MARU TECHNICAL REPORT: LONG-TERM EVALUATION OF BIOASSESSMENT INDICES IN SAN DIEGO REGION STREAMS

Photo: Agua Caliente Creek, an Intermittent Reference Sampling Site

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CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

Table of Contents

1. Bioassessment Background and Report Purpose

Biological assessment, or bioassessment, is the science of evaluating the health or integrity of an ecosystem by assessing the organisms that live within it. Bioassessment allows for the California Water Quality Control Board San Diego Region (San Diego Water Board) to better protect and restore waters by facilitating a broader evaluation of the cumulative effects of stressors beyond analyzing for individual chemicals. Unlike traditional chemistry-based monitoring, which provides only limited information about a relatively narrow portion of the environment at a discrete point in time, bioassessment can account for living organisms exposed to multiple chemicals and other stressors (such as altered habitats and changes in water-flow patterns) over extended time periods. Consequently, bioassessment has the potential to provide a more integrated reflection of the condition of an aquatic ecosystem; bioassessment also is more closely tied to environmental managers' end-goal focus on ecosystem protection and serves as an important way to monitor and protect the populations of endangered species and fisheries.

Bioassessment condition is typically interpreted through the development of bioassessment indices, often referred to as indices of biological or biotic integrity. The use of biological indices to determine ecosystem integrity under the United States Clean Water Act is widespread, with indices developed across the United States using a variety of organisms (e.g. fish, example: Vile 2012, benthic macroinvertebrates, example: ADEQ 2007, and algae, see Paul et al. 2017) across multiple habitat types, such as streams, estuaries, and lakes. USEPA also "recommends the use of biological assessments as a measure of true environmental results" (USEPA 2013).

In California, stream bioassessment programs sample benthic macroinvertebrates (BMI) and benthic algae (diatoms, soft algae, cyanobacteria) to assess biological integrity, relying on a state-wide California Stream Condition Index for BMI (CSCI, Mazor et al. 2016) and Algal Stream Condition Index for algae (ASCI, Theroux et al. 2020). These are predictive indices (i.e., they measure biological condition as deviation between observed biological composition and composition predicted from reference models; see Stoddard et al. 2006, Hawkins et al. 2010). Scores from the CSCI and ASCI can be assessed with thresholds derived from reference distributions, such as a percentile of the reference dataset, to identify where stream biological communities are likely or clearly impacted. Percentiles of reference are commonly used by States to set thresholds for determining biological integrity and impairment under the Clean Water Act, with California relying upon the 10th percentile of the CSCI as both an impairment threshold (e.g. San Diego Water Board 2016) and biological water quality objective (San Diego Water Board 2020a).

The applicability of these indices in California's intermittent streams for purposes of assessment and regulatory application (e.g. stream protection and restoration) has been questioned statewide. However, the use of the CSCI in intermittent streams was previously examined in the San Diego Region, with the CSCI determined to be appropriate (Mazor et al. 2015). Intermittent streams were therefore included in the San Diego Water Board's biological objectives, adopted in December of 2020 (San Diego Water Board 2020a). Scientific peer review conducted as part of the biological objective development, supported and highlighted the inclusion of intermittent streams as a positive aspect of the water quality objective (San Diego Water Board 2020b).

The purpose of this technical report is to expand upon the Mazor et al. 2015 report through the inclusion of additional BMI data from perennial and intermittent sites, in addition to evaluating algal ASCI scores at R9 reference perennial and intermittent sites for the first time.

1.1. Stream Bioassessment Background

The State of California has been conducting bioassessment on wadeable streams in the San Diego region since the 1990's, with early sampling conducted by the San Diego Water Board, the State Water Board's Surface Water Ambient Monitoring Program (SWAMP), and USEPA. Early efforts used solely benthic macroinvertebrates to assess biological integrity, though by the early 2000's benthic algae had begun to be incorporated into sampling. Current stream bioassessment includes the following measurements:

- Benthic macroinvertebrates
- Benthic algae: cyanobacteria, diatoms, soft algae
- Physical habitat: instream and riparian
- Water chemistry
- Flow

SWAMP has developed standard operating procedures (SOPs) for bioassessment field sampling, laboratory identification of specimens, quality assurance/control, data management, and reporting. The San Diego Water Board follows SWAMP SOPs when conducting bioassessment. These can all be found on the SWAMP bioassessment website:
https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/

All San Diego Water Board bioassessment data is available to the public via the CEDEN database.

California biological assessment programs historically targeted perennial or presumed perennial stream systems for BMI and algae, with sampling traditionally occurring from late March through October, but typically targeting the spring to early summer period in southern California (Mazor et al. 2016). These programs, many of which used a probabilistic monitoring design, historically did not monitor streamflow duration when evaluating benthic community composition due to the potential cost and uncertainties in flow assessment (e.g., Nadeau et al. 2015).

1.2. Indices of Biological Integrity

1.2.1. Biological Indices

In order to assess bioassessment data, biological scoring tools are needed to translate complex species identification information into a condition determination. The development of these biological scoring tools, often referred to as indices or metrics of biological integrity, has been on-going since the 1990's, with various regional IBIs developed throughout the State. Mazor et al. (2014) provided preliminary evidence of the applicability of a 2005 Southern California specific Index of Biotic Integrity (Ode et al. 2005) in intermittent streams: IBI scores at reference sites (i.e., those with limited human disturbance in the upstream catchment) with intermittent flow were similar to scores observed at perennial reference sites, and IBI scores also decreased as human disturbance in upstream catchments increased. However, the number of study sites was limited (n = 14, only 3 of which were reference sites), and the IBI scoring was based on a non-predictive index. Consequently, that study had limited ability to account for the diversity of flow regimes that occur in non-perennial streams in the San Diego Region.

In 2015 the State of California released a peer-reviewed statewide California Stream Condition Index (CSCI, Mazor et al. 2016) for assessing the biological condition of wadeable streams throughout the State based on benthic macroinvertebrates. The CSCI utilizes a combined-reference-site approach to determine the site-specific benthic community expected to be present at any sampled site. The classification of sites as "reference" and "non-reference" for the purposes of the CSCI was also scientifically peer-reviewed (see Ode et al. 2016). An evaluation of the appropriateness of the CSCI for streams in the San Diego Region was conducted concurrent with CSCI publication (Mazor et al. 2015), which determined the CSCI to be appropriate for use in San Diego Region intermittent streams.

In 2020 an additional peer-reviewed statewide Algal Stream Condition Index (ASCI, Theroux et al. 2020) was released. The ASCI utilizes an approach that mirrors the CSCI, except multiple indices were developed, with a diatom-specific index (d-ASCI) and combined "hybrid" diatom/soft algae index (h-ASCI). The ASCI is currently being updated to use a molecular taxonomic identification approach as an alternative to traditional laboratory microscopic identifications (Theroux et al. 2023).

Both the CSCI and ASCIs are indices that score from a scale of 0-1. As the indices use a reference-approach, a score of 1 indicates a sampled stream site is scoring equivalent to reference, while lower scores show deviation from expected condition, indicating degradation. The CSCI and ASCI publications calculated thresholds for scores which would show a site to be considered in an impacted condition. These thresholds are included in Table 1, below. The CSCI threshold of 0.79 was adopted by the San Diego Water Board as a water quality objective in 2020 (San Diego Water Board 2020a).

Table 1. Table of 10th Percentile Thresholds in Mazor et al. 2016 and Theroux et al. 2020.

Index	Threshold
CSCI	0.79
d-ASCI	0.86
h-ASCI	0.86

1.3. Study Purpose

The purpose of this technical report is to assess additional data from perennial and intermittent reference streams (per Ode et al. 2016) sampled in the San Diego Region for both BMI and algae to further evaluate the applicability of the CSCI and ASCIs.

While the Mazor et al. 2015 study remains accurate, some “reference” sites used in the study have been excluded from the State of California’s reference site pool due to discovery of upstream anthropogenic impacts (e.g. flow modification, illegal cannabis cultivation) or more recent GIS data showing they do not meet screening thresholds from Ode et al. 2016. In addition, more intermittent and perennial reference sites have been identified and sampled in the nine years since report publication. For algae, the ASCI was not included in the original Mazor et al. 2015 publication. This technical report evaluates ASCI performance in R9 intermittent and perennial reference streams relative to published thresholds to assist with data interpretation for assessment purposes.

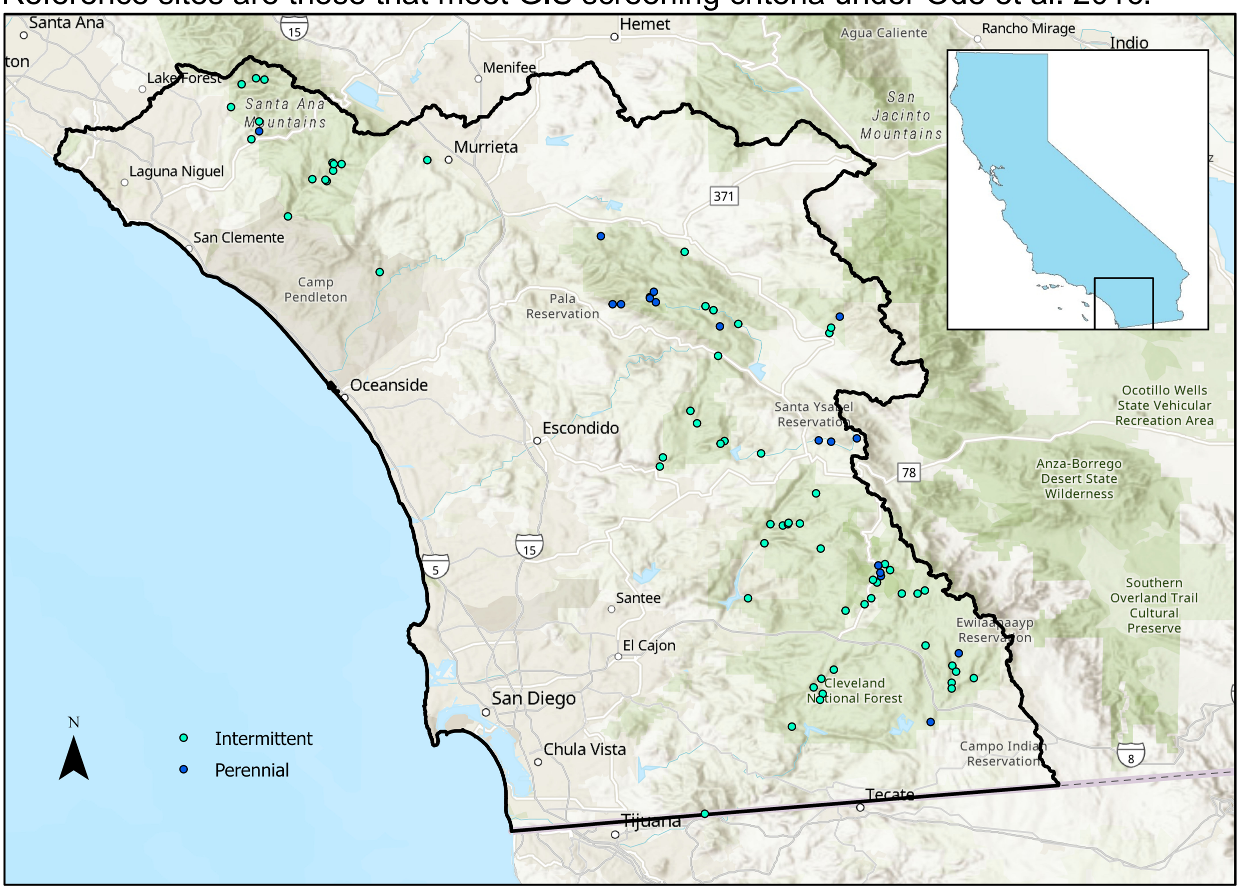
2. Methods

2.1. Site Selection: Reference Condition Management Program Sites/Reference Sites

Sites used in this report are those considered to be “reference” under the State of California’s SWAMP program. The SWAMP program conducts long-term monitoring at a set of sites that meet GIS reference screening criteria (Ode et al. 2016) as part of the state’s reference condition management program (RCMP). This network of sites, and their on-going monitoring, is critical for establishing reference conditions, which are “healthy” or “biologically intact” conditions in streams with little to no upstream anthropogenic stressors. The development of the CSCI and ASCI relied heavily upon data collected by the RCMP.

In addition to sampling sites from the RCMP dataset, the San Diego Water Board regularly identifies and samples new reference sites to add to the potential State reference site pool (Figure 1, also section 2.4 below). Finally, new reference sites are also associated with sampling conducted by the Stormwater Monitoring Coalition (SMC), which samples randomly selected (“probabilistic”) sites that can, by chance, be located in location that meet Ode et al. 2016 reference screens. Sampling results from RCMP sites and new reference sites were used in this report.

Figure 1. Map of Sampling Sites in the San Diego Region that meet reference screens. Reference sites are those that meet GIS screening criteria under Ode et al. 2016.



2.2. Bioassessment Monitoring

Stream bioassessment sampling was collected in accordance with SWAMP SOPs for bioassessment field sampling (Ode et al. 2016b) and laboratory identification of specimens for BMI and algae (Woodard et al. 2012, Stancheva et al. 2015, respectively), and quality assurance/control, data management, and reporting under the SWAMP Quality Assurance Program Plan (SWAMP QAPP). These can all be found on the SWAMP bioassessment website:
https://www.waterboards.ca.gov/water_issues/programs/swamp/bioassessment/

This report also contains monitoring results collected under National Pollutant Discharge Elimination System (NPDES) permits by the SMC, which follow the above SOPs and have project QAPPs required to be consistent with the SWAMP QAPP. Bioassessment results were obtained from the California Environmental Data Exchange Network (CEDEN) and the Stormwater Monitoring Coalition (SMC) databases.

2.3. Site Flow Classification

For the purposes of this report, streams have been classified into two flow-type categories: 1) perennial or 2) intermittent (see Figures 2 and 3 for examples). Perennial streams are those streams that have flowing surface water year-round during all known years. Intermittent streams are those streams that have surface flows during a portion of the year and lack flows for a portion of the year. Note that intermittent streams may flow year-round in wet years and retain disconnected perennial pools in dry years (e.g. Figure 4).

The classification of streams or stream segments as perennial or intermittent relied on multiple methods used independently or in combination. These include:

1) Permanent flow gages (e.g. United States Geologic Survey [USGS])
2) Deployment of water level or water presence loggers in stream runs or glides
3) Actual site visits during the wet and dry season
4) Firsthand accounts from on-site resource staff

Figure 2. Example of Reference Intermittent Stream During the Dry Phase (Bluewater Canyon)

Figure 3. Example of Reference Perennial Stream (French Creek)

Figure 4. San Mateo Creek, which can exhibit intermittent surface flows during dry years but retains permanent disconnected perennial pools. San Mateo may flow year-round during wet years.

2.4. Statistical Analysis

Comparison of San Diego Region Reference Site Thresholds to CSCI and ASCI Thresholds

CSCI and ASCI scores used for analysis were calculated using ArcGIS and a custom R package (see Boyle et al. 2020). Traditional simple statistical analysis (mean, standard deviation, quantiles) was conducted on scores for qualitative comparison to CSCI and ASCI development results. As the dataset available includes repeat sampling at the same sites in different years, this analysis was conducted two ways:

1) All sites+years were included in statistical calculation. This was done to incorporate all interannual variability within the dataset.

2) A single sample was selected from each site for use in statistical calculations. For this approach, a random site+year was selected and statistical calculations were conducted. This was then repeated 1,000 times ("bootstrapped" or "modeled"), and the resulting median and the 95th percentile confidence interval of calculated general statistics was reported.

As the study's primary purpose was to further evaluate CSCI thresholds in addition to exploring ASCI thresholds applicability across stream flow types, statistical comparison was conducted in two ways. First, a simple ANOVA, or Welch's t-test if variances were unequal, was conducted to compare means between R9 stream flow types and those from the developmental dataset from Mazor et al. 2016 and Theroux et al. 2020. Second, an evaluation of differences in the percentile scoring thresholds from R9 stream flow types and those used by Mazor et al. 2016 and Theroux et al. 2020 to identify stream condition classes was conducted (see Table 2 below). Quantile estimation (Wilcox et al. 2014) was used to compare thresholds for the 1st, 10th, and 30th percentiles across stream flow regimes. This comparison was conducted using the *WRS2* package in R (Mair and Wilcox 2020). A modeled approach consistent with the general statistics was conducted for all statistical tests, except ANOVA and quantile estimation was repeated 10 times to evaluate p-value consistency.

Table 2. Thresholds of Stream Condition for the CSCI (Mazor et al. 2016) and ASCI (Theroux et al. 2020)

	CSCI	ASCI - Diatoms	ASCI – Hybrid
Likely Intact, ≥30th percentile	0.92	0.94	0.94
Possibly Altered, ≥10th percentile	0.79	0.86	0.86
Likely Altered, ≥1st percentile	0.63	0.75	0.75
Very Likely Altered, <1st percentile	0.63	0.75	0.75

3. Results

3.1. Sites Sampled

Available data for analysis included 230 samples collected from 2000 to 2024 for the CSCI, and 168 samples from 2008-2024 for the ASCI. Table 3 below provides basic information on the sites included. Not all sites had both CSCI and ASCI scores, as algae sampling did not begin in the region until 2008. Notably, additional reference site sampling events were available but were purposely excluded because sampling was:

- In years immediately following a fire event in the tributary watershed.
- Not conducted in a manner that met the SWAMP SOP. Some sampling events were conducted during no-flow conditions and/or on stream segments that contained excessive dry transects.

Sampled reference sites in the region were predominantly intermittent, with n = 17 sampling sites classified as perennial.

Table 3. Bioassessment Sites Sampled and Flow Classification.

Station	Stream	Latitude	Longitude	Flow Status
901ATCAAS	Upper Arroyo Trabuco	33.682	-117.502	Intermittent
901BELOLV	Upper Bell Creek	33.640	-117.553	Intermittent
901DCCDCx	Devil's Canyon	33.473	-117.466	Intermittent
901DCCSMC	Devil's Canyon	33.473	-117.466	Intermittent
901LIONCN	Lion Canyon	33.603	-117.460	Intermittent
901NP9BWR	Bluewater Canyon	33.530	-117.429	Intermittent
901NP9CSC	Cold Spring Canyon	33.591	-117.522	Intermittent
901NP9FLC	Falls Canyon	33.675	-117.537	Intermittent
901NP9HJC	Holy Jim Canyon	33.684	-117.515	Intermittent
901NP9TNC	Tenaja Canyon	33.527	-117.407	Intermittent
901S00469	San Mateo	33.529	-117.409	Intermittent
901S01705	Hot Spring Canyon	33.603	-117.510	Perennial
901S01849	Tenaja Falls	33.555	-117.398	Intermittent
901S02873	Upper San Mateo	33.543	-117.397	Intermittent
901S06969	Tenaja Falls	33.553	-117.396	Intermittent
901SCCA74	Upper Hot Spring Canyon	33.618	-117.510	Intermittent
901SMCWHC	Wildhorse Canyon Creek	33.553	-117.384	Intermittent
901UNTLCC	Unnamed Tributary Long Canyon Creek	33.618	-117.435	Intermittent
901USMFCP	Unnamed Tributary San Mateo	33.536	-117.401	Intermittent

Station	Stream	Latitude	Longitude	Flow Status
902NP9CWC	Cottonwood Canyon	33.419	-116.860	Intermittent
902R9CC1	Cole Canyon	33.559	-117.253	Intermittent
902ASTRLC	Arroyo Seco	33.443	-116.988	Perennial
902SMROB8	Roblar Creek	33.388	-117.326	Intermittent
903ACPCT1	Lower Agua Caliente	33.295	-116.639	Intermittent
903ACPCT2	Middle Agua Caliente	33.303	-116.636	Intermittent
903DCBDCP	Doane Creek	33.342	-116.904	Perennial
903FCPSPx	French Creek	33.350	-116.913	Perennial
903FRC	Upper French Creek Meadow	33.358	-116.907	Intermittent
903NP9LWF	Lower West Fork San Luis Rey	33.309	-116.778	Intermittent
903NP9PRC	Prisoner Creek	33.260	-116.809	Intermittent
903NP9UAC	Upper Agua Caliente	33.320	-116.623	Perennial
903PCPMPx	Upper Pauma Creek	33.348	-116.913	Perennial
903R9PPCD	Middle Pauma Creek	33.339	-116.957	Perennial
903REFPC	Lower Pauma Creek	33.339	-116.970	Perennial
903S01413	Pine Valley	33.305	-116.806	Perennial
903WE0798	West Fork San Luis Rey	33.336	-116.828	Intermittent
903WE0900	West Fork San Luis Rey	33.330	-116.816	Intermittent
905BCC	Black Canyon Creek	33.130	-116.799	Intermittent
905BCN1xx	Upper Boden	33.105	-116.893	Intermittent
905BMCCGx	Black Canyon Creek	33.126	-116.805	Intermittent
905CE0512	South	33.111	-116.743	Intermittent
905DGCC1x	Carney Canyon	33.157	-116.841	Intermittent
905DGUT1x	Upper Temescal	33.176	-116.851	Intermittent
905S02561	Upper Santa Ysabel	33.129	-116.636	Perennial
905SDBDN9	Lower Boden Canyon	33.091	-116.898	Intermittent
905SDISS2	Iron Springs	33.134	-116.597	Perennial
905WE0679	Middle Santa Ysabel	33.131	-116.655	Perennial
906UPCUQC	Upper Poway Creek	32.961	-116.995	Intermittent
907BCT	Boulder Creek Tributary	32.966	-116.652	Intermittent
907CCCR02	Cedar Creek	33.001	-116.710	Intermittent
907CONECR	Conejos Creek	32.890	-116.763	Intermittent
907M23330	Cedar Creek	33.003	-116.702	Intermittent
907M23346	Upper Cedar Creek	33.004	-116.684	Intermittent
907S03210	Upper San Diego River	33.003	-116.729	Intermittent
907SDB016	Cedar Creek	33.005	-116.701	Intermittent
907SDB035	Lower Boulder Creek	32.974	-116.738	Intermittent

Station	Stream	Latitude	Longitude	Flow Status
907TEMREF	Temescal Creek	33.050	-116.659	Intermittent
909CCCSPx	Upper Cold Stream	32.940	-116.564	Perennial
909FC00260	Descanso Creek	32.891	-116.574	Intermittent
909HPCASR	Harper Creek	32.933	-116.546	Intermittent
909JQCASR	Juaquapin Creek	32.914	-116.566	Intermittent
909JPCH79	Japacha Creek	32.918	-116.572	Intermittent
909LCSASR	Lower Cold Stream at Sweetwater Confluence	32.924	-116.560	Perennial
909NP9DCC	Descanso Creek	32.890	-116.575	Intermittent
909NP9UNT	Unnamed Tributary to Descanso Creek	32.881	-116.585	Intermittent
909S00282	Sweetwater	32.871	-116.614	Intermittent
909SWCASR	Stonewall	32.942	-116.554	Intermittent
909WE0780	Middle Cold Stream	32.929	-116.561	Perennial
911COPPER	Copper Canyon	32.561	-116.829	Intermittent
911FCCAKC	Fred Canyon	32.760	-116.451	Intermittent
911GSCAPV	Granite Springs	32.897	-116.528	Intermittent
911HCCSMR	Horse Canyon	32.757	-116.467	Intermittent
911KCKCRx	Upper Kitchen Creek	32.787	-116.451	Intermittent
911M24934	Upper Horsethief	32.767	-116.651	Intermittent
911NP9ATC	Antone Canyon	32.768	-116.418	Intermittent
911NP9EPC	Espinosa Creek	32.744	-116.649	Intermittent
911NP9HTC	Horsethief Canyon	32.754	-116.663	Intermittent
911NP9LPC	La Posta Creek	32.701	-116.484	Perennial
911NP9UCW	Upper Cottonwood	32.818	-116.492	Intermittent
911PVCAEC	Pine Valley above Espinosa	32.735	-116.653	Intermittent
911S00538	Pine Valley	32.781	-116.632	Intermittent
911S00858	Indian Creek	32.902	-116.493	Intermittent
911S01142	Lower Pine Valley	32.735	-116.653	Intermittent
911TCCTCx	Troy Canyon	32.806	-116.441	Perennial
911TJIND2	Indian Creek	32.897	-116.504	Intermittent
911TJKC1x	Middle Kitchen	32.761	-116.452	Intermittent
911TJKTC5	Kitchen Creek	32.752	-116.452	Intermittent
911TJLCC2	Long Canyon Creek	32.778	-116.445	Intermittent
911TJWIL3	Wilson Creek	32.694	-116.696	Intermittent

3.2. General Statistics (Mean and Standard Deviation) and ANOVA/t-tests

General statistics for the CSCI and ASCI for R9 Perennial and R9 Intermittent are included in Table 4 below for all sites+years. Modeled general statistics are presented in Table 5.

Table 4. General statistics for the CSCI from the development dataset and R9 reference sites (all sites+years).

Index	Published Mean	Published SD	R9 Perennial Mean All	R9 Perennial SD All	R9 Intermittent Mean All	R9 Intermittent SD All
CSCI	1.01	0.16	1.02	0.11	0.99	0.12
ASCI-Diatoms	1.0	0.11	0.96	0.16	0.98	0.15
ASCI-Hybrid	1.0	0.11	0.97	0.16	1.00	0.15

Table 5. Modeled general statistics for the CSCI from the development dataset and R9 reference sites for all sites + years.

Index	Published Mean	Published SD	R9 Perennial (Modeled Median Mean, 95% CI)	R9 Perennial (Modeled Median SD, 95% CI)	R9 Intermittent (Modeled Median Mean, 95% CI)	R9 Intermittent (Modeled Median SD, 95% CI)
CSCI	1.01	0.16	1.04, 1.02 – 1.06	0.12, 0.10 – 0.13	0.98, 0.96 – 0.99	0.12, 0.11 – 0.13
ASCI-Diatoms	1.0	0.11	0.91, 0.87 – 0.95	0.16, 0.10 – 0.19	0.99, 0.98 – 1.01	0.15, 0.14 – 0.16
ASCI-Hybrid	1.0	0.11	0.93, 0.89 – 0.98	0.15, 0.10 – 0.19	1.0, 0.98 – 1.02	0.15, 0.14 – 0.16

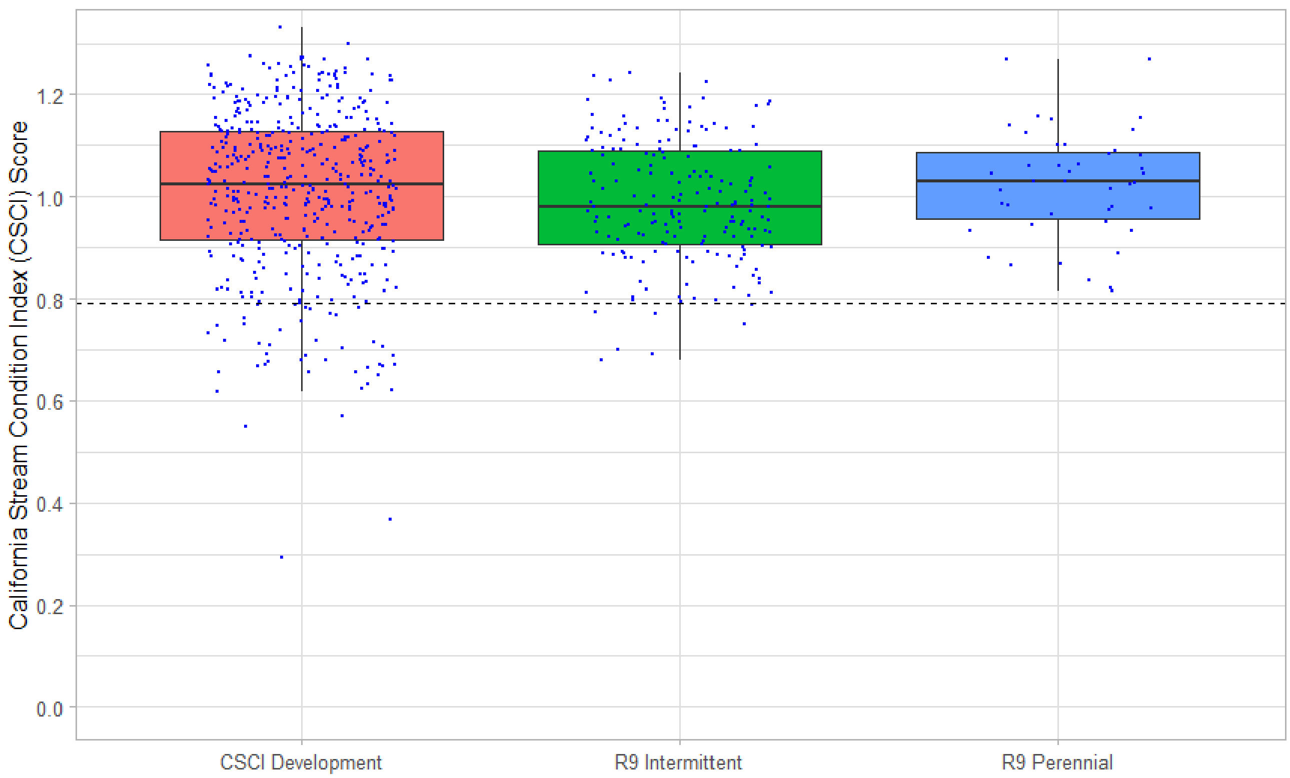
Welch’s t-test results for the CSCI for all sites+years did find a slightly significant difference in mean CSCI between the CSCI development dataset and R9 intermittent sites (t = -2.04, DF= 451, p = 0.0417), though there was no difference between the CSCI development dataset and R9 perennial sites (t = 0.77, DF = 65, p = 0.4428). Modeled t-test results (n = 10 runs) were somewhat similar to results for all sites+years, with a slightly significant difference to significant difference observed for R9 intermittent in 7/10 runs and no difference observed for R9 perennial in all runs.

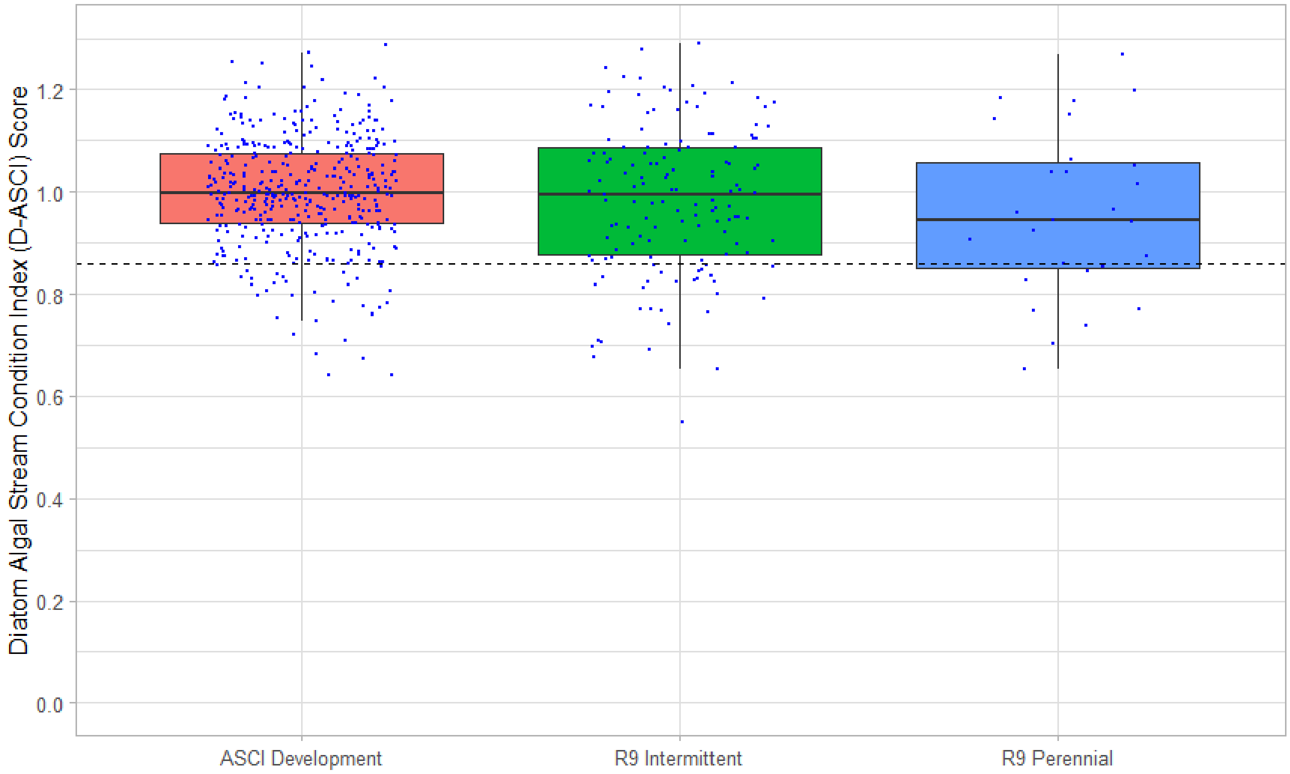
Welch’s t-test results for the diatom ASCI for all sites+years found no significant difference in mean Diatom ASCI between the ASCI development dataset and R9 perennial sites (t = -1.318, DF = 27, p = 0.1983) and no difference between the ASCI development dataset and R9 intermittent (t = -1.1768, DF =191, p = 0.2407). Modeled t-test results (n = 10 runs) found no significant difference between the ASCI development dataset and R9 intermittent in all runs and no difference observed for R9 perennial in 6/10 runs.

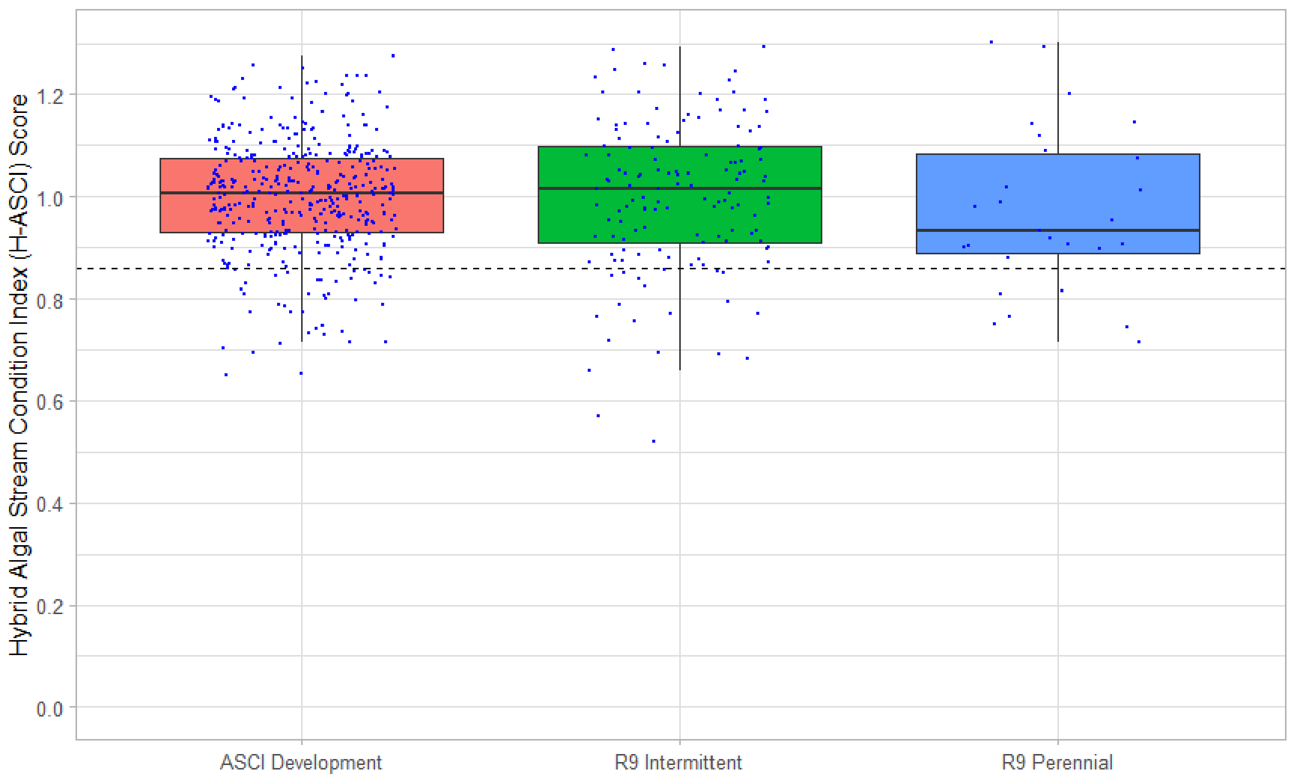
Welch's t-test results for the hybrid ASCI for all sites+years found no significant difference in mean hybrid ASCI between the ASCI development dataset and R9 perennial sites (t = -0.977, DF = 27, p = 0.3369) and for R9 intermittent as well (t = -.012, DF = 191, p = 0.9897). Modeled t-test results (n = 10 runs) mirrored results for all sites+years, with no significant difference observed between the ASCI development dataset and R9 intermittent in all runs, and no significant difference in 7/10 perennial runs.

A graphical representation of the population of scores for both the CSCI and ASCIs compared to development datasets is included in Figure 5, below.

Figure 5. Boxplots showing CSCI and ASCI results for reference sites from the index development datasets, R9 intermittent reference sites, and R9 perennial reference sites. Box definitions: outer = 25th,75th percentiles, solid line = median




Diatom Algal Stream Condition Index (D-ASCI) Score
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ASCI Development
R9 Intermittent
R9 Perennial


Hybrid Algal Stream Condition Index (H-ASCI) Score
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ASCI Development
R9 Intermittent
R9 Perennial

3.3. Percentile Statistical Comparisons

3.3.1. CSCI Comparisons

When all sampling sites+years are included, all calculated percentiles for both R9 Perennial and R9 Intermittent stream segments meet or exceed those from the CSCI development dataset (Table 6). When randomly selecting a site+year from repeat sites, median calculated percentiles (n = 10 random runs) also meet or exceed the percentiles from CSCI development, except for the 30th percentile for R9 intermittent, which is slightly lower (Table 7).

Table 6. Calculated Percentiles for R9 Perennial and R9 Intermittent Sites for all Sampling Sites+Years. CSCI Development thresholds are from Mazor et al. 2016.

Classification	CSCI - Development	R9 Perennial	R9 Intermittent
Likely Intact, ≥30th percentile	0.92	0.98	0.92
Possibly Altered, ≥10th percentile	0.79	0.87	0.83
Likely Altered, ≥1st percentile	0.63	0.82	0.70
Very Likely Altered, <1st percentile	0.63	0.82	0.70

Table 7. Calculated 95th percentile range for percentile thresholds for R9 Perennial and R9 Intermittent Sites when a Sampling Site is randomly selected (n = 1000 percentile runs). CSCI Development thresholds are from Mazor et al. 2016.

Classification	CSCI - Development	R9 Perennial	R9 Intermittent
Likely Intact, ≥30th percentile	0.92	0.97 – 1.03	0.89 – 0.93
Possibly Altered, ≥10th percentile	0.79	0.85 – 0.97	0.80 – 0.82
Likely Altered, ≥1st percentile	0.63	0.81 – 0.88	0.69 – 0.76
Very Likely Altered, <1st percentile	0.63	0.81 – 0.88	0.69 – 0.76

Comparison of all sampling sites+years for R9 Perennial Sites with the CSCI development dataset found no difference between the two populations for the 30th percentile, but the 10th and 1st percentiles for R9 Perennial were significantly higher than the CSCI development dataset (Table 8).

Table 8. Results of percentile comparisons for the CSCI development dataset and R9 perennial reference sites for all sites+years.

Classification	CSCI Development (n = 473)	R9 Perennial (n = 45)	p-value
Likely Intact, ≥30th percentile	0.92	0.97	0.255
Possibly Altered, ≥10th percentile	0.79	0.87	< 0.01
Likely Altered, ≥1st percentile	0.63	0.82	< 0.001
Very Likely Altered, <1st percentile	0.63	0.82	< 0.001

Statistical comparison of all sampling sites+years for R9 Intermittent Sites with the CSCI development dataset found no difference between the two populations for the 30th percentile and 10th percentile, but the 1st percentile for R9 Intermittent sites was significantly higher than the CSCI development dataset (Table 9).

Table 9. Results of percentile comparisons for the CSCI development dataset and R9 intermittent reference sites for all sites+years.

Classification	CSCI Development (n = 473)	R9 Intermittent (n = 185)	p-value
Likely Intact, ≥30th percentile	0.92	0.92	0.144
Possibly Altered, ≥10th percentile	0.79	0.83	0.06
Likely Altered, ≥1st percentile	0.63	0.70	< 0.001
Very Likely Altered, <1st percentile	0.63	0.70	< 0.001

Statistical comparison of a randomly selected sampling year among repeat sites for R9 Perennial Sites (n = 10 runs) with the CSCI development dataset found no difference between the two populations for the 30th percentile in 9 out of 10 runs, while the 10th and 1st percentiles for R9 Perennial was significantly higher than the CSCI development dataset in all runs (Table 10).

Table 10. Results of modeled percentile comparisons for the CSCI development dataset and R9 perennial reference sites.

Classification	CSCI Development (n = 473)	R9 Perennial* (n = 18)	p-value
Likely Intact, ≥30th percentile	0.92	0.96	0.043 – 0.965
Possibly Altered, ≥10th percentile	0.79	0.88	< 0.01
Likely Altered, ≥1st percentile	0.63	0.86	< 0.001
Very Likely Altered, <1st percentile	0.63	0.86	< 0.001

*examples given from a single percentile calculation out of ten.

Statistical comparison of a randomly selected sampling year (n = 10 runs) for R9 Intermittent Sites with the CSCI development dataset found a slight difference ($p < 0.05$) between the two populations for the 30th percentile (R9 Intermittent were slightly lower) in 4/10 runs, while the 1st percentile for R9 Intermittent sites was always significantly higher than the CSCI development dataset. The 10th percentile was not significantly different in any runs (Table 11).

Table 11. Results of modeled percentile comparisons for the CSCI development dataset and R9 intermittent reference sites.

Classification	CSCI Development (n = 473)	R9 Intermittent* (n = 69)	p-value
Likely Intact, ≥30th percentile	0.92	0.92	0.011 – 0.208
Possibly Altered, ≥10th percentile	0.79	0.82	0.204 – 0.843
Likely Altered, ≥1st percentile	0.63	0.72	< 0.001
Very Likely Altered, <1st percentile	0.63	0.72	< 0.001

*examples given from a single percentile calculation out of ten.

3.3.2. ASCI Comparisons – Diatom Index

When all sampling sites+years are included, calculated percentiles for both R9 Perennial and R9 Intermittent stream segments are below all of those from the ASCI development dataset (Table 12). When randomly selecting a site+year from repeat sites, the range of calculated thresholds (n = 1000 random runs) mirror the results from all sampling sites+years (Table 13).

Table 12. Calculated percentile thresholds for R9 Perennial and R9 Intermittent Sites for all Sampling Sites+Years. ASCI Development thresholds are from Theroux et al. 2020.

Classification	ASCI - Diatom Development	R9 Perennial ASCI-Diatom	R9 Intermittent ASCI-Diatom
Likely Intact, ≥30th percentile	0.94	0.86	0.90
Possibly Altered, ≥10th percentile	0.86	0.76	0.81
Likely Altered, ≥1st percentile	0.75	0.67	0.66
Very Likely Altered, <1st percentile	0.75	0.67	0.66

Table 13. Calculated 95th percentile range for percentile thresholds for R9 Perennial and R9 Intermittent Sites when a Sampling Site is randomly selected (n = 1000 percentile runs). ASCI Development thresholds are from Theroux et al. 2020.

Classification	ASCI - Diatom Development	R9 Perennial ASCI-Diatom	R9 Intermittent ASCI-Diatom
Likely Intact, ≥30th percentile	0.94	0.79 – 0.85	0.88 – 0.93
Possibly Altered, ≥10th percentile	0.86	0.71 – 0.78	0.77 – 0.82
Likely Altered, ≥1st percentile	0.75	0.66 - 0.71	0.61 – 0.72
Very Likely Altered, <1st percentile	0.75	0.66 - 0.71	0.61 – 0.72

Statistical comparison of all sampling sites+years for R9 Perennial Sites with the ASCI Diatom development dataset found a significant difference between the two populations for the 30th and 10th percentile, but not the 1st percentiles (Table 14).

Table 14. Results of percentile comparisons for the ASCI diatom development dataset and R9 perennial reference sites for all sites+years.

Classification	ASCI Diatom Development (n = 404)	R9 Perennial (n = 27)	p-value
Likely Intact, ≥30th percentile	0.94	0.86	0.013
Possibly Altered, ≥10th percentile	0.86	0.74	< 0.01
Likely Altered, ≥1st percentile	0.75	0.66	0.59
Very Likely Altered, <1st percentile	0.75	0.66	0.59

Statistical comparison of all sampling sites+years for R9 Intermittent Sites with the ASCI Diatom development dataset mirrored the results for R9 Perennial, with the 30th and 10th percentiles both being significantly less than the development dataset (Table 15).

Table 15. Results of percentile comparisons for the ASCI diatom development dataset and R9 intermittent reference sites for all sites+years.

Classification	ASCI Diatom Development (n = 404)	R9 Intermittent (n = 139)	p-value
Likely Intact, ≥30th percentile	0.94	0.86	< 0.05
Possibly Altered, ≥10th percentile	0.86	0.74	< 0.01
Likely Altered, ≥1st percentile	0.75	0.66	0.585
Very Likely Altered, <1st percentile	0.75	0.66	0.585

Statistical comparison of a randomly selected sampling year among repeat sites for R9 Perennial Sites (n = 10 runs) with the ASCI Diatom development dataset found R9 Perennial Sites to be significantly lower than the ASCI Diatom development dataset in all runs between the two populations for both the 30th and 10th percentile in 10 out of 10 runs. The 1st percentile was not significantly different in 10/10 runs (Table 16).

Table 16. Results of modeled percentile comparisons for the ASCI diatom development dataset and R9 perennial reference sites

Classification	ASCI Development (n = 404)	R9 Perennial* (n = 12)	p-value
Likely Intact, ≥30th percentile	0.94	0.82	0.032 - < 0.01
Possibly Altered, ≥10th percentile	0.86	0.74	0.015 - < 0.01
Likely Altered, ≥1st percentile	0.75	0.71	0.51*
Very Likely Altered, <1st percentile	0.75	0.71	0.51*

*examples given from a single percentile calculation out of ten.

Comparison of a randomly selected sampling year among repeat sites for R9 Intermittent Sites (n = 10 runs) with the ASCI Diatom development dataset found no difference between the two populations for the 1st percentile in all runs, while the 10th and 30th percentiles for R9 Intermittent were significantly lower than the ASCI Diatom development dataset in 10/10 and 3/10 runs, respectively (Table 17).

Table 17. Results of modeled percentile comparisons for the ASCI diatom development dataset and R9 intermittent reference sites

Classification	ASCI Development (n = 404)	R9 Intermittent* (n = 67)	p-value
Likely Intact, ≥30th percentile	0.94	0.91	0.224 – < 0.05
Possibly Altered, ≥10th percentile	0.86	0.78	< 0.05 – < 0.01
Likely Altered, ≥1st percentile	0.75	0.68	0.817*
Very Likely Altered, <1st percentile	0.75	0.68	0.817*

*examples given from a single percentile calculation out of ten.

3.3.3. ASCI Comparisons – Hybrid Index

When all sampling sites+years are included, calculated percentiles for both R9 Perennial and R9 Intermittent stream segments are lower than those from the ASCI development dataset (Table 18). When randomly selecting a site+year from repeat sites, median calculated percentiles (n = 1000 random runs) are lower, except for the 30th and 10th percentiles for R9 Intermittent (Table 19).

Table 18. Calculated Percentiles for R9 Perennial and R9 Intermittent Sites for all Sampling Sites+Years. ASCI Development thresholds are from Theroux et al. 2020.

Classification	ASCI - Hybrid Development	R9 Perennial ASCI-Hybrid	R9 Intermittent ASCI-Hybrid
Likely Intact, ≥30th percentile	0.94	0.90	0.92
Possibly Altered, ≥10th percentile	0.86	0.76	0.84
Likely Altered, ≥1st percentile	0.75	0.72	0.60
Very Likely Altered, <1st percentile	0.75	0.72	0.60

Table 19. Calculated 95th percentile range for percentile thresholds for R9 Perennial and R9 Intermittent Sites when a Sampling Site is randomly selected (n = 1000 percentile runs). ASCI Hybrid Development thresholds are from Theroux et al. 2020.

Classification	ASCI - Hybrid Development	R9 Perennial ASCI-Hybrid	R9 Intermittent ASCI-Hybrid
Likely Intact, ≥30th percentile	0.94	0.81 – 0.90	0.90 – 0.94
Possibly Altered, ≥10th percentile	0.86	0.74 – 0.82	0.78 – 0.86
Likely Altered, ≥1st percentile	0.75	0.72 – 0.73	0.55 – 0.74
Very Likely Altered, <1st percentile	0.75	0.72 – 0.73	0.55 – 0.74

Statistical comparison of all sampling sites+years for R9 Perennial Sites with the ASCI Hybrid development dataset found no difference between the two populations for the 1st percentile, but the 10th and 30th percentiles for R9 Perennial were lower than the ASCI Hybrid development dataset (Table 20).

Table 20. Results of percentile comparisons for the ASCI hybrid development dataset and R9 perennial reference sites for all sites+years.

Classification	ASCI Hybrid Development (n = 418)	R9 Perennial (n = 27)	p-value
Likely Intact, ≥30th percentile	0.94	0.88	< 0.01
Possibly Altered, ≥10th percentile	0.86	0.76	0.033
Likely Altered, ≥1st percentile	0.75	0.72	0.239
Very Likely Altered, <1st percentile	0.75	0.72	0.239

Statistical comparison of all sampling sites+years for R9 Intermittent Sites with the ASCI Hybrid development dataset found no difference between the two populations for the 10th percentile and 30th percentile, but the 1st percentile was slightly significantly different than the ASCI Hybrid development dataset (Table 21).

Table 21. Results of percentile comparisons for the ASCI hybrid development dataset and R9 intermittent reference sites for all sites+years.

Classification	ASCI Hybrid Development (n = 418)	R9 Intermittent (n = 139)	p-value
Likely Intact, ≥30th percentile	0.94	0.93	0.256
Possibly Altered, ≥10th percentile	0.86	0.82	0.094
Likely Altered, ≥1st percentile	0.75	0.57	0.044
Very Likely Altered, <1st percentile	0.75	0.57	0.044

Statistical comparison of a randomly selected sampling year among repeat sites for R9 Perennial Sites (n = 10 runs) with the ASCI development dataset found no difference between the two populations for the 1st percentile in all runs, while the 10th percentile was not significantly different in 7/10 runs and 30th percentiles was not significantly different in 3/10 runs (Table 22).

Table 22. Results of modeled percentile comparisons for the ASCI hybrid development dataset and R9 perennial reference sites

Classification	ASCI Hybrid Development (n = 418)	R9 Perennial* (n = 12)	p-value
Likely Intact, ≥30th percentile	0.94	0.91	0.065 – < 0.001
Possibly Altered, ≥10th percentile	0.86	0.78	0.262 – < 0.01
Likely Altered, ≥1st percentile	0.75	0.72	0.254*
Very Likely Altered, <1st percentile	0.75	0.72	0.254*

*examples given from a single percentile calculation out of ten.

Statistical comparison of a randomly selected sampling year among repeat sites for R9 Intermittent Sites (n = 10 runs) with the ASCI development dataset found no difference between the two populations for the 1st and 30th percentile in 9/10 and 10/10 runs, respectively, while the 10th percentile was not significantly different in 6/10 runs (Table 23).

Table 23. Results of modeled percentile comparisons for the ASCI hybrid development dataset and R9 intermittent reference sites.

Classification	ASCI Hybrid Development (n = 418)	R9 Intermittent* (n = 60)	p-value
Likely Intact, ≥30th percentile	0.94	0.93	0.616*
Possibly Altered, ≥10th percentile	0.86	0.83	0.400 – 0.018
Likely Altered, ≥1st percentile	0.75	0.71	0.551 – 0.038
Very Likely Altered, <1st percentile	0.75	0.71	0.551 – 0.038

*examples given from a single percentile calculation out of ten.

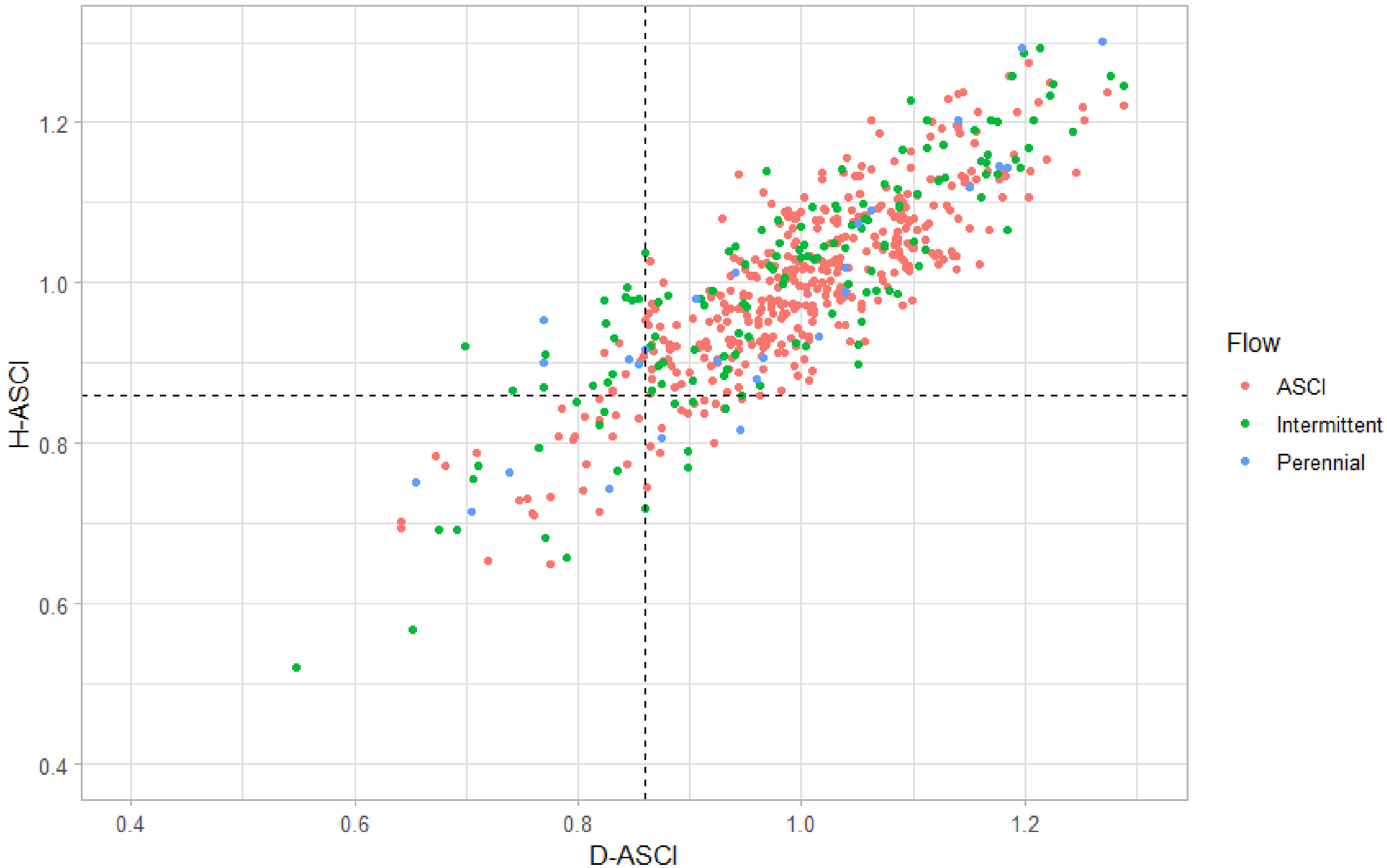
3.4. ASCI Diatom and Hybrid Combined Evaluation

Based on the results from statistical and percentile comparison, which found means to be similar but 10th percentiles to vary by both ASCI and stream flow type, with modeled results showing variability run to run, further evaluation of the R9 Perennial and Intermittent sites was done with BOTH indices included and compared to the ASCI development dataset (see Table 24 and Figure 6 below). This was done to assist with interpretation of scores for impairment and protection purposes. For sites with multiple sampling events, failure was counted when the repeat sampling confirmed scores below the 10th percentile.

Table 24. Percentages of 10th percentile failures for ASCI diatoms, hybrid, and diatoms+hybrid in the development dataset and R9 reference sites.

Status	ASCI Development Dataset	R9 Perennial (n = 12 sites)	R9 Intermittent (n = 67 sites)
Fails Diatoms	31/404 (7.67%)	5/12 (41.66%)	12/67 (17.91%)
Fails Hybrid	38/418 (9.09%)	2/12 (25.00%)	8/67 (11.94%)
Fails Both	25/404 (6.19%)	1/12 (8.33%)	5/67 (7.46%)

Figure 6. Scatterplot showing paired diatom and hybrid scores from stations from the ASCI development dataset (“ASCI”), and R9 perennial (“Perennial”) and intermittent (“Intermittent”) reference sites. Dashed lines denote the 10th percentile thresholds from Theroux et al. 2020.



4. Discussion

4.1. Threshold Comparisons

4.1.1. CSCI

Analysis of CSCI scores for R9 Perennial and R9 Intermittent reference sites found their distribution to be somewhat similar to that of the CSCI development dataset, with some deviations that do not impact assessment use at this time, specifically the 10th percentile calculated by Mazor et al. (0.79, 2016) which is used for impairment determinations and as a water quality objective (San Diego Water Board).

For perennial streams the mean R9 Perennial score was not found to be different than the CSCI development dataset. However, the calculated percentiles were found to differ, with both the 10th and 1st percentiles (0.87 and 0.82 respectively) significantly higher than the 0.79 threshold, indicating the 0.79 threshold may be insufficient for protection purposes. However, only 18 perennial reference sample sites were included in the dataset due to the paucity of reference perennial streams in the region (n = 12 sites). Additional efforts to identify perennial reference sites in the region would help bolster the dataset.

For intermittent streams the mean R9 Intermittent score was slightly lower than the CSCI development dataset in some modeled runs, but population percentiles met or exceeded those calculated by Mazor et al. (2016) for alteration determination. R9 intermittent streams did have a slightly lower 30th percentile when quantile comparisons were conducted in some runs, though that percentile is not used by the State for assessment purposes.

In summary, these results match prior findings during San Diego Water Board water quality biological objective development, which found the 0.79 threshold to be adequate for use in intermittent and perennial streams. Additional work is warranted on perennial reference sites from the region and nearby southern CA reference streams with similar natural characteristics to determine if the 0.79 threshold is sufficiently protective or if a higher threshold is warranted for naturally perennial streams.

4.1.2. ASCI

Analysis of ASCI scores for R9 Perennial and R9 Intermittent reference sites differed from CSCI scores. While mean ASCI Diatom and Hybrid scores did not differ, there were differences observed in R9 reference site score percentiles that warrant consideration for assessment purposes. The ASCI Hybrid appears to be the better option for use in both R9 Perennial and Intermittent streams in this regard.

For R9 Perennial streams, the mean ASCI diatom and hybrid scores were found to not be significantly different than the ASCI development dataset. However, diatom 10th percentile perennial scores were significantly lower than the ASCI development dataset, though sample size was low. Hybrid score analysis differed from diatom-only, as most hybrid model runs did not find a difference in ASCI development and R9 Perennial hybrid 10th percentile scores (70 percent), though the full dataset had a slightly significant difference. 1st percentile scores were not found to be significantly different for all sites+years and for modeled results. Altogether this indicates that the population distributions are similar, the results show the 10th percentile threshold for diatoms was variable for repeat site visits in some years.

For R9 Intermittent streams the mean ASCI diatom and hybrid scores were found to not be significantly different than the ASCI development dataset. Similar to perennial, the modeled 10th percentile scores were significantly different for diatoms. However, R9 Intermittent were not significantly different in most model runs (60 percent) as well as when compared to the development dataset. Also mirroring perennial, 1st percentile scores were not found to be significantly different for all sites+years and for modeled results (9/10 runs). Altogether this indicates that the population distributions between the ASCI development dataset and R9 Intermittent reference sites for hybrid were similar and performed better than diatoms when looking at the 10th percentile threshold.

In summary, mean ASCI scores at R9 perennial and intermittent did not differ from the ASCI development dataset, but the modeled population distributions did differ for the 10th percentile for diatoms in both stream flow types and for hybrid in just perennial for the full dataset. However, 1st percentile scores were not statistically different. The variability around the 10th percentile potentially increases the possibility of a type I error during site condition assessment, defined here as falsely assessing a stream as impaired when it may not be dissimilar from reference, especially when only assessing diatoms. However, the sample size for perennial was limited (n =12 sites), and the 10th percentile for R9 intermittent did not significantly differ from the development dataset for the ASCI hybrid in most model runs. These varied results can present complications for threshold use across stream flow types and indices, especially if stream flow modification is present (e.g. dewatering, flow augmentation) that makes stream flow type difficult to discern.

Given that the results show that the hybrid ASCI to be more precise than the diatom index when looking at each index independently, the initial recommendation is to be preferentially use the hybrid. However, use of the hybrid requires the identification of both diatoms and soft algae in the benthic algal sample (the diatom ASCI only requires diatoms). This then makes the diatom ASCI available for use for every sample identified for hybrid ASCI calculation.

As a result of having both indices available, the recommendation to avoid a type I error for data assessment is to preferentially conduct assessment using a combined score assessment when both diatom and hybrid scores are available for a sampling site. If one of the two ASCI scores is equal to or above the published ASCI 10th percentile threshold (0.86), then the site should be considered to be protected. This paired site/score approach is favored as R9 reference site results for both ASCIs failing had similar rates to those observed in the ASCI development dataset (see Table 24).

Finally, we recommend that sampling a stream only once should not be considered sufficient for impairment assessment purposes, especially when only ASCI diatom scores alone are available, as this would make a Type I error more likely. Assessment that relies on multiple samples is also consistent with the current Integrated Report Listing Policy (State Water Board 2015) which requires more than one sample for impairment determination. It is recommended that sites with multiple diatom-only results that are consistently below the 10^{th} percentile threshold be considered impaired in the interim and require supplemental sampling that includes both diatoms and soft algae to further confirm impairment. Alternatively, such sites could be sampled and assessed using algal molecular methods, when available (Theroux et al. 2023).

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