Instructions for Application of the Rapid Screening Casual Assessment (RSCA) Tools v 2.0 in California's Streams

David J. Gillett

Southern California Coastal Water Research Project
3535 Harbor Blvd
Costa Mesa, CA 92626

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This report describes a set of tools that are the product of an ongoing dialog amongst a wide array of researchers working in and around the field of causal assessment. Specifically, these concepts of rapid causal assessment have been improved by the input of Sue Norton, Rafi Mazor, Eric Stein, Scot Hagerthey, Phil Markel, and Josh Westfall. Refinement of data visualizations and summaries were provided by Anne Holt, Robert Butler, Rafi Mazor, and the San Gabriel River Regional Monitoring Program. The readability of the report was improved by comments from Eric Stein, Rafi Mazor, Heather Boyd, and Doug Shibberu. Funding for this work was provided under agreement 17-070-270 between the California State Water Resources Control Board, the Santa Ana Regional Water Quality Control Board, and SCCWRP. Subsequent funding was provided by the San Gabrial River Regional Monitoring Group.

Preface

The goal of this document is to provide instructions on how to perform and interpret a screening-level (i.e., Tier 1) casual assessment with data typically collected as part of freshwater bioassessment monitoring in California. More specifically, it will provide guidance using the data produced by SCCWRP's Rapid Screening Causal Assessment (RSCA) dashboard developed by SCCWRP staff. The underlying tools of the dashboard are an ongoing area of research and as such, the mechanics and breadth of the RSCA tools will continue to evolve. Consequently, it is important to note that these instructions refer to the 01/28/2022 version of the RSCA tools.

Glossary of Terms

Analyte – the actual stressor measurement or calculated metric that represents the presence and magnitude of a given stressor in a stream

Bray-Curtis Dissimilarity – a measure of dissimilarity between two samples based upon the shared identity and abundance of the organism present in the samples. Values can range from 1 – no shared organisms between samples – to 0 – both samples are identical.

Cannot be Evaluated – a stressor module-specific overall result indicating that No Evidence was the result of every line of analysis for that stressor at the test site or that no data were available from the test site for any of the analytes in that stressor module

Comparator Site – a stream location that could support the same biotic assemblage(s) as the test site in the absence of disturbance (i.e., ecologically similar) and therefore a source of biotic and abiotic data that can be used to evaluate the potential impact of a stressor on the biota.

Indeterminate Cause – a stressor module-specific overall result indicating that the integrated lines of analysis do not support the conclusion that the stressor is linked to observed biological condition at the test site, but they do not counter the conclusion either

Indeterminate Evidence – an analyte-specific line of analysis score that indicates that the results of the analysis neither support, nor weaken the notion that the analyte could be responsible for the observed biological condition at the test site

Likely Cause – a stressor module-specific overall result indicating that the integrated lines of analysis support the conclusion that the stressor is linked to observed biological condition at the test site

Module – the organizational unit of stressors within the RSCA toolbox, constructed around broad classes of stressors known to impact streams in California and comprised of specific measures of those stressors typically collected in bioassessment monitoring programs

No Evidence – an analyte-specific line of analysis score that indicates that the analysis could not be conducted at the test site due to, among other things, missing data from comparator sites, or insufficient data to meet the analytical requirements of the line of analysis

No Test Data – an analyte-specific line of analysis score that indicates that the analysis could not be conducted at the test site due to a lack of analyte data at the test site

Passing CSCI – a stressor module-specific overall result indicating that biological conditions at the test site met their management target and therefore causal assessment is not interpretable nor appropriate

Supporting Evidence – an analyte-specific line of analysis score that indicates that the results of the analysis support the notion that the stressor could be responsible for the observed biological condition at the test site

Test Site – the stream location with degraded biotic assemblages and the focus of the causal assessment

Unlikely Cause – a stressor module-specific overall result indicating that the integrated lines of analysis support the conclusion that the stressor is not linked to observed biological condition at the test site

Weakening Evidence - an analyte-specific line of analysis score that indicates that the results of the analysis do not support the notion that the stressor could be responsible for the observed biological condition at the test site

A Short Causal Assessment Summary

Environmental causal assessment is the study of how to diagnose the potential cause or causes that may have led to degraded biological conditions in a waterbody. The goal of a causal assessment is not to characterize general stressors to biology, but to identify the specific stressors that are likely impacting the resident biota of a specific waterbody. Causal assessment is a natural extension of bioassessment, where the condition of resident biota is used to infer the health and integrity of a system (i.e., condition assessment). When an assessment of condition indicates degraded biological conditions, the next step is to identify the reasons potential reasons why (i.e., causal assessment).

A three-tier approach to causal assessment building upon the US EPA Causal Analysis Diagnosis Decision Information System (CADDIS) framework has been developed to inform management of California's aquatic resources:

> **Tier One – Screening Causal Assessment**: An evaluation configured to provide a relatively quick overview assessment and summary of the stressors impacting a system using a standard set of potential stressors, a standardized suite of analytical techniques, and a standardized interpretation framework to characterize the relationship between stressor exposure and biological response. Given its ease of use and relatively quick turnaround time, the screening-level assessment is designed to be applied at a large number of monitoring sites as soon as standardized monitoring data are collected and analyzed. This level of causal assessment could therefore be used to help mangers prioritize remediation efforts within their region of responsibility. This tier produces an assessment of the causality for the most common stressors to a waterbody to better inform and streamline more detailed follow-on analyses.
>
> **Tier Two – Detailed Causal Assessment**: A more involved assessment configured to provide a more thorough investigation of the "standard" stressors identified as likely causes during a screening casual assessment, as well as stressors and environmental characteristics unique to a given location. This level of causal assessment is a stakeholder informed process that uses site-specific data and analyses, with the goal of providing greater confidence on the likelihood of a stressor as a cause. The detailed casual assessment is the appropriate point to incorporate site-specific or less-widely collected data types like those from long-term data loggers, unique analytes, or toxicity identification and evaluation studies as a supplement to the standard bioassessment monitoring data. This tier produces a detailed, rigorous investigation of select stressors impacting a waterbody, providing insight into sources and potential management actions to improve waterbody conditions.

Tier Three – Confirmatory Causal Assessment: An assessment configured to provide the stakeholder and management community with confidence that remediating a given stressor will have a good likelihood of improving the condition of the resident biota in specific system. This level of causal assessment is a very situation-dependent process. It involves experimental manipulations and modelling to demonstrate the effectiveness of potential management actions to improve biotic conditions at a location, as well as set expectations for improvement before large-scale implementation. This tier produces a demonstration of how specific stressors are impacting the biota of a specific waterbody and how their amelioration may be expected to improve conditions there.

The RSCA Conceptual Approach

This document presents guidance on using a series of tools that have been created for conducting a Tier 1 screening causal assessment in a quick, automated fashion. These Rapid Screening Causal Assessment (RSCA) tools work with standardized data types typically collected with protocols developed by the California-wide Surface Water Ambient Monitoring Program (SWAMP) by monitoring surveys like the Perennial Stream Assessment (PSA) program and the Southern California Stormwater Monitoring Coalition (SMC) stream survey. This suite of tools uses multiple lines of evidence (termed lines of analysis) to evaluate the likelihood that one or more of a broad class of stressors could be the cause associated with degraded biological conditions observed within a system (Table 1).

As presently constructed, these tools use California Stream Condition Index (CSCI) (Mazor et al. 2016) scores as the measure of a stream's of biological condition. The CSCI is an index that uses stream benthic macroinvertebrate composition to infer the health and integrity of wadeable streams. The CSCI scores determine whether a site is a candidate for causal assessment (e.g., scores below the designated target for the stream) and serve as the biological endpoint for many of the lines of analysis. It is our goal that in future iterations these tools will be adapted for use with other biotic indicators (e.g., stream algae) and in other environments (e.g., coastal embayments).

Stressors are organized into **modules** that represent broad classes of stressors known to typically affect streams across southern California (Mazor 2015). At present, we have developed modules for eutrophication, elevated conductivity, elevated temperature, and altered habitat. Each of these stressor modules is comprised of a series of **analytes**, which are variables typically measured in streams during routine monitoring and are chosen to represent the expression of the stressor to the resident biota (Table 1). The analytes represent a mix of direct (e.g., % sands and fines or benthic AFDM) and indirect stressors (e.g., total nitrogen or riparian cover) to stream benthic macroinvertebrates and may evolve or expand as additional biological endpoints are incorporated into the framework (e.g., benthic algae).

Each analyte is evaluated with multiple analytical approaches at each **test site** (i.e., the location with degraded biology). All of the analyses utilize a comparative approach, where biotic and abiotic conditions at the test site are compared to the patterns observed at other ecologically similar sites from across California. These **comparator sites** are sites that would be expected to support the same biotic community as the test site in the absence of disturbance and comprise a gradient in biological condition, as well as exposure to stress. Comparator sites are not the same thing as reference sites (sensu Ode et al. 2016), though some California reference sites may be included as comparator sites. Comparator sites are locations with different biological condition and stressor exposure than the test site, but a similar physical and biogeographic setting (Gillett et al. 2019). Using comparator sites to provide data for analysis helps to ensure that the natural, underlying characteristics of a stream are accounted for when identifying the influence of stressors on the resident biota (e.g., the amount of fine grain sediment that may be deleterious to fauna typical to a low-land coastal stream may be different than to fauna typical to a high-elevation mountain stream).

Table 1 – A description of the stressor modules currently covered by the RSCA tools, their component analytes, and the expected, albeit simplified, relationship of those analytes to biological condition in streams.

Module	Direction	Analyte Name
Elevated Conductivity	Negative	Chloride
	Negative	Specific Conductivity
	Negative	Sulfate
	Negative	Total Dissolved Solids
Eutrophication	Negative	Benthic OM Ash Free Dry Mass
	Negative	Benthic Chlorophyll a
	Positive	Dissolved Oxygen
	Negative	Total Nitrogen
	Negative	Phosphorus as P
Altered Habitat	Positive	Evenness of Flow Habitats
	Positive	Diversity of Aquatic Life Habitats
	Positive	Diversity of Natural Substrate
	Negative	Percent Sands and Fines
Elevated Temperature	Negative	Temperature
	Positive	Riparian Cover

Using comparator site and test site data, each analyte is scored as providing **supporting, indeterminate,** or **weakening** evidence that a specific class of stressors could be a cause of the degraded conditions. An analyte can also be scored as **no evidence** if the analysis cannot be conducted due to lack of data or failure of the data to meet underlying assumptions of the line of analysis. **No test data** scores are produced when there are no measurements for the analyte at the test site. The analyte scores within a given line of analysis are then aggregated to provide causal

assessment score for each line of evidence for the stressor module. The scores from each line of analysis are then aggregated to provide an overall causal assessment for that stressor of either **likely cause**, **indeterminate cause**, **unlikely cause**, or **cannot be evaluated** at each test site.

There are four basic steps in the RSCA process (Figure 1):

1. Identifying the test site(s) one is interested in assessing and gathering the required biotic and abiotic data for each one;
2. Identifying comparator sites and gathering the required biotic and abiotic data for each;
3. Evaluating each line of analysis for each analyte;
4. Aggregating the individual scores and lines of analysis to provide an overall causal assessment result.

We have created a web-based dashboard to automate these steps. The dashboard can be accessed from https://rsca.sccwrp.org/sgrrmp#

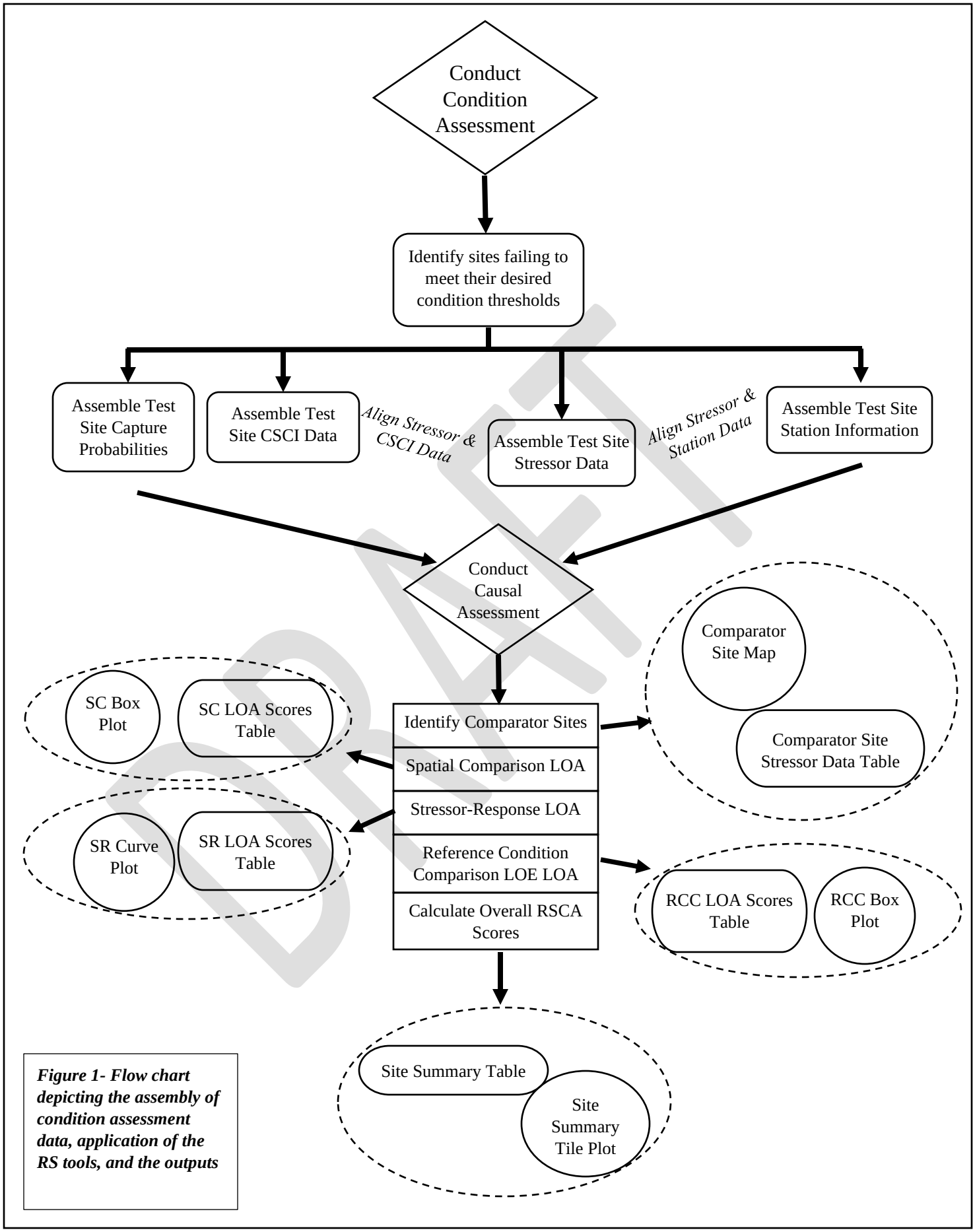

Figure 1- Flow chart depicting the assembly of condition assessment data, application of the RS tools, and the outputs

Using the Tools

Getting started

At its heart, causal assessment is a site-specific analysis. As such, the initial interface with the RSCA dashboard is a map of sites and streamlines across the region (Figure 2). Sites are locations where a condition assessment sample (stream benthic macroinvertebrates (BMI) at present) has been collected, data uploaded to the SMC data portal, and for which a CSCI score has subsequently been calculated (provided GIS metrics required for CSCI calculations have already been submitted to the SMC data portal). Causal assessment results are only interpretable at sites which have sites that have degraded biology. Currently, degraded biology can be defined as a CSCI score below its Stream Classification And Priority Explorer (SCAPE) expectation (Beck 2020) or below the 10th percentile of reference sites (i.e., 0.79) as defined in Mazor et al. (2016). Only sites with degraded biology will have causal assessment results associated with them. Sites with non-degraded biology will be displayed, but all results are categorized as **passing CSCI**.

The RSCA dashboard is presented with several tabs that, proceeding from left to right, provide increasing levels of detail on the causal assessment results and the underlying data used to evaluate a given test site. Selecting a site from the Overview Map tab will populate the

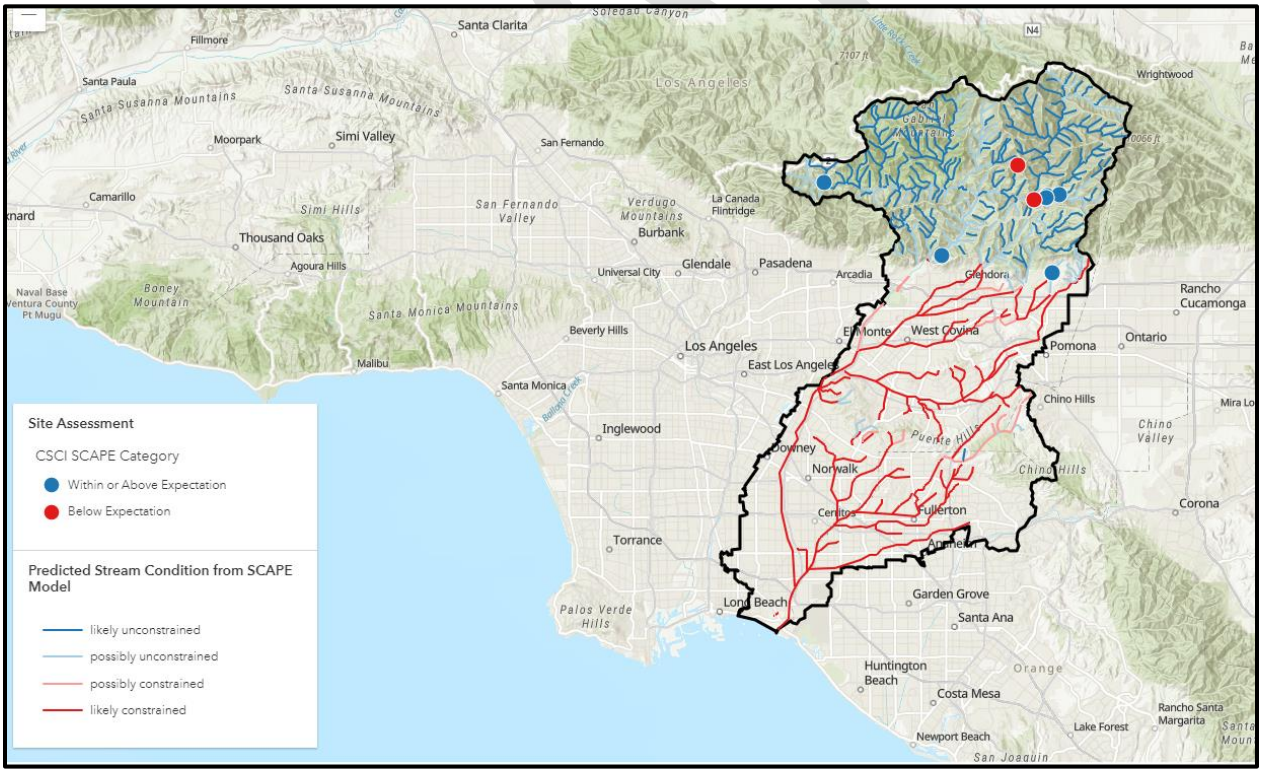

Figure 2 An example of the RSCA Overview Map tab displaying a series of bioassessment sites and streamlines. Sites are categorized by their most recent observed CSCI score relative to their SCAPE expectation and streamlines are categorized by their SCAPE expectation

subsequent tabs with the appropriate information. To see the results from a different site, simply return to the Overview Map tab and click a new site.

Test site data

Though site-specific in nature, the RSCA evaluations are actually done at the sample level. Many potential test sites only have one sample associated with them, so site and sample are equivalent. However, if multiple samples have been collected at a site through time, results are presented sequentially through time and the overview map displays the most recent sample. Only synoptically collected stressor and biological data are associated with each other.

Some sites may have biological or stressor data collected multiple times on the same date (i.e., Field Replicates). If replicate stressor data were collected from a site, then the values are averaged and associated with the single CSCI score. If replicate BMI data were collected, then the sample with the numerically higher CSCI score is used for the RSCA evaluation. However, the data from the replicate BMI samples and their interpretation within the RSCA framework can be viewed on the Line of Analysis (LOA) Results tab by selecting the replicate of interest.

Identifying comparator sites

Comparator sites are identified following the approach described in Gillett et al. (2019) that uses expected biological similarity to measure the ecological similarity between potential comparator sites and the test site. In short, **Bray-Curtis dissimilarity** values are calculated between the expected taxa at the test site and the expected taxa at potential comparator site. Expected taxa lists are obtained from the Observed:Expected model within the CSCI. A site is retained as a comparator if it has a dissimilarly of <0.1 to the test site.

Comparator sites are displayed on a map of California on the Comparator Sites tab (Figure 3). A spreadsheet containing the basic station information – site ID, common name, latitude, longitude, expected similarity to the test site – can be downloaded from the tab as well (Table 2).

The comparator sites are the source of the stressor and biology data used to diagnose the causal relationships at the test site. All of the stressor data used in casual assessment lines of analysis associated with each comparator sites can be downloaded in a spreadsheet from the Comparator Site tab (Table 3).

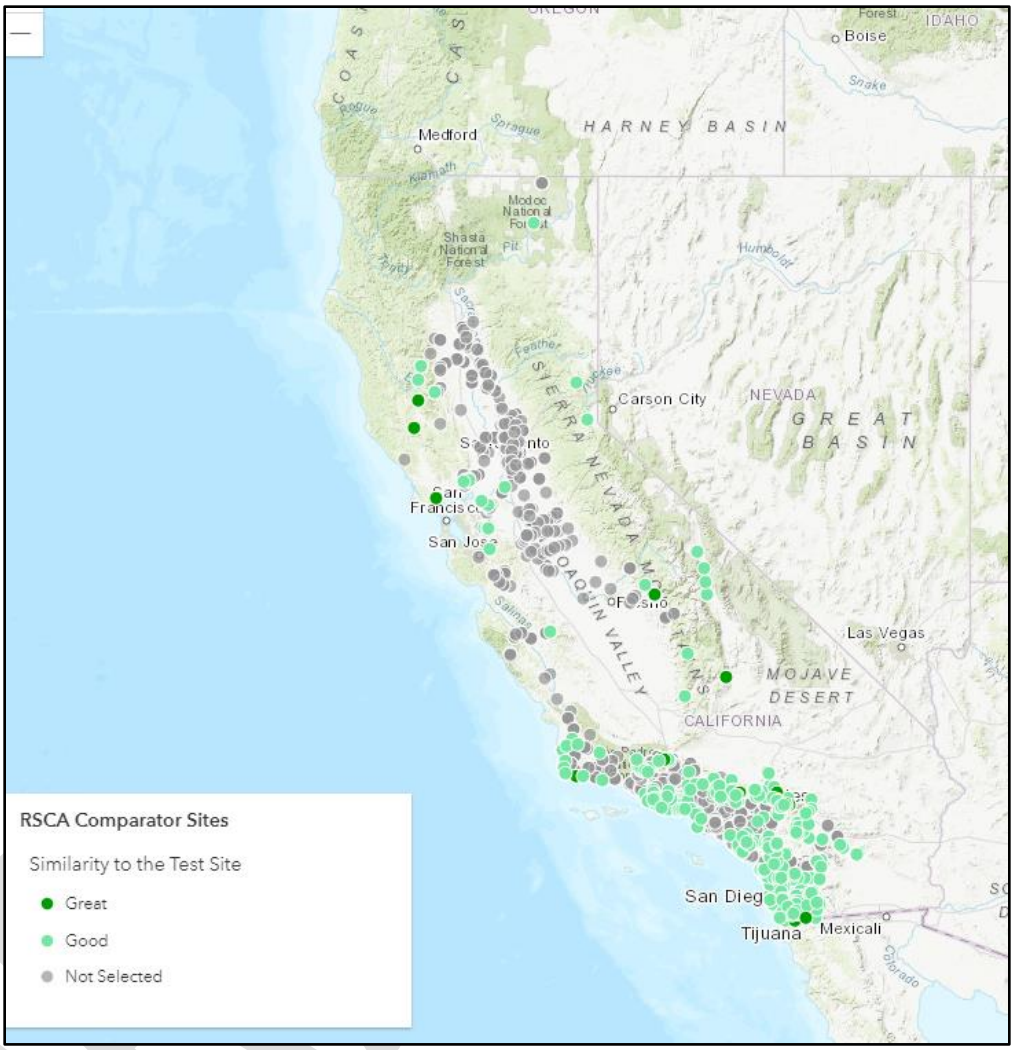

Figure 3 An example of the comparator site map produced for each test site. The map depicts the locations of all potential comparators and highlights those with Great Comparability (≤0.05 BC distance) or Good Comparability (≤0.1 BC distance), which were used in the causal analyses. For context, it also shows not selected for analysis.

Table 2 – A partial example of the Comparator Site Inventory table listing the different comparator sites associated with the selected test site. These tables include the location of the test site, the name and location of the comparator sites and the ecological similarity of the that site to the test site, based upon expected Bray-Curtis dissimilarities.

Test Site	Test Latitude	Test Longitude	Comparator	Dissimilarity	Comparator Quality	Comparator Latitude	Comparator Longitude	County
SMC01096	34.28465	-118.29343	705PCCPCP	0.0694	Good	34.16679	-116.57254	San Bernardino
SMC01096	34.28465	-118.29343	719BMCPRE	0.0330	Great	34.03657	-116.56747	San Bernardino
SMC01096	34.28465	-118.29343	719CE0696	0.0559	Good	33.56833	-116.10667	Riverside
SMC01096	34.28465	-118.29343	719CVSC52	0.0197	Great	33.67242	-116.14923	Riverside
SMC01096	34.28465	-118.29343	719CVSCOT	0.0559	Good	33.52444	-116.07778	Riverside
SMC01096	34.28465	-118.29343	719MISSCK	0.0289	Great	34.00778	-116.62242	Riverside
SMC01096	34.28465	-118.29343	719NP7AZC	0.0786	Good	33.85612	-116.80592	Riverside
SMC01096	34.28465	-118.29343	719NP7BNC	0.0921	Good	33.86821	-116.77257	Riverside
SMC01096	34.28465	-118.29343	719NP7DPC	0.0688	Good	33.63367	-116.39182	Riverside
SMC01096	34.28465	-118.29343	719NP7LBC	0.0179	Great	33.89109	-116.69468	Riverside

Table 3 – A partial example of the Comparator Site Data table listing the stressor module, the Comparator Site station and sample ID, the analyte name, analyte value with units, and the CSCI score of the sample.

Module	Comparator	Comparator Sample ID	Analyte	Analtye Result	Units	Comparator CSCI Score
Eutrophication	705PCCPCP	705PCCPCP_2013-05-21_1_BMI_RWB	AFDM	30.1	g/m2	0.8079
Eutrophication	719BMCPRE	719BMCPRE_2013-05-22_1_BMI_RWB	AFDM	278	g/m2	0.9159
Eutrophication	719BMCPRE	719BMCPRE_2014-04-07_1_BMI_RWB	AFDM	402	g/m2	0.7439
Eutrophication	719BMCPRE	719BMCPRE_2015-04-06_1_BMI_RWB	AFDM	36.7	g/m2	0.7690
Eutrophication	719MISSCK	719MISSCK_2013-05-21_1_BMI_RWB	AFDM	41.8	g/m2	0.9561
Eutrophication	719MISSCK	719MISSCK_2015-04-06_1_BMI_RWB	AFDM	2.03	g/m2	0.6159
Eutrophication	719NP7AZC	719NP7AZC_2014-04-09_1_BMI_RWB	AFDM	4.98	g/m2	0.9399
Eutrophication	719NP7AZC	719NP7AZC_2015-04-07_1_BMI_RWB	AFDM	17.8	g/m2	0.8627
Eutrophication	719NP7AZC	719NP7AZC_2016-05-12_1_BMI_RWB	AFDM		g/m2	0.8920
Eutrophication	719NP7AZC	719NP7AZC_2017-05-16_1_BMI_RWB	AFDM	2.48	g/m2	0.7777
Eutrophication	719NP7BNC	719NP7BNC_2014-04-09_1_BMI_RWB	AFDM	25.9	g/m2	1.0010
Eutrophication	719NP7BNC	719NP7BNC_2015-04-07_1_BMI_RWB	AFDM	18.3	g/m2	0.8262

Spatial Co-Occurrence Line of Analysis

This first line of analysis is designed to compare levels of stressor exposure at the test site to comparator sites in better condition (i.e., higher CSCI scores) than the test site. Like all lines of analysis, Spatial Co-Occurrence is a sample-specific analysis. For each module, this line of analysis produces a table summarizing the pertinent pieces of information used in evaluating each analyte from the Spatial Co-Occurrence LOA perspective and a schematic box plot to illustrate the process visually. If a sample event included a BMI field replicate, the results for replicate are presented separately.

Data are scored for causal assessment within this line of analysis in the following fashion. Within the context of each test site sample, population estimates are created for each analyte based upon values observed at comparator sites with CSCI scores greater than the test site. Analyte values observed at the test site are compared to different population estimates from the comparator sites.

For those analytes that are expected to have a positive relationship to biotic condition:

The test site data are scored as “Supporting” evidence if the value observed at the test site is less than the 25th percentile.

The test site data are scored as “Indeterminate” if the value observed at the test site is between the 25th and 50th percentiles

The test site data are scored as “Weakening” if the value observed at the test site is greater than the 50th percentile.

If there are no data observed at the test site, it is scored as "No Evidence".

For those analytes that are expected to have a negative relationship to biotic condition:

The test site data are scores as “Supporting” evidence if the value observed at the test site is greater than the 75th percentile.

The test site data are scored as “Indeterminate” if the value observed at the test site is between the 75th and 50th percentiles

The test site data are scored as “Weakening” if the value observed at the test site is less than the 50th percentile

If there are no data observed at the test site, it is scored as "No Evidence".

The summarizing table details the scoring of each individual analyte within a given stressor module. The table contains estimates of the 25th, 50th, and 75th percentiles of the analyte observed at better condition comparator sites, the number of better condition comparator sites (n), the line of analysis score (SCO Score). The table also contains the test site SampleID and the measured value of the analyte at the test site (Table 4).

Table 4 – An example of Spatial Co-Occurrence line of analysis summary output.

Module	Direction	Test SampleID	Analyte Name	Test Result	Unit	p25	p50	p75	n	SCO Score
Conductivity	Negative	SMC01096_2010-06-07_1_BMI_RWB	Chloride	8	mg/L	11.5	80	210	1,712	Weakening
Conductivity	Negative	SMC01096_2010-06-07_1_BMI_RWB	SpecificConductivity		uS/cm	415.3	995.2	1600.8	1,712	No Test Data
Conductivity	Negative	SMC01096_2010-06-07_1_BMI_RWB	Sulfate	23.8	mg/L	65.3	251	396.25	1,712	Weakening
Conductivity	Negative	SMC01096_2010-06-07_1_BMI_RWB	Total Dissolved Solids		mg/L	575	830	1158.5	1,712	No Test Data

The data visualization (Figure 4) is a schematic box plot illustrating the position of the test site analyte observation along the distribution of analyte values at the comparator sites. The dashed, horizontal line indicates the analyte value observed at the test site, the color of which represents the SCO Score: a dark grey line indicates No Evidence, red indicates Supporting evidence, blue indicates Weakening evidence, and green indicates Indeterminate evidence. Yellow indicates the test site had a CSCI >0.79 and a causal assessment would not be relevant. No dashed line indicates that the test site was missing data for that analyte. Any instance where there are fewer than five comparator site data points is scored as No Evidence, as the box plot cannot be meaningfully interpreted.

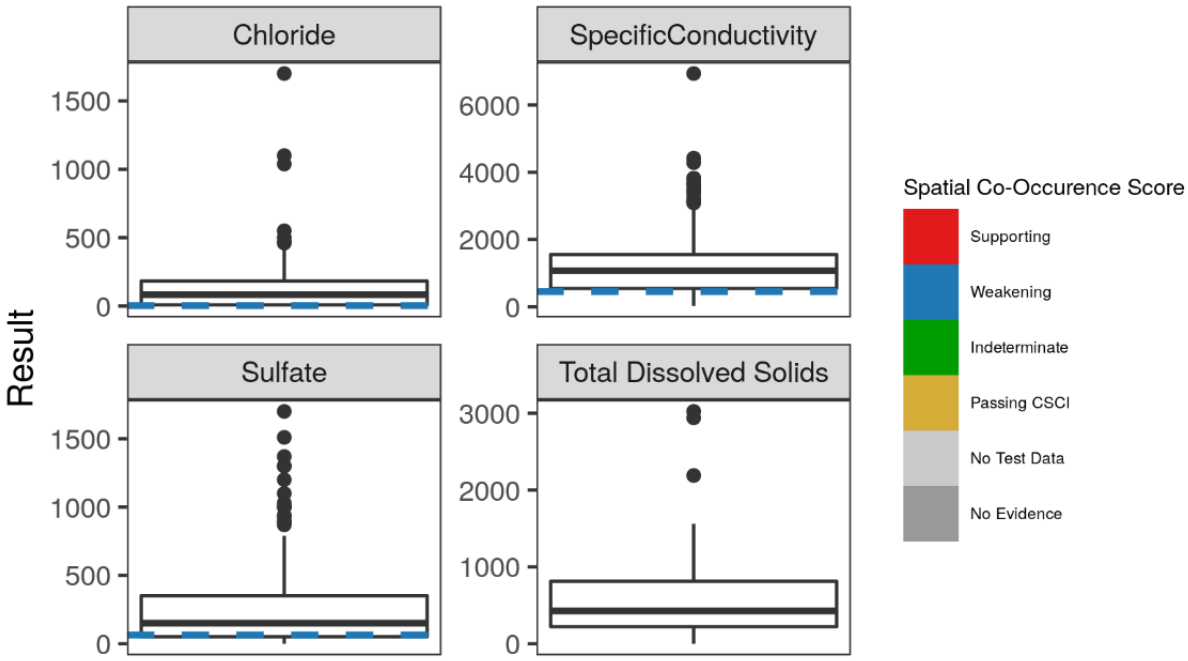

Figure 4 An illustration of the data visualizations that accompany the Spatial Co-Occurrence line of analysis. Plots are schematic box plots of the analyte measured at comparator sites in better condition to the test site. The dashed line indicates the value of the analyte at the test site and is colored to correspond to the causal inference derived from the relationship.

Stressor Response

This second line of analysis is designed to compare the observed measure of stressor exposure and biological response (CSCI score, as currently constructed) at each test site to an expected level of biological response inferred from the pattern in that same combination of stressor and response observed at appropriate comparator sites. Our approach is based upon logistic regression relationships where the probability of observing a CSCI score below 0.79 is predicted given the observed level of stressor exposure. This approach is built around a predicted outcome (i.e., degraded biotic conditions) that is directly related to management of the waterbody. However, if the comparator site dataset has a low number of sites with complete data or the distribution of that data does not cover the full range of biological response and stressor exposure, the resulting logistic regression model may not provide meaningful interpretability. From the causal assessment perspective, this situation would be evaluated as "No Evidence"

Stressor Response is a sample-specific analysis. For each module, this line of analysis produces a table summarizing the pertinent pieces of information used in evaluating each analyte from the Stressor Response LOA perspective and a logistic regression plot to illustrate the process visually. If a sample event included a field replicate, the results for replicate are presented separately.

Data are scored for causal assessment within this line of analysis in the following fashion. A logistic regression model predicting the probability of poor biological condition for a given stressor is created using data from all comparator sites identified for the test site. The suitability of the model for purposes of causal assessment is first evaluated by comparing the direction of the model (positive or negative) with the expected direction of the stressor response model for that analyte (Table 1). If the direction of the model is contrary to the expectation, then the model is rejected as not suitable for the LOA. Secondly, the p-value of the model is used to filter out poorly fitting models. As with any statistical inference using frequentist statistics across multiple different parameters, there is a chance of incorrectly rejecting the null hypothesis (the regression slope (β) =0 in the present example). However, as the p-value is not being used to make the causal association, but only as a manner of quickly filtering out poorly fitted logistic models, we have chosen to not adjust the results for multiple comparisons. Models with p-values greater than 0.1 are considered non-informative and are rejected as not suitable to the LOA. If the model is informative, it is then used to predict the probability of poor condition biota at the test site given the level of the stressor observed at the test site.

Any analyte with a rejected logistic model (wrong direction or poor model fit) are scored as "No Evidence"

If the test site analyte value has a predicted probability >=0.6, it is scored as "Supporting" evidence

If the test site analyte value has a predicted probability between 0.6 and 0.4, it is scored as "Indeterminate" evidence

If test site analyte value has a predicted probability <=0.4, it is scored as "Weakening" evidence.

If the test site is missing data for the analyte, it is scored as "No Test Site Data"

The Stressor Response summary table will contain the predicted probability of degraded biotic conditions (Probability of Poor Condition), the standard error of that prediction (SE of Probability), the overall logistic model p-value (Model *p*-value), and the line of analysis score (SR Score). The table will also contain the test SampleID, the value of the analyte measured at the test site, and the expected direction of the stressor response relationship (Table 5).

Table 4 An example of the summary table associated with the Stressor Response LOA.

Module	Direction	Test Sample ID	Analyte name	Test Value	Unit	Probablity of Poor Condition	SE of Probability	Model *p* -value	SR Score
Conductivity	Negative	SMC00710_2009-05-19_1_BMI_RWB	Chloride	516.09	mg/L	0.965	0.010	4.16E-28	Supporting
Conductivity	Negative	SMC00710_2009-05-19_1_BMI_RWB	SpecificConductivity		uS/cm			2.20E-40	No Test Data
Conductivity	Negative	SMC00710_2009-05-19_1_BMI_RWB	Sulfate	500.44	mg/L	0.682	0.019	5.03E-14	Supporting
Conductivity	Negative	SMC00710_2009-05-19_1_BMI_RWB	Total Dissolved Solids		mg/L			4.14E-09	No Test Data

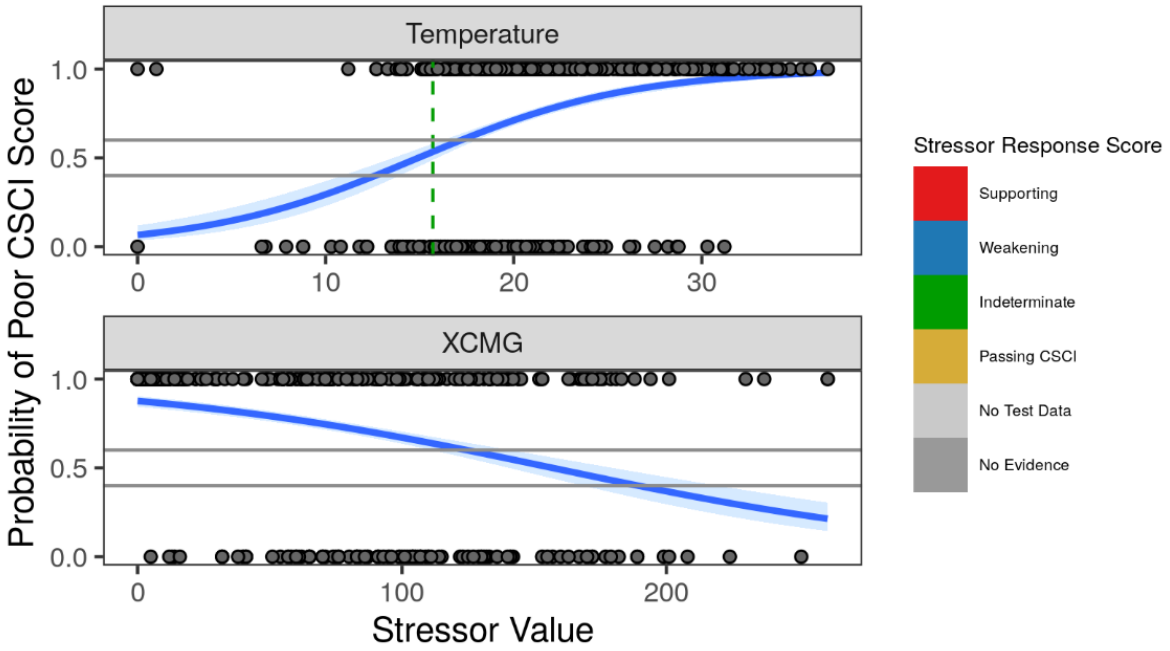

Figure 5 An illustration of the data visualization that is produced by the Stressor Response line of analysis. The graphic is a series of logistic regression plots depicting the relationship between the probability of poor condition biology and different levels of each analyte. The curves are generated from comparator site data. The shaded blue area represents the standard error of the predicted probabilities. The vertical line indicates the analyte value observed at the test site colored to correspond to the causal inference derived from the relationship.

The data visualization (Figure 5) is a logistic regression plot illustrating the position of the test site analyte observation along the logistic stressor response curve derived from the comparator sites. The dots in the rug at 1 and 0 represent the analyte value observed at comparator sites used in the regression with poor or good biology, respectively. The solid, horizontal lines indicate the 0.6 and 0.4 probability of observing poor condition biology based upon the patterns observed at the comparator sites. The dashed, vertical line indicates the analyte value observed at the test

site, the color of which represents the SR Score: a dark grey line indicates No Evidence, red indicates Supporting evidence, blue indicates Weakening evidence, and green indicates Indeterminate evidence. Yellow indicates the test site had a CSCI >0.79 and a causal assessment would not be relevant. No dashed line indicates that the test site was missing data for that analyte.

Reference Condition Comparison

The third line of analysis is designed to compare levels of stressor exposure at the test site to comparator sites that have reference condition biology (CSCI ≥ 0.79). Though conceptually similar to the Spatial Co-Occurrence LOA, this analysis is more sensitive to disturbances and explicitly links levels of stressor exposure to a biological target often used in management of streams. The Reference Condition Comparison LOA is a sample-specific analysis. For each module, this line of analysis produces a table summarizing the pertinent pieces of information used in evaluating each analyte from the Reference Condition Comparison LOA perspective and a schematic box plot to illustrate the process visually. If a sample event included a field replicate, the results for the BMI replicate are presented separately.

Data are scored for causal assessment within this line of analysis in the following fashion. For each test site sample, population estimates are created for each analyte based upon values observed at reference condition comparator sites. Analyte values observed at the test site are compared to different population estimates from the comparator sites. The comparator site data set for each analyte needs to have five or more data points to be considered informative for the causal assessment

With those analytes that are expected to have a positive relationship to biotic condition:

> If the analyte value observed at the test site is less than the 10th percentile, it is scored as "Supporting" evidence.
>
> If the analyte value observed at the test site is between the 10th and 25th percentiles, it is scored as "Indeterminate" evidence.
>
> If the analyte value observed at the test site is greater than the 25th percentile, it is scored as "Weakening" evidence.
>
> If there are no analyte data observed at the test site, it is scored as "No Test Data".
>
> If there are less than five analyte measurements from the comparator sites, it is scored as “No Evidence”.

With those analytes that are expected to have a negative relationship to biotic condition:

> If the analyte value observed at the test site is greater than the 90th percentile, it is scored as "Supporting" evidence.
>
> If the analyte value observed at the test site is between the 90th and 75th percentiles, it is scored as "Indeterminate" evidence.

If the analyte value observed at the test site is less than the 75th percentile, it is scored as "Weakening" evidence.

If there are no data observed at the test site, it is scored as "No Test Data".

If there are less than five analyte measurements from the comparator sites, it is scored as “No Evidence”.

The summarizing table details the scoring of each individual analyte within a given stressor module. The table contains estimates of the 10^{th}, 25^{th}, 75^{th}, and 90^{th} percentiles of the analyte observed at reference condition comparator sites, the number of reference condition comparator sites (n), and the line of analysis score (RCC Score). The table also contains the test site SampleID and the measured value of the analyte at the test site (Table 4).

Table 6 - An example of the summary table associated with the Reference Condition Comparison line of analysis

Module	Direction	Test SampleID	Analyte Name	Test Value	Unit	p10	p25	p75	p90	n	RCC Score
Habitat	Positive	SMC01320_2010-06-07_1_BMI_RWB	Ev_FlowHab	0.36	none	0.074	0.4	0.75	0.88	884	Indeterminate
Habitat	Positive	SMC01320_2010-06-07_1_BMI_RWB	H_AqHab	1.42	none	0.76	1.21	1.65	1.772	884	Weakening
Habitat	Positive	SMC01320_2010-06-07_1_BMI_RWB	H_SubNat	1.5	none	1.057	1.4	1.83	1.92	884	Weakening
Habitat	Negative	SMC01320_2010-06-07_1_BMI_RWB	PCT_SAFN	25	%	10.7	20	50	70	884	Weakening

The data visualization (Figure 6) is a schematic box plot illustrating the position of the test site analyte observation along the distribution of analyte values at the reference condition comparator sites. The dashed, horizontal line indicates the analyte value observed at the test site, the color of which represents the RCC Score: a grey line indicates No Evidence, red indicates Supporting evidence, blue indicates Weakening evidence, and green indicates Indeterminate evidence. Yellow indicates the test site had a CSCI >0.79 and a causal assessment would not be relevant. No dashed line indicates that the test site was missing data for that analyte. Any instance where there are fewer than five comparator site data points is scored as No Evidence, as the box plot cannot be meaningfully interpreted.

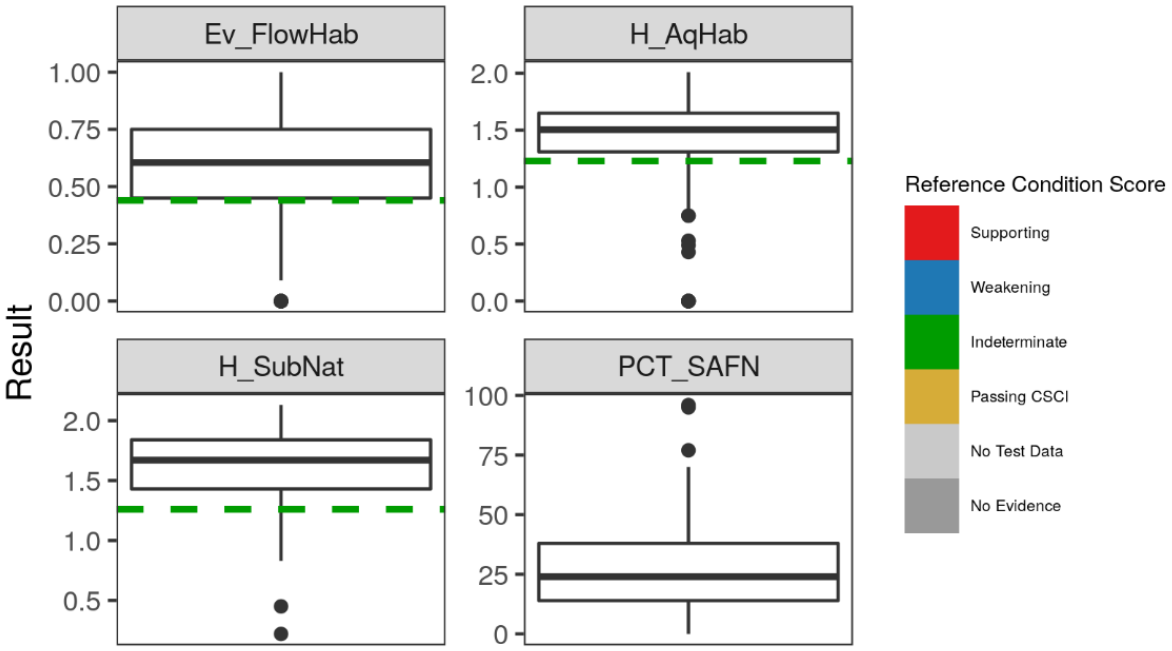

Figure 6 - An illustration of the data visualizations that accompany the Reference Condition Comparison line of analysis. Plots are schematic box plots of the analyte measured at comparator sites in reference condition. The dashed line indicates the value of the analyte at the test site and is colored to correspond to the causal inference derived from the relationship.

Integrating Lines of Analysis Scores

Summary scores for each line of analysis within a stressor module are created by aggregating the scores for the individual analytes associated with that module. These line of analysis-level summary scores are then combined to produce an overall causal assessment result for each stressor module (see below). The line of analysis summaries are sample-specific. If multiple samples on different dates have been collected from a site, they will be concatenated and displayed sequentially (Figure 7).

The summary score for a given line of analysis within each stressor module is determined from the scores of the individual analytes that comprise the module:

- If any analyte within the module was scored as Supporting, then the line of analysis is scored as *Supporting.*
- If no analyte within the module was scored as Supporting and at least one analyte was scored as Weakening, then the line of analysis is scored as *Weakening*
- If all of the analytes within the module were scored as Indeterminate or a mix of Indeterminate, No Test Data, or No Evidence, then the line of analysis is scored as *Indeterminate.*
- If all of the analytes within a module were scored as No Evidence, then the line of analysis is scored as *No Evidence*.
- If all of the analytes within a module were scores No Test Data, then the line analysis is scored as *No Test Data*

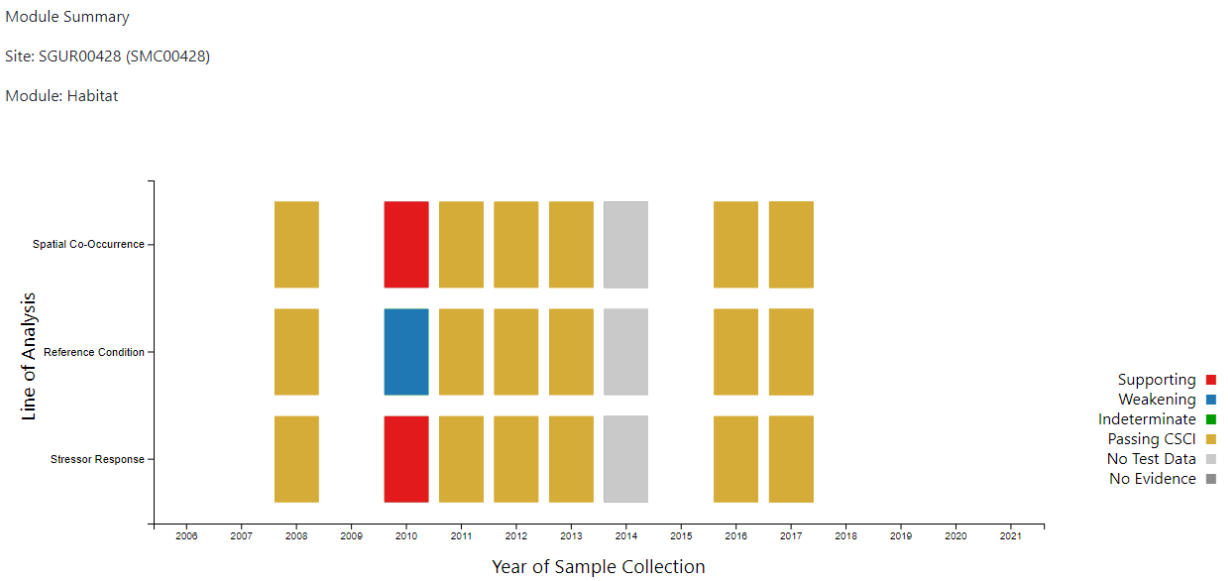

Figure 7 – An example illustration of Module Summary graphics illustrating the summarization for three lines of analysis for a single stressor module at a site that was sampled two times. The color of the cell corresponds to the Line of Analysis score.

The overall RSCA result for each stressor module is determined by integrating the Line of Analysis scores from the summary scores of the lines of analysis analyzed for that stressor module. The RSCA results are sample- and stressor-specific. If multiple samples have been at multiple dates have been collected from one site, the results are concatenated and presented sequentially together through time (Figure 8). Similarly, each stressor is considered discrete from the others to allow the user to consider or prioritize each result as they see fit. Overall, module-level results are scored as follows:

- If there are more lines of analysis with summary scores of Supporting evidence than there are with scores of Weakening evidence, then the overall result is that the stressor is a *Likely Cause* of the observed biological condition.
- If there are more lines of analysis with summary scores of Weakening evidence than there are with Supporting evidence, then the overall result is that the stressor is an *Unlikely Cause* of the observed biological condition.
- If there are the same number of lines of analysis scored as Supporting and Weakening evidence, then the overall result is that the stressor is an *Indeterminate Cause*
- If all of the lines of analysis are scored as Indeterminate evidence or a mix of Indeterminate and No evidence, then the overall result is that the stressor is an *Indeterminate Cause*.
- If all the lines of analysis are scored as No Evidence or No Test Data, then the overall result is that the stressor *Cannot be Evaluated*.

A tabular version of the summarized module and line of analysis results (Table 7) can be downloaded in a spreadsheet workbook from the Site Summary tab within the RSCA dashboard. Bundled with these tables will also be the summary results for the Spatial Co-Occurrence (Table

4), Stressor Response (Table 5), and Reference Condition Comparison (Table 6) lines of analysis. Additionally, a table of Monitoring Recommendations can be downloaded from the Site Summary tab. The Monitoring Recommendations present the data inventory for all analytes at the test site and the comparator sites used in the RSCA analysis. It will highlight any potential data gaps where a single analyte (high priority to follow up) or all analytes (very high priority to follow up) from each stressor module may be missing from the test site. The Monitoring Recommendations report similarly summarizes the data density for each analyte among comparator sites to highlight any gaps that, if filled, may produce a more accurate causal assessment.

Table 7 – An example of the Site Summary table detailing the RSCA results for four stressor modules at a site that was sampled twice. Additionally, Site name, comid, latitude, longitude, and sample IDs are presented with the CSCI score from each sampling event and the SCAPE expectations for the site.

Test Site	Comid	Latitude	Longitude	Sample Date	Test SampleID	Test CSCI	10th Precentile SCAPE	50th Percentile SCAPE	90th Percentile SCAPE	CSCI Relative to Scape	CSCI Relative to Reference	Conductivity	Eutrophication	Habitat	Temperature
SMC01096	22514542	34.28465	-118.29343	6/7/2010	SMC01096_2010-06-07_1_BMI_RWB	0.583	0.636	0.832	1.041	Below SCAPE Expectation	Failing CSCI	Unlikely Cause	Unlikely Cause	Unlikely Cause	Likely Cause
SMC01096	22514542	34.28465	-118.29343	6/14/2016	SMC01096_2016-06-14_1_BMI_RWB	0.770	0.636	0.832	1.041	Meeting SCAPE Expectation	Failing CSCI	Indeterminate Cause	Indeterminate Cause	Likely Cause	Indeterminate Cause

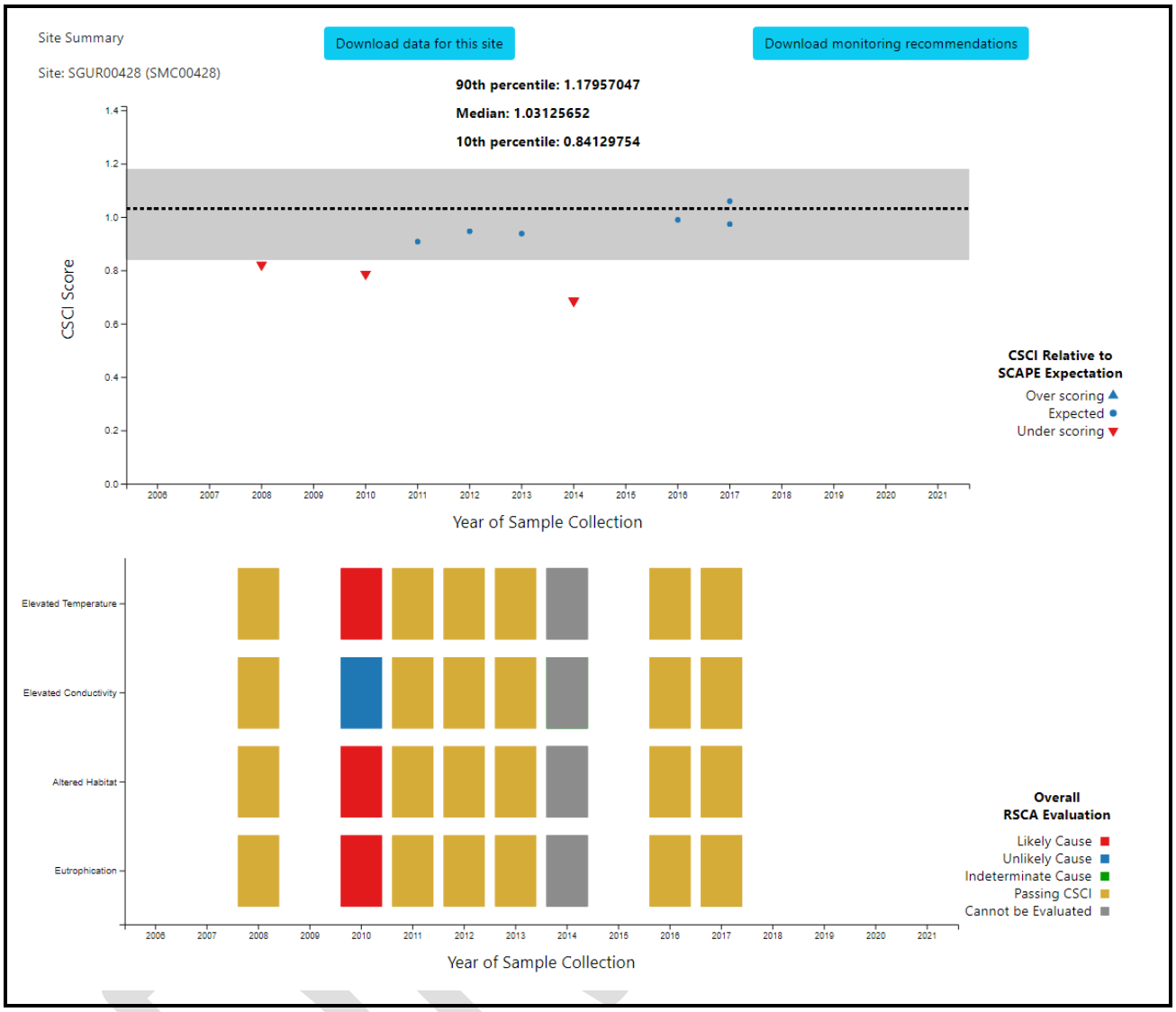

Figure 8 – An example illustration of Site Summary graphics illustrating the summarization for the RSCA results for four stressor modules at a site that was sampled two times. The color of the cell corresponds to the Overall RSCA Result. The top panel depicts CSCI scores observed at the test site in the context of their SCAPE expectation (grey polygon) and the 0.79 reference/non-reference threshold (black dashed line).

Guidance on using RSCA outputs

The outputs of the RSCA dashboard were designed to be useful to multiple end-users, but also evolve as new analyses and visualization techniques are incorporated. The dashboard and the RSCA outputs provide multiple levels of detail and complexity that can be used in a variety of ways: 1. The site-level summary provides the high-level snapshot of the potential problems at a test site; 2. The analysis of different stressor metrics at the test site, provides detailed insight into the magnitude and nature of stressors contextualized within the different lines of evidence; 3. The underlying data downloads, provides the biotic and abiotic monitoring data from a test site

and its comparators in a thoughtful way to facilitate further exploration of the data beyond the RSCA construct.

It is important to consider that these are screening-level results designed to standardize the causal assessment process in a quick and easy to communicate manner, as well as set the stage for more efficient follow-on causal assessment actions than the traditional causal assessment process could. These results are not necessarily the ultimate causal diagnoses of poor-quality biology at a location, but rather should be used to guide further exploration or planning at a site to eventually improve its condition.

The user can work with these data in any fashion that they see fit, but the following represents our suggestions on how to maximize the RSCA outputs to understand a given site and work towards improving its condition. Our suggestions are framed around the flow of events in the idealized bioassessment of a given site: 1. Collection of monitoring data; 2. Assessment of biological condition; 3. Application of the RSCA tools at sites failing to meet their designated condition goals; 4. Conducting a follow-on, detailed causal assessment to refine the identity of the causative agents; 5. Characterization of stressor sources and potential mitigation/remediation options for causative agents; 6. Conducting a confirmatory causal assessment based on potential mitigation options.

One of the first things to consider when interpreting the site-level summary of causality at the test site is the time frame of the result. If the RSCA results are based upon test site data that was collected more than 5 years ago, we would strongly suggest revisiting the site to re-evaluate the biological condition and the levels of stressor exposure at the test site before any further follow-on actions are taken. Similarly, if there were any notable changes in the watershed of a site (e.g., fire, drought, hydromodification) between the collection of data used in the RSCA and the present day, we would strongly suggest revisiting the site to confirm the biological condition and the levels of stressor exposure at the test site before any further follow-on actions are taken. If the results are based on data from less than 5 years ago and the watershed of the site is relatively stable, we would suggest that revisiting a site is at the discretion of the vested parties and the strength of the causal results.

The temporal context of site summary RSCA results can also extend to the repeated sampling of a site through time. At present, there is no consensus approach on how to best integrate RSCA results at a site through time, or even if they should be integrated. Options can range from making decisions based upon the most recent set of results, weighting results based upon their proximity to the present day, focusing on the most frequently observed result for a stressor, or focusing on the most contiguous result. If a test site has been sampled multiple times, we suggest investigating the consistency of results through time before considering follow-on actions at a test site. If the patterns in how a given stressor type is evaluated through time is highly variable (e.g., switching between likely or unlikely), we would suggest further investigating if the causal evaluation coincided with external environmental variables that could exacerbate or mute the stressor impact (rainfall, air temperature, etc.) (e.g., Beck and Mazor 2020). If the patterns in how a given stressor type is evaluated is less variable (e.g., switching from indeterminate to likely or unlikely), we would suggest looking at the analyte-to-analyte, line of analysis results to

characterize the stability and magnitude of the stressor exposure measurements to guide any follow-on actions.

When reviewing the site-level results and determining the next steps of action, it can be useful to review the patterns within the individual lines of analysis. Understanding the causal patterns among the individual analytes of a given stressor module can provide insight into the nature of the stressor (e.g., eutrophication stress supported by total nitrogen, but not phosphorus). Reviewing the individual analytes may also provide insight into how any follow-on monitoring and detailed causal assessment could be structured – e.g., eutrophication was supported by total nitrogen and phosphorus, but the test site lacked data on chlorophyll a, benthic organic matter, and dissolved oxygen. Collection of the unsampled metrics could provide a more well-rounded picture of the site's status. Deployment of dissolved oxygen data loggers, collection of harmful algal bloom toxins, or profiling of the algal community could provide stronger links to BMI-based impacts.

The basic level of interpretation with the RSCA outputs is at discrete sites. However, it can be instructive to view the results of multiple causal assessments across a region together on the Overview Map tab. Visualizing spatial patterns of the results may help illustrate patterns across or between watersheds that may lend themselves to different remediation options – Does only one site within a watershed have poor condition biological assemblages? Do multiple sites? Do the sites have similar likely/unlikely stressor diagnoses? Are they contiguous along a series of stream reaches or are they dispersed across a watershed?

The individual analytes that comprise the different stressor modules of the RSCA were selected to balance their ability to characterize different aspects of the stressor classes with the widespread availability of those data types in the region and the state's monitoring programs. There may be other measures that more perfectly capture the exposure of a given stressor to the biotic community, but that are not commonly included in bioassessment monitoring programs (e.g., spot measures of temperature vs. long term data obtained with deployed loggers). As such, reviewing the individual analyte patterns that comprise the RSCA result may inform the application of other data from the site (or nearby locales) to support or weaken the case for a given stressor. Alternatively, analyte patterns could inform the collection of other stressor related data from the test site in a follow-on monitoring or causal assessment action (Table 8).

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