be used in regularly flowing intermittent streams without modification, as long as the sampling protocol is used without alteration. Because the data set demonstrates applicability in regions 7 and 9, we believe regions 4 and 8 are likely similar enough with respect to the CSCI models. However, future data analysis should verify this assumption. Similarly, more data are needed to assess the CSCI's applicability in seldomly flowing intermittent streams in Southern California.
- Consider alternative approaches to interpret CSCI scores in regularly flowing intermittent streams in Northern California. We showed that scores are depressed at about half of intermittent streams in region 2, as well most streams in other regions in northern California. In these regions, we recommend the following:
  - Do not modify the SOPs for routine monitoring applications. Continue to collect biological data wherever aquatic life assessments are needed.
  - Changes in CSCI scores may be interpreted as changes in condition at intermittent reaches. That is, if scores go down following a disturbance, the stream is likely impacted. Conversely, if scores go up following restoration, the stream's condition has likely improved. Declines in scores can be used to assess the effectiveness of watershed protection programs or efforts to protect highquality waters.

- The 0.79 threshold for the CSCI is likely to misidentify some healthy intermittent streams in northern California as degraded. Thresholds presented in Table 21 (Part 3) are appropriate for provisional use, although values may be updated. Even with updated thresholds, calibration of a new index may be preferable. If an intermittent stream scores below this alternative threshold, consider hydrology as a potential cause of degradation. Alternatively, if resources allow, collect data from nearby intermittent reference stream and use their scores as comparison for sites of interest.
- Seek out multiple sources of hydrologic information when determining a stream's flow status, particularly at ungaged locations. Not only is information needed on the present-day hydrology, but information is also needed to determine historical conditions, as the CSCI can be used to assess the impacts of hydrologic modification.
- Use ASCI scores in both perennial and regularly flowing intermittent streams in both Northern and Southern California. Flow duration did not influence ASCI scores in either region.

## **Part 1 Summary**

#### **Key Questions:**

- How well do the data sets used to develop bioassessment tools represent the environmental conditions in the Central Valley and Modoc Plateau?
- How do bioassessment models perform in the Central Valley?
- How does streamflow duration and intermittency affect model performance?

Assessing the biological integrity of wadeable streams in the Central Valley of California presents a number of challenges, such as the lack of reference sites that may be used to calibrate models. Bioassessment tools, such as the California Stream Condition Index (CSCI; Mazor et al. 2016), the Algal Stream Condition Indices (ASCIs; Theroux et al. 2020), and to a lesser extent, eutrophication response models use reference sites to characterize natural conditions and to set benchmarks that measure changes from natural conditions.

In this report, we evaluated the fitness of bioassessment tools for use in the Central Valley. We found that reference data sets used to calibrate the CSCI and ASCI differed from typical sites in the Central Valley floor. For example, several Central Valley floor sites had larger watersheds and different soil properties compared to reference sites. In contrast, the data sets were representative of environmental conditions in the Modoc Plateau (another ecoregion in the

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Water Board's jurisdiction where reference sites are scarce). Because eutrophication response models do not rely as heavily on reference sites as the CSCI or ASCI models, the representativeness of its development data set was good for both ecoregions.

It was not possible to determine with certainty whether the lack of representativeness means that the indices are too biased to use in this region because the evidence was equivocal. Evidence favoring their use was the number of sites that were able to achieve reference-like scores for the indices (especially the ASCI). These sites were often — but not exclusively — at sites with riparian corridors located close to the Sierra Nevada foothills. Evidence suggesting that the indices may be biased was that scores at less-disturbed sites were lower than at standard reference sites. We cannot say for certain if these scores were low due to bias or due to the amount of disturbance these sites experience. Eutrophication response models showed that performance levels within the Central Valley and Modoc Plateau ecoregions were similar to those observed elsewhere in California.

Another challenge for assessing biological integrity in the Central Valley — as well as many other parts of California — is the preponderance of intermittent streams. The tools we evaluated were developed with data collected primarily from perennial sites (although an unknown number of these sites may be intermittent). We determined that CSCI scores and ASCI scores at intermittent reference sites are comparable to perennial reference sites in Southern California, but not in Northern California. Due to natural factors like their small size and higher water temperatures, intermittent streams may be particularly sensitive to eutrophication, and their management may influence the likelihood of eutrophication in downstream waterbodies.

### Recommendations and conclusions

- The CSCI and ASCI may be used to assess biological integrity in perennial streams in the Central Valley and Modoc Plateau ecoregions. For the Modoc Plateau, reference-based thresholds may be used to identify potentially degraded conditions in perennial streams. However, an alternative implementation framework may be necessary to reduce the potential for biased assessments in the Central Valley, as described below.
- Technical evidence is equivocal about the need for alternatives to traditional reference-based biointegrity thresholds for the Central Valley. Although high scores are attainable in the Central Valley, reference sites used to calibrate the CSCI and ASCIs poorly represent the region's natural conditions. CSCI and ASCI thresholds based on best-available scores at less-disturbed sites may account for potential bias in the Central Valley, and also for limitations to biointegrity associated with channel modification. Collect data at additional less-disturbed Central Valley sites to make sure that best-available scores are properly characterized. Best-attainable scores determined through

modeling and causal assessment may provide a better option for identifying site-specific thresholds.

- The CSCI and ASCI may be used to assess regularly flowing intermittent sites, even though they may require different interpretation in Northern California. Summary statistics of CSCI scores at intermittent reference sites can be used as thresholds, rather than thresholds derived from perennial reference sites. However, these alternative thresholds should be used as an interim measure until an appropriately calibrated index is available; this conclusion is applicable to intermittent streams throughout Northern California (not just within the Central Valley).
- At both perennial and intermittent sites in the Central Valley (and other parts of California), changes in CSCI and ASCI may be interpreted to indicate improving or degrading conditions.
- Eutrophication response models may be used to inform management of the
  environmental drivers of these conditions in the Central Valley. Due to their outsized
  importance in nutrient cycling and organic matter processing, including intermittent
  streams in nutrient and eutrophication management programs will likely contribute to
  success.

# PART 2: EUTROPHICATION AND BIOINTEGRITY INDICATORS FOR MODIFIED CHANNELS

### **Definition of a modified channel**

A modified channel is a stream or waterway that either has an artificial origin (i.e., dug from upland areas), or is a natural stream whose morphology has undergone one or more deliberate modifications, such as hardening, straightening, or lining with resistant material. These changes are intended to support flood control, transportation, conveyance of water (both supply and return flows), water recycling, or other uses, and the modifications are expected to remain in place as long as those uses are required. Both natural waterways and artificial channels excavated from terrestrial upland habitats can be considered a modified channel. Unintentional changes to a channel morphology (e.g., incision due to hydromodification or bank destabilization due to excessive cattle grazing or human recreation) do not meet this definition channel modification, nor do modifications that eliminate the channel altogether (e.g., impoundments, or diversion of a stream to an underground pipe). This definition is not intended to be used to reflect or supersede definitions of jurisdictional waterbodies; some modified channels fitting this definition may be considered jurisdictional waterbodies, while others may not.

## Determining if a channel is natural or modified

Determining whether a stream-reach is a modified channel typically requires direct observation of bed and bank material, and other morphological features. Knowledge of the historic condition of the channel can help, but this information may not always be available for every stream; in these cases, examining the stream network topography can be helpful, as described below. Use the flow chart in Figure 28 to determine if a channel is modified. The questions in this flowchart are summarized below. Regulatory and management applications may require more information than is necessary for the process described here. Restored channels may meet the definitions of modified or constructed channels, but depending on the management needs, it may be appropriate to treat them as natural.

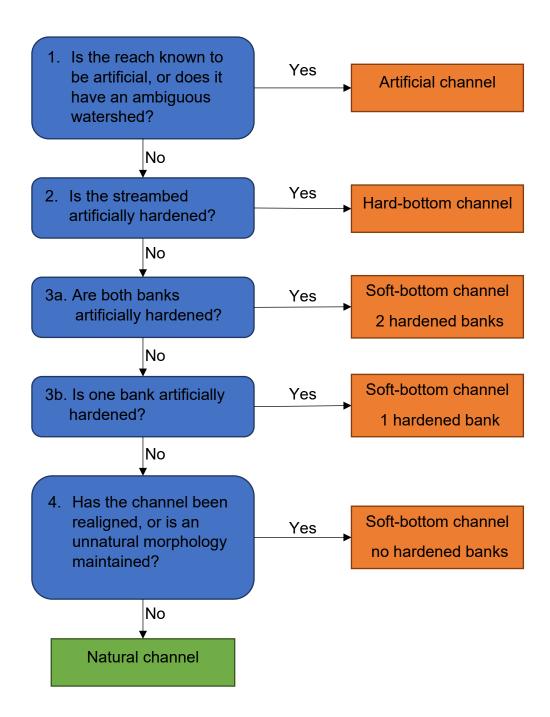


Figure 29. A flow chart to classify modified channels.

Note that channel modification can occur in many different ways, and the classification system described in this report not account for the diversity of modified channel types in California. For example, the presence or absence of low-flow channels, the use of smooth concrete versus more rough-textured substrate, and the extent of channel recontouring are not considered in this classification system. In addition, this classification does not consider the varied purposes

#### Part 2: Eutrophication and biointegrity indicators

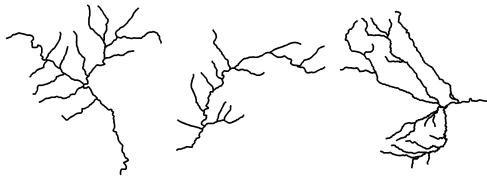
of channel modification (e.g., conveyance of irrigation water versus flood protection). Further research is needed to determine if additional categories or an alternative classification method for modified channels are needed.

# Question 1: Is the reach known to be artificial, or does it have an ambiguous watershed?

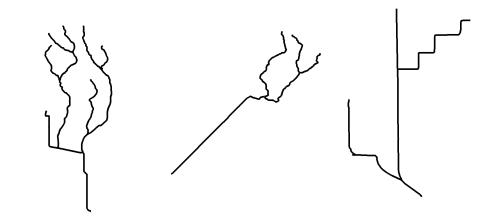
Artificial channels are created by digging ditches in formerly terrestrial, upland habitats. In contrast, other types of modified channels were historically natural channels or wetlands.

Historical records or imagery are good sources of information in determining if a reach is artificial or natural. However, these sources of data may not always be available. In some cases, the artificial status of a channel may be determined by investigating the landscape in a GIS and examining the shape of the stream network (Figure 29). Natural channels are typically found within stream networks that exhibit dendritic patterns, in which numerous tributaries coalesce into a single stream. The direction of flow can be inferred network topology alone. Modified but historically natural channels may have straight contours and angular rather than meandering turns, but will often have stream networks with typical dendritic patterns. Constructed channels will also have straight contours and angular turns, and also lack typical dendritic stream networks. They lack obvious connections to natural headwaters, and it may be difficult to determine surface areas that could potentially contribute runoff, nor can the direction of flow be determined by examining the network topology.

#### A. Natural dendritic networks



#### B. Modified dendritic networks



## C. Nondendritic networks



Figure 30. Examples of stream networks. A. Natural channels are typically found in networks with branching, dendritic topology. Stream contours are wiggly, rather than straight, and sharp angles are rare or absent. B. Modified but historically natural channels often occur in channels with straightened contours and sharp angles, but are found within networks that exhibit branching, dendritic topology. The overall direction of flow may be discerned from the network topology. C. Constructed channels are often also straightened, and are often found in networks that lack dendritic topology. It is not possible to discern the direction of flow from network topology alone.

Field observations can help inform a classification, but they are rarely sufficient to determine if a reach is natural or artificial, as artificial channels may appear to be morphologically identical to natural but heavily modified channels (e.g., Figure 30 and Figure 31).





Figure 31. Examples of artificial channels. Left: Tulare Lake Canal (Tulare County). Right: Campbell-Moreland Ditch 374s (Tulare County).





Figure 32. Historically natural modified channels (Strong Ranch Slough, Sacramento County, left) may be difficult to distinguish from artificial channels (unnamed channel, Sacramento County, right) based solely on field observations.

Because predictive indices such as the CSCI and ASCI require delineations of contributing watersheds to characterize a reach's environmental setting (Mazor et al. 2016, Boyle et al. 2020, Theroux et al. 2020), index scores cannot be calculated for some artificial channels using traditional methods. In cases where a traditional watershed cannot be delineated, a circular buffer extending 5 km from the sampling location should be used to characterize its environmental setting.

Note that constructed channels may or may not have hardened streambed and banks.

## Question 2: Is the streambed hardened?

Streambeds may be hardened by lining a reach with concrete, riprap, or other resistant material. At least 25% of the length of an assessed reach should be hardened to meet this classification; this minimum extent of hardening is assumed to be sufficient to potentially impact hydrologic and ecologic processes in a channel. Streambed hardening most often occurs in urban areas where high watershed imperviousness raises the risk of incision. Typically, both banks are also hardened where a streambed is hardened, but exceptions to this pattern occur (Figure 32).

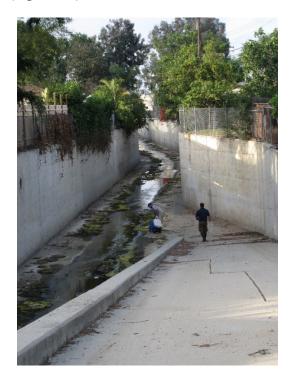




Figure 33. Examples of reaches with hardened streambeds. Left: Fullerton Creek (Orange County) has hardened bed and banks. Right: Morrison Creek (Sacramento County) has a concrete-lined streambed but vegetated soft sediment on the banks.

## Question 3: Is one or both banks hardened?

Banks may be armored with or without streambed hardening. Concrete, riprap, wood pilings, and other resistant material may be used to resist bank erosion. Whereas streambed hardening most often occurs in urban areas, bank hardening also frequently occurs in rural and undeveloped areas to protect roads and other streamside property. Armoring may occur on one bank (Figure 33) or two (Figure 32, left; Figure 34).





Figure 34. Examples of reaches with one hardened bank. Left: The Arroyo Seco (Los Angeles County). Right: Conejo Creek (Ventura County).





Figure 35. Examples of reaches with two hardened banks. Left: San Leandro Creek (Alameda County). Right: Morrison Creek (Sacramento County).

# Question 4: Has the channel been realigned, or is an unnatural morphology maintained?

Modified earthen channels may have been realigned or straightened from their historic path, and processes that generate diverse microhabitats in the reach (e.g., riffles and pools) are

disrupted. In extreme cases, earthen modified channels are easy to distinguish from natural channels (e.g., Tulare Lake Canal in Figure 30). But in some cases, such as channels with extensive floodplains that have set-back levees, the distinction can be debatable.

# **Evaluating biointegrity indicators for use in modified channels**

Aquatic community measures such as the CSCI and ASCI provide measures of biological integrity, which respond to eutrophication stress, as well as other types of stress (such as habitat quality and flow modification). Thus, they are not solely an indicator of eutrophication, but rather provide a comprehensive measure of the ability of a waterbody to support aquatic life (Karr 1991). Recent studies show that bioassessment scores are typically depressed in modified channels (e.g., Mazor 2015, Mazor et al. 2018, Taniguchi-Quan et al. 2020, Dusterhoff et al. 2021), and that index scores may be less responsive to eutrophication stress in these systems. The CSCI appears to have lowest scores in hard-bottom channels, while the ASCI appears to have lowest scores in soft-bottom channels.

# Conceptual model for the impacts of channel modification on wadeable stream biointegrity

As part of its Causal Assessment/Diagnosis Decision Information System (CADDIS), the US EPA provides conceptual models to help managers understand the impacts of different stressors on aquatic ecosystems (U.S. Environmental Protection Agency 2017). We adapted the conceptual model for habitat degradation for channel modification, which sets up three tiers of factors to evaluate: Human activities, Stressors, and Ecosystem responses (Figure 35).

Part 2: Eutrophication and biointegrity indicators

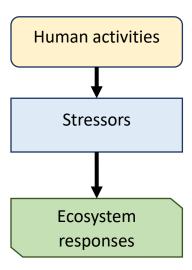


Figure 36. Simplified conceptual model to link human activities (such as channel modification) with ecosystem responses (such as loss of biointegrity).

#### Human activities

Human activities associated with channel modification were identified as follows (Figure 36):

- Channel construction
- Streambed hardening
- Hardening of one or both banks
- · Channel regrading or realignment
- Changing hydrologic regime (wet or dry at different periods; human-altered source of water including ag drainage and treated wastewater)
- Operations and maintenance activities, including:
  - Sediment removal
  - Vegetation removal by mowing, herbicidal application, fire, or grazing
  - Infrastructural repair
  - Vector control (e.g., pesticide application or mosquitofish introduction)
  - Flooding or diversions
  - Removal of dead wood and driftwood (habitat)
  - Homogenization of channel (removes habitat diversity)

Part 2: Eutrophication and biointegrity indicators

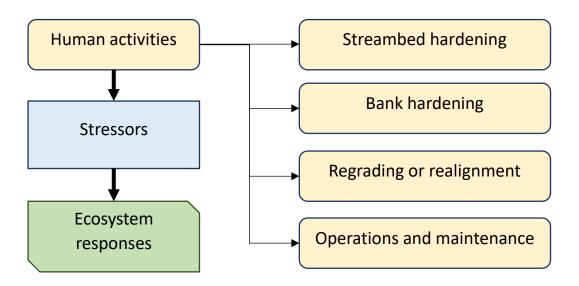


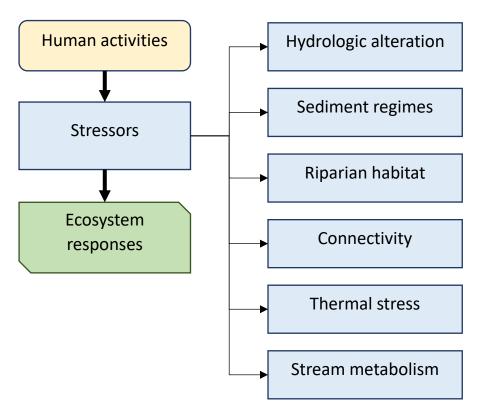
Figure 37. Human activities related to channel modification.

#### Stressors

Once human activities were identified, we evaluated how each activity would influence different classes of stressors (Figure 37).

- Hydrologic alteration, including changes to flow regimes, streamflow duration, peak magnitude, velocity, recession rates, baseflow rates, shea stress, groundwater interactions, and flow habitat diversity (e.g., loss of riffles or pools)
  - Example for streambed hardening: Increased velocity, peak magnitude, and shear stress. Accelerated baseflow and stormflow recession rates. Greatly reduced groundwater interaction. Loss of riffles and pools.
- Altered sediment regimes, including abundance large particles, substrate size diversity, interstitial habitats, hyporheic refugia, microtopographic complexity, channel roughness, and bank habitat.
  - Example for streambed hardening: Loss of large particles, lack of substrate diversity, elimination of interstitial habitats and hyporheic refugia, loss of microtopographic complexity, and loss of channel roughness. No change to bank habitat.
- Changes to riparian habitat, including changes in vegetation density, hydrophyte abundance, long-lived species, shading, overhanging vegetation, allochthonous inputs, and large woody debris imports and exports.

- Example for streambed hardening: Few direct changes, although export of woody debris or other allochthonous material would be accelerated.
- Loss of connectivity, including lateral (floodplain access), longitudinal (barriers to instream passage), and vertical (access to hyporheic zone).
  - Example for streambed hardening: Large, negative impacts to longitudinal and vertical connectivity. No direct changes to lateral connectivity.
- Thermal stress related to changes in shading, water depth, and groundwater influence.
  - Example for streambed hardening: Increased thermal stress largely driven by loss of water depth and reduction of groundwater inputs.
- Changes in stream metabolism, including altered production, retention, and export of organic matter from both allochthonous and autochthonous sources.
  - Example for streambed hardening: Increased production due to thermal stress and increased substrate for mat-forming macroalgae. Limited retention and accelerated export of organic matter.



#### Figure 38. Stressors related to channel modification.

#### Ecosystem responses

Changes in each stressor were then related to potential ecosystem responses based on theoretical relationships with environmental conditions associated with channel modification. The examples provided here may or may not have demonstrated support in the scientific literature.

#### Benthic macroinvertebrates

 Example for streambed hardening. These groups could increase in abundance or diversity: filterers, taxa that attach to hard substrates, taxa with high temperature tolerance, taxa with hypoxia tolerance, aerial dispersers, swimmers, drifters, and taxa with rapid reproduction. These groups could decrease in abundance or diversity: burrowers, low temperature requirements, high dissolved oxygen requirements, and semivoltine taxa. These changes would be reflected in reduced CSCI scores.

#### Benthic algae

 Example for streambed hardening. These groups could increase in abundance or diversity: mat-forming species (such as *Cladophora*), taxa that attach to hard substrate, taxa that tolerate high temperatures, taxa with rapid growth rates, planktonic forms. These taxa could decrease in abundance or diversity: sedimentation-tolerant taxa. These changes would be reflected in reduced ASCI scores.

#### Fish

Example for streambed hardening. Hardened reaches create barriers to
migration that lead to a loss of anadromous species. Loss of spawning or foraging
habitats, and changes in food sources could affect both anadromous and
resident fish species. Cold-water species could also be reduced due to increased
thermal stress. These changes would be reflected in a loss of most native taxa,
with some invasive species proliferating (e.g., those tolerant of warmer
conditions).

#### Amphibians

 Example for streambed hardening. Loss of spawning or foraging habitat, changes to quality or accessibility of upland habitat, and changes in food sources could

lead to an overall reduction in amphibian populations. These changes would be reflected in a loss of most native taxa, with some invasive species proliferating.

#### Aquatic macrophytes

 Example for streambed hardening: Rooted species would be unable to grow due to lack of soft substrate. Floating species could be reduced due to increased velocity. Invasive species would be expected to proliferate. These changes would lead to an overall loss of abundance and diversity of native species.

#### Riparian vegetation assemblages

Example for streambed hardening: Few direct impacts to overall cover, species
diversity, number of hydrophytes, vertical or horizontal structural complexity, or
age diversity. (Impacts from bank hardening likely much greater and more
direct.) Potential indirect impacts due to changes in groundwater interactions
could lead to a reduction in the overall vigor of riparian plants.

#### Eutrophication processes

Example for streambed hardening: Autochthonous algal and microbial production might increase due to increased light and thermal stress, leading to increased organic matter accumulation. Increased velocity could increase scour and downstream export of organic matter. These changes could lead to increased likelihood of harmful algal toxin production in situ or in downstream waterbodies, increased diel DO variability, and shift in food web, among other things.

Part 2: Eutrophication and biointegrity indicators

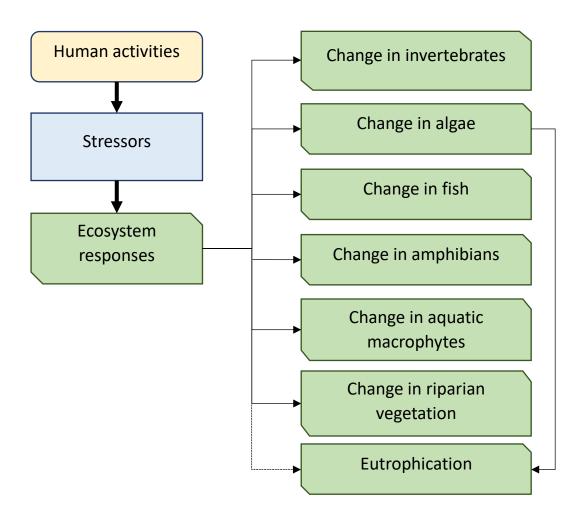


Figure 39. Potential ecosystem responses to channel modification.

Eutrophication is not a direct response to channel modification but rather a response mediated by the potential impacts of channel modification on algae.

# Decision process for selecting biointegrity indicators for classes of modified streams

Reference-based bioassessment indices (like the CSCI and ASCIs) provide direct measurements of biological integrity, and they are effective at doing so in both natural and modified stream channels (Reynoldson et al. 1997, Hawkins et al. 2010). The presence or severity of channel modification has no bearing on the ability to use these indices to understand biological conditions relative to reference conditions. However, the ability to interpret these indices and use them in certain applications can be affected by severe channel modifications that

overwhelm the ability of biotic communities to reflect the impacts of other stressors, such as poor water quality or flow alteration.

Scores may be interpreted in a relative sense (e.g., increases in a score are interpreted to indicate improving conditions), and in an absolute sense (e.g., scores above a reference-based threshold indicate that a reach is in reference condition). Scores may be compared to reference-based thresholds to determine if a stream has a biotic composition similar to natural, undisturbed streams (provided that the stream's environmental setting is adequately represented in the index's development data set, as explored in Part 1). Modified channel types that rarely achieve scores above reference thresholds can be compared to other thresholds (e.g., best observed thresholds) to assess interim management goals.

We took a data-driven approach to evaluating biointegrity indicators measures and their recommended uses for each class of modified channels.

Specifically, we evaluated criteria for two factors:

- 1. High score attainability: Is there evidence that high bioassessment index scores are attainable in the modified channel class?
- 2. Responsiveness: Is there evidence that the index responds to stress in an expected manner?

Evaluation of the attainability of high bioassessment scores based on the frequency of observing scores above a reference-based threshold. A low value for this criterion would reflect the fact that high score attainment was uncommon for modified channel types, but not so low that atypical sites (e.g., modified channels in undeveloped watersheds) could greatly influence the outcomes. A numeric value of 10% was selected for this criterion, although other numeric values may be appropriate for certain applications. Note that this criterion is based on the assumption that the data sets used in this analysis represent the range of conditions of modified channels found in California. Evaluation of the index responsiveness was based on the statistical significance (p<0.1) of linear regression models of the index response to stress gradients. For both factors, we also evaluated if data were sufficient for analysis based on the number of sites ( $n \ge 20$ ) and statistical power of non-significant relationships (power  $\ge 80\%$ ). These criteria for number of sites, statistical significance, and power were based on the technical judgment of the authors. Criteria are summarized in Table 14. The criteria presented in this table should not be interpreted as recommendations for developing policy or setting thresholds for modified channels in California, but rather as one potential application of this approach.

Table 14. Criteria for evaluating evidence of high score attainability or responsiveness for biointegrity indices in classes of modified channels.

Factor	Criteria	Conclusion
High score attainability	<20 sites have data	Insufficient data
High score attainability	≥10% of sites have scores above the 10 <sup>th</sup> percentile of reference	High scores are attainable.
High score attainability	<10% of sites have scores above the 10 <sup>th</sup> percentile of reference	High scores are rarely attainable.
Responsiveness	<20 sites have data	Insufficient data
Responsiveness	Linear regression between stressor and index score is significantly negative (or positive for dissolved oxygen concentration), with p<0.10	There is evidence of responsiveness
Responsiveness	Linear regression between stressor and index score is not significantly negative (or positive for dissolved oxygen concentration), with p>0.10 and power ≥ 0.8	No evidence of responsiveness
Responsiveness	Linear regression between stressor and index score is not significantly negative (or positive for dissolved oxygen concentration), with p>0.10 but power < 0.8	Insufficient data

Part 2: Eutrophication and biointegrity indicators

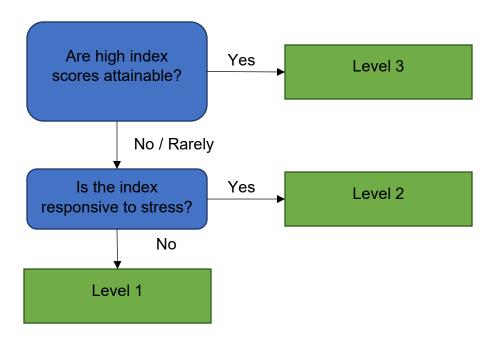


Figure 40. Decision process for making recommendations about the use of bioassessment indices in classes of modified channels.

Based on these criteria, we developed guidelines for how bioassessment indices could be used in each class of modified channel (Figure 39). In cases where insufficient data are available for analysis, we recommend additional data collection targeting the class of modified channel. These guidelines do not supersede conclusions about the applicability of tools to Central Valley Floor streams described in Part 1 of this report.

• Level 1 usage is the core application of biointegrity indices, and is appropriate for all stream types where bioassessment protocols apply (as described in Ode et al. 2016a and elaborated in Beck and Mazor 2020). Indices may be used to establish baseline conditions. For these streams, reference-based thresholds provide a way to assess similarity to natural conditions, and to identify streams that have been biologically degraded. However, stream types with a Level 1 designation have diminished responsiveness to stressor gradients, and additional tools may be needed to track the impacts of water quality or flow management changes. Although the index still provides a direct measure of aquatic life, it may not provide all the desired or necessary information about the effectiveness of management strategies intended to reduce pollution assuming the channel modification remains in place.

- Level 2 usage is appropriate for stream types where indices have been demonstrated to respond to all stressor gradients. This level includes all the same uses of Level 1. In addition, indices with Level 2 usage are appropriate for use in tracking the impacts of water quality or flow management changes. Reference thresholds are the appropriate way to determine if a stream has been degraded from natural conditions. However, the additional use of best-observed thresholds may be beneficial for benchmarking improvements and setting interim goals. There is evidence that strategies for improving biological conditions focused on reducing water quality or flow stress have potential to succeed, even if the channel modification remains in place.
- Level 3 usage is appropriate for stream types that frequently attain high scores (e.g., scores above reference thresholds). It includes all the same uses described in Level 1 and Level 2. Reference thresholds are the appropriate way to determine if a stream has been degraded from natural conditions and may be used to set meaningful management goals. However, best-observed thresholds are not suitable management goals for streams with Level 3 usage. (Numerically, best-observed thresholds will be higher and than comparable reference-based thresholds). The index can be used to track changes in condition over time, and to assess the effectiveness of water quality improvements or other management activities aimed at reducing the risk to aquatic life via the trajectory of response.

These usage categories are presented to support the Water Boards in the development of policies for modified channels but are not intended to identify specific policy decisions that the Water Boards should make. Decisions about whether to require an indicator when assessing discharges into a modified channel or applying alternative thresholds for modified versus unmodified channels should be made in consideration of Water Board priorities.

# Evidence of high score attainability

We queried the CEDEN database for bioassessment samples and found 4457 sites collected between 1994 and 2022 for use in the study. Of these, 1029 had co-occurring eutrophication indicator data. Eutrophication indicators used in the analysis include two measures of nutrient concentration (i.e., Total N and Total P, in mg/L) and three measures of algal biomass (i.e., benthic Chlorophyll-a [Chl-a] in mg/m², benthic ash-free dry mass [AFDM] in g/m², and percent macroalgal cover on the streambed). Study sites were distributed throughout all major regions of the state. We evaluated three approaches towards classifying modified channels: on 1) channels classified as "C (constructed)" in the Central Valley Inland Surface Water Plan (ISWP; Regional Water Quality Control Board-Central Valley 1992); 2) channels with ambiguous

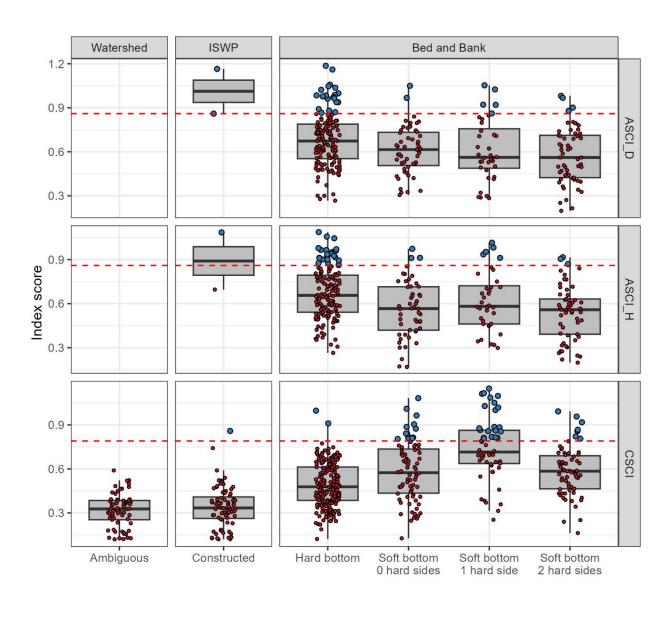
watersheds; and 3) and classes based bed and bank material (as described above). Of the 1029 sites with bioassessment and eutrophication data, 344 occurred in one of these modified channel classes; all had CSCI scores available, and 281 also had ASCI scores available (Table 2). When multiple samples were available, we evaluated the highest score because it represents the highest score that site attained.

We evaluated the percent of sites attaining scores above the  $10^{th}$  percentile of reference (i.e.,  $\geq$  0.79 for the CSCI and  $\geq$  0.86 for the ASCIs, derived for perennial streams outside the Central Valley) in each class of modified channel. If at least 10% of sites had scores above the  $10^{th}$  percentile of reference, we determined that there was evidence of high score attainability for an index in a class of modified channel; if fewer than 10% of sites had high scores, we determined that there was no evidence of high score attainability. If data were available at fewer than 20 sites, we determined that more data was needed to assess evidence of high score attainability.

For hard-bottom channels, we determined that high scores are attainable for the ASCIs, but not the CSCI (where only 1% of sites had scores above 0.79; Table 15, Figure 40). In contrast, there was evidence that high CSCI scores are attainable in all classes of soft-bottom channels (especially in those with one hardened side, where 40% of sites had CSCI scores above 0.79). In contrast, there was evidence that high ASCI scores are attainable in one-sided soft-bottom channels, but not in the other classes of soft-bottom channel. Among sites with ambiguous watersheds and constructed channels, there was no evidence that high CSCI scores are attainable. There was insufficient data to evaluate ASCI scores in these classes of modified channel.

Maps of sites with classifications based on bed and bank material are presented in <u>Supplement S1-3</u>. A map of sites designated as constructed channels is presented in <u>Supplement S1-4</u>.

Part 2: Eutrophication and biointegrity indicators



High score? • No ● Yes

Figure 41. Distribution of bioassessment index scores in different classes of modified channels. Red dashed lines indicate a threshold based on the 10<sup>th</sup> percentile of scores at reference sites. Large blue dots indicate sites with scores above that threshold.

Table 15. Assessment of high score attainability in classes of modified channels. ISWP: Classification in the Inland Surface Water Plan (ISWP; Regional Water Quality Control Board-Central Valley 1992). Blank cells indicate that no data were available for analysis.

Classification	Class	Index	# sites	# sites passing	% sites passing	Evidence of attainability
ISWP	Constructed	ASCI_D	2	2	100	Need more data
ISWP	Constructed	ASCI_H	2	1	50	Need more data
ISWP	Constructed	CSCI	75	1	1	No
Watershed	Ambiguous	ASCI_D	0			Need more data
Watershed	Ambiguous	ASCI_H	0			Need more data
Watershed	Ambiguous	CSCI	65	0	0	No
Bed & Bank	Hard bottom	ASCI_D	152	20	13	Yes
Bed & Bank	Hard bottom	ASCI_H	152	23	15	Yes
Bed & Bank	Hard bottom	CSCI	203	2	1	No
Bed & Bank	Soft bottom-0 hard sides	ASCI_D	51	2	4	No
Bed & Bank	Soft bottom-0 hard sides	ASCI_H	51	3	6	No
Bed & Bank	Soft bottom-0 hard sides	CSCI	78	13	17	Yes
Bed & Bank	Soft bottom-1 hard side	ASCI_D	36	5	14	Yes
Bed & Bank	Soft bottom-1 hard side	ASCI_H	36	5	14	Yes
Bed & Bank	Soft bottom-1 hard side	CSCI	52	21	40	Yes
Bed & Bank	Soft bottom-2 hard sides	ASCI_D	57	4	7	No
Bed & Bank	Soft bottom-2 hard sides	ASCI_H	57	3	5	No
Bed & Bank	Soft bottom-2 hard sides	CSCI	67	7	10	Yes

## Evidence of responsiveness

We calculated slopes of linear regressions between index scores and selected stressors within each class of modified channel where data from at least 20 sites were available (Figure 41). The stressors included several related to eutrophication (specifically, total nitrogen [TN; mg/L], total phosphorus [TP; mg/L], benthic chlorophyll-a [Chla; mg/m²], benthic ash-free dry mass [AFDM; g/m²], percent macroalgal cover on the streambed [PCT\_MAP; %], dissolved oxygen [DO; mg/L], and temperature [Temp; °C]), as well as specific conductivity (SpCond;  $\mu$ S/cm) and percent sands and fines on the streambed (PCT\_SAFN; %). We were only able to analyze classifications based on bed and bank material because there was insufficient data (<20 sites) to analyze channels with ambiguous watersheds or constructed channels. Note that DO and Temp were analyzed as "snapshot" spot measurements taken during bioassessment sample collection, and thus may not reflect the most stressful (i.e., low DO or high Temp) conditions experienced at a reach.

We concluded that there was evidence of index responsiveness in a class if slopes were significantly negative (or significantly positive in the case of dissolved oxygen) with p<0.1 for any of the evaluated stressors. We concluded that there was no evidence of responsiveness if slopes were not significantly different from zero ( $p\ge0.1$ ) and power was sufficient ( $\ge0.8$ ). If power was insufficient, there was insufficient data to draw a conclusion.

There was no evidence of responsiveness for any index within hard-bottom channels. The CSCI was responsive to multiple stressors in all classes of soft-bottom channels. The ASCIs were responsive to multiple stressors in soft-bottom channels with 0 or 1 hardened side, but data were insufficient to draw conclusions about many stressors in soft-bottom channels with 2 hard sides (Figure 41, Table 16, Table 17).

The overall weakness of the responses to stress within classes of modified channels is unsurprising and should not be interpreted to mean that the stressor has no impact on biointegrity within these streams, particularly given the relatively small number of sites with data to explore some of these relationships (particularly for soft-bottom channels). Co-occurring stressors can also complicate the interpretation of these relationships. The wedge-shaped relationships are evidence of a strong relationship: Index scores are low when the stress indicator levels are high, whereas scores are more variable (and sometimes high) when stress indicator levels are low.

Part 2: Eutrophication and biointegrity indicators

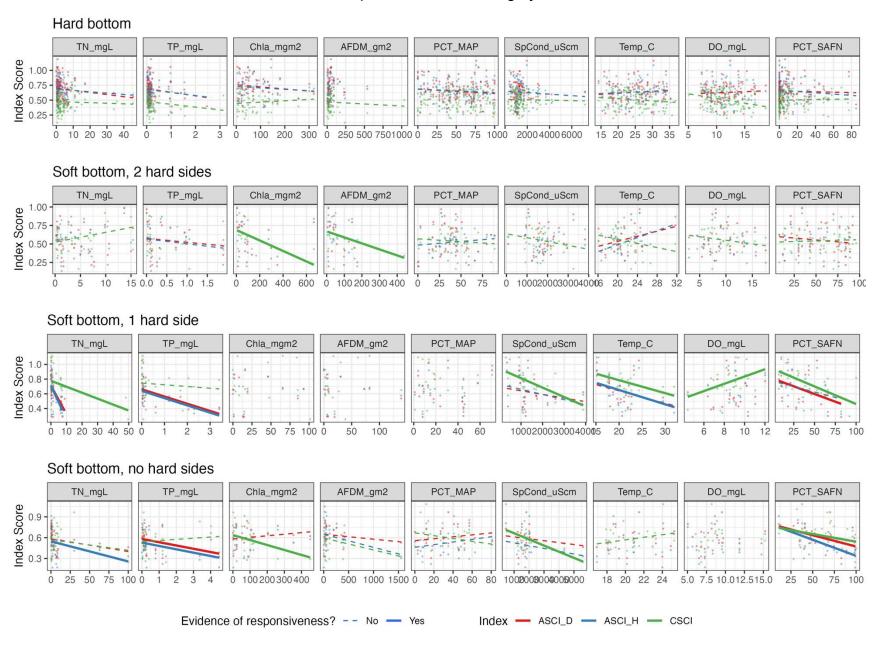


Figure 42. Analysis of responsiveness of biointegrity index scores to selected stressors in different classes of modified channels. Each dot represents an index score for a site (a single site may be represented by up to 3 dots). Lines represent slopes of linear regressions. [TN: total nitrogen in mg/L. TP: total phosphorus in mg/L. Chla: benthic chlorophyll-a in mg/m². AFDM: benthic ash-free dry mass in g/m². PCT\_MAP: percent macroalgal cover on the streambed. DO: dissolved oxygen in mg/L. Temp: water temperature in °C. SpCond: specific conductivity in  $\mu$ S/cm. PCT\_SAFN: percent sands and fines on the streambed.

Table 16. Analysis of biointegrity index response to stress in classes of modified channels. n: Number of sites with data. L90: Lower bound of the 90% confidence interval. U90: Upper bound of the 90% confidence interval.

Class	Index	Stressor	n	Slope	L90	U90	Power	Do linear models provide evidence of index responsiveness to stress?
Hard bottom	ASCI_D	TN	107	-0.004	-0.008	0.001	1	No
Hard bottom	ASCI_D	TP	90	-0.06	-0.138	0.017	1	No
Hard bottom	ASCI_D	Chla	52	0	0	0	1	No
Hard bottom	ASCI_D	AFDM	54	0	0	0	0.13	Insufficient data
Hard bottom	ASCI_D	PCT_MAP	110	0	0	0	1	No
Hard bottom	ASCI_D	SpCond	85	0	0	0	0.41	Insufficient data
Hard bottom	ASCI_D	Temp	87	0.003	-0.002	0.009	1	No
Hard bottom	ASCI_D	DO	85	0.004	-0.008	0.015	1	No
Hard bottom	ASCI_D	PCT_SAFN	114	0	0	0	0.99	No
Hard bottom	ASCI_H	TN	107	-0.002	-0.007	0.002	1	No
Hard bottom	ASCI_H	TP	90	-0.051	-0.125	0.022	1	No
Hard bottom	ASCI_H	Chla	52	0	0	0	1	No
Hard bottom	ASCI_H	AFDM	54	0	0	0	0.13	Insufficient data
Hard bottom	ASCI_H	PCT_MAP	109	0	0	0	1	No
Hard bottom	ASCI_H	SpCond	84	0	0	0	1	No
Hard bottom	ASCI_H	Temp	86	0.002	-0.004	0.008	1	No
Hard bottom	ASCI_H	DO	84	0.002	-0.01	0.014	0.78	Insufficient data
Hard bottom	ASCI_H	PCT_SAFN	113	0	0	0	1	No
Hard bottom	CSCI	TN	121	-0.001	-0.004	0.002	1	No
Hard bottom	CSCI	TP	101	-0.047	-0.101	0.008	1	No
Hard bottom	CSCI	Chla	60	0	0	0	1	No
Hard bottom	CSCI	AFDM	62	0	0	0	0.99	No
Hard bottom	CSCI	PCT_MAP	134	0	0	0	0.64	Insufficient data
Hard bottom	CSCI	SpCond	112	0	0	0	0.81	No
Hard bottom	CSCI	Temp	113	-0.004	-0.008	0	1	No
Hard bottom	CSCI	DO	111	-0.016	-0.024	-0.007	1	No
Hard bottom	CSCI	PCT_SAFN	153	0	0	0	0.99	No
Soft bottom-0 hard sides	ASCI_D	TN	37	-0.002	-0.004	0	1	No
Soft bottom-0 hard sides	ASCI_D	TP	32	-0.047	-0.082	-0.013	1	Yes
Soft bottom-0 hard sides	ASCI_D	Chla	22	0	0	0	0.98	No
Soft bottom-0 hard sides	ASCI_D	AFDM	25	0	0	0	0.94	No
Soft bottom-0 hard sides	ASCI_D	PCT_MAP	26	0	0	0	1	No
Soft bottom-0 hard sides	ASCI_D	SpCond	21	0	0	0	0.98	No
Soft bottom-0 hard sides	ASCI_D	Temp	21	0.006	-0.019	0.032	0.59	Insufficient data

Part 2: Eutrophication and biointegrity indicators

01	Index	Stressor	n	Slope	L90	U90	Power	Do linear models provide evidence of
Class Soft bottom-0 hard sides	ASCI D	DO	21	0.006	-0.018	0.029	0.6	index responsiveness to stress? Insufficient data
Soft bottom-0 hard sides	ASCI_D ASCI_D	PCT_SAFN	28	0.000	0.010	0.029	1	Yes
Soft bottom-0 hard sides	ASCI_D ASCI_H	TN	37	-0.003	-0.006	0	1	Yes
Soft bottom-0 hard sides	ASCI_H ASCI_H	TP	32	-0.003	-0.096	0	1	Yes
Soft bottom-0 hard sides	<del>-</del>	Chla	22	0.048	0.090	0	0.65	Insufficient data
Soft bottom-0 hard sides	ASCI_H	AFDM	25				1	No
Soft bottom-0 hard sides	ASCI_H		26	0	0	0	1	No
Soft bottom-0 hard sides	ASCI_H	PCT_MAP	21	0	0	0	1	No
Soft bottom-0 hard sides	ASCI_H	SpCond	21	0.002	-0.028	0.032	0.13	Insufficient data
	ASCI_H	Temp						
Soft bottom-0 hard sides	ASCI_H	DO CAEN	21	0.003	-0.025	0.031	0.21	Insufficient data
Soft bottom-0 hard sides	ASCI_H	PCT_SAFN	28	0	0	0	1	Yes
Soft bottom-0 hard sides	CSCI	TN	40	-0.002	-0.004	0.001	1	No
Soft bottom-0 hard sides	CSCI	TP	34	0.017	-0.03	0.063	0.97	No
Soft bottom-0 hard sides	CSCI	Chla	24	0	0	0	1	Yes
Soft bottom-0 hard sides	CSCI	AFDM	27	0	0	0	1	No
Soft bottom-0 hard sides	CSCI	PCT_MAP	27	0	0	0	1	No
Soft bottom-0 hard sides	CSCI	SpCond	22	0	0	0	1	Yes
Soft bottom-0 hard sides	CSCI	Temp	22	0.019	-0.007	0.044	1	No
Soft bottom-0 hard sides	CSCI	DO	22	0.005	-0.021	0.03	0.4	Insufficient data
Soft bottom-0 hard sides	CSCI	PCT_SAFN	36	0	0	0	1	Yes
Soft bottom-1 hard side	ASCI_D	TN	31	-0.039	-0.056	-0.021	1	Yes
Soft bottom-1 hard side	ASCI_D	TP	22	-0.098	-0.156	-0.04	1	Yes
Soft bottom-1 hard side	ASCI_D	PCT_MAP	20	0	0	0	0.16	Insufficient data
Soft bottom-1 hard side	ASCI_D	SpCond	22	0	0	0	0.99	No
Soft bottom-1 hard side	ASCI_D	Temp	22	-0.017	-0.036	0.002	1	No
Soft bottom-1 hard side	ASCI_D	DO	22	0.011	-0.037	0.059	0.55	Insufficient data
Soft bottom-1 hard side	ASCI_D	PCT_SAFN	22	0	0	0	1	Yes
Soft bottom-1 hard side	ASCI_H	TN	29	-0.045	-0.065	-0.026	1	Yes
Soft bottom-1 hard side	ASCI_H	TP	21	-0.099	-0.155	-0.043	1	Yes
Soft bottom-1 hard side	ASCI_H	PCT_MAP	20	0	0	0	0.1	Insufficient data
Soft bottom-1 hard side	ASCI_H	SpCond	21	0	0	0	1	No
Soft bottom-1 hard side	ASCI_H	Temp	21	-0.02	-0.037	-0.002	1	Yes
Soft bottom-1 hard side	ASCI H	DO	21	0.01	-0.038	0.057	0.46	Insufficient data
	, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,							
Soft bottom-1 hard side	ASCI_H	PCT_SAFN	22	0	0	0	1	No
Soft bottom-1 hard side Soft bottom-1 hard side	_	PCT_SAFN TN	22 36	0 -0.008	0 -0.013	0 -0.003	1	No Yes

Part 2: Eutrophication and biointegrity indicators

Class	Index	Stressor	n	Slope	L90	U90	Power	Do linear models provide evidence of index responsiveness to stress?
Soft bottom-1 hard side	CSCI	PCT_MAP	22	0	0	0	0.5	Insufficient data
Soft bottom-1 hard side	CSCI	SpCond	24	0	0	0	1	Yes
Soft bottom-1 hard side	CSCI	Temp	24	-0.018	-0.034	-0.002	1	Yes
Soft bottom-1 hard side	CSCI	DO	24	0.05	0.013	0.086	1	Yes
Soft bottom-1 hard side	CSCI	PCT_SAFN	29	0	0	0	1	Yes
Soft bottom-2 hard sides	ASCI_D	TN	39	0.001	-0.012	0.013	0.18	Insufficient data
Soft bottom-2 hard sides	ASCI_D	TP	26	-0.054	-0.188	0.08	0.96	No
Soft bottom-2 hard sides	ASCI_D	PCT_MAP	43	0	0	0	0.13	Insufficient data
Soft bottom-2 hard sides	ASCI_D	SpCond	34	0	0	0	0.14	Insufficient data
Soft bottom-2 hard sides	ASCI_D	Temp	33	0.017	0.002	0.032	1	No
Soft bottom-2 hard sides	ASCI_D	DO	33	0.001	-0.014	0.016	0.14	Insufficient data
Soft bottom-2 hard sides	ASCI_D	PCT_SAFN	44	0	0	0	1	No
Soft bottom-2 hard sides	ASCI_H	TN	39	0.001	-0.011	0.014	0.32	Insufficient data
Soft bottom-2 hard sides	ASCI_H	TP	26	-0.066	-0.2	0.069	0.99	No
Soft bottom-2 hard sides	ASCI_H	PCT_MAP	43	0	0	0	1	No
Soft bottom-2 hard sides	ASCI_H	SpCond	33	0	0	0	0.19	Insufficient data
Soft bottom-2 hard sides	ASCI_H	Temp	32	0.024	0.009	0.038	1	No
Soft bottom-2 hard sides	ASCI_H	DO	32	-0.002	-0.016	0.013	0.28	Insufficient data
Soft bottom-2 hard sides	ASCI_H	PCT_SAFN	44	0	0	0	0.41	Insufficient data
Soft bottom-2 hard sides	CSCI	TN	40	0.013	0.003	0.023	1	No
Soft bottom-2 hard sides	CSCI	TP	27	-0.015	-0.125	0.095	0.32	Insufficient data
Soft bottom-2 hard sides	CSCI	Chla	20	0	0	0	1	Yes
Soft bottom-2 hard sides	CSCI	AFDM	20	0	0	0	1	Yes
Soft bottom-2 hard sides	CSCI	PCT_MAP	43	0	0	0	0.99	No
Soft bottom-2 hard sides	CSCI	SpCond	37	0	0	0	1	No
Soft bottom-2 hard sides	CSCI	Temp	36	-0.014	-0.029	0.001	1	No
Soft bottom-2 hard sides	CSCI	DO	36	-0.01	-0.023	0.004	1	No
Soft bottom-2 hard sides	CSCI	PCT_SAFN	49	0	0	0	0.85	No

Table 17. Summary of evidence of biointegrity index responsiveness in classes of modified channels. There was insufficient data to analyze constructed channels and channels with ambiguous watersheds.

Class	Index	Evidence of responsiveness	No evidence of responsiveness	Insufficient data
Hard bottom	ASCI_D	None	TN, TP, Chla, PCT_MAP, Temp, DO, PCT_SAFN	AFDM, SpCond
Hard bottom	ASCI_H	None	TN, TP, Chla, PCT_MAP, SpCond, Temp, PCT_SAFN	AFDM, DO
Hard bottom	CSCI	None	TN, TP, Chla, AFDM, SpCond, Temp, DO, PCT_SAFN	PCT_MAP
Soft bottom-0 hard sides	ASCI_D	TP, PCT_SAFN	TN, Chla, AFDM, PCT_MAP, SpCond	Temp, DO
Soft bottom-0 hard sides	ASCI_H	TN, TP, PCT_SAFN	AFDM, PCT_MAP, SpCond	Chla, Temp, DO
Soft bottom-0 hard sides	CSCI	Chla, SpCond, PCT_SAFN	TN, TP, AFDM, PCT_MAP, Temp	DO
Soft bottom-1 hard side	ASCI_D	TN, TP, PCT_SAFN	SpCond, Temp	Chla, AFDM, PCT_MAP, DO
Soft bottom-1 hard side	ASCI_H	TN, TP, Temp	SpCond, PCT_SAFN	Chla, AFDM, PCT_MAP, DO
Soft bottom-1 hard side	CSCI	TN, SpCond, Temp, DO, PCT_SAFN	TP	Chla, AFDM, PCT_MAP
Soft bottom-2 hard sides	ASCI_D	None	TP, Temp, PCT_SAFN	TN, Chla, AFDM, PCT_MAP, SpCond, DO
Soft bottom-2 hard sides	ASCI_H	None	TP, PCT_MAP, Temp	TN, Chla, AFDM, SpCond, DO, PCT_SAFN
Soft bottom-2 hard sides	CSCI	Chla, AFDM	TN, PCT_MAP, SpCond, Temp, DO, PCT_SAFN	TP

# Guidelines for index usage categories for classes of modified channels

Application of the decision process in Figure 39 resulted in at least one index recommended for standard usage in every class of modified channel based on bed and bank material. The CSCI was recommended for standard usage in all classes of soft-bottom channels, and the ASCI indices were recommended for standard usage in hard-bottom channels. All three indices were recommended for standard usage in soft-bottom channels with one hardened side. Due to a lack of data, we could not make recommendations for constructed channels. These guidelines are summarized in Table 18.

Table 18. Summary of guidelines for aquatic community indicators in different classes of modified channels.

Modified channel class	Level 3	Level 2	Level 1	Insufficient data
Hard bottom	ASCI-D ASCI-H	None	CSCI	None
Soft bottom-0 hard sides	CSCI	ASCI-D ASCI-H	None	None
Soft bottom-1 hard side	ASCI-D ASCI-H CSCI	None	None	None
Soft bottom-2 hard sides	CSCI	None	ASCI-D* ASCI-H*	None
Constructed channels and channels with ambiguous watersheds	None	None	None	CSCI** ASCI-D** ASCI-H**

<sup>\*:</sup> There was no evidence of response for ASCI-D in this class of channels to gradients in total phosphorus, temperature or percent sands and fines, but there were insufficient data to assess other stress gradients. There was no evidence of response for ASCI-H in this class of channels to gradients in total phosphorus, percent macroalgal cover, or temperature, but there were insufficient data to assess other stress gradients.

<sup>\*\*</sup>There was no evidence CSCI scores could attain high scores in this class of channel, but there were insufficient data to assess whether standard or supplemental usage was recommended. There was insufficient data to make any recommendations about algal indices for this class of channel.

# Decision process for selecting biointegrity indicators for specific sites

The decision process described above (Figure 39) results in general guidelines for classes of modified channels. However, we recommend first investigating site-specific circumstances that might modify these general guidelines.

Figure 42 provides an approach for evaluating whether changing these guidelines is appropriate for a modified channel.

This approach focuses on two factors. First, an initial screening is recommended to determine whether good biological conditions (as indicated by CSCI or ASCI scores greater than the 10<sup>th</sup> percentile of reference) are already supported. Although rare, high index scores have been observed in some modified channels, and management activities should focus on maintaining these good conditions. Second, modified channels with largely undeveloped watersheds should also be monitored and managed similar to unmodified channels. Based on observed relationships between index scores and watershed development, we recommend a minimum cutoff of 90% natural landcover (i.e., no more than 10% urban or agricultural landcover, corresponding to classes 21 to 24 and 81 to 82 in the National Landcover Dataset [NLCD]; Jon Dewitz 2024) to identify channels with undeveloped watersheds. This number was based on the authors' best professional judgment and is consistent with a study on the influence of landcover on biological conditions in modified channels in the Bay Area (Dusterhoff et al. 2021, Figure 43).

Part 2: Eutrophication and biointegrity indicators

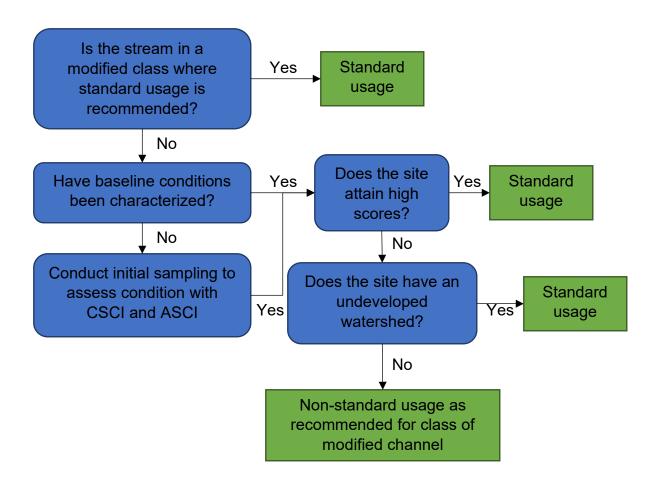


Figure 43. Decision process for determining if non-standard usages are appropriate for specific sites in classes of modified channels where non-standard usage is generally recommended.

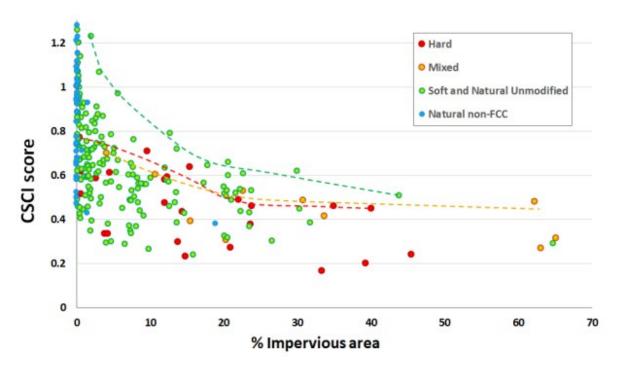


Figure 44. Relationship between watershed landcover (% impervious area) and biological conditions in Bay Area modified channels (from Dusterhoff et al. 2021). Flood control channels and Natural non-flood control channel (non-FCC) CSCI scores in Santa Clara County plotted against percent impervious cover within each point's contributing watershed. CSCI score ceilings (dashed lines with colors matching channel type) are defined by the 90th percentile of scores within discrete 10% impervious area bins (i.e., 0-10%, 10-20%, etc.).

# Caveats and limitations of these guidelines and recommendations

We applied "bright line" numeric criteria to evaluate evidence about high score attainability and responsiveness. These numeric criteria reflect the authors' collective best professional judgment, but others may find stricter or looser criteria are more appropriate, resulting in a different set of guidelines and recommendations. For example, requiring a higher percent of sites to demonstrate high score attainability would likely result in recommendations for non-standard usage of the CSCI in soft-bottom channels.

Evidence of high score attainability was based on the percent of sites in each channel class able to attain high scores. This criterion assumes that our data sets are an unbiased representation of the conditions found in each channel class in California. If our dataset overrepresents the most severely degraded modified channels, then we may have underestimated high score attainability.

Our analysis of responsiveness was limited to a handful of stressors at a subset of sites where data were available, and it is possible that we could observe strong responses to other stressors. Similarly, Temp and DO were analyzed as "snapshot" measurements that typically underestimate the most stressful conditions a reach may experience. A selection of different water quality stress indicators (and analysis of continuous DO or Temp data), as well as measures of hydrologic alteration, could result in different recommendations. Many of the modeled responses are based on relatively small data sets, and it is possible that conclusions about responsiveness could change with additional data collection.

Another limitation of the responsiveness analysis is use of simple linear models. As evident in Figure 41, many of these stressors have non-linear relationships with index scores. More sophisticated statistical models, such as general additive models or additive quantile regression (Fasiolo et al. 2021), could have more power to characterize responses.

Assessing responsiveness requires that the datasets include a large stressor gradient, and there are few modified channels with low levels of stress (meaning that these data sets may have inadequate representation of stress gradients). Thus, our evaluations likely underestimate the responsiveness of biointegrity indices to gradients in stress. Efforts to identify and sample modified channels with relatively good water quality would be challenging but worthwhile.

# **Evaluating eutrophication indicators for use in modified channels**

# Review of eutrophication indicators for wadeable streams

Sutula et al. (2022) identified several eutrophication indicators for use in California wadeable streams (Table 19). That study did not distinguish between modified and unmodified channels. Candidate indicators included drivers of eutrophication (e.g., nutrient concentrations) as well as eutrophication responses, including measures of: 1) Organic matter accumulation (e.g., benthic chlorophyll-a, ash-free dry mass), 2) Water and benthic chemistry (e.g., diel range of pH or dissolved oxygen), 3) Aquatic community structure (e.g., CSCI or ASCI scores), and 4) Harmful algal blooms (e.g., cyanotoxin concentration in tissues).

Sutula et al. (2022) identified whether the indicators were primary or supporting, depending on the degree to which they met a set of criteria for evaluating the potential use of these indicators for assessing eutrophication risks to aquatic life or human uses, including:

Have a clear link to beneficial uses

- Must be able to show a trend toward increasing or decreasing eutrophication with an acceptable signal-to-noise ratio
- Response indicators should have a predictive relationship with causal factors that can be modeled empirically (as a statistical relationship or mechanistic tools like dynamic simulation models)
- Have a scientifically sound and practical measurement process, with a readily available
   SOP
- Have a scientific basis for a numeric threshold of risk of impact to beneficial uses

For a complete description of the evaluation process, see Sutula et al. (2022).

In addition to the recommendations in Table 19, Sutula et al. (2022) recommended aquatic community measures based on algal and benthic macroinvertebrate composition (e.g., the CSCI and ASCIs) as aquatic life goals for eutrophication management purposes.

Table 19. Summary of eutrophication indicators recommended for use in wadeable streams eutrophication risk assessment. NR: No recommendation.

Eutrophication Indicator	Aquatic life uses	Other uses
DRIVERS OF EUTROPHICATION		
Nutrient concentrations	Primary	Primary
Hydrology	Supporting	Supporting
Light exposure	Supporting	Supporting
Temperature	Supporting	Supporting
RESPONSES TO EUTROPHICATION		
Organic Matter Accumulation		
Benthic algal biomass (benthic chl- a, water column chl-a)	Primary	Primary
Planktonic algal biomass (benthic chl-a, water column chl-a)	Primary	Primary
Benthic or floating macroalgal percent cover	Supporting	Supporting
Benthic or planktonic AFDM, or organic C, N, P	Primary	Primary
Aquatic macrophytes: biomass, shoot height, density	NR	NR
Aquatic macrophyte percent cover	NR	NR
Water and Benthic Chemistry		
Continuous DO and pH; Diel range	Primary	NR

Part 2: Eutrophication and biointegrity indicators

Eutrophication Indicator	Aquatic life uses	Other uses
Water column or sediment oxygen demand	Primary	NR
Ecosystem metabolism and trophic state	Supporting	NR
Dissolved organic carbon, trihalomethane	NR	Supporting
Harmful Algal Blooms		
Benthic cyanoHAB toxin	Supporting	Supporting
Benthic cyanoHAB cell density	Supporting	Supporting
Particulate cyanoHAB toxin	Primary	Primary
Particulate cyanoHAB cell density	Supporting	Primary
CyanoHAB toxin concentration in tissue	Primary	Primary
SPATT toxin concentration	Supporting	Supporting

# Applicability of eutrophication indicators for modified channels

## Drivers of eutrophication

Drivers of eutrophication (e.g., nutrient concentrations) should be measured in modified channels in the same way that they are measured in modified channels. Channel modification does not affect the ability to measure nutrient concentrations. Light and temperature are frequently elevated in modified channels, thus increasing the likelihood of eutrophication. Hydrology may also increase the likelihood if the channel modification increases residence time or leads to stagnation, but it can also mitigate the likelihood if velocity is increased.

Recommended revisions for modified channels: None.

# Organic matter accumulation

Most measures of organic matter accumulation can be applied to modified channels the same as to natural streams. Planktonic biomass is largely unaffected by direct channel modification. The loss of riparian shading could stimulate growth; however, the high velocities associated with channel modification may limit planktonic biomass. However, many benthic measures could be suppressed by management activities (e.g., recent streambed regrading), and measurements collected within 2 weeks of such activities may be temporarily depressed. Measures based on rooted aquatic macrophytes are unsuitable in hard-bottom streams because they lack soft substrate in which rooted plants can grow.

Recommended revisions for modified channels: Sampling should be timed to avoid the direct impacts of channel maintenance activities (e.g., by delaying sampling for 3-4 weeks after disturbance). Rooted aquatic macrophyte measures not recommended in hard-bottom channels.

## Water and benthic chemistry

Most measures of water chemistry are unaffected by channel modification and may be used as indicators in modified channels. Channel modification may affect dissolved oxygen, both positively (by creating artificially shallow or turbulent portions) or negatively (by elevating temperature). Measures of sediment oxygen demand are unsuitable in hard-bottom channels that lack accumulated sediment.

Recommended revisions for modified channels: None.

## Harmful algal blooms

Most measures of harmful algal blooms apply to modified channels the same as they do to natural streams. However, benthic measures may give a false negative result if they are sampled shortly after certain management activities that remove algal growth from the streambed (e.g., streambed regrading).

Recommended revisions for modified channels: Sampling should be timed to avoid the direct impacts of channel maintenance activities (e.g., by delaying sampling for 3-4 weeks after disturbance).

# **Part 2 Summary**

#### Key Questions:

- How can modified channels be classified?
- How does channel modification affect measurements of biointegrity or eutrophication?
- How might biointegrity or eutrophication indicators be interpreted differently in different classes of modified streams?

This report describes a process for selecting biointegrity and eutrophication indicators in different classes of modified channels. First, we present a flow chart for classifying modified channels based primarily on bed and bank material. Second, we evaluate the factors that could alter the selection, measurement, or interpretation of biointegrity and eutrophication indicators in different classes of modified channels. With a few exceptions, most eutrophication indicators can be treated the same whether they are assessed in modified or unmodified channels. In contrast, two lines of evidence point to different usages of biointegrity indices in certain classes of modified channels: 1) limited evidence that high index scores are attainable, and 2) evidence that index scores have limited responses to stress. Based on these lines of evidence, we propose three categories of usage for each index in different classes of modified channels: standard usage with traditional thresholds (i.e., same sampling and interpretation as in unmodified channels), standard usage with alternative thresholds (i.e., index recommended with alternative interpretation framework, such as different assessment thresholds in different management applications), or supplemental usage (i.e., index recommended as a supplement to other measures of biological integrity). At least one index was recommended for standard usage with traditional thresholds in all classes defined by bed and bank material; algal indices were recommended for hard-bottom channels, and the benthic macroinvertebrate index was recommended in soft-bottom channels. Finally, we present a process for considering sitespecific circumstances that may modify these guidelines (e.g., protecting high quality conditions in modified channels able to attain high biointegrity index scores).

# PART 3: CANDIDATE BIOINTEGRITY AND EUTROPHICATION THRESHOLDS FOR MODIFIED CHANNELS, INTERMITTENT STREAMS, AND STREAMS ON THE CENTRAL VALLEY FLOOR

#### **Introduction**

In this study, we first present a variety of options for selecting assessment thresholds for biointegrity and eutrophication indicators for different types of wadeable streams that occur in the Central Valley region where traditional approaches for setting thresholds may be challenging due to their natural environmental settings. Parts 1 and 2 of this report demonstrate that streams in certain environmental settings (e.g., the Central Valley floor and in northern California intermittent streams) may receive lower than expected scores using the California Stream Condition Index (CSCI; Mazor et al. 2016) due to poor representation of these settings in the index's calibration data. Thus, alternative thresholds may help interpret the CSCI in these natural settings.

Secondly, studies also demonstrate that certain classes of anthropogenically modified channels rarely attain biointegrity scores indicative of reference conditions when assessed with certain bioassessment indices (e.g., CSCI scores in hard-bottom channels). Furthermore, some of these indices show weakened responses to stress gradients, suggesting that managing these stressors may not benefit aquatic life. Thus, alternative thresholds may help managers prioritize among modified channels or set interim targets while modifications remain in place. This study provides a range of numeric values managers can use to set these thresholds for biointegrity and eutrophication indicators.

Although this study focuses on the Central Valley, we used data from all parts of California. Except where noted to the contrary, the findings are applicable to all parts of the state.

This study is not intended to endorse the use of specific thresholds or waterbody classifications in policy or regulatory programs. Rather, the intention is to illuminate how natural factors (like streamflow duration, and the environmental settings within the Central Valley) and channel modification can influence decisions regarding the boundaries between how poor biointegrity conditions are defined.

# **Biointegrity and eutrophication indicators**

We focus on three biointegrity indicators:

- The California Stream Condition Index (CSCI) for benthic macroinvertebrates (Mazor et al. 2016)
- The Algal Stream Condition Index (ASCI) for Diatoms (D-ASCI) (Theroux et al. 2020)
- The ASCI for a hybrid of diatoms and soft-bodied algal taxa (H-ASCI) (Theroux et al. 2020)

In addition, we focus on five eutrophication indicators where thresholds have previously been identified for wadeable streams (Mazor et al. 2022):

- Total Nitrogen in mg/L (TN)
- Total Phosphorus in mg/L (TP)
- Benthic chlorophyll-a in mg/m<sup>2</sup> (chl-a)
- Benthic ash-free dry mass in g/m<sup>2</sup> (AFDM)
- Percent macroalgal cover on the streambed (% cover)

# Stream types where alternative thresholds may be useful

In Parts 1 and 2 of this report, several stream types were identified where the applicability of bioassessment indices was called into question (e.g., Central Valley floor streams, northern California intermittent streams), or there was little evidence that scores above traditional thresholds were attainable (e.g., CSCI scores in hard-bottom channels). We present alternative approaches to identifying thresholds for these stream types (Table 20).

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Table 20. Classes of wadeable streams for which alternative thresholds were evaluated.

Stream type	Abbreviation	Reason why alternative approaches may be useful
Central Valley floor	CVF	Natural factors: Poor representation of natural conditions in reference data sets used to calibrate the CSCI and ASCIs
Regularly flowing intermittent streams in xeric portions of northern California	RFI-N	Natural factors: Poor representation of natural conditions in reference data sets used to calibrate the CSCI and ASCIs
Regularly flowing intermittent streams in southern California	RFI-S	No justification. No evidence of bias in CSCI or ASCI scores.
Hard-bottom channels	НВ	Anthropogenic factors: No evidence of high CSCI score attainability.
Soft-bottom channels with 2 hardened sides	SB2	Anthropogenic factors: No evidence of high ASCI score attainability.
Soft-bottom channels with 1 hardened side	SB1	No justification. No evidence that high CSCI or ASCI scores are unattainable.
Soft-bottom channels with no hardened sides	SB0	Anthropogenic factors: No evidence of high ASCI score attainability.
Constructed channels/ channels with ambiguous watersheds	CC	Anthropogenic factors: No evidence of high CSCI score attainability. (ASCI not evaluated.)

Alternative thresholds are not appropriate for soft-bottom channels with one hardened side (SB1) because we found evidence that high scores for all three indices were attainable in this class of channel (Part 2 of this report). Data were insufficient to draw conclusions about thresholds in seldomly flowing intermittent streams (SFI) in northern (SFI-N) or southern (SFI-S) California (Part 1 of this report).

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# Approaches to identifying thresholds

There are three general approaches to identifying thresholds for biointegrity indicators. A fourth approach is also possible for identifying thresholds for eutrophication indicators. In order to provide the Regional Board with a range of options, we present high-, intermediate-, and low-stringency numbers for all types of thresholds. The level of stringency to use is a policy decision based on the desired confidence in achieving a biointegrity outcome. This study does not endorse a specific level of stringency, and policymakers may want to consider levels of stringency other than those included in this study. For biointegrity indicators, high-stringency thresholds have *higher* numeric values than low-stringency thresholds. For eutrophication indicators, high-stringency thresholds have *lower* numeric values than low-stringency thresholds.

## Reference thresholds

Streams with minimal levels of human disturbance exhibit a range of values for biointegrity and eutrophication indicators (Stoddard et al. 2006, Hawkins et al. 2010). Thresholds based on these statistical distributions are useful for distinguishing natural variability from impacts related to human activity. Numbers representing the *low end* of the distribution of biointegrity scores (such as the 1<sup>st</sup>, 10<sup>th</sup>, or 30<sup>th</sup> percentiles) or *high end* of eutrophication indicator concentrations (such as the 70<sup>th</sup>, 90<sup>th</sup>, or 99<sup>th</sup> percentiles) are usually used to identify reference-based thresholds (Hawkins et al. 2010), including as water quality criteria (U.S. Environmental Protection Agency 2000, Heiskary and Bouchard 2015).

The selection of a percentile of reference to use as a threshold is typically reflective of the confidence in the reference data set (i.e., the confidence that a site identified as reference is truly undisturbed), as well as the tolerance for potentially misclassifying a site as having good or poor conditions. Selecting less stringent reference-based thresholds (e.g., the 1<sup>st</sup> percentile of biointegrity index scores) may reflect a high confidence in the quality of the reference sites from which data were collected (that is, that few truly degraded sites were mistakenly included in the reference data set). Alternatively, it may reflect a low tolerance for misidentifying a site as having poor conditions (perhaps because the social or economic costs of misidentify a site as degraded are viewed as unacceptable). Conversely, selecting more stringent thresholds (e.g., 30<sup>th</sup> for biointegrity index scores) is appropriate if many reference sites are influenced by human activity (and thus, reference distributions are skewed by undetected degradation in the reference dataset), or if there is a low tolerance for misidentifying a site as having good conditions.

For regions like the Central Valley, where reference sites are largely absent, this approach is unsuitable for identifying thresholds. Best-observed thresholds (described in the next section)

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provide an alternative approach for characterizing natural biointegrity or eutrophication conditions in pervasively altered regions.

## Best-observed thresholds

The highest biointegrity scores (or lowest eutrophication indicator concentrations) observed in classes of streams represent conditions that are, at least in certain circumstances, attainable (U.S. Environmental Protection Agency 2000, Dodds and Welch 2000, Heiskary and Bouchard 2015). These may be considered a form of reference-based thresholds when reference conditions are themselves unknowable due to pervasive disturbances characteristic of the Anthropocene, and are most appropriate for novel, human dominated aquatic ecosystems (Kopf et al. 2015). Note that in its 2000 report, the U.S. Environmental Protection Agency describes best-observed thresholds as "reference thresholds" when applied to probabilistic samples of streams; this terminology is not followed in the present study.

Whereas reference-based thresholds are derived from statistical distribution points to describe the low end of biointegrity index scores (e.g., the 1<sup>st</sup>, 10<sup>th</sup>, or 30<sup>th</sup> percentiles) or the high end of eutrophication indicator levels (e.g., the 70<sup>th</sup>, 90<sup>th</sup>, or 99<sup>th</sup> percentiles), best-observed thresholds characterize the *high end* of biointegrity index scores and the *low end* of eutrophication indicators. Selecting more stringent thresholds (e.g., the 99<sup>th</sup> percentile for biointegrity indicators, or the 1<sup>st</sup> percentile of eutrophication indicators) is appropriate when there is low risk tolerance for misidentifying a site as having poor conditions, or when there is low confidence that the best-observed conditions are close to reference conditions. Conversely, selecting less stringent thresholds (e.g., 70<sup>th</sup> or 30<sup>th</sup> percentiles for biointegrity and eutrophication indicators, respectively) is appropriate when there is low risk for misidentifying a site as having good conditions, or when there is high confidence that the best-observed conditions are close to reference conditions.

For the Central Valley, where the lack of reference sites has the potential to introduce bias in biointegrity tools, best-observed thresholds provide a way to eliminate the impacts of that bias.

Note that best-observed thresholds may or may not be attainable at any given site and that site-specific issues may determine which management strategies to reduce eutrophication stress are feasible. For example, a hard-bottom channel that abuts fully developed parcels may not have as many opportunities to increase shading as another hard-bottom channel that traverses a public right-of-way where riparian trees can be planted.

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## Best-attainable thresholds

Thresholds based on best-attainable conditions represent the theoretical best state a reach can achieve, given appropriate management within long-term constraints that are unlikely to change in the near future. Such constraints may include watershed development, channel modification, upstream dams or diversions, or discharge of effluent achieving the maximum technically feasible treatment. Although determining the expected improvements at a site given proper management is a technical task, determining what constitutes a long-term constraint is a policy decision.

Best-attainable thresholds are site-specific, and may be identified through modeling exercises, review of historical data, or through thoughtful selection of site-specific sites to serve as benchmarks for degraded sites (Olson and Hawkins 2013). Although they are typically used to account for natural factors that influence water quality or aquatic life, they may also account for constraints related to human activities, such as technology-based standards (Barbour et al. 1996).

# Response models

A fourth approach to identifying thresholds, response models, is appropriate for eutrophication indicators, which can drive changes in biointegrity (U.S. Environmental Protection Agency 2000, Heiskary and Bouchard 2015). These models can take the form of logistic models, which predict the likelihood of attaining biointegrity goals as stress increases, or the form of continuous models, which directly predict the likely biointegrity index score as stress increases. Thresholds derived from response models have already been identified for California's wadeable streams (Mazor et al. 2022).

# **Threshold calculations**

We calculated reference and best-observed thresholds for biointegrity and eutrophication indicators, as shown in Table 21 and Figure 44. For eutrophication thresholds derived from response models, we followed the approach described in Mazor et al. (2022) and used the same data set described in that study. However, instead of calculating logistic regressions for three separate biointegrity goals, we calculated continuous models (specifically, general additive models; GAMs) to predict index scores from increasing eutrophication stress. Thus, a single model could be used to identify levels of stress associated with a wide range of biointegrity goals (such as the 24 unique biointegrity goals identified in Table 21). These models were calibrated with the same statewide datasets used by Mazor et al. (2022). For model training, we did not limit calibration datasets to the different stream classes shown in Table 20

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because we have previously demonstrated that relationships between biointegrity and stress are weakened in many modified channel classes (Part 2 of this report).

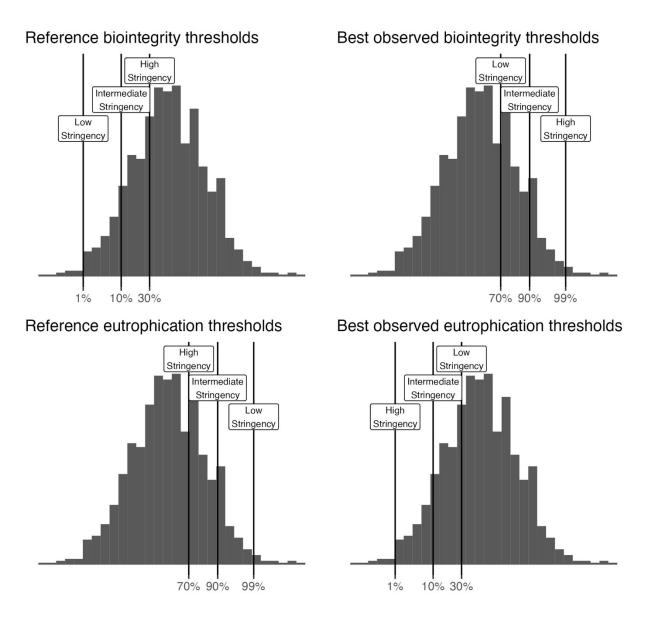


Figure 45. Examples of deriving high-, intermediate-, and low-stringency thresholds from reference or best-observed distributions for biointegrity and eutrophication indicators

Table 21. High-, intermediate-, and low-stringency options for percentiles are used to identify thresholds for different classes of streams associated with different levels of stringency. Abbreviations are defined in Table 20. SB1\*: Soft-bottom channels with one hardened side should use standard thresholds derived from statewide reference streams; best-observed thresholds are presented for informational purposes.

Stream class	Approach	Indicator Type	High stringency	Intermediate stringency	Low stringency
Wadeable (Standard)	Reference	Biointegrity	30 <sup>th</sup>	10 <sup>th</sup>	<b>1</b> st
Wadeable (Standard)	Reference	Eutrophication	70 <sup>th</sup>	90 <sup>th</sup>	99 <sup>th</sup>
RFI-N	Reference	Biointegrity	30 <sup>th</sup>	10 <sup>th</sup>	1 <sup>st</sup>
RFI-N	Reference	Eutrophication	70 <sup>th</sup>	90 <sup>th</sup>	99 <sup>th</sup>
CVF	Best observed	Biointegrity	99 <sup>th</sup>	90 <sup>th</sup>	70 <sup>th</sup>
CVF	Best observed	Eutrophication	1 <sup>st</sup>	10 <sup>th</sup>	30 <sup>th</sup>
НВ	Best observed	Biointegrity	99 <sup>th</sup>	90 <sup>th</sup>	70 <sup>th</sup>
НВ	Best observed	Eutrophication	1 <sup>st</sup>	10 <sup>th</sup>	30 <sup>th</sup>
SB2	Best observed	Biointegrity	99 <sup>th</sup>	90 <sup>th</sup>	70 <sup>th</sup>
SB2	Best observed	Eutrophication	1 <sup>st</sup>	10 <sup>th</sup>	30 <sup>th</sup>
SB1*	Best observed	Biointegrity	99 <sup>th</sup>	90 <sup>th</sup>	70 <sup>th</sup>
SB1*	Best observed	Eutrophication	1 <sup>st</sup>	10 <sup>th</sup>	30 <sup>th</sup>
SB0	Best observed	Biointegrity	99 <sup>th</sup>	90 <sup>th</sup>	70 <sup>th</sup>
SB0	Best observed	Eutrophication	1 <sup>st</sup>	10 <sup>th</sup>	30 <sup>th</sup>
CC	Best observed	Biointegrity	99 <sup>th</sup>	90 <sup>th</sup>	70 <sup>th</sup>
CC	Best observed	Eutrophication	1 <sup>st</sup>	10 <sup>th</sup>	30 <sup>th</sup>

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### Biointegrity thresholds

We examined distributions of biointegrity index scores in different stream classes (Figure 45) to identify potential thresholds (Table 22). Results are presented for all index/stream class combinations, even if standard index usage was supported in Parts 1 or 2 of this report. Thresholds for constructed channels could not be assessed for constructed channels due to a lack of data.

We calculated potential thresholds for classes of modified channels using only data from the Central Valley ecoregion if at least 10 sites were available, specifically: hard-bottom channels, soft-bottom channels with 0 hard sides, and constructed channels (CSCI only). CSCI thresholds declined for hard-bottom channels (e.g., intermediate thresholds dropped from 0.67 to 0.50) and soft-bottom channels with 0 hard sides (e.g., intermediate thresholds dropped from 0.78 to 0.48). Thus, the pattern where CSCI scores are higher in soft-bottom channels may be reversed in the Central Valley. These changes may reflect the more severe disturbance affecting modified channels in the Central Valley, or the combined impacts of channel alteration with potential bias in the CSCI. In contrast, the ASCI thresholds increased substantially (e.g., low-stringency thresholds for the ASCI\_D in hard-bottom channels was 0.99). Due to the low numbers of sites used to calculate these thresholds, we advise caution in using these numbers in place of those calculated from larger statewide datasets. The applicability of statewide thresholds in the Central Valley ecoregion could be re-assessed following additional data collection focused on modified channels (especially soft-bottom channels) in the Central Valley.

### Note about thresholds for intermittent streams in Northern California

As noted above, samples from intermittent reference streams in northern California are currently being evaluated to determine if samples were collected appropriately (e.g., under conditions with sufficient flow for sampling); therefore, these results should be considered provisional while analyses are being updated.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

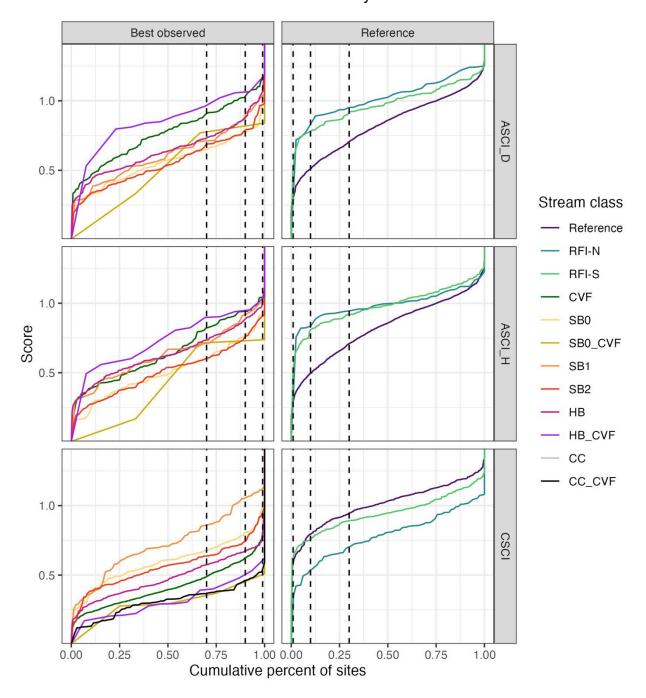


Figure 46. Cumulative distributions of biointegrity index scores in different classes of streams. Dashed lines represent high-, intermediate-, and low-stringency thresholds for best-observed (i.e., 70<sup>th</sup>, 90<sup>th</sup>, and 99<sup>th</sup> percentiles) and reference (i.e., 1<sup>st</sup>, 10<sup>th</sup>, and 30<sup>th</sup> percentiles) distributions. Other abbreviations are shown in Table 20. Classes with the "CVF" suffix represent subpopulations of each stream class restricted to sites on the Central Valley Floor.

Table 22. Potential biointegrity index thresholds for different classes of streams. Abbreviations of stream classes are shown in Table 20. Percentiles used to calculate high-, intermediate-, and low-stringency thresholds are shown in Table 21. n: number of sites used to calculate percentiles. RFI-N: Regularly flowing intermittent streams in northern xeric California. ND: Insufficient data. Thresholds were also calculated for sites restricted to the Central Valley floor (i.e., stream classes ending in "\_CVF"), although these typically had insufficient data for evaluation. Reference-based thresholds were calculated assuming normal distributions, following Mazor et al. (2016) and Theroux et al. (2020).

type			score attainability?		High stringency	Intermediate stringency	Low stringency
	Vadeable standard)	ASCI_D	Yes	418	0.94	0.86	0.75
	Vadeable standard)	ASCI_H	Yes	418	0.94	0.86	0.75
	Vadeable standard)	CSCI	Yes	473	0.92	0.79	0.63
Reference F	RFI-N	ASCI_D	Yes	16	0.98	0.90	0.78
Reference F	RFI-N	ASCI_H	Yes	16	0.96	0.90	0.81
Reference F	RFI-N	CSCI	No	38	0.73	0.61	0.44
Reference F	RFI-S	ASCI_D	Yes	45	0.91	0.80	0.66
Reference F	RFI-S	ASCI_H	Yes	45	0.91	0.80	0.65
Reference F	RFI-S	CSCI	Yes	53	0.89	0.82	0.71
Best observed C	CVF	ASCI_D	Yes	89	1.13	1.02	0.92
Best observed C	CVF	ASCI_H	Yes	86	1.05	0.94	0.80
Best observed C	CVF	CSCI	No	301	0.85	0.67	0.52
Best observed S	SB0	ASCI_D	No	51	1.01	0.77	0.68
Best observed S	SB0	ASCI_H	No	51	0.94	0.79	0.64
Best observed S	SB0	CSCI	Yes	78	0.99	0.78	0.66
Best observed S	SB0 (CVF only)	ASCI_D	ND	3	0.84	0.83	0.80
Best observed S	SB0 (CVF only)	ASCI_H	ND	3	0.74	0.73	0.72

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Threshold type	Stream class	Index	Evidence of high score attainability?	n	High stringency	Intermediate stringency	Low stringency
Best observed	SB0 (CVF only)	CSCI	ND	4	0.51	0.47	0.38
Best observed	SB1	ASCI_D	Yes	36	1.01	0.85	0.68
Best observed	SB1	ASCI_H	Yes	36	0.97	0.86	0.67
Best observed	SB1	CSCI	Yes	52	1.10	1.00	0.81
Best observed	SB2	ASCI_D	No	57	0.93	0.77	0.64
Best observed	SB2	ASCI_H	No	57	0.88	0.76	0.60
Best observed	SB2	CSCI	Yes	67	0.96	0.75	0.64
Best observed	HB	ASCI_D	Yes	152	1.05	0.88	0.74
Best observed	HB	ASCI_H	Yes	152	1.02	0.87	0.74
Best observed	HB	CSCI	No	203	0.74	0.67	0.55
Best observed	HB (CVF only)	ASCI_D	ND	8	1.05	1.03	0.96
Best observed	HB (CVF only)	ASCI_H	ND	8	0.95	0.93	0.80
Best observed	HB (CVF only)	CSCI	ND	10	0.57	0.49	0.39
Best observed	CC	ASCI_D	ND	ND	ND	ND	ND
Best observed	CC	ASCI_H	ND	ND	ND	ND	ND
Best observed	CC	CSCI	No	65	0.53	0.45	0.37
Best observed	CC_CVF	CSCI	No	65	0.53	0.45	0.37

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### Eutrophication thresholds

#### Reference and best-observed thresholds

We examined distributions of eutrophication indicators in different stream classes to identify potential thresholds (Table 20). Reference-based thresholds for wadeable streams are derived from analyses reported in Mazor et al. (2022) using the datasets described in that study, whereas other thresholds are derived from analyses in Parts 1 and 2 of this report. Thresholds for constructed channels could not be assessed for constructed channels due to a lack of data. Thresholds are presented in Table 23.

Table 23. Potential eutrophication thresholds for different classes of streams. Stream class abbreviations are provided in Table 20. Percentiles used to calculate high-, intermediate-, and low-stringency thresholds are shown in Table 21. n: number of sites used to calculate percentiles. Insufficient data were available to assess constructed channels or channels with ambiguous watersheds.

Indicator	Threshold type	Stream class	n	High stringency	Intermediate stringency	Low stringency
TN (mg/L)	Reference	Wadeable (standard)	529	0.131	0.233	1.639
TN (mg/L)	Reference	RFI-N	24	0.330	0.579	2.229
TN (mg/L)	Reference	RFI-S	44	0.254	0.351	0.558
TN (mg/L)	Best observed	CVF	99	0.037	0.143	0.32
TN (mg/L)	Best observed	SB0	59	0.341	0.497	0.781
TN (mg/L)	Best observed	SB1	44	0	0.261	0.379
TN (mg/L)	Best observed	SB2	59	0.163	0.436	0.924
TN (mg/L)	Best observed	НВ	158	0.029	0.43	1.109
TP (mg/L)	Reference	Wadeable (standard)	529	0.024	0.058	0.202
TP (mg/L)	Reference	RFI-N	24	0.184	0.495	0.547
TP (mg/L)	Reference	RFI-S	44	0.055	0.175	0.267
TP (mg/L)	Best observed	CVF	99	0.012	0.028	0.055
TP (mg/L)	Best observed	SB0	54	0.01	0.039	0.075
TP (mg/L)	Best observed	SB1	43	0.007	0.011	0.048

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Indicator	Threshold type	Stream class	n	High stringency	Intermediate stringency	Low stringency
TP (mg/L)	Best observed	SB2	48	0	0.009	0.05
TP (mg/L)	Best observed	НВ	141	0	0.028	0.068
Chl-a (mg/m2)	Reference	Wadeable (standard)	529	14.3	29.7	59.9
Chl-a (mg/m2)	Reference	RFI-N	21	15.4	38.0	85.3
Chl-a (mg/m2)	Reference	RFI-S	43	17.9	33.9	131.4
Chl-a (mg/m2)	Best observed	CVF	86	1.1	4.2	7.6
Chl-a (mg/m2)	Best observed	SB0	46	1.3	9	43.7
Chl-a (mg/m2)	Best observed	SB1	39	1	5.5	26.6
Chl-a (mg/m2)	Best observed	SB2	55	7.7	18.4	61.4
Chl-a (mg/m2)	Best observed	НВ	133	4.8	18.8	34.7
AFDM (g/m2)	Reference	Wadeable (standard)	529	11.4	27.3	95.4
AFDM (g/m2)	Reference	RFI-N	19	13.3	26.2	32.3
AFDM (g/m2)	Reference	RFI-S	42	26.1	56.2	95.8
AFDM (g/m2)	Best observed	CVF	87	2.1	4.3	9.2

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Indicator	Threshold type	Stream class	n	High stringency	Intermediate stringency	Low stringency
AFDM (g/m2)	Best observed	SB0	49	3.2	15.6	30
AFDM (g/m2)	Best observed	SB1	39	2.2	7.2	25
AFDM (g/m2)	Best observed	SB2	56	3.7	14.6	28.4
AFDM (g/m2)	Best observed	НВ	136	4.1	9.5	22
% cover	Reference	Wadeable (standard)	385	18	39	68
% cover	Reference	RFI-N	16	24	31	42
% cover	Reference	RFI-S	47	39	55	87
% cover	Best observed	CVF	71	0	0	2
% cover	Best observed	SB0	37	0	5	16
% cover	Best observed	SB1	34	1	4	9
% cover	Best observed	SB2	52	0	10	26
% cover	Best observed	НВ	155	0	10	35

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#### Response model thresholds

We followed Mazor et al. (2022) in modeling the response of biointegrity indices to increasing levels of eutrophication stress. However, instead of developing logistic regression models to predict the likelihood of meeting/not meeting each threshold identified in Table 23, we instead developed just a single continuous model to predict index score, one model per index and eutrophication indicator (15 models total). We used the same statewide dataset from Mazor et al. (2022).

We calibrated shape-constrained additive models using monotonic decreasing splines. We chose this shape because it is consistent with our conceptual model of a monotonic, non-linear decrease in index scores as stress increases. All analyses were conducted with the shape-constrained additive models (scam) package in R (Pya and Wood 2015, R Core Team 2022). To confirm that the eutrophication response models showed meaningful and significant relationships between index scores and eutrophication stress, we compared AIC (Akaike Information Criterion) values with those derived from null models (i.e., models that ignore levels of eutrophication stress and instead predict based on the mean observed index score). Substantially lower AIC values indicate that eutrophication response models were superior to null models.

To validate the assumptions about the negative relationships between biointegrity index scores and eutrophication stress, we also modeled responses using cubic splines. Cubic splines are unconstrainted models, and are well suited to identifying monotonic as well as non-monotonic non-linear responses to stress. These results are presented in Supplement 3.

Consistent with other studies (Biggs 2000, Heiskary and Bouchard 2015, Mazor et al. 2022), our eutrophication response models showed a steep decline in index scores at low levels of stress, followed by a "flattening out" at high levels of stress (Figure 46). The macroalgal cover indicator was an exception: ASCI scores had a relatively linear relationship with stress, and CSCI scores showed the steepest decline at levels above 75% cover. Overall, these models suggest that reductions to very low levels of eutrophication stress may be necessary to see a response in bioassessment index scores. Models calibrated at ecoregional (e.g., the Central Valley) or aggregated ecoregional scales (e.g., Xeric California) are presented in <u>Supplement S3</u>.

To assist with threshold identification from these models, we developed a web tool that allows users to specify a biointegrity goal (e.g., values shown in Table 22), and determine eutrophication indicator levels these models predict will achieve these goals (<a href="https://sccwrp.shinyapps.io/ModifiedChannelThresholds/">https://sccwrp.shinyapps.io/ModifiedChannelThresholds/</a>). Results for intermediate "best observed" biointegrity goals are shown in Table 24; a complete list of thresholds is presented in <a href="mailto:Supplement S4">Supplement S4</a>. For comparison, we provided results based on standard statewide reference-

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based thresholds (i.e., 0.79 for the CSCI and 0.86 for the ASCIs) derived from SCAM models, as well as results from logistic regression models reported by Mazor et al. (2022). A major difference between the thresholds derived from the SCAM and logistic regression models is that the latter are adjusted to reflect an 80% relative probability of achieving biointegrity goals, whereas the former are not adjusted, and therefore reflect a 50% probability.

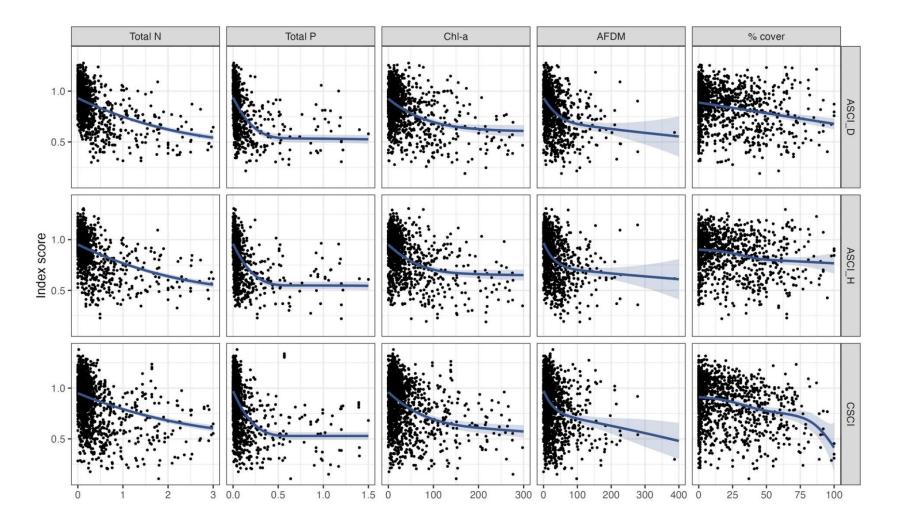


Figure 47. Modeled relationships between biointegrity index scores and eutrophication stress. Blue lines indicate fits from shape-constrained general additive models. Blue ribbons indicate the 95% confidence around the model fit. Black dots indicate observed values (values above the highest value on the x-axes not shown). Total N: total nitrogen (mg/L). Total P: total phosphorus (mg/L). Chl-a: benthic chlorophyll-a (mg/m²). AFDM: benthic ash-free dry mass (g/m²). % cover: percent macroalgal cover on the streambed.

Table 24. Potential eutrophication thresholds derived from response models. The first three rows show thresholds reported by Mazor et al. (2022), derived from logistic regressions. The subsequent rows show thresholds derived from SCAM models in the present study based on intermediate-stringency "best observed" biointegrity thresholds shown in Table 23; eutrophication thresholds derived from SCAM models based on statewide reference biointegrity goals are provided for comparative purposes. Total N: total nitrogen (mg/L). Total P: total phosphorus (mg/L). Chl-a: benthic chlorophyll-a (mg/m²). AFDM: benthic ash-free dry mass (g/m²). % cover: percent macroalgal cover on the streambed. SCAM: shape-constrained general additive model. NI: No eutrophication threshold identified because the biointegrity goal was outside the range predicted by the model.

Stream class	Index	Standard usage with traditional thresholds supported?	Biointegrity goal	Total N	Total P	Chl-a	AFDM	% cover
Wadeable streams (logistic regression, Mazor et al. 2022)	ASCI_D	Yes	0.86	0.33	0.054	46.8	24.8	27
Wadeable streams (logistic regression, Mazor et al. 2022)	ASCI_H	Yes	0.86	0.24	0.054	44.1	24.8	31
Wadeable streams (logistic regression, Mazor et al. 2022)	CSCI	Yes	0.79	1.189	0.194	48.9	41.3	31
Wadeable streams (SCAM, present study)	ASCI_D	Yes	0.86	0.348	0.044	19.5	15.2	13
Wadeable streams (SCAM, present study)	ASCI_H	Yes	0.86	0.453	0.056	24.3	17.6	27
Wadeable streams (SCAM, present study)	CSCI	Yes	0.79	1.021	0.102	51.1	37.2	45
CVF	ASCI_D	Yes	1.02	NI	NI	NI	NI	NI
CVF	ASCI_H	Yes	0.94	0.054	0.009	2.4	3.6	NI
CVF	CSCI	No	0.67	2.108	0.203	116.2	139.7	82
SB0	ASCI_D	No	0.77	0.862	0.107	55.0	40.4	56
SB0	ASCI_H	No	0.79	0.856	0.104	49.2	35.6	66

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Stream class	Index	Standard usage with traditional thresholds supported?	Biointegrity goal	Total N	Total P	Chl-a	AFDM	% cover
SB0	CSCI	Yes	0.78	1.099	0.110	55.0	41.2	48
SB1	ASCI_D	Yes	0.85	0.402	0.051	23.1	17.6	18
SB1	ASCI_H	Yes	0.86	0.453	0.056	24.3	17.6	27
SB1	CSCI	Yes	1.00	NI	NI	NI	NI	NI
SB2	ASCI_D	No	0.77	0.862	0.107	55.0	40.4	56
SB2	ASCI_H	No	0.76	1.045	0.128	63.1	47.6	NI
SB2	CSCI	Yes	0.75	1.339	0.132	67.9	56.1	63
НВ	ASCI_D	Yes	0.88	0.246	0.032	13.2	10.8	4
НВ	ASCI_H	Yes	0.87	0.402	0.050	21.3	15.6	23
НВ	CSCI	No	0.67	2.108	0.203	116.2	139.7	82
СС	CSCI	No	0.45	NI	NI	NI	NI	98

Biointegrity and eutrophication indicators and thresholds for modified channels, intermittent streams, and streams on the Central Valley floor

### Limits of biointegrity-based eutrophication thresholds

As noted in Sutula et al. (2022) and Mazor et al. (2022), these approaches to identifying eutrophication thresholds have notable limitations in that they are focused on protecting aquatic life within an assessment reach. Thus, the ability of these thresholds to protect downstream waterbodies or to prevent adverse impacts to human health is not addressed here. These considerations may be particularly important when managers consider approaches that result in increases in eutrophication thresholds.

## An example approach for using thresholds to make assessments

This section illustrates an example of how to use the thresholds identified in this report to make assessments of biointegrity and eutrophication risk. The approach described below is presented as an example, and it should not be interpreted as an endorsement of one specific approach in Water Board programs.

### Querying and synthesizing thresholds

The full set of high-, intermediate-, and low-stringency biointegrity and eutrophication thresholds are presented in <u>Supplement S4</u>. Analysts seeking to identify thresholds can use the supplemental material to generate a range of numbers that can inform threshold determination. Below, we describe a stepwise process for selecting thresholds for a given stream:

- 1. Identify the relevant class or classes of streams.
  - Typically, standard thresholds for the "wadeable streams" class should be queried as baseline for comparison with other thresholds.
  - If the stream is naturally intermittent, select "RFI-N" (if the stream is in northern California) or "RFI-S" (if the stream is in southern California).
  - If the stream is on the Central Valley floor, select "CVF"
  - If the reach has a modified channel, select the appropriate category (i.e., HB, SB2, SB1, SB0, or CC). Refer to Part 2 for details on classifying modified channels.
- 2. Select the desired level of stringency (high, intermediate, or low) based on management goals and priorities. The level of stringency to use is a policy decision based in part on the confidence required in achieving a biointegrity outcome.

### Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

- 3. Select the indicators of interest. Analysts should determine whether indicators should be evaluated independently or treated as combined lines of evidence about biointegrity conditions or eutrophication of a site.
- 4. Evaluate the range of resulting numbers to identify a final threshold. In general, biointegrity thresholds that are above reference thresholds with equivalent stringency (and eutrophication thresholds that are below equivalent reference thresholds) may not be achievable in some natural settings.

Once a range of thresholds are identified, analysts can select a threshold by any number of methods (such as selecting the mean, median, or most conservative number) consistent with their management goals. We have developed a dashboard that automatically plots all queried thresholds. The dashboard (<a href="https://sccwrp.shinyapps.io/ModifiedChannelThresholds/">https://sccwrp.shinyapps.io/ModifiedChannelThresholds/</a>) also presents means of unflagged thresholds for each group (e.g., hard-bottom modified channels), as well as for the overall mean. In addition, dashboard users can provide data to compare observed indicator values with these thresholds.

After going through this process, analysts may want to consider whether site-specific bestattainable thresholds are warranted or feasible.

### Case studies in assessments of Central Valley streams

We selected three sites in the Central Valley region where bioassessment has occurred to illustrate how to apply thresholds to assess biointegrity and eutrophication stress.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

### Elder Creek (519PS0134) Sacramento County (38.48281 - 121.39559)



Figure 48. A photograph of Elder Creek taken in July of 2008.

Elder Creek is a soft-bottom modified channel with no hardened sides that has been channelized and leveed for flood management (Figure 47). It is located in an urban watershed Riparian vegetation is limited. It is presumed to be perennial, although historically it may have been intermittent. We queried bioassessment data from this site, which was collected on July 7, 2008 (Table 25).

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

Setting (Google Maps). Red pin indicates monitoring site.



Table 25. Biointegrity and eutrophication indicator values from Elder Creek.

Indicator	Observed value
CSCI	0.39
ASCI_D	0.97
ASCI_H	1.02
Total N (mg/L)	0.79
Total P (mg/L)	1.03
Benthic chlorophyll-a (mg/m²)	13.26
Benthic ash-free dry mass (g/m²)	9.2

Based on this stream's classifications, the following intermediate-stringency thresholds were extracted from the table in Supplement S4:

- Wadeable streams
- Central Valley Floor (CVF)
- Soft-bottom modified channels with 0 hardened sides (SB0)

Intermediate-stringency thresholds were selected for illustrative purposes, and we do not recommend a specific level of stringency for Elder Creek.

Biointegrity indices have three potential thresholds, corresponding to the three stream classes listed above, based on reference distributions for wadeable streams, and best-observed scores for the other classes. For the CSCI, thresholds were applicable for all three classes, because best-observed thresholds for CVF and SBO streams was lower than reference thresholds for wadeable streams. In contrast, for the ASCIs, the best-observed thresholds for CVF streams were *higher* than the reference thresholds with equivalent stringency, and they would

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therefore be inappropriate for assessing ASCI scores in Central Valley Floor streams. These higher-than-reference biointegrity thresholds, plus any eutrophication thresholds based on these biointegrity thresholds are flagged and should be interpreted with caution. These numbers are presented with other results below, but we gave them no weight when making assessments.

Although thresholds for intermittent streams are not included in this example, it is a policy decision whether to consider perennial versus intermittent thresholds for a stream that has undergone conversion from intermittent to perennial flow duration. If the decision is made to assess Elder Creek as an intermittent stream, the CSCI thresholds for CVF and SB0 streams do not apply, as these thresholds (0.67 and 0.78, respectively) are higher than the reference-based CSCI threshold for intermittent streams (i.e., 0.61).

Comparison to intermediate-stringency thresholds for these stream classes shows that Elder Creek was in poor condition for benthic macroinvertebrates, while algal index scores were similar to reference condition. Total phosphorus was substantially higher than all applicable thresholds, no matter how they were derived. However, benthic ash-free dry mass was lower than most thresholds, apart from the best-observed threshold for Central Valley Floor streams (9.2 vs 4.3.g/m²). Similarly, benthic chlorophyll-a met all applicable thresholds from response models or reference distributions, exceeding only the two best-observed thresholds. Total nitrogen concentration met thresholds based on CSCI response models, yet it exceeded all other applicable thresholds (Figure 48).

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

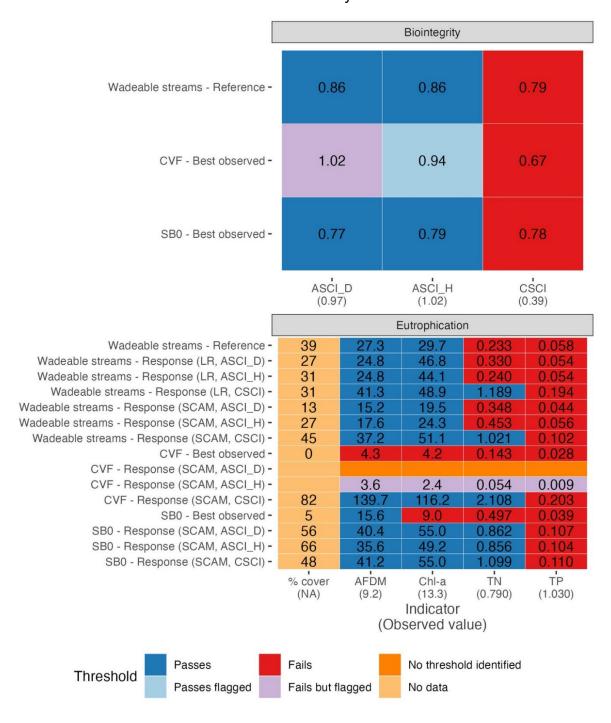


Figure 49a. Observed values and applicable intermediate-stringency thresholds of biointegrity and eutrophication indicators for Elder Creek. Each row represents an approach for identifying thresholds. Numbers in cells are the threshold identified for that column's indicator. Numbers in parentheses in the x-axis labels are the values observed at Elder Creek. LR: Logistic regression. SCAM: shape-constrained additive models. CVF: Central Valley Floor. SB0: Soft-bottom engineered channels with no hardened sides.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

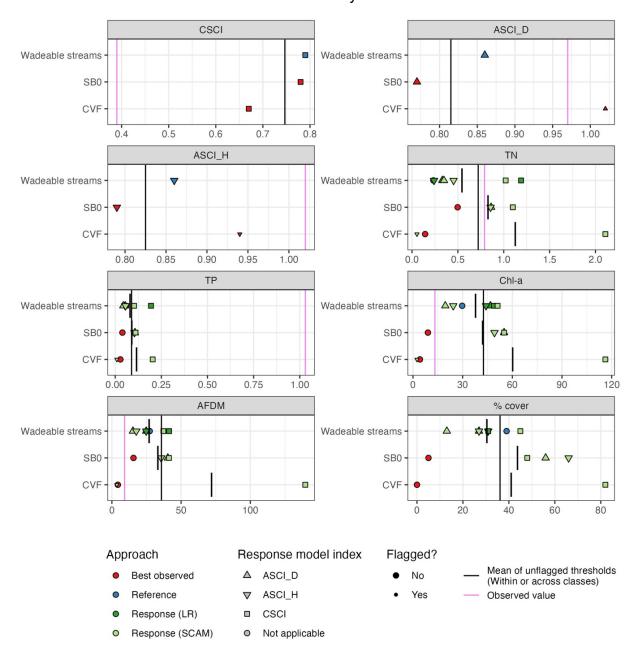


Figure 48b. Observed values and applicable thresholds of biointegrity and eutrophication indicators for Elder Creek. Each point represents an intermediate-stringency threshold. Flagged thresholds are shown as smaller symbols and should generally be disregarded. Vertical black lines represent means of unflagged thresholds (calculated for each class of stream as well as the overall mean). Observed values, where available, are shown as vertical violet lines. LR: Logistic regression. SCAM: shape-constrained additive models. CVF: Central Valley Floor. SB0: Soft-bottom engineered channels with no hardened sides.

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### Magpie Creek (519PS0002) Sacramento County (38.65573 - 121.38781)



Figure 50. A photo of Magpie Creek taken in July of 2009.

Magpie Creek is a hard-bottom modified channel in Sacramento (Figure 49). Magpie Creek is channelized and managed for flood control in an urban watershed. It is presumed to be perennial, although streamflow duration information was not available, and it was likely historically intermittent. We queried bioassessment data from this site, which was collected on August 5, 2010, 2010 (Table 26).

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Setting (Google Maps). Red pin indicates monitoring site.



Table 26. Biointegrity and eutrophication indicator values from Magpie Creek.

Indicator	Observed value
CSCI	0.21
ASCI_D	0.92
ASCI_H	0.82
Total N (mg/L)	0.64
Total P (mg/L)	0.20
Benthic chlorophyll-a (mg/m²)	154
Benthic ash-free dry mass (g/m²)	83
Macroalgal cover (%)	41

Based on this stream's classifications, the following intermediate-stringency thresholds were extracted from the table in <u>Supplement S4</u>:

- Wadeable streams
- Central Valley Floor (CVF)
- Hard-bottom engineered channels (HB)

Intermediate-stringency thresholds were selected for illustrative purposes, and we do not recommend a specific level of stringency for Magpie Creek.

For the CSCI, thresholds were applicable for all three classes listed above. For the ASCIs, only one of the three thresholds were applicable (i.e., wadeable streams). In contrast to Elder Creek, where there was evidence that the class of channel modification cannot support reference-level ASCI scores, hard-bottom channels like Magpie Creek frequently attain reference-level scores.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

As above, higher-than-reference biointegrity thresholds, plus any eutrophication thresholds based on these biointegrity thresholds are flagged and should be interpreted with caution.

Although thresholds for intermittent streams are not included in this example, it is a policy decision whether to consider perennial versus intermittent thresholds for a stream that has undergone conversion from intermittent to perennial flow duration. If the decision is made to assess Magpie Creek as an intermittent stream, the CSCI thresholds for CVF and HB streams do not apply, as these thresholds (0.67 for both categories) are higher than the reference-based CSCI threshold for intermittent streams (i.e., 0.61).

Comparison to relevant intermediate-stringency thresholds for these stream classes shows that Magpie Creek was in poor condition for both the CSCI (0.21) and for ASCI\_H (0.82), while the ASCI\_D score (0.92) indicated good conditions. Benthic chlorophyll-a (154 mg/m²) exceeded all applicable thresholds, and ash-free dry mass (83 g/m²) exceeded all thresholds but two based on CSCI response models. Percent macroalgal cover (41%), total nitrogen (0.64 mg/L), and total phosphorus (0.20 mg/L) exceeded most thresholds, only meeting those derived from response models based on the CSCI (which were typically much higher than other thresholds; Figure 50).

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

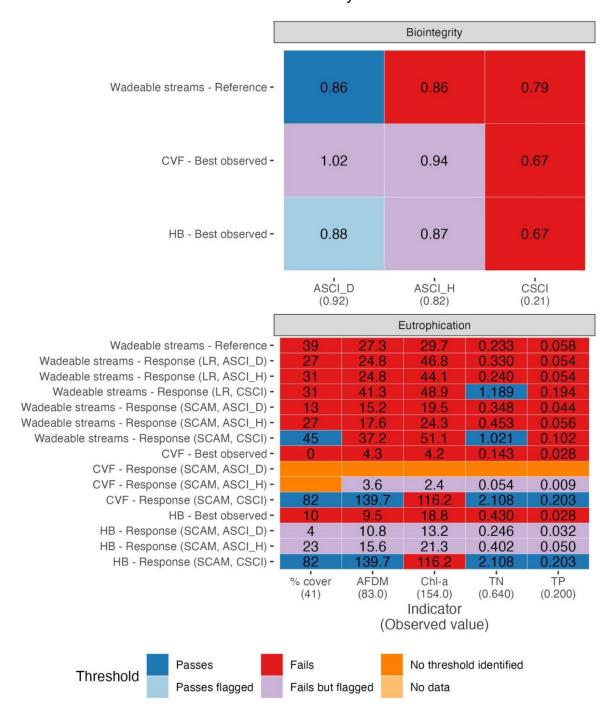


Figure 51a. Observed values and applicable intermediate-stringency thresholds of biointegrity and eutrophication indicators for Magpie Creek. Each row represents an approach for identifying thresholds. Numbers in cells are the threshold identified for that column's indicator. Numbers in parentheses in the x-axis labels are the values observed at Magpie Creek. LR: Logistic regression. SCAM: shape-constrained additive models. CVF: Central Valley Floor. HB: Hard-bottom engineered channels.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

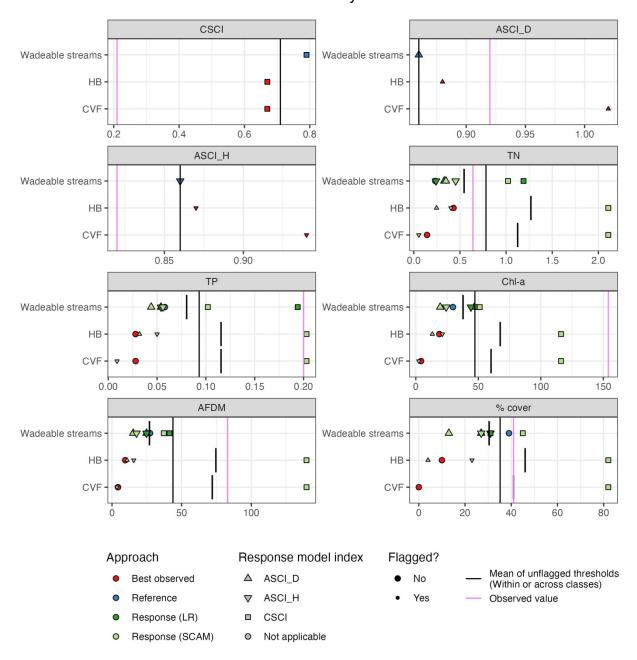


Figure 50b. Observed values and applicable thresholds of biointegrity and eutrophication indicators for Magpie Creek. Each point represents an intermediate-stringency threshold. Flagged thresholds are shown as smaller symbols and should generally be disregarded. Vertical black lines represent means of unflagged thresholds (calculated for each class of stream as well as the overall mean). Observed values, where available, are shown as vertical violet lines. LR: Logistic regression. SCAM: shape-constrained additive models. CVF: Central Valley Floor. HB: Hard-bottom engineered channels.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

Pine Creek (504PS0574) Tehama County (39.8958 121.9236)



Figure 52. An aerial image of Pine Creek taken in August of 2013. The blue line represents the flowline from the National Hydrography Plus data set. The dot represents the downstream end of the sampling reach.

Pine Creek is a natural, intermittent stream in Tehama County, within the Central Valley ecoregion but close to the foothills of the Sierra Nevada (Figure 51). The watershed is lightly developed. We presume that the intermittency is natural, and is not caused by diversions, increased watershed imperviousness, or other anthropogenic activities. We queried bioassessment data from this site, which was collected on July 7, 2008 (Table 27).

Table 27. Biointegrity and eutrophication indicator values from Pine Creek.

Indicator	Observed value
CSCI	0.83
ASCI_D	0.90
ASCI_H	0.94
Total N (mg/L)	0.07
Total P (mg/L)	0.013
Benthic chlorophyll-a (mg/m²)	33.3
Benthic ash-free dry mass (g/m²)	11

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

Based on this stream's classifications, the following high-stringency thresholds were extracted from the table in <u>Supplement S4</u>:

- Wadeable streams
- Central Valley Floor (CVF)
- Regularly flowing intermittent streams in Northern California (RFI-N)

High-stringency thresholds were selected for illustrative purposes, and we do not recommend a specific level of stringency for Pine Creek.

For the CSCI, thresholds were applicable for all three classes listed above. For the ASCIs, only one of the three thresholds were applicable (i.e., wadeable streams), because alternative thresholds for RFI-N streams were, like CVF streams, greater than the wadeable streams reference thresholds with equivalent stringency. As above, higher-than-reference biointegrity thresholds, plus any eutrophication thresholds based on these biointegrity thresholds are flagged and should be interpreted with caution.

The CSCI (0.83) did not meet the high-stringency thresholds for wadeable streams (0.92) or CVF streams (0.85), but it did meet the threshold for RFI-N streams (which was 0.73), suggesting that intermittency may account for the lower score. Although the ASCI\_H (0.94) met its relevant threshold, the ASCI\_D (0.90) did not. Total phosphorus (0.013 mg/L) and total nitrogen (0.07 mg/L) exceeded best-observed thresholds, but they met most reference thresholds and thresholds from response models (with the exception of the ASCI\_H model for wadeable streams). Benthic chlorophyll-a (33.3 mg/m²) and ash-free dry mass (11 g/m²) exceeded several thresholds. Because high-stringency biointegrity thresholds for ASCIs were relatively high, response models were sometimes unable to identify eutrophication thresholds (e.g., orange rows in Figure 52).

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

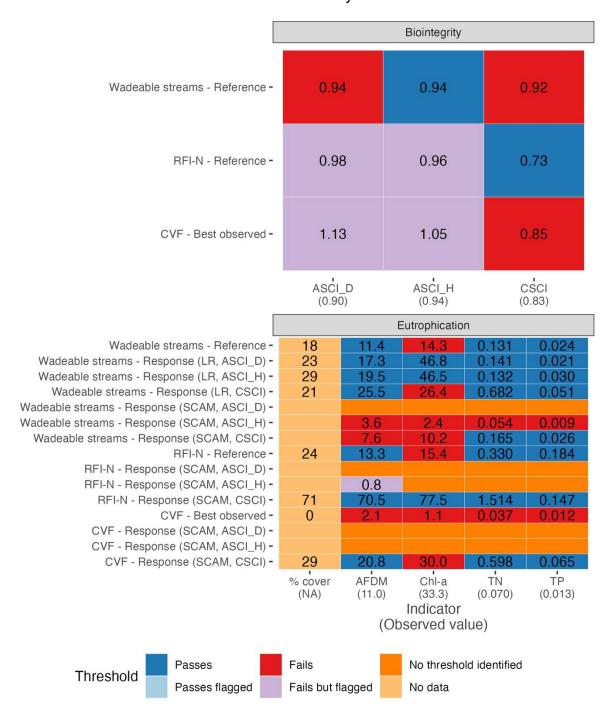


Figure 53a. Observed values and applicable high-stringency thresholds of biointegrity and eutrophication indicators for Pine Creek. Each row represents an approach for identifying thresholds. Numbers in cells are the threshold identified for that column's indicator. Numbers in parentheses in the x-axis labels are the values observed at Pine Creek. LR: Logistic regression. SCAM: shape-constrained additive models. CVF: Central Valley Floor. RFI-N: Regularly flowing intermittent streams in Northern California.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

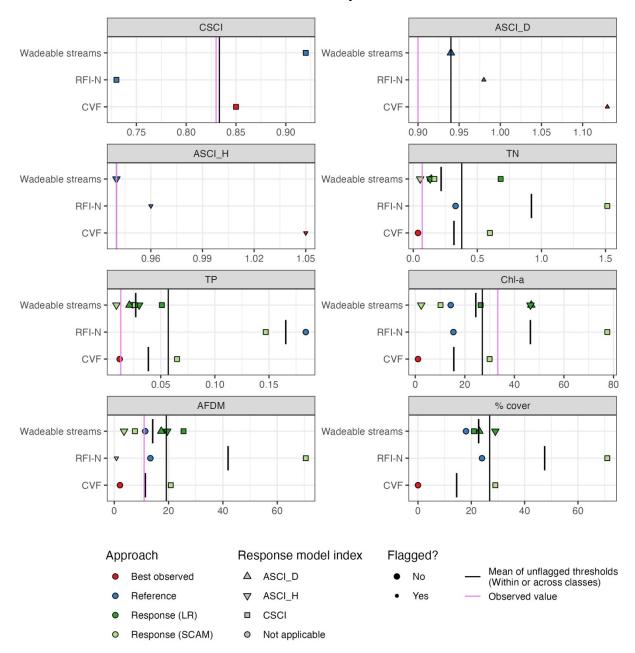


Figure 52b. Observed values and applicable thresholds of biointegrity and eutrophication indicators for Pine Creek. Each point represents a high-stringency threshold. Flagged thresholds are shown as smaller symbols and should generally be disregarded. Vertical black lines represent means of unflagged thresholds (calculated for each class of stream as well as the overall mean). Observed values, where available, are shown as vertical violet lines. LR: Logistic regression. SCAM: shape-constrained additive models. RFI-N: Regularly flowing intermittent streams in Northern California. CVF: Central Valley Floor.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

# Comparison of best-observed biointegrity thresholds with other non-reference-based thresholds

### Biological Condition Gradient models (BCG)

BCGs are conceptual models that describe structural and functional changes in ecosystems as increasing human activity causes biological degradation, and they serve to link numeric measures of biointegrity to narrative statements about loss of diversity or function (Davies and Jackson 2006). Paul et al. (2020) developed a BCG model for California streams based on benthic macroinvertebrate and algal assemblage composition. Experts evaluated bioassessment data from over 200 sites representing a wide range of conditions in California, and assigned them to one of six pre-determined condition categories, ranging from BCG1 (most intact) to BCG6 (most degraded; Table 28). Paul et al. (2020) then linked these categories to numeric values of CSCI and ASCI scores.

By comparing the alternative "best observed" thresholds for modified channels or channels in the Central Valley Floor (Table 22) to the BCG model, we are able to link these thresholds to narratives about ecosystem function that these the threshold represent (Figure 53). (The California BCG model was not developed to account for natural intermittency, so intermittent thresholds were excluded from the analysis.) Across all three indices, most of the alternative intermediate thresholds corresponded to BCG4 (i.e., moderate changes in structure; replacement of sensitive taxa; and minor changes in function). CSCI thresholds for constructed channels corresponded to BCG5 (i.e., major changes in structure; conspicuously unbalanced; reduced functional complexity). Overall, stringency, rather than channel modification status, had a larger effect on the BCG category that matched a given threshold. Thus, while standard thresholds tend to correspond to the high end of biointegrity index scores within a BCG category, thresholds for modified channels tend to match the low end of that same category.

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

### Table 28. Biological condition gradient (BCG) categories from Davies and Jackson (2006).

BCG category	Description
1	Natural structural, functional, and taxonomic integrity maintained
2	Minor taxonomic changes; no detectable functional change
3	Evident changes in structure; loss of some highly sensitive taxa; minimal changes in function
4	Moderate changes in structure; replacement of many sensitive taxa; minor changes in function
5	Major changes in structure; conspicuously unbalanced; reduced functional complexity
6	Severe changes throughout; wholesale changes in composition and function

Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor

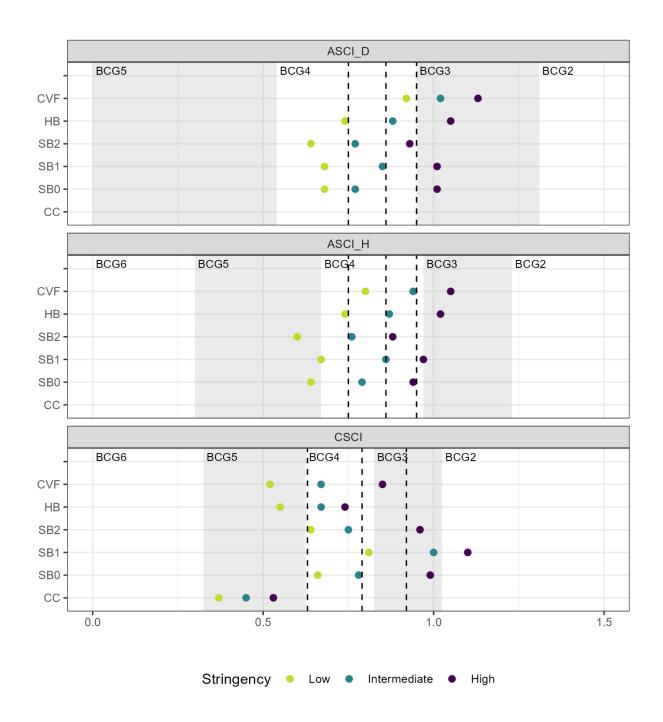


Figure 54. Comparison of best-observed thresholds for classes of streams with biological condition gradient categories. The vertical dashed lines represent the low-, intermediate-, and high-stringency reference-based thresholds for wadeable streams.

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### Landscape models (SCAPE)

Beck et al. (2019) developed the SCAPE (Stream Classification and Prioritization Explorer) model, a statistical model to predict ranges of likely CSCI scores based on land use in the watershed. For that model, land-use metrics were acquired from the StreamCat dataset (Hill et al. 2016), an nation-wide dataset of GIS metrics calculated for all watersheds and local catchments represented in the National Hydrography Dataset Plus (NHD+; McKay et al. 2014). These metrics include factors related to human activity, such as percent urban or agricultural land cover, or road density. The model was then used to predict ranges of likely scores for every NHD+ stream segment in California.

Much like the alternative best-observed thresholds presented in this study, the ranges predicted by the SCAPE model have been proposed as a way for identifying streams that may be unlikely to achieve reference-based thresholds, and for identifying interim goals (Beck et al. 2020). We compared best-observed thresholds from this study to the ranges predicted by the SCAPE model. Specifically, we compared low-stringency thresholds to the 70<sup>th</sup> percentile of the SCAPE's predicted range, and the intermediate-stringency thresholds to the 90<sup>th</sup> percentile (the 99<sup>th</sup> percentile, which would be the appropriate comparison for high-stringency thresholds, were not available).

Although the two methods are based on different types of information (i.e., reach-scale bed and bank material vs. watershed-scale land use), they largely provided similar information about likely upper limits of biointegrity scores in modified channels (Figure 54). For most channel types, the best-observed thresholds were within the interquartile range of the SCAPE predictions. Notably, the best-observed thresholds for CVF streams were slightly below the interquartile range, and the thresholds for CC streams were far below the range. Because SCAPE is based on StreamCat data, and because the StreamCat dataset excludes most constructed channels and stream segments with ambiguous watersheds, it is likely that the ranges presented in Figure 54 are unrepresentative subset of constructed channels and channels in the Central Valley.

We recommend that predictions from SCAPE models be used as an additional line of evidence in setting goals for modified channels. Whereas best-observed thresholds account for the likely constraints imposed by channel alteration, the SCAPE model accounts for the likely constraints imposed by watershed development.

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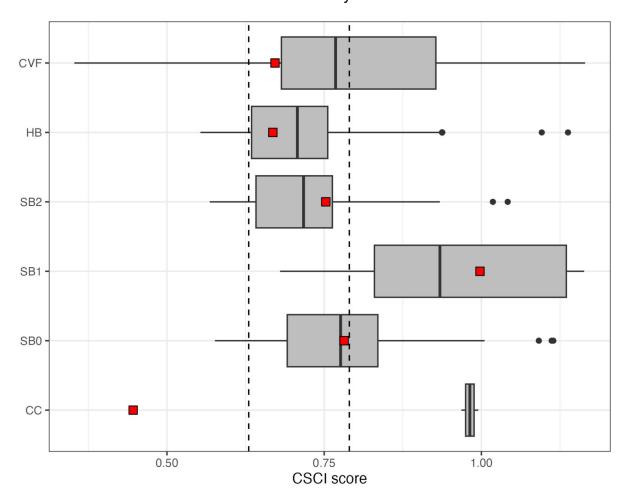


Figure 55. Comparison of segment-specific predictions of the upper range of likely CSCI scores from a landscape model (SCAPE; Beck et al. 2019) and best-observed thresholds for different channel types. Boxplots represent the range of predictions from the SCAPE model, and red squares represent best-observed thresholds. The vertical dashed lines represent reference-based thresholds. Only intermediate-stringency thresholds (i.e., 90<sup>th</sup> percentiles) are shown; low-stringency thresholds showed a similar pattern.

### Causal assessment in modified channels

A central question about bioassessment in modified channels is not simply whether conditions are poor, or whether these conditions are associated with channel modification, but rather, which stressors are likely further affecting conditions on top of the impacts of channel modification alone? Causal assessment tools have the potential to answer this question. Causal assessment is the science of evaluating data to determine which stressors are likely causes of poor biological conditions, and which are unlikely causes (Norton et al. 2014, U.S.

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Environmental Protection Agency 2017). Thus, causal assessment is an essential step in determining how to restore or rehabilitate degraded streams. Rapid screening causal assessment (RSCA) tools have been developed for California to standardize and expedite the process of associating stressors with sites in poor condition (Gillett et al. 2023). Because causal assessment tools have largely been developed for use in natural (unmodified) streams, their application to modified channels has not been thoroughly evaluated, and some enhancements or adjustments may be helpful in these systems. For example, most lines of evidence within RSCA are based on comparisons between a test site and healthier sites with similar natural environmental settings. It may be useful to complement these lines of evidence with lines derived from subsets of healthier sites with similar types of modifications (e.g., comparisons of a hard-bottom channel with healthier hard-bottom channel). Pilot investigations into causal assessments of modified channels are underway in southern California (e.g., Mazor 2024), although additional studies are needed in other parts of the state.

#### **Part 3 Conclusions**

#### **Key Questions**

- What are ranges of biointegrity index scores and eutrophication indicators associated with different types of streams?
  - What are natural ranges at minimally disturbed reference sites? When are these ranges different from ranges observed at typical reference perennial wadeable streams?
  - What are the best-observed conditions (i.e., highest biointegrity index scores or lowest eutrophication indicator levels) at types of streams defined by human activity (e.g., modified channels)? When are these best-observed conditions outside the range at reference sites?
- How do biointegrity index scores respond to increasing levels of eutrophication indicators?
  - What eutrophication indicator levels are associated with different biointegrity goals?

### Biointegrity thresholds

Reference-based biointegrity thresholds are an approach for assessing aquatic life in
wadeable streams and determining whether these reaches support a natural balance of
native organisms. Except in cases where indices are suspected to be biased (specifically,

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intermittent streams in northern California, and possibly wadeable streams on the Central Valley Floor), reference-based thresholds provide a valid measure of aquatic life, and index scores should be considered strong evidence of whether a wadeable stream (modified or otherwise) supports a natural balance of native benthic macroinvertebrates (for the CSCI) or algae (for the ASCIs). Thus, for most *assessment* applications, reference-based thresholds are useful in both natural and modified streams.

- Naturally intermittent streams in northern California should not be assessed using CSCI thresholds derived from perennial streams, based on provisional analyses. Ideally, the CSCI should be recalibrated or modified for use in these streams. However, index recalibration requires a great deal of data, which may take several years to generate. Until an updated index is available, the thresholds presented in Table 22 (or updated versions of those thresholds) provide an interim solution. These thresholds can be interpreted the same as reference-based thresholds. However, due to their low numeric values (e.g., 0.54 vs 0.79 for intermediate stringency), these alternative thresholds may not provide an adequate level of sensitivity for identifying biological degradation (meaning that truly degraded sites may go undetected).
- There are several cases where standard thresholds may be considered for use in intermittent streams:
  - Alternative thresholds are not needed for <u>intermittent streams in</u> southern California.
  - Nor are they needed for <u>ASCI scores</u> in intermittent streams in any part of the state.
  - Historically perennial streams that have been converted to intermittent or ephemeral through human activity can be assessed with standard thresholds calibrated from perennial reference streams to evaluate the impacts of hydrologic alteration and other disturbances.
- Although the evidence is less conclusive, alternative CSCI thresholds could be useful for <u>Central Valley Floor</u> streams. But because there is no way to derive reference-based thresholds in this pervasively altered region, it is difficult to identify thresholds that confer an equivalent level of protection. Thus, the best-observed thresholds presented in Table 22 may be an alternative to reference-based thresholds in the Central Valley Floor (except when they result in higher numeric values than reference-based thresholds of equivalent stringency).

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- Best-observed biointegrity thresholds are an alternative to reference-based thresholds, and they may be appropriate for stream classes where reference conditions are determined to be an impractical goal due to costs, timelines, or incompatible uses of a waterbody. This determination is fundamentally a matter of priorities and policy that cannot be made on a strictly technical basis. Although reference-based thresholds are well suited for assessment applications in these streams, best-observed thresholds may be more helpful for prioritizations and setting interim goals while constraints are in place.
  - Some modified streams may not be able to meet the best-observed thresholds in Table 22, even if state-of-the-art management practices are implemented.
     Factors such as watershed development, upstream dams, legacy mines, and other stressors that are outside the typical control of watershed managers may impose a more severe constraint on stream condition than channel modification.
  - A stream that meets best-observed thresholds may not support aquatic life at a level consistent with its designated beneficial uses. With the possible exception of Central Valley floor streams, best-observed thresholds are not useful for assessment applications.
  - Narrative descriptions from biological condition gradient models can help communicate the ecological state of streams that meet these non-reference thresholds.
  - Best-observed thresholds should be used in tandem with segment-specific ranges of expected conditions derived from the SCAPE landscape model to provide a comprehensive understanding of the likely constraints from reachscale and watershed-scale disturbances.

#### Eutrophication thresholds

- Where lower-than-normal biointegrity thresholds are desired, the eutrophication thresholds derived from the response models (Table 24) provide a 50% likelihood of achieving these goals related to aquatic life. However, downstream waterbodies and human health uses may not be protected by these higher eutrophication indicator levels. Best-observed and reference-based eutrophication thresholds are best used as checks to ensure that whatever targets are ultimately selected are practical to achieve.
- A previous report on wadeable streams suggests that % cover is not suitable for use as an indicator of eutrophication due to high variability in its measurement and weak

- Part 3: Candidate thresholds for modified channels, intermittent stream, and streams on the Central Valley floor
  - relationships with aquatic life measures (Sutula et al. 2022). This study does not contradict those findings.
  - Mazor et al. (2022) recommended the use of multiple eutrophication indicators for causal assessment applications. Those recommendations are also applicable to modified channels or other stream types evaluated in this study.

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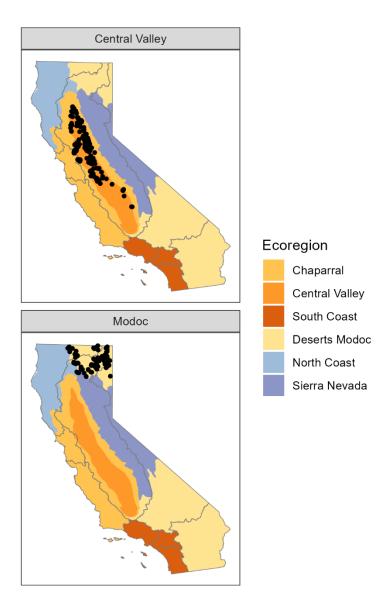
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### **SUPPLEMENTAL MATERIAL**

### Supplement S1: Maps of sites used in selected data analyses

### Supplement S1-1: Central Valley Floor and Modoc Plateau sites

These sites were used as "test sites" to evaluate the representativeness of bioassessment tool calibration data sets in Part 1: Bioassessment data and model fitness review.

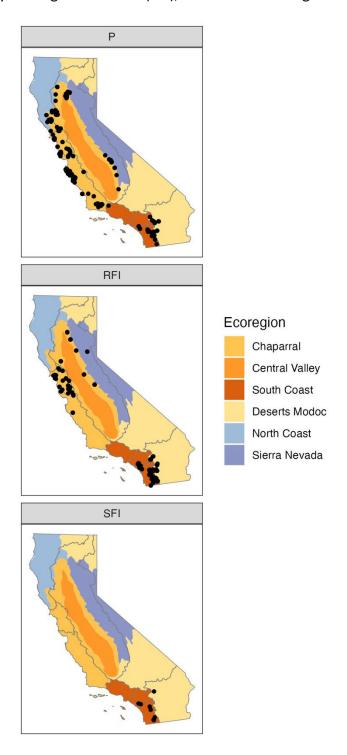


Supplement S3: Regional response models

Regional Board	Study area	# sites
Central Valley	Central Valley	306
Central Valley	Modoc	104
Lahontan	Modoc	2
North Coast	Modoc	20
San Francisco Bay	Central Valley	5

# Supplement S1-2: Perennial, regularly flowing, and seldomly flowing intermittent streams

These sites were used to evaluate the applicability of bioassessment indices in perennial (P), regularly flowing intermittent (RFI), and seldom flowing intermittent (SFI) reference sites.

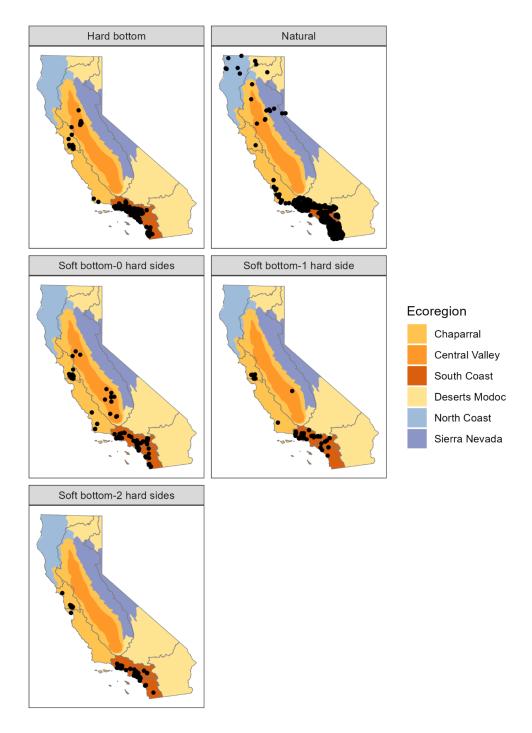


#### Supplement S3: Regional response models

Ecoregion	Flow status	# reference sites
Chaparral	Perennial	123
Chaparral	Regularly flowing intermittent	35
South Coast	Perennial	19
South Coast	Regularly flowing intermittent	53
South Coast	Seldom flowing intermittent	8
<b>Deserts Modoc</b>	Perennial	10
<b>Deserts Modoc</b>	Regularly flowing intermittent	10
<b>Deserts Modoc</b>	Seldom flowing intermittent	1
Sierra Nevada	Perennial	1
Sierra Nevada	Regularly flowing intermittent	1

#### Supplement S1-3: Bed and bank material

These sites were used to evaluate biointegrity and eutrophication indicators in streams classified based on bed and bank material.



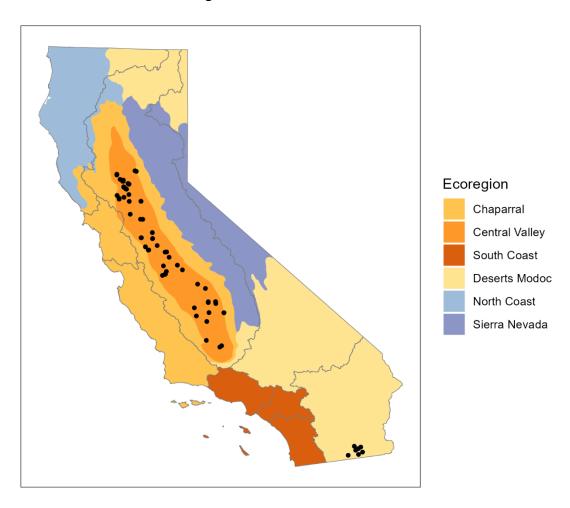
Note: Most soft-bottom channels in the Central Valley are also considered "constructed channels", as shown in Supplement S1-4.

Supplement S3: Regional response models

Ecoregion	Class	# sites
Chaparral	Hard bottom	16
Chaparral	Natural	17
Chaparral	Soft bottom-0 hard sides	29
Chaparral	Soft bottom-1 hard side	9
Chaparral	Soft bottom-2 hard sides	7
Central Valley	Hard bottom	11
Central Valley	Natural	4
Central Valley	Soft bottom-0 hard sides	13
Central Valley	Soft bottom-1 hard side	1
South Coast	Hard bottom	208
South Coast	Natural	664
<b>South Coast</b>	Soft bottom-0 hard sides	56
South Coast	Soft bottom-1 hard side	50
South Coast	Soft bottom-2 hard sides	64
<b>Deserts Modoc</b>	Natural	3
<b>North Coast</b>	Natural	6
Sierra Nevada	Natural	5

### Supplement S1-4: Constructed channels

These sites were used to evaluate biointegrity and eutrophication indicators in constructed channels or channels with ambiguous watersheds.



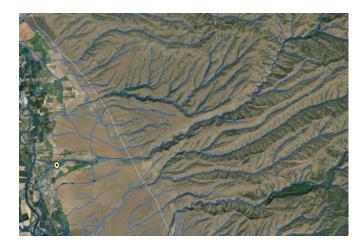
Ecoregion	# sites
Central Valley	65
<b>Deserts Modoc</b>	7

# Supplement S2: Aerial imagery of high-scoring sites in the Central Valley ecoregion

Mill Creek ~0.6mi above Shasta Blvd (Tehama County)

Site code: 504PS0739. 40.0493117 -122.0868802

Sample date: 2015-05-26. CSCI: 0.83





Watershed-scale (left) and reach-scale (right) aerial imagery

#### Deer Creek above Leininger Rd. (Tehama County)

504PS0227 (~3mi above Leininger Rd.) 39.98518 -121.96753

Sample date: 2009-08-12. CSCI: 0.97.

504FC1115 (~2.8mi above Leininger Rd) 39.98053 -121.97234

Sample date: 2014-06-19. CSCI: 0.91





Watershed-scale (left) and reach-scale (right) aerial imagery





Field photos of site 504PS0227, taken on 2009-08-12.

### Pine Creek ~0.2mi below Meridian Rd. (Tehama County)

504PS0574 39.8958 -121.9236

Sample date: 2013-06-06. CSCI: 0.83





Watershed-scale (left) and reach-scale (right) aerial imagery.

#### Butte Creek ~0.7 mi above Midway Rd. (Butte County)

520FCA019 39.61321 -121.7794

Sample date: 2019-06-19. CSCI: 0.88

Sample date: 2019-07-08. CSCI: 0.59





Watershed-scale (left) and reach-scale (right) aerial imagery.

### Dry Creek at Hwy 149. (Butte County)

520DRC149 39.6080556 -121.6711111

Sample date: 2004-04-07. CSCI: 0.94





Watershed-scale (left) and reach-scale (right) aerial imagery.

#### Yuba River at Marysville (Yuba County)

515YRMxxx 39.17583 -121.52389

Sample date: 1997-10-03. CSCI: 0.68

Sample date: 1998-10-08. CSCI: 0.96

Sample date: 1999-10-13. CSCI: 0.86

Sample date: 2000-10-27. CSCI: 0.75

Note: This site is likely boatable (i.e., non-wadeable). The CSCI is not intended for use in

boatable streams.





Watershed-scale (left) and reach-scale (right) aerial imagery.

# American River ~1.4mi above Watt Ave (Sacramento County)

519FCA067 38.57225 -121.35765

Sample date: 2008-09-09. CSCI: 0.86

Note: This site is considered boatable (i.e., non-wadeable). The CSCI is not intended for use in boatable streams.





Watershed-scale (left) and reach-scale (right) aerial imagery.

### Cosumnes River 0.25 mi. below Hwy 16 crossing (Sacramento County)

531Site14 38.48611 -121.09694

Sample date: 1995-10-12. CSCI: 0.85





Watershed-scale (left) and reach-scale (right) aerial imagery.

This site is within the 1987 boundary of the Central Valley ecoregion, but outside the 2016 boundary. It is within the Central California Foothills and Coastal Mountains ecoregion (also known as the Chaparral ecoregion) for the 2016 boundary set.

# Tuolumne River ~4mi above Hickman Rd. (Stanislaus County)

535PS0265 37.64451 -120.6902

Sample date: 2009-08-20. CSCI: 0.80





Watershed-scale (left) and reach-scale (right) aerial imagery





Field photos of site 535PS0265, taken on 2009-08-20.

### Byrd Slough above Hwy 180. (Fresno County)

551PS0308 36.72368 -119.45941

Sample date: 2010-07-27. CSCI: 0.81





Watershed-scale (left) and reach-scale (right) aerial imagery





Field photos of site 551PS0308, taken on 2010-07-27.

### Supplement S3: Additional investigations into response models

Shape-constrained additive models were used to evaluate the responses of bioassessment indices to eutrophication stress at the statewide level, with results presented in Figure 46. In part 1 of this supplement, we validated the assumption of a negative relationship by creating unconstrained models. In part 2 of this supplement, we compare statewide response models to models based on data from regions within California.

#### Supplement 3 Part 1: Cubic spline models

The models presented in this study were constrained to be monotonic and negative, which reflect the conceptual model of a negative relationship between increasing eutrophication stress and decreasing biointegrity. To validate this assumption, we created models that lack these assumptions, and compare the results of those unconstrained models to the shape-constrained models that were subsequently used to identify eutrophication thresholds.

Cubic regression spline models were generated with the gam() function from the mgcv R package with the default cubic spline smoothing penalty (Wood 2017). Knots of the spline were placed evenly throughout the range of each eutrophication stress variable, and cubic polynomials are fit to neighboring knots along this range such that the resulting spline is continuous through the values at each of the knots.

The cubic splines were very similar to the shape-constrained models, validating the assumption of a negative relationship between biointegrity index scores and increasing eutrophication values (Figure 56). Cubic splines show a sharper decrease with TN and TP compared to the constrained fits, and they leveled off at slightly higher values. The cubic splines were also monotonic or nearly monotonic.

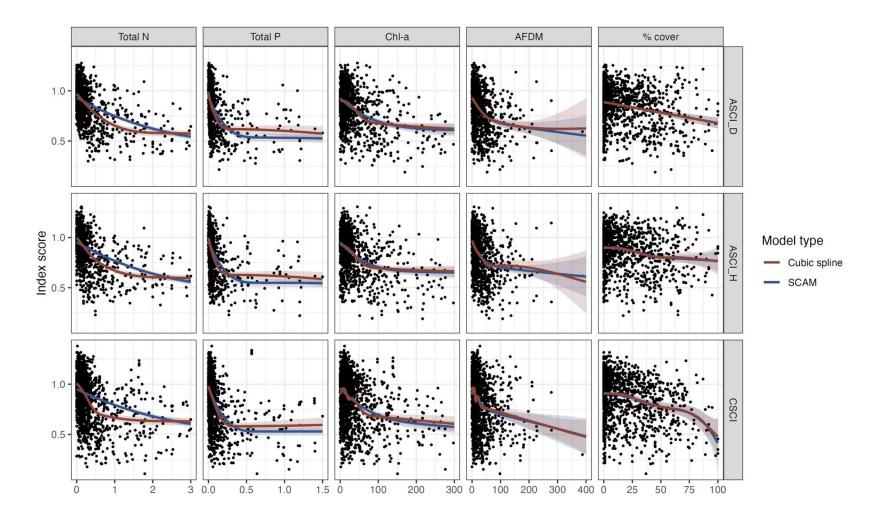
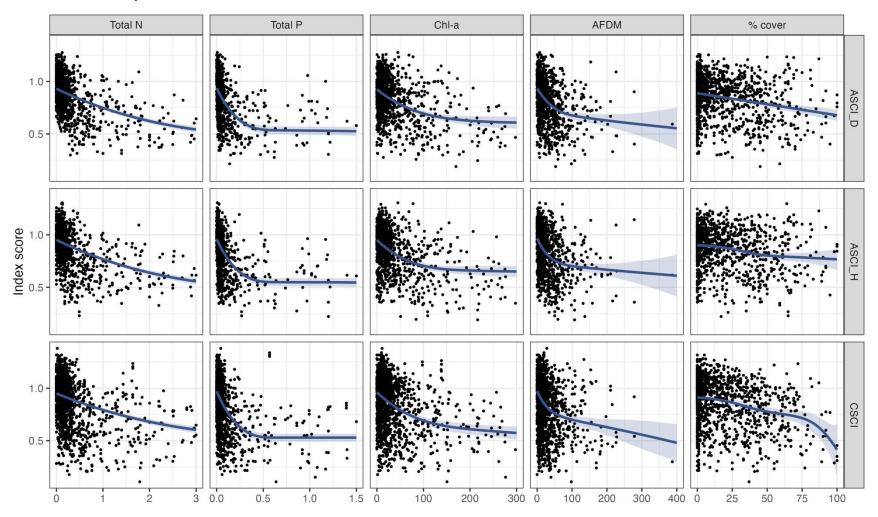


Figure 56. Comparison of shape-constrained and unconstrained (i.e., cubic spline) models that show the response of biointegrity index scores to increasing eutrophication indicator values.

#### Supplement 3 Part 2: Regional response models

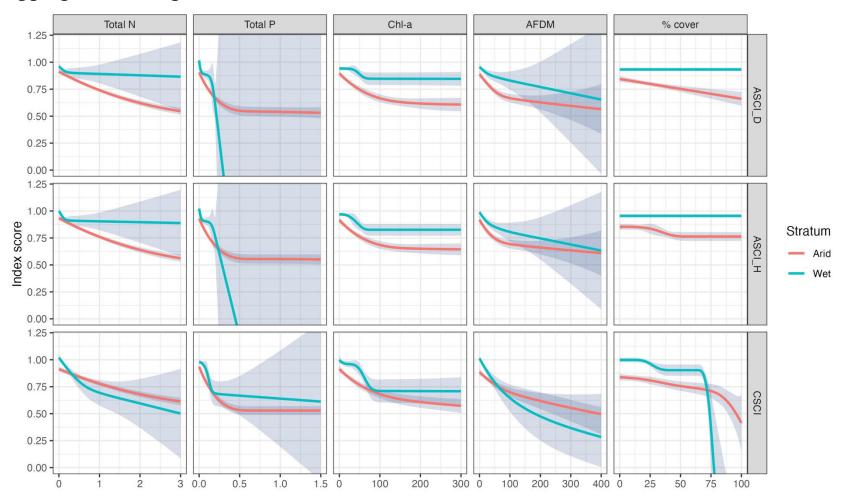
Below we present models calculated for aggregated ecoregions (i.e., xeric vs. wet) as well as individual ecoregions (i.e., North Coast, Chaparral, South Coast, Central Valley, Sierra Nevada, and Desert/Modoc).

#### Statewide response models



Supplement S3: Regional response models

#### Aggregated ecoregional models

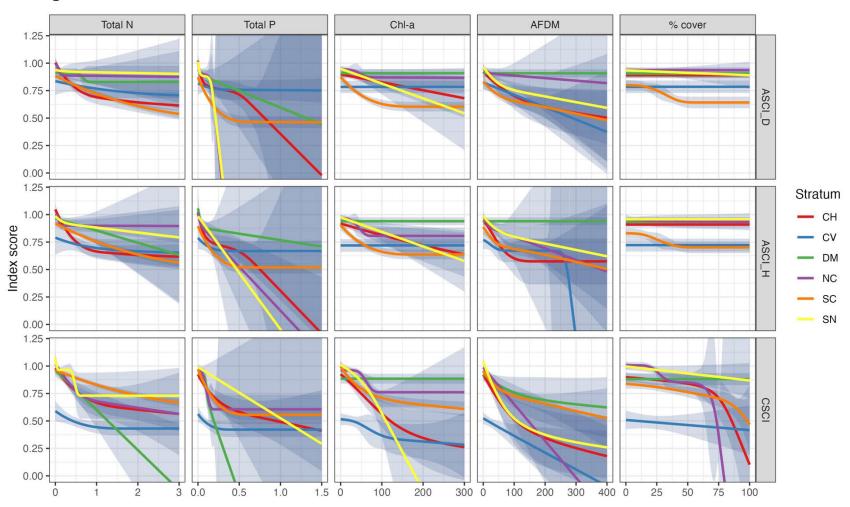


Arid aggregated ecoregion: Chaparral, South Coast, Central Valley, and Desert/Modoc

Wet aggregated ecoregion: North Coast, Sierra Nevada

#### Supplement S3: Regional response models

#### Ecoregional models



#### Supplement S3: Regional response models

Summaries of shape-constrained additive models developed at the statewide (Stratum = California), aggregated ecoregion (Stratum = Arid, Wet), or ecoregion (Stratum = Chaparral [CH], Central Valley [CV], Desert/Modoc [DM], North Coast [NC], South Coast [SC]) levels. AIC: Akaike information criterion. AIC\_null: AIC for null model (i.e., a model based on mean index scores and not related to eutrophication levels). Lower AIC values indicate that the model extracts more information from the data. AIC values that are substantially lower than AIC\_null values indicate eutrophication indicator values are useful for predicting index scores. Deviance explained: The percent of total deviance explained by the model. Higher values indicate that the model explains a higher percent of the variability in index scores. R<sup>2</sup>: Adjusted r-squared of the model. Higher values indicate a stronger relationship between index scores and eutrophication indicator levels. F: F-statistic associated with the eutrophication indicator coefficient in the model. Higher values indicate a stronger relationship between index scores and eutrophication indicator levels. Blank cells indicate that a model could not be calibrated. p: p-value associated with the eutrophication indicator coefficient in the model. p-values are the probability of observing the data assuming that there is no relationship between index scores and eutrophication indicator levels. Lower values indicate a low probability that the observed relationship is due to random chance. Blank cells indicate that a model could not be calibrated. n: Number of samples used to calibrate the model.

Eutrophication			_		Deviance	_			
indicator	Index	Stratum	AIC	AIC_null	explained	R <sup>2</sup>	F	p	<u>n</u>
Total N	ASCI_D	California	-648	-271.5	37.9	0.378	483.4	5.66E-97	794
Total N	ASCI_H	California	-661.1	-263.1	39.7	0.396	244.1	1.34E-99	794
Total N	CSCI	California	-318.6	-16.4	22.8	0.227	119	7.54E-67	1186
Total P	ASCI_D	California	-546.6	-271.5	29.8	0.296	99.4	8.48E-63	794
Total P	ASCI_H	California	-557.5	-263.1	31.5	0.312	115.5	1.38E-69	794
Total P	CSCI	California	-338.3	-16.4	23.9	0.238	371.7	5.65E-77	1186
Chl-a	ASCI_D	California	-424.9	-271.5	18	0.178	86.7	1.08E-35	794
Chl-a	ASCI_H	California	-419.1	-263.1	18.2	0.18	86.1	7.41E-36	794
Chl-a	CSCI	California	-201.3	-16.4	14.8	0.146	63.6	2.47E-40	1186
AFDM	ASCI_D	California	-409.4	-271.5	16.5	0.162	51.3	4.35E-31	794
AFDM	ASCI_H	California	-410.4	-263.1	17.5	0.172	54.6	3.76E-33	794
AFDM	CSCI	California	-162	-16.4	12	0.118	45.1	5.08E-31	1186
% cover	ASCI_D	California	-308.9	-271.4	5.4	0.053	40.4	3.49E-10	708
% cover	ASCI_H	California	-307.5	-283.8	4	0.036	9.5	3.45E-06	708

Supplement S3: Regional response models

Eutrophication					Deviance	_			
indicator	Index	Stratum	AIC	AIC_null	explained	R <sup>2</sup>	F	р	n
% cover	CSCI	California	-108.4	-51.6	8.1	0.077	15.5	1.13E-12	756
Total N	ASCI_D	Arid	-343.4	-100.6	37.6	0.375	311.4	5.69E-62	519
Total N	ASCI_D	Wet	-336.3	-335	1.6	0.01	2.2	0.11732588	275
Total N	ASCI_H	Arid	-340.1	-87.4	39	0.388	150.1	1.92E-61	519
Total N	ASCI_H	Wet	-362.4	-353.7	4.3	0.037	6.1	0.00269288	275
Total N	CSCI	Arid	-104.4	69	21.8	0.215	68.2	3.21E-39	728
Total N	CSCI	Wet	-269	-235.8	7.8	0.074	15.7	4.44E-08	458
Total P	ASCI_D	Arid	-260.3	-100.6	27.3	0.269	56.6	1.17E-35	519
Total P	ASCI_D	Wet	-359.9	-335	10.9	0.098	8.5	3.24E-06	275
Total P	ASCI_H	Arid	-262.8	-87.4	29.4	0.291	68.7	1.08E-40	519
Total P	ASCI_H	Wet	-370.4	-353.7	8.1	0.07	6.5	0.0001065	275
Total P	CSCI	Arid	-130.4	69	24.2	0.241	231.3	6.22E-48	728
Total P	CSCI	Wet	-258.1	-235.8	5.7	0.052	9.9	6.39E-06	458
Chl-a	ASCI_D	Arid	-191.2	-100.6	16.7	0.164	51.6	1.76E-21	519
Chl-a	ASCI_D	Wet	-341.6	-335	3.4	0.029	5.6	0.00667092	275
Chl-a	ASCI_H	Arid	-169.3	-87.4	15.1	0.148	47.9	2.78E-18	519
Chl-a	ASCI_H	Wet	-375.9	-353.7	9.1	0.084	13.6	2.30E-06	275
Chl-a	CSCI	Arid	-27.2	69	13	0.127	34.9	1.37E-21	728
Chl-a	CSCI	Wet	-258.5	-235.8	5.9	0.054	9.8	5.09E-06	458
AFDM	ASCI_D	Arid	-163.1	-100.6	12.1	0.117	25	4.21E-14	519
AFDM	ASCI_D	Wet	-341.8	-335	3.7	0.031	4.7	0.00841059	275
AFDM	ASCI_H	Arid	-147	-87.4	11.7	0.112	23.6	1.13E-13	519
AFDM	ASCI_H	Wet	-374.7	-353.7	8.6	0.08	11.1	1.06E-05	275
AFDM	CSCI	Arid	24.7	69	6.5	0.062	18.4	4.06E-10	728
AFDM	CSCI	Wet	-279.8	-235.8	9.9	0.096	22.5	1.27E-10	458
% cover	ASCI_D	Arid	-128	-109.8	4.3	0.041	20.4	7.77E-06	461
% cover	ASCI_D	Wet	-298.4	-298.4	0	0			247
% cover	ASCI_H	Arid	-119.1	-109.5	2.9	0.025	6.9	0.0011374	461
% cover	ASCI_H	Wet	-325.2	-325.2	0	0			247

Supplement S3: Regional response models

Eutrophication					Deviance				
indicator	Index	Stratum	AIC	AIC_null	explained	R <sup>2</sup>	F	р	n
% cover	CSCI	Arid	-23.4	-4.3	5.1	0.045	7	4.28E-05	481
% cover	CSCI	Wet	-212.6	-193	8.2	0.076	12.2	8.35E-06	275
Total N	ASCI_D	СН	-152.3	-109.7	28.1	0.27	21.5	4.92E-10	142
Total N	ASCI_D	CV	-20	-21.3	4.4	0.004	0.7	0.4623678	39
Total N	ASCI_D	DM	-57.9	-58.7	2.1	0.004	1.2	0.27896479	57
Total N	ASCI_D	NC	-146	-141.3	7.1	0.055	3.6	0.0226716	120
Total N	ASCI_D	SC	-140	-6.2	38.3	0.381	173.3	3.38E-34	281
Total N	ASCI_D	SN	-187.1	-189.8	0.1	-0.008	0.1	0.8514072	155
Total N	ASCI_H	CH	-136.3	-84.1	32.6	0.317	29.1	2.37E-12	142
Total N	ASCI_H	CV	-16.1	-18.3	4.9	-0.006	0.7	0.51114213	39
Total N	ASCI_H	DM	-60.4	-60.5	3.4	0.016	1.9	0.17291549	57
Total N	ASCI_H	NC	-127.6	-122.2	7	0.057	4.4	0.01451495	120
Total N	ASCI_H	SC	-158	-8.9	42.2	0.417	79.5	1.54E-35	281
Total N	ASCI_H	SN	-237.9	-237.2	2.4	0.014	2.2	0.11326062	155
Total N	CSCI	CH	-60.2	-6.4	23.5	0.226	21.2	1.59E-12	220
Total N	CSCI	CV	-42.7	-38.9	12.7	0.095	3.9	0.02535436	57
Total N	CSCI	DM	-38.5	-28.5	16.3	0.15	12.5	0.00067214	68
Total N	CSCI	NC	-134	-109.8	11.1	0.103	11.6	3.39E-06	241
Total N	CSCI	SC	-126.1	15.6	31.9	0.314	55.1	1.14E-32	383
Total N	CSCI	SN	-133.8	-125.1	5.9	0.049	5.4	0.00297314	217
Total P	ASCI_D	CH	-137.5	-109.7	21.4	0.196	10	9.48E-07	142
Total P	ASCI_D	CV	-18.8	-21.3	1.7	-0.026	0.4	0.68205312	39
Total P	ASCI_D	DM	<b>-</b> 57.9	-58.7	3	0.008	1	0.32183977	57
Total P	ASCI_D	NC	-149.1	-141.3	10.7	0.085	4.4	0.00493397	120
Total P	ASCI_D	SC	-117.5	-6.2	34	0.333	47.8	1.33E-26	281
Total P	ASCI_D	SN	-201.9	-189.8	11.5	0.095	5.3	0.00083004	155
Total P	ASCI_H	CH	-121.9	-84.1	26.8	0.251	13.8	6.08E-09	142
Total P	ASCI_H	CV	-17	-18.3	6.8	0.016	1.3	0.28327397	39
Total P	ASCI_H	DM	-64.8	-60.5	12.8	0.1	3.8	0.02693483	57

Supplement S3: Regional response models

Eutrophication					Deviance	_			
indicator	Index	Stratum	AIC	AIC_null	explained	R <sup>2</sup>	F	р	n
Total P	ASCI_H	NC	-127.8	-122.2	7.6	0.061	4.3	0.013168	120
Total P	ASCI_H	SC	-111.8	-8.9	31.6	0.311	64.3	4.63E-25	281
Total P	ASCI_H	SN	-244.7	-237.2	5.9	0.053	9.4	0.00242295	155
Total P	CSCI	CH	-31.9	-6.4	12.5	0.117	13.3	1.39E-06	220
Total P	CSCI	CV	-43.1	-38.9	13.4	0.102	4.2	0.02037424	57
Total P	CSCI	DM	-44.1	-28.5	26.4	0.234	7.8	0.00016591	68
Total P	CSCI	NC	-137	-109.8	12.1	0.114	16.4	1.95E-07	241
Total P	CSCI	SC	-122.4	15.6	30.6	0.304	168.1	3.44E-34	383
Total P	CSCI	SN	-124.2	-125.1	0.5	0	1.1	0.30158492	217
Chl-a	ASCI_D	CH	-112.8	-109.7	3.6	0.029	5.2	0.02455087	142
Chl-a	ASCI_D	CV	-21.3	-21.3	0	0			39
Chl-a	ASCI_D	DM	-58.7	-58.7	0	0			57
Chl-a	ASCI_D	NC	-141.5	-141.3	2.9	0.015	1.9	0.15475995	120
Chl-a	ASCI_D	SC	-51.2	-6.2	15.4	0.151	50.6	7.59E-12	281
Chl-a	ASCI_D	SN	-193.5	-189.8	3.7	0.03	5.6	0.01858452	155
Chl-a	ASCI_H	CH	-88.7	-84.1	4.8	0.04	5.8	0.01116813	142
Chl-a	ASCI_H	CV	-18.3	-18.3	0	0			39
Chl-a	ASCI_H	DM	-60.5	-60.5	0	0			57
Chl-a	ASCI_H	NC	-134.6	-122.2	12.8	0.113	8.6	0.00032717	120
Chl-a	ASCI_H	SC	-52.7	-8.9	15	0.147	49.3	1.41E-11	281
Chl-a	ASCI_H	SN	-243	-237.2	4.9	0.042	7.6	0.00622824	155
Chl-a	CSCI	CH	-35.4	-6.4	14.1	0.132	13.4	3.49E-07	220
Chl-a	CSCI	CV	-41.4	-38.9	10.6	0.074	2.6	0.07164369	57
Chl-a	CSCI	DM	-28.5	-28.5	0	0			68
Chl-a	CSCI	NC	-115.8	-109.8	3.3	0.029	8.1	0.0047279	241
Chl-a	CSCI	SC	-79.4	15.6	23.1	0.225	34.4	3.23E-21	383
Chl-a	CSCI	SN	-146.6	-125.1	10.9	0.102	12.1	6.89E-06	217
AFDM	ASCI_D	CH	-119.9	-109.7	9.2	0.081	6.8	0.00136115	142
AFDM	ASCI_D	CV	-23.8	-21.3	10.9	0.085	4.5	0.0399126	39

Supplement S3: Regional response models

Eutrophication					Deviance	- 0	_		
indicator	Index	Stratum	AIC	AIC_null	explained	R <sup>2</sup>	F	р	<u>n</u>
AFDM	ASCI_D	DM	-58.7	-58.7	0	0			57
AFDM	ASCI_D	NC	-140.4	-141.3	1.7	0.005	1.3	0.26617225	120
AFDM	ASCI_D	SC	-21.4	-6.2	6.5	0.059	8.2	0.00018516	281
AFDM	ASCI_D	SN	-198.5	-189.8	7.5	0.065	6.1	0.0029287	155
AFDM	ASCI_H	CH	<b>-</b> 99.8	-84.1	13	0.117	10.4	6.17E-05	142
AFDM	ASCI_H	CV	-20.2	-18.3	14.1	0.093	2.9	0.0648751	39
AFDM	ASCI_H	DM	-60.5	-60.5	0	0			57
AFDM	ASCI_H	NC	-129.5	-122.2	8.7	0.073	4.9	0.00753803	120
AFDM	ASCI_H	SC	-28	-8.9	7.9	0.072	9.6	2.76E-05	281
AFDM	ASCI_H	SN	-248.1	-237.2	8.8	0.078	7.2	0.00097871	155
AFDM	CSCI	CH	-26.4	-6.4	10.2	0.094	10.7	1.89E-05	220
AFDM	CSCI	CV	-42.7	-38.9	9.8	0.081	5.7	0.0186826	57
AFDM	CSCI	DM	-30.1	-28.5	6.8	0.046	2.2	0.12309623	68
AFDM	CSCI	NC	-119.3	-109.8	4.7	0.043	11.3	0.00081376	241
AFDM	CSCI	SC	-21	15.6	10.4	0.098	13.5	9.67E-09	383
AFDM	CSCI	SN	-164.9	-125.1	18.2	0.175	22.1	7.62E-10	217
% cover	ASCI_D	CH	-106.1	-106.1	0	0			132
% cover	ASCI_D	CV	-19.7	-19.7	0	0			38
% cover	ASCI_D	DM	-55.4	-55.4	0	0			54
% cover	ASCI_D	NC	-126.1	-126.1	0	0			106
% cover	ASCI_D	SC	-32.9	-16.9	8.1	0.073	10.3	4.91E-05	237
% cover	ASCI_D	SN	-166.9	-168.5	0.3	-0.004	0.4	0.52327819	141
% cover	ASCI_H	CH	-93.5	-93.5	0	0			132
% cover	ASCI_H	CV	-17.2	-17.2	0	0			38
% cover	ASCI_H	DM	-56.3	-56.3	0	0			54
% cover	ASCI_H	NC	-108.8	-111.3	0.2	-0.011	0.2	0.75388785	106
% cover	ASCI_H	SC	-33	-23.3	5.6	0.048	7	0.00115246	237
% cover	ASCI_H	SN	-217.1	-217.1	0	0			141
% cover	CSCI	CH	-26	-18.5	8.3	0.068	4.9	0.00541892	135

Supplement S3: Regional response models

<b>Eutrophication</b>					Deviance				
indicator	Index	Stratum	AIC	AIC_null	explained	$\mathbb{R}^2$	F	р	n
% cover	CSCI	CV	-15.2	-16.7	1.2	-0.016	0.4	0.51473041	38
% cover	CSCI	DM	-13.5	-13.5	0	0			55
% cover	CSCI	NC	-111.3	-87.9	24.1	0.22	10.4	3.78E-06	105
% cover	CSCI	SC	-50.7	-38.5	6.9	0.058	5.7	0.00072257	253
% cover	CSCI	SN	-103	-103	1.2	0.006	2	0.15361957	170

## Supplement S4: Biointegrity and eutrophication thresholds for intermittent streams, modified channels, and streams on the Central Valley floor derived at multiple levels of stringency

Potential biointegrity and eutrophication thresholds for different classes of streams. RFI-N: Regularly flowing intermittent streams in northern xeric California. RFI-S: Regularly flowing intermittent streams in southern California. CVF: Central Valley Floor. HB: Hard-bottom streams. SB2: Soft-bottom streams with two hardened banks. SB1: Soft-bottom streams with one hardened bank. SB0: Soft-bottom streams with no hardened banks. CC: Constructed channels. NA: Threshold could not be identified.

Note: This table may also be accessed from the threshold query dashboard (https://sccwrp.shinyapps.io/ModifiedChannelThresholds/)

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Reference	30th percentile of reference values	High	Biointegrity	CSCI	0.92	
Wadeable streams	Reference	10th percentile of reference values	Intermediate	Biointegrity	CSCI	0.79	
Wadeable streams	Reference	1st percentile of reference values	Low	Biointegrity	CSCI	0.63	
RFI-N	Reference	30th percentile of reference values	High	Biointegrity	CSCI	0.73	
RFI-N	Reference	10th percentile of reference values	Intermediate	Biointegrity	CSCI	0.61	
RFI-N	Reference	1st percentile of reference values	Low	Biointegrity	CSCI	0.44	
RFI-S	Reference	30th percentile of reference values	High	Biointegrity	CSCI	0.89	
RFI-S	Reference	10th percentile of reference values	Intermediate	Biointegrity	CSCI	0.82	Biointegrity goal is higher than reference.
RFI-S	Reference	1st percentile of reference values	Low	Biointegrity	CSCI	0.71	Biointegrity goal is higher than reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CVF	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.85	
CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.67	
CVF	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.52	
НВ	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.74	
НВ	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.67	
НВ	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.55	
SB2	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.96	Biointegrity goal is higher than reference.
SB2	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.75	
SB2	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.64	Biointegrity goal is higher than reference.
SB1	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	1.1	Biointegrity goal is higher than reference.
SB1	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	1	Biointegrity goal is higher than reference.
SB1	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.81	Biointegrity goal is higher than reference.
SB0	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.99	Biointegrity goal is higher than reference.
SB0	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.78	
SB0	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.66	Biointegrity goal is higher than reference.
CC	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.53	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CC	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.45	
CC	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.37	
SB0_CVF	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.51	
SB0_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.47	
SB0_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.38	
HB_CVF	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.57	
HB_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.49	
HB_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.39	
CC_CVF	Best observed	99th percentile of observed values	High	Biointegrity	CSCI	0.53	
CC_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	CSCI	0.45	
CC_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	CSCI	0.37	
Wadeable streams	Reference	30th percentile of reference values	High	Biointegrity	ASCI_D	0.94	
Wadeable streams	Reference	10th percentile of reference values	Intermediate	Biointegrity	ASCI_D	0.86	
Wadeable streams	Reference	1st percentile of reference values	Low	Biointegrity	ASCI_D	0.75	
RFI-N	Reference	30th percentile of reference values	High	Biointegrity	ASCI_D	0.98	Biointegrity goal is higher than reference.
RFI-N	Reference	10th percentile of reference values	Intermediate	Biointegrity	ASCI_D	0.9	Biointegrity goal is higher than reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-N	Reference	1st percentile of reference values	Low	Biointegrity	ASCI_D	0.78	Biointegrity goal is higher than reference.
RFI-S	Reference	30th percentile of reference values	High	Biointegrity	ASCI_D	0.91	
RFI-S	Reference	10th percentile of reference values	Intermediate	Biointegrity	ASCI_D	0.8	
RFI-S	Reference	1st percentile of reference values	Low	Biointegrity	ASCI_D	0.66	
CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	1.13	Biointegrity goal is higher than reference.
CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	1.02	Biointegrity goal is higher than reference.
CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	0.92	Biointegrity goal is higher than reference.
НВ	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	1.05	Biointegrity goal is higher than reference.
НВ	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	0.88	Biointegrity goal is higher than reference.
НВ	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	0.74	
SB2	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	0.93	
SB2	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	0.77	
SB2	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	0.64	
SB1	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	1.01	Biointegrity goal is higher than reference.
SB1	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	0.85	
SB1	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	0.68	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB0	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	1.01	Biointegrity goal is higher than reference.
SB0	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	0.77	
SB0	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	0.68	
CC	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	NA	Insufficient data
CC	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	NA	Insufficient data
CC	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	NA	Insufficient data
SB0_CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	0.84	
SB0_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	0.83	
SB0_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	8.0	Biointegrity goal is higher than reference.
HB_CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	1.05	Biointegrity goal is higher than reference.
HB_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	1.03	Biointegrity goal is higher than reference.
HB_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	0.96	Biointegrity goal is higher than reference.
CC_CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_D	NA	Insufficient data
CC_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_D	NA	Insufficient data
CC_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_D	NA	Insufficient data
Wadeable streams	Reference	30th percentile of reference values	High	Biointegrity	ASCI_H	0.94	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Reference	10th percentile of reference values	Intermediate	Biointegrity	ASCI_H	0.86	
Wadeable streams	Reference	1st percentile of reference values	Low	Biointegrity	ASCI_H	0.75	
RFI-N	Reference	30th percentile of reference values	High	Biointegrity	ASCI_H	0.96	
RFI-N	Reference	10th percentile of reference values	Intermediate	Biointegrity	ASCI_H	0.90	Biointegrity goal is higher than reference.
RFI-N	Reference	1st percentile of reference values	Low	Biointegrity	ASCI_H	0.81	Biointegrity goal is higher than reference.
RFI-S	Reference	30th percentile of reference values	High	Biointegrity	ASCI_H	0.91	
RFI-S	Reference	10th percentile of reference values	Intermediate	Biointegrity	ASCI_H	0.8	
RFI-S	Reference	1st percentile of reference values	Low	Biointegrity	ASCI_H	0.65	
CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	1.05	Biointegrity goal is higher than reference.
CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	0.94	Biointegrity goal is higher than reference.
CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	0.8	Biointegrity goal is higher than reference.
НВ	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	1.02	Biointegrity goal is higher than reference.
НВ	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	0.87	Biointegrity goal is higher than reference.
НВ	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	0.74	
SB2	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	0.88	
SB2	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	0.76	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB2	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	0.6	
SB1	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	0.97	Biointegrity goal is higher than reference.
SB1	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	0.86	
SB1	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	0.67	
SB0	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	0.94	
SB0	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	0.79	
SB0	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	0.64	
CC	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	NA	Insufficient data
CC	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	NA	Insufficient data
CC	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	NA	Insufficient data
SB0_CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	0.74	
SB0_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	0.73	
SB0_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	0.72	
HB_CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	0.95	Biointegrity goal is higher than reference.
HB_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	0.93	Biointegrity goal is higher than reference.
HB_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	0.8	Biointegrity goal is higher than reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CC_CVF	Best observed	99th percentile of observed values	High	Biointegrity	ASCI_H	NA	Insufficient data
CC_CVF	Best observed	90th percentile of observed values	Intermediate	Biointegrity	ASCI_H	NA	Insufficient data
CC_CVF	Best observed	70th percentile of observed values	Low	Biointegrity	ASCI_H	NA	Insufficient data
Wadeable streams	Reference	70th percentile of reference values	High	Eutrophication	TN	0.131	
Wadeable streams	Reference	90th percentile of reference values	Intermediate	Eutrophication	TN	0.233	
Wadeable streams	Reference	99th percentile of reference values	Low	Eutrophication	TN	1.639	
RFI-N	Reference	70th percentile of reference values	High	Eutrophication	TN	0.33	
RFI-N	Reference	90th percentile of reference values	Intermediate	Eutrophication	TN	0.579	
RFI-N	Reference	99th percentile of reference values	Low	Eutrophication	TN	2.229	
RFI-S	Reference	70th percentile of reference values	High	Eutrophication	TN	0.254	
RFI-S	Reference	90th percentile of reference values	Intermediate	Eutrophication	TN	0.351	
RFI-S	Reference	99th percentile of reference values	Low	Eutrophication	TN	0.558	
CVF	Best observed	1st percentile of observed values	High	Eutrophication	TN	0.037	
CVF	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TN	0.143	
CVF	Best observed	30th percentile of observed values	Low	Eutrophication	TN	0.32	
НВ	Best observed	1st percentile of observed values	High	Eutrophication	TN	0.029	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
НВ	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TN	0.43	
НВ	Best observed	30th percentile of observed values	Low	Eutrophication	TN	1.109	
SB2	Best observed	1st percentile of observed values	High	Eutrophication	TN	0.163	
SB2	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TN	0.436	
SB2	Best observed	30th percentile of observed values	Low	Eutrophication	TN	0.924	
SB1	Best observed	1st percentile of observed values	High	Eutrophication	TN	0	
SB1	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TN	0.261	
SB1	Best observed	30th percentile of observed values	Low	Eutrophication	TN	0.379	
SB0	Best observed	1st percentile of observed values	High	Eutrophication	TN	0.341	
SB0	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TN	0.497	
SB0	Best observed	30th percentile of observed values	Low	Eutrophication	TN	0.781	
CC	Best observed	1st percentile of observed values	High	Eutrophication	TN	NA	Insufficient data
CC	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TN	NA	Insufficient data
CC	Best observed	30th percentile of observed values	Low	Eutrophication	TN	NA	Insufficient data
Wadeable streams	Reference	70th percentile of reference values	High	Eutrophication	TP	0.024	
Wadeable streams	Reference	90th percentile of reference values	Intermediate	Eutrophication	TP	0.058	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Reference	99th percentile of reference values	Low	Eutrophication	TP	0.202	
RFI-N	Reference	70th percentile of reference values	High	Eutrophication	TP	0.184	
RFI-N	Reference	90th percentile of reference values	Intermediate	Eutrophication	TP	0.495	
RFI-N	Reference	99th percentile of reference values	Low	Eutrophication	TP	0.547	
RFI-S	Reference	70th percentile of reference values	High	Eutrophication	TP	0.055	
RFI-S	Reference	90th percentile of reference values	Intermediate	Eutrophication	TP	0.175	
RFI-S	Reference	99th percentile of reference values	Low	Eutrophication	TP	0.267	
CVF	Best observed	1st percentile of observed values	High	Eutrophication	TP	0.012	
CVF	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TP	0.028	
CVF	Best observed	30th percentile of observed values	Low	Eutrophication	TP	0.055	
НВ	Best observed	1st percentile of observed values	High	Eutrophication	TP	0	
НВ	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TP	0.028	
НВ	Best observed	30th percentile of observed values	Low	Eutrophication	TP	0.068	
SB2	Best observed	1st percentile of observed values	High	Eutrophication	TP	0	
SB2	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TP	0.009	
SB2	Best observed	30th percentile of observed values	Low	Eutrophication	TP	0.05	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB1	Best observed	1st percentile of observed values	High	Eutrophication	TP	0.007	
SB1	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TP	0.011	
SB1	Best observed	30th percentile of observed values	Low	Eutrophication	TP	0.048	
SB0	Best observed	1st percentile of observed values	High	Eutrophication	TP	0.01	
SB0	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TP	0.039	
SB0	Best observed	30th percentile of observed values	Low	Eutrophication	TP	0.075	
CC	Best observed	1st percentile of observed values	High	Eutrophication	TP	NA	Insufficient data
CC	Best observed	10th percentile of observed values	Intermediate	Eutrophication	TP	NA	Insufficient data
CC	Best observed	30th percentile of observed values	Low	Eutrophication	TP	NA	Insufficient data
Wadeable streams	Reference	70th percentile of reference values	High	Eutrophication	Chl-a	14.3	
Wadeable streams	Reference	90th percentile of reference values	Intermediate	Eutrophication	Chl-a	29.7	
Wadeable streams	Reference	99th percentile of reference values	Low	Eutrophication	Chl-a	59.9	
RFI-N	Reference	70th percentile of reference values	High	Eutrophication	Chl-a	15.4	
RFI-N	Reference	90th percentile of reference values	Intermediate	Eutrophication	Chl-a	38	
RFI-N	Reference	99th percentile of reference values	Low	Eutrophication	Chl-a	85.3	
RFI-S	Reference	70th percentile of reference values	High	Eutrophication	Chl-a	17.9	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-S	Reference	90th percentile of reference values	Intermediate	Eutrophication	Chl-a	33.9	
RFI-S	Reference	99th percentile of reference values	Low	Eutrophication	Chl-a	131.4	
CVF	Best observed	1st percentile of observed values	High	Eutrophication	Chl-a	1.1	
CVF	Best observed	10th percentile of observed values	Intermediate	Eutrophication	Chl-a	4.2	
CVF	Best observed	30th percentile of observed values	Low	Eutrophication	Chl-a	7.6	
НВ	Best observed	1st percentile of observed values	High	Eutrophication	Chl-a	4.8	
НВ	Best observed	10th percentile of observed values	Intermediate	Eutrophication	Chl-a	18.8	
НВ	Best observed	30th percentile of observed values	Low	Eutrophication	Chl-a	34.7	
SB2	Best observed	1st percentile of observed values	High	Eutrophication	Chl-a	7.7	
SB2	Best observed	10th percentile of observed values	Intermediate	Eutrophication	Chl-a	18.4	
SB2	Best observed	30th percentile of observed values	Low	Eutrophication	Chl-a	61.4	
SB1	Best observed	1st percentile of observed values	High	Eutrophication	Chl-a	1	
SB1	Best observed	10th percentile of observed values	Intermediate	Eutrophication	Chl-a	5.5	
SB1	Best observed	30th percentile of observed values	Low	Eutrophication	Chl-a	26.6	
SB0	Best observed	1st percentile of observed values	High	Eutrophication	Chl-a	1.3	
SB0	Best observed	10th percentile of observed values	Intermediate	Eutrophication	Chl-a	9	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB0	Best observed	30th percentile of observed values	Low	Eutrophication	Chl-a	43.7	
CC	Best observed	1st percentile of observed values	High	Eutrophication	Chl-a	NA	Insufficient data
CC	Best observed	10th percentile of observed values	Intermediate	Eutrophication	Chl-a	NA	Insufficient data
CC	Best observed	30th percentile of observed values	Low	Eutrophication	Chl-a	NA	Insufficient data
Wadeable streams	Reference	70th percentile of reference values	High	Eutrophication	AFDM	11.4	
Wadeable streams	Reference	90th percentile of reference values	Intermediate	Eutrophication	AFDM	27.3	
Wadeable streams	Reference	99th percentile of reference values	Low	Eutrophication	AFDM	95.4	
RFI-N	Reference	70th percentile of reference values	High	Eutrophication	AFDM	13.3	
RFI-N	Reference	90th percentile of reference values	Intermediate	Eutrophication	AFDM	26.2	
RFI-N	Reference	99th percentile of reference values	Low	Eutrophication	AFDM	32.3	
RFI-S	Reference	70th percentile of reference values	High	Eutrophication	AFDM	26.1	
RFI-S	Reference	90th percentile of reference values	Intermediate	Eutrophication	AFDM	56.2	
RFI-S	Reference	99th percentile of reference values	Low	Eutrophication	AFDM	95.8	
CVF	Best observed	1st percentile of observed values	High	Eutrophication	AFDM	2.1	
CVF	Best observed	10th percentile of observed values	Intermediate	Eutrophication	AFDM	4.3	
CVF	Best observed	30th percentile of observed values	Low	Eutrophication	AFDM	9.2	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
НВ	Best observed	1st percentile of observed values	High	Eutrophication	AFDM	4.1	
НВ	Best observed	10th percentile of observed values	Intermediate	Eutrophication	AFDM	9.5	
НВ	Best observed	30th percentile of observed values	Low	Eutrophication	AFDM	22	
SB2	Best observed	1st percentile of observed values	High	Eutrophication	AFDM	3.7	
SB2	Best observed	10th percentile of observed values	Intermediate	Eutrophication	AFDM	14.6	
SB2	Best observed	30th percentile of observed values	Low	Eutrophication	AFDM	28.4	
SB1	Best observed	1st percentile of observed values	High	Eutrophication	AFDM	2.2	
SB1	Best observed	10th percentile of observed values	Intermediate	Eutrophication	AFDM	7.2	
SB1	Best observed	30th percentile of observed values	Low	Eutrophication	AFDM	25	
SB0	Best observed	1st percentile of observed values	High	Eutrophication	AFDM	3.2	
SB0	Best observed	10th percentile of observed values	Intermediate	Eutrophication	AFDM	15.6	
SB0	Best observed	30th percentile of observed values	Low	Eutrophication	AFDM	30	
CC	Best observed	1st percentile of observed values	High	Eutrophication	AFDM	NA	Insufficient data
CC	Best observed	10th percentile of observed values	Intermediate	Eutrophication	AFDM	NA	Insufficient data
CC	Best observed	30th percentile of observed values	Low	Eutrophication	AFDM	NA	Insufficient data
Wadeable streams	Reference	70th percentile of reference values	High	Eutrophication	% cover	18	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Reference	90th percentile of reference values	Intermediate	Eutrophication	% cover	39	
Wadeable streams	Reference	99th percentile of reference values	Low	Eutrophication	% cover	68	
RFI-N	Reference	70th percentile of reference values	High	Eutrophication	% cover	24	
RFI-N	Reference	90th percentile of reference values	Intermediate	Eutrophication	% cover	31	
RFI-N	Reference	99th percentile of reference values	Low	Eutrophication	% cover	42	
RFI-S	Reference	70th percentile of reference values	High	Eutrophication	% cover	39	
RFI-S	Reference	90th percentile of reference values	Intermediate	Eutrophication	% cover	55	
RFI-S	Reference	99th percentile of reference values	Low	Eutrophication	% cover	87	
CVF	Best observed	1st percentile of observed values	High	Eutrophication	% cover	0	
CVF	Best observed	10th percentile of observed values	Intermediate	Eutrophication	% cover	0	
CVF	Best observed	30th percentile of observed values	Low	Eutrophication	% cover	2	
НВ	Best observed	1st percentile of observed values	High	Eutrophication	% cover	0	
НВ	Best observed	10th percentile of observed values	Intermediate	Eutrophication	% cover	10	
НВ	Best observed	30th percentile of observed values	Low	Eutrophication	% cover	35	
SB2	Best observed	1st percentile of observed values	High	Eutrophication	% cover	0	
SB2	Best observed	10th percentile of observed values	Intermediate	Eutrophication	% cover	10	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB2	Best observed	30th percentile of observed values	Low	Eutrophication	% cover	26	
SB1	Best observed	1st percentile of observed values	High	Eutrophication	% cover	1	
SB1	Best observed	10th percentile of observed values	Intermediate	Eutrophication	% cover	4	
SB1	Best observed	30th percentile of observed values	Low	Eutrophication	% cover	9	
SB0	Best observed	1st percentile of observed values	High	Eutrophication	% cover	0	
SB0	Best observed	10th percentile of observed values	Intermediate	Eutrophication	% cover	5	
SB0	Best observed	30th percentile of observed values	Low	Eutrophication	% cover	16	
CC	Best observed	1st percentile of observed values	High	Eutrophication	% cover	NA	Insufficient data
CC	Best observed	10th percentile of observed values	Intermediate	Eutrophication	% cover	NA	Insufficient data
CC	Best observed	30th percentile of observed values	Low	Eutrophication	% cover	NA	Insufficient data
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	TN	0.141	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	TN	0.33	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	TN	1.081	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	TN	0.132	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	TN	0.24	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	TN	1.258	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.92.	High	Eutrophication	TN	0.682	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	TN	1.189	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.63.	Low	Eutrophication	TN	NA	No threshold identified
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	TP	0.021	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	TP	0.054	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	TP	0.141	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	TP	0.03	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	TP	0.054	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	TP	0.156	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.92.	High	Eutrophication	TP	0.051	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	TP	0.194	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.63.	Low	Eutrophication	TP	0.77	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	Chl-a	46.8	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	Chl-a	46.8	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	Chl-a	83.2	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	Chl-a	46.5	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	Chl-a	44.1	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	Chl-a	77.8	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.92.	High	Eutrophication	Chl-a	26.4	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	Chl-a	48.9	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.63.	Low	Eutrophication	Chl-a	98.5	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	AFDM	17.3	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	AFDM	24.8	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	AFDM	42	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	AFDM	19.5	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	AFDM	24.8	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	AFDM	45.8	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.92.	High	Eutrophication	AFDM	25.5	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	AFDM	41.3	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.63.	Low	Eutrophication	AFDM	68.3	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	% cover	23	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	% cover	27	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	% cover	37	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	% cover	29	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	% cover	31	
Wadeable streams	Response	Logistic model, 80% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	% cover	41	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.92.	High	Eutrophication	% cover	21	
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	% cover	31	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Logistic model, 80% probability of attaining CSCI score above 0.63.	Low	Eutrophication	% cover	53	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	TN	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	TN	0.348	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	TN	0.991	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	TN	0.054	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	TN	0.453	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	TN	1.114	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.92.	High	Eutrophication	TN	0.165	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	TN	1.021	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.63.	Low	Eutrophication	TN	2.601	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.98.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.9.	Intermediate	Eutrophication	TN	0.147	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.78.	Low	Eutrophication	TN	0.802	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.96.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.9.	Intermediate	Eutrophication	TN	0.246	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.81.	Low	Eutrophication	TN	0.736	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.73.	High	Eutrophication	TN	1.514	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.61.	Intermediate	Eutrophication	TN	2.895	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.44.	Low	Eutrophication	TN	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.91.	High	Eutrophication	TN	0.099	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.8.	Intermediate	Eutrophication	TN	0.682	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.66.	Low	Eutrophication	TN	1.658	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.91.	High	Eutrophication	TN	0.198	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Intermediate	Eutrophication	TN	0.796	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.65.	Low	Eutrophication	TN	1.895	
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.89.	High	Eutrophication	TN	0.345	
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.82.	Intermediate	Eutrophication	TN	0.802	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.71.	Low	Eutrophication	TN	1.697	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.13.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.02.	Intermediate	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 0.92.	Low	Eutrophication	TN	0.051	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 1.05.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	Intermediate	Eutrophication	TN	0.054	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Low	Eutrophication	TN	0.796	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.85.	High	Eutrophication	TN	0.598	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	TN	2.108	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.52.	Low	Eutrophication	TN	NA	No threshold identified
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 1.05.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.88.	Intermediate	Eutrophication	TN	0.246	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.74.	Low	Eutrophication	TN	1.057	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 1.02.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.87.	Intermediate	Eutrophication	TN	0.402	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.74.	Low	Eutrophication	TN	1.18	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.74.	High	Eutrophication	TN	1.423	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	TN	2.108	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.55.	Low	Eutrophication	TN	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.93.	High	Eutrophication	TN	0.003	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	TN	0.862	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.64.	Low	Eutrophication	TN	1.835	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.88.	High	Eutrophication	TN	0.348	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.76.	Intermediate	Eutrophication	TN	1.045	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.6.	Low	Eutrophication	TN	2.411	
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.96.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.75.	Intermediate	Eutrophication	TN	1.339	
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.64.	Low	Eutrophication	TN	2.468	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.85.	Intermediate	Eutrophication	TN	0.402	
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	TN	1.492	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.97.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	TN	0.453	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.67.	Low	Eutrophication	TN	1.718	
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.1.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.	Intermediate	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 0.81.	Low	Eutrophication	TN	0.874	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	TN	0.862	
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	TN	1.492	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	TN	0.054	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.79.	Intermediate	Eutrophication	TN	0.856	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.64.	Low	Eutrophication	TN	1.991	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.99.	High	Eutrophication	TN	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.78.	Intermediate	Eutrophication	TN	1.099	
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.66.	Low	Eutrophication	TN	2.222	Eutrophication threshold is set for a biointegrity goal that is above reference.
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	High	Eutrophication	TN	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Intermediate	Eutrophication	TN	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Low	Eutrophication	TN	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	High	Eutrophication	TN	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Intermediate	Eutrophication	TN	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Low	Eutrophication	TN	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.53.	High	Eutrophication	TN	NA	No threshold identified

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.45.	Intermediate	Eutrophication	TN	NA	No threshold identified
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.37.	Low	Eutrophication	TN	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	TP	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	TP	0.044	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	TP	0.122	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	TP	0.009	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	TP	0.056	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	TP	0.135	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.92.	High	Eutrophication	TP	0.026	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	TP	0.102	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.63.	Low	Eutrophication	TP	0.249	
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.98.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.9.	Intermediate	Eutrophication	TP	0.02	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.78.	Low	Eutrophication	TP	0.099	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.96.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.9.	Intermediate	Eutrophication	TP	0.032	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.81.	Low	Eutrophication	TP	0.09	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.73.	High	Eutrophication	TP	0.147	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.61.	Intermediate	Eutrophication	TP	0.276	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.44.	Low	Eutrophication	TP	NA	No threshold identified

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.91.	High	Eutrophication	TP	0.015	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.8.	Intermediate	Eutrophication	TP	0.084	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.66.	Low	Eutrophication	TP	0.21	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.91.	High	Eutrophication	TP	0.026	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Intermediate	Eutrophication	TP	0.096	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.65.	Low	Eutrophication	TP	0.24	
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.89.	High	Eutrophication	TP	0.041	
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.82.	Intermediate	Eutrophication	TP	0.083	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.71.	Low	Eutrophication	TP	0.165	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.13.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.02.	Intermediate	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 0.92.	Low	Eutrophication	TP	0.009	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 1.05.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	Intermediate	Eutrophication	TP	0.009	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Low	Eutrophication	TP	0.096	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.85.	High	Eutrophication	TP	0.065	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	TP	0.203	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.52.	Low	Eutrophication	TP	NA	No threshold identified
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 1.05.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.88.	Intermediate	Eutrophication	TP	0.032	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.74.	Low	Eutrophication	TP	0.131	
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 1.02.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.87.	Intermediate	Eutrophication	TP	0.05	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.74.	Low	Eutrophication	TP	0.144	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.74.	High	Eutrophication	TP	0.14	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	TP	0.203	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.55.	Low	Eutrophication	TP	0.408	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.93.	High	Eutrophication	TP	0.003	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	TP	0.107	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.64.	Low	Eutrophication	TP	0.236	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.88.	High	Eutrophication	TP	0.044	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.76.	Intermediate	Eutrophication	TP	0.128	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.6.	Low	Eutrophication	TP	0.323	
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.96.	High	Eutrophication	TP	0.005	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.75.	Intermediate	Eutrophication	TP	0.132	
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.64.	Low	Eutrophication	TP	0.236	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.85.	Intermediate	Eutrophication	TP	0.051	
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	TP	0.188	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.97.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	TP	0.056	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.67.	Low	Eutrophication	TP	0.215	
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.1.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.	Intermediate	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 0.81.	Low	Eutrophication	TP	0.089	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	TP	0.107	
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	TP	0.188	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	TP	0.009	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.79.	Intermediate	Eutrophication	TP	0.104	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.64.	Low	Eutrophication	TP	0.254	
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.99.	High	Eutrophication	TP	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.78.	Intermediate	Eutrophication	TP	0.11	
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.66.	Low	Eutrophication	TP	0.213	Eutrophication threshold is set for a biointegrity goal that is above reference.
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	High	Eutrophication	TP	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Intermediate	Eutrophication	TP	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Low	Eutrophication	TP	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	High	Eutrophication	TP	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Intermediate	Eutrophication	TP	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Low	Eutrophication	TP	NA	Insufficient data

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.53.	High	Eutrophication	TP	0.557	
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.45.	Intermediate	Eutrophication	TP	NA	No threshold identified
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.37.	Low	Eutrophication	TP	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	Chl-a	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	Chl-a	19.5	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	Chl-a	64.9	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	Chl-a	2.4	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	Chl-a	24.3	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	Chl-a	68.2	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.92.	High	Eutrophication	Chl-a	10.2	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	Chl-a	51.1	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.63.	Low	Eutrophication	Chl-a	160.1	
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.98.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.9.	Intermediate	Eutrophication	Chl-a	6.9	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.78.	Low	Eutrophication	Chl-a	50.2	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.96.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.9.	Intermediate	Eutrophication	Chl-a	12.6	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.81.	Low	Eutrophication	Chl-a	41.4	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.73.	High	Eutrophication	Chl-a	77.5	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.61.	Intermediate	Eutrophication	Chl-a	197.6	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.44.	Low	Eutrophication	Chl-a	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.91.	High	Eutrophication	Chl-a	4.2	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.8.	Intermediate	Eutrophication	Chl-a	41.7	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.66.	Low	Eutrophication	Chl-a	134.5	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.91.	High	Eutrophication	Chl-a	10.2	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Intermediate	Eutrophication	Chl-a	45	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.65.	Low	Eutrophication	Chl-a	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.89.	High	Eutrophication	Chl-a	18.3	
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.82.	Intermediate	Eutrophication	Chl-a	39.9	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.71.	Low	Eutrophication	Chl-a	88.6	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.13.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.02.	Intermediate	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 0.92.	Low	Eutrophication	Chl-a	1.2	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 1.05.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	Intermediate	Eutrophication	Chl-a	2.4	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Low	Eutrophication	Chl-a	45	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.85.	High	Eutrophication	Chl-a	30	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	Chl-a	116.2	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.52.	Low	Eutrophication	Chl-a	NA	No threshold identified
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 1.05.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.88.	Intermediate	Eutrophication	Chl-a	13.2	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.74.	Low	Eutrophication	Chl-a	70.3	
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 1.02.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.87.	Intermediate	Eutrophication	Chl-a	21.3	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.74.	Low	Eutrophication	Chl-a	73.9	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.74.	High	Eutrophication	Chl-a	72.7	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	Chl-a	116.2	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.55.	Low	Eutrophication	Chl-a	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.93.	High	Eutrophication	Chl-a	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	Chl-a	55	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.64.	Low	Eutrophication	Chl-a	166.1	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.88.	High	Eutrophication	Chl-a	18.3	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.76.	Intermediate	Eutrophication	Chl-a	63.1	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.6.	Low	Eutrophication	Chl-a	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.96.	High	Eutrophication	Chl-a	0	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.75.	Intermediate	Eutrophication	Chl-a	67.9	
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.64.	Low	Eutrophication	Chl-a	146.5	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.85.	Intermediate	Eutrophication	Chl-a	23.1	
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	Chl-a	112.6	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.97.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	Chl-a	24.3	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.67.	Low	Eutrophication	Chl-a	152.6	
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.1.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.	Intermediate	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 0.81.	Low	Eutrophication	Chl-a	43.5	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	Chl-a	55	
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	Chl-a	112.6	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	Chl-a	2.4	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.79.	Intermediate	Eutrophication	Chl-a	49.2	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.64.	Low	Eutrophication	Chl-a	NA	No threshold identified
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.99.	High	Eutrophication	Chl-a	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.78.	Intermediate	Eutrophication	Chl-a	55	
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.66.	Low	Eutrophication	Chl-a	124.9	Eutrophication threshold is set for a biointegrity goal that is above reference.
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	High	Eutrophication	Chl-a	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Intermediate	Eutrophication	Chl-a	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Low	Eutrophication	Chl-a	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	High	Eutrophication	Chl-a	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Intermediate	Eutrophication	Chl-a	NA	Insufficient data

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Low	Eutrophication	Chl-a	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.53.	High	Eutrophication	Chl-a	NA	No threshold identified
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.45.	Intermediate	Eutrophication	Chl-a	NA	No threshold identified
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.37.	Low	Eutrophication	Chl-a	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	AFDM	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	AFDM	15.2	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	AFDM	48	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	AFDM	3.6	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	AFDM	17.6	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	AFDM	52.9	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.92.	High	Eutrophication	AFDM	7.6	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	AFDM	37.2	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.63.	Low	Eutrophication	AFDM	197.8	
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.98.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.9.	Intermediate	Eutrophication	AFDM	6.4	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.78.	Low	Eutrophication	AFDM	37.2	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.96.	High	Eutrophication	AFDM	0.8	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.9.	Intermediate	Eutrophication	AFDM	10	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.81.	Low	Eutrophication	AFDM	29.6	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.73.	High	Eutrophication	AFDM	70.5	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.61.	Intermediate	Eutrophication	AFDM	226.2	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.44.	Low	Eutrophication	AFDM	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.91.	High	Eutrophication	AFDM	4.4	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.8.	Intermediate	Eutrophication	AFDM	30.8	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.66.	Low	Eutrophication	AFDM	127.3	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.91.	High	Eutrophication	AFDM	8.4	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Intermediate	Eutrophication	AFDM	32.4	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.65.	Low	Eutrophication	AFDM	257.9	
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.89.	High	Eutrophication	AFDM	12.8	
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.82.	Intermediate	Eutrophication	AFDM	28	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.71.	Low	Eutrophication	AFDM	88.5	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.13.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.02.	Intermediate	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 0.92.	Low	Eutrophication	AFDM	2.4	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 1.05.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	Intermediate	Eutrophication	AFDM	3.6	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Low	Eutrophication	AFDM	32.4	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.85.	High	Eutrophication	AFDM	20.8	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	AFDM	139.7	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.52.	Low	Eutrophication	AFDM	348.3	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 1.05.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.88.	Intermediate	Eutrophication	AFDM	10.8	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.74.	Low	Eutrophication	AFDM	52.5	
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 1.02.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.87.	Intermediate	Eutrophication	AFDM	15.6	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.74.	Low	Eutrophication	AFDM	58.9	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.74.	High	Eutrophication	AFDM	62.5	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	AFDM	139.7	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.55.	Low	Eutrophication	AFDM	308.7	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.93.	High	Eutrophication	AFDM	0.4	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	AFDM	40.4	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.64.	Low	Eutrophication	AFDM	171	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.88.	High	Eutrophication	AFDM	13.6	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.76.	Intermediate	Eutrophication	AFDM	47.6	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.6.	Low	Eutrophication	AFDM	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.96.	High	Eutrophication	AFDM	1.6	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.75.	Intermediate	Eutrophication	AFDM	56.1	
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.64.	Low	Eutrophication	AFDM	183.4	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.85.	Intermediate	Eutrophication	AFDM	17.6	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	AFDM	95.3	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.97.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	AFDM	17.6	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.67.	Low	Eutrophication	AFDM	191.4	
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.1.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.	Intermediate	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 0.81.	Low	Eutrophication	AFDM	30.8	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	AFDM	40.4	
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	AFDM	95.3	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	AFDM	3.6	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.79.	Intermediate	Eutrophication	AFDM	35.6	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.64.	Low	Eutrophication	AFDM	293.5	
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.99.	High	Eutrophication	AFDM	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.78.	Intermediate	Eutrophication	AFDM	41.2	
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.66.	Low	Eutrophication	AFDM	154.2	Eutrophication threshold is set for a biointegrity goal that is above reference.
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	High	Eutrophication	AFDM	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Intermediate	Eutrophication	AFDM	NA	Insufficient data
СС	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Low	Eutrophication	AFDM	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	High	Eutrophication	AFDM	NA	Insufficient data

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Intermediate	Eutrophication	AFDM	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Low	Eutrophication	AFDM	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.53.	High	Eutrophication	AFDM	335.1	
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.45.	Intermediate	Eutrophication	AFDM	NA	No threshold identified
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.37.	Low	Eutrophication	AFDM	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.94.	High	Eutrophication	% cover	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.86.	Intermediate	Eutrophication	% cover	13	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_D score above 0.75.	Low	Eutrophication	% cover	66	
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	% cover	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	% cover	27	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
Wadeable streams	Response	Additive model, 50% probability of attaining ASCI_H score above 0.75.	Low	Eutrophication	% cover	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.92.	High	Eutrophication	% cover	NA	No threshold identified
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.79.	Intermediate	Eutrophication	% cover	45	
Wadeable streams	Response	Additive model, 50% probability of attaining CSCI score above 0.63.	Low	Eutrophication	% cover	87	
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.98.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.9.	Intermediate	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_D score above 0.78.	Low	Eutrophication	% cover	51	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.96.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.9.	Intermediate	Eutrophication	% cover	1	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-N	Response	Additive model, 50% probability of attaining ASCI_H score above 0.81.	Low	Eutrophication	% cover	45	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.73.	High	Eutrophication	% cover	71	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.61.	Intermediate	Eutrophication	% cover	88	
RFI-N	Response	Additive model, 50% probability of attaining CSCI score above 0.44.	Low	Eutrophication	% cover	99	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.91.	High	Eutrophication	% cover	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.8.	Intermediate	Eutrophication	% cover	42	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_D score above 0.66.	Low	Eutrophication	% cover	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.91.	High	Eutrophication	% cover	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Intermediate	Eutrophication	% cover	53	
RFI-S	Response	Additive model, 50% probability of attaining ASCI_H score above 0.65.	Low	Eutrophication	% cover	NA	No threshold identified
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.89.	High	Eutrophication	% cover	15	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.82.	Intermediate	Eutrophication	% cover	36	Eutrophication threshold is set for a biointegrity goal that is above reference.
RFI-S	Response	Additive model, 50% probability of attaining CSCI score above 0.71.	Low	Eutrophication	% cover	76	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.13.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 1.02.	Intermediate	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_D score above 0.92.	Low	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 1.05.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	Intermediate	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining ASCI_H score above 0.8.	Low	Eutrophication	% cover	53	Eutrophication threshold is set for a biointegrity goal that is above reference.
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.85.	High	Eutrophication	% cover	29	
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	% cover	82	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CVF	Response	Additive model, 50% probability of attaining CSCI score above 0.52.	Low	Eutrophication	% cover	94	
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 1.05.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.88.	Intermediate	Eutrophication	% cover	4	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_D score above 0.74.	Low	Eutrophication	% cover	70	
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 1.02.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.87.	Intermediate	Eutrophication	% cover	23	Eutrophication threshold is set for a biointegrity goal that is above reference.
НВ	Response	Additive model, 50% probability of attaining ASCI_H score above 0.74.	Low	Eutrophication	% cover	NA	No threshold identified
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.74.	High	Eutrophication	% cover	67	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.67.	Intermediate	Eutrophication	% cover	82	
НВ	Response	Additive model, 50% probability of attaining CSCI score above 0.55.	Low	Eutrophication	% cover	92	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.93.	High	Eutrophication	% cover	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	% cover	56	
SB2	Response	Additive model, 50% probability of attaining ASCI_D score above 0.64.	Low	Eutrophication	% cover	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.88.	High	Eutrophication	% cover	18	
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.76.	Intermediate	Eutrophication	% cover	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining ASCI_H score above 0.6.	Low	Eutrophication	% cover	NA	No threshold identified
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.96.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.75.	Intermediate	Eutrophication	% cover	63	
SB2	Response	Additive model, 50% probability of attaining CSCI score above 0.64.	Low	Eutrophication	% cover	86	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.85.	Intermediate	Eutrophication	% cover	18	
SB1	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	% cover	99	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.97.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.86.	Intermediate	Eutrophication	% cover	27	
SB1	Response	Additive model, 50% probability of attaining ASCI_H score above 0.67.	Low	Eutrophication	% cover	NA	No threshold identified
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.1.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 1.	Intermediate	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB1	Response	Additive model, 50% probability of attaining CSCI score above 0.81.	Low	Eutrophication	% cover	39	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 1.01.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.77.	Intermediate	Eutrophication	% cover	56	

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
SB0	Response	Additive model, 50% probability of attaining ASCI_D score above 0.68.	Low	Eutrophication	% cover	99	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.94.	High	Eutrophication	% cover	NA	No threshold identified
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.79.	Intermediate	Eutrophication	% cover	66	
SB0	Response	Additive model, 50% probability of attaining ASCI_H score above 0.64.	Low	Eutrophication	% cover	NA	No threshold identified
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.99.	High	Eutrophication	% cover	NA	Eutrophication threshold is set for a biointegrity goal that is above reference.
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.78.	Intermediate	Eutrophication	% cover	48	
SB0	Response	Additive model, 50% probability of attaining CSCI score above 0.66.	Low	Eutrophication	% cover	84	Eutrophication threshold is set for a biointegrity goal that is above reference.
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	High	Eutrophication	% cover	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Intermediate	Eutrophication	% cover	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_D score above NA.	Low	Eutrophication	% cover	NA	Insufficient data

Supplement S4: Thresholds at multiple levels of stringency

Class	Approach	Details	Stringency	Indicator type	Indicator	Threshold	Flag
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	High	Eutrophication	% cover	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Intermediate	Eutrophication	% cover	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining ASCI_H score above NA.	Low	Eutrophication	% cover	NA	Insufficient data
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.53.	High	Eutrophication	% cover	94	
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.45.	Intermediate	Eutrophication	% cover	98	
CC	Response	Additive model, 50% probability of attaining CSCI score above 0.37.	Low	Eutrophication	% cover	NA	No threshold identified