Causal Assessment Evaluation and Guidance for California Appendix C - San Diego River Causal Assessment Case Study

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EXECUTIVE SUMMARY

A causal assessment was conducted in the lower reaches of the San Diego River in San Diego, California. This assessment was conducted to determine the causes behind observed biological impairments in the stream. Specifically, the impairment in the San Diego River was a very low (7.14 out of 100) Southern California Index of Biotic Integrity (IBI) (Ode et al. 2005) score observed in 2010 at the long-term monitoring site designated as the Mass Loading Station (MLS). Four upstream monitoring sites along the San Diego River (TWAS 1, TWAS 2, TWAS 3, and Cedar Creek) were selected as the comparator sites. All of the sites, with the exception of Cedar Creek, had equally poor IBI scores to the test site. To better differentiate among the sites, three submetrics of the Southern California IBI: 1.) The % abundance of collector-gatherer taxa (e.g., *Baetis* spp); 2.) The % of non-insect taxa (e.g., oligochaetes); and 3.) The % of tolerant taxa (e.g., *Physa* spp.), as well as the relative abundance of amphipods, were used as biological endpoints in a number of the analyses.

This causal assessment was performed following the USEPA's CADDIS causal assessment framework (USEPA 2000). In brief, this approach consists of: 1.) Identifying a site with biological impairment (test site); 2.) Selecting similar sites within the same stream network for comparison (comparator sites); 3.) Identifying the potential stressors to the stream (candidate causes); 4.) Analyzing differences in stressors, biology, and their interaction at the test and comparator sites (within the case); 5.) Comparing stressors, biology, and their interaction at the test site to similar data from elsewhere (outside the case); and 6.) Summarizing these results into a narrative classifying the potential stressors as likely, unlikely, or uncertain causes to the biological impairment.

This assessment was conducted as a partnership between the Southern California Coastal Water Research Project, the City of San Diego, the County of San Diego, and the San Diego Regional Water Quality Control Board. The assessment partners decided to focus on five candidate cause stressors potentially responsible for the biological impairment observed at the MLS site in San Diego River: 1.) Altered physical habitat; 2.) Metals; 3.) Elevated conductivity; 4.) Increased nutrients; and 5.) Pesticides. These stressors were chosen by the group based upon input from the local stakeholders familiar with the stream, the watershed characteristics, and potential anthropogenic disturbances to the system. Each one of these candidate causes was comprised of a number of proximate stressors (e.g., dissolved metals, sediment-bound metals, periphyton-bound metals), which represent the potential direct insult to the biota from the candidate cause. Data were not available for every proximate stressor within each candidate cause at every site (e.g., herbicides or sediment-bound metals), but enough data were available for some degree of evaluation for all five of the candidate causes.

The causal assessment was conducted with the preexisting data provided by the different partners. All of the sites are part of the City and County of San Diego's National Pollutant Discharge Elimination System (NPDES) monitoring sites established by the San Diego County Municipal Stormwater Permit from 2007 for San Diego River. The chemical, biological (benthic macroinvertebrates), and physical habitat data from this program provided the bulk of the information needed for the within the case portion of the causal assessment. These data were also augmented with data from additional studies conducted at the test and comparator sites in 2010 looking at algal community structure and sediment-bound synthetic pyrethroids. Data used in the outside the case portion of the causal assessment were assembled from a variety of sources, including :the State of California's Reference Condition Monitoring Program (RCMP), the Surface Water Ambient Monitoring Program (SWAMP) ,various probabilistic stream biomonitoring programs (e.g.,

Perennial Stream Assessment [PSA] and Stormwater Monitoring Coalition [SMC]), and appropriate examples from the scientific literature.

Within the CADDIS causal assessment framework, there are a number of potential types of evidence (i.e., analyses) that can be brought to bear in the within the case and the outside of the case portions of the assessment. The spatial temporal co-occurrence and stressor-response types of evidence were used in the within the case step. The field stressor-response, laboratory stressor-response, and reference condition comparison evidence types were used in the outside the case step.

The overall results from the causal assessment are summarized in Table ES-1. Of the five candidate causes, there was supporting evidence that elevated conductivity and pesticides (specifically, synthetic pyrethroids) may be partially responsible for the impaired biological condition at the test site. Conversely, the evidence indicated that dissolved metals in the water column were not a cause for the impairment. There was inconsistent or contradicting evidence for both nutrients and altered physical habitat from within the case. Furthermore, there were limited data available for these candidate causes in the outside the case portion of the assessment. Consequently they were ruled as indeterminate; not excluded, but not confirmed as causes for the observed biological impairment.

The most confident conclusions that could be made about candidate causes were those examples where both within the case and outside the case data were available. For the stressor-response and reference condition comparison outside of the case evidence types, data were selected from sites with similar geographic/environmental characteristics to the MLS site. Sites were selected to reduce the variability in the observed biological communities due to non-anthropogenic forcing factors (e.g., elevation, slope, or underlying geology) known to have an influence on benthic macroinvertebrate community structure (e.g., Allan 2004, Mykrä et al. 2008). These outside of the case evidence types were extremely valuable in the causal assessment process, as there was pervasive impairment, not only at the test site, but at nearly all of the comparator sites. This pattern of impairment weakened our confidence in the diagnostic power of the within the case data used by itself. Contextualizing the stressors and observed biotic response(s) with data from outside the case allowed us to come to more definitive conclusions about the role of conductivity and metals in the observed impairments. Conversely, the lack of these types of evidence was one of the contributing factors to our uncertainty about the roles of nutrients and alteration of the physical habitat.

The assessment provided enough evidence that, based upon the available data, allowed us to exclude one candidate cause (metals [dissolved metals]) and indicate two others (conductivity and pesticides [synthetic pyrethroids]). As noted previously, this success was due in large part to the ability to bring in data from environmentally similar streams from outside the watershed to compare against data from within the stream.

Table ES-1. Summary outcome of the potential influence of the five candidate cause stressors on the impaired benthic macroinvertebrate community observed at the MLS site in the lower San Diego River in 2010.

Outcome	Candidate Cause	Evidence & Comments
Probable Stressors	High Conductivity	Elevated conductivity and total dissolved solids at MLS compared to within and outside the case sites. Consistent stressor response relationship w/ non-insect taxa and amphipods within and outside the case.
	Pesticides	Elevated levels of water column cyhalothrin-λ, fenvalerate, and sediment bifenthrin at MLS compared to comparator sties. Stressor response relationship w/ non-insect taxa within the case. No data were available for herbicides.
Unlikely Stressors	Heavy Metals	Elevated levels of some dissolved metals at MLS compared to comparator sites, but not at toxic levels and inverse stressor response relationships from within the case. No data were available for inference about sediment- or periphyton-bound metals and they could not be refuted.
Unresolved Stressors	Altered Phyical Habitat	Mixed levels at MLS compared to comparator sites. Inconsistent/indeterminant stressor response relationship within and outside the case. Some evidence for sands & fines and habitat simplification. Limted data from outside the case.
	Nutrients	Mixed levels of nutrient responses at MLS compared to within and outside the case. Weakening evidence of stressor-relationship with amphipods within the case. No data from outside the case were available.

CASE DEFINITION

This causal assessment was conducted as a response to very low Southern California Index of Biotic Integrity (IBI) (Ode et al. 2005) scores observed in the lower reaches of the San Diego River in 2010. The assessment was conducted as a partnership between the Southern California Coastal Water Research Project, the City of San Diego, the County of San Diego, and the San Diego Regional Water Quality Control Board. The actual test site for the assessment was the long-term monitoring site (MLS [mass loading station]) located east of US Interstate 5 and west of Route 163 in San Diego, CA, adjacent to the Fashion Valley shopping center. The Southern California IBI is a multi-metric index that uses community structure of benthic macroinvertebrates to evaluate the condition of a stream. During the 2010 sampling, the MLS site had a score of 7.14; well below the existing threshold of two standard deviations of the mean score of reference sites (39).

The MLS test site was within the most downstream portions of the river that are above tidal influence (D. Gillett, per obs) and the comparator sites, TWAS-1 (Temporary Watershed Assessment Station), TWAS-2, TWAS-3, and CC (Cedar Creek), were located progressively upstream (Figure 1). The test and comparator sites comprised the within the case portion of the assessment. The MLS and comparator sites were part of the San Diego County Municipal Storm Water National Pollutant Discharge Elimination System (NPDES) monitoring sites as established by the San Diego County Municipal Stormwater Permit from 2007. As part of the NPDES monitoring network, the test and comparator sites had biological, chemical, and physical habitat data from the 2010 period of interest (Table 1). Duplicate samples were collected at the TWAS 2 site (designated TWAS 2-2) and were kept as a distinct comparator. As such, there were five potential comparisons that could be made in the within the case portion of the assessment. Additionally, there was also monthly chemistry/water quality data collected from most of the sites in the time prior to the collection of the biological data. These data formed the core of the comparative analyses that made up the within case lines of evidence.

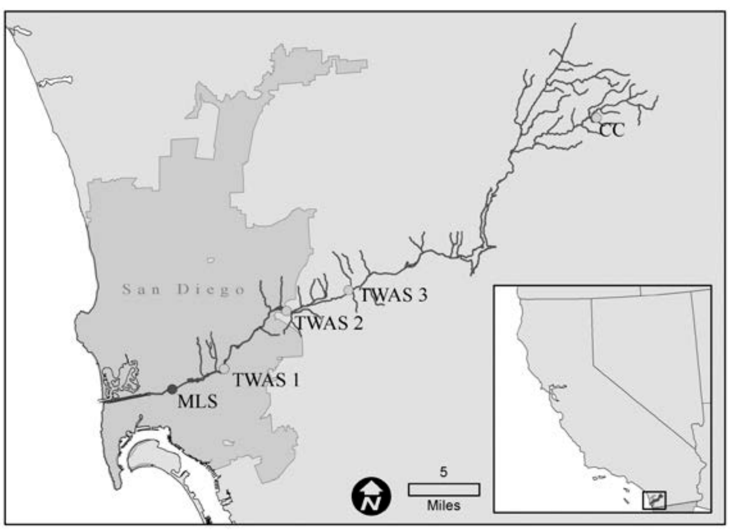

Figure 1. A map of the San Diego River showing the location of the MLS test site and the comparator sites TWAS 1, TWAS 2, TWAS 3, and CC. Inset with a map of the west coast of US for reference.

Table 1. Inventory of the type of data and its original source used in the analysis of each candidate cause and their component proximate stressors for each line of evidence used in the causal assessment.

Candidate Cause/ Conceptual Diagram	Proximate Stressor	Data Available	Data Source	Data Within the Case Lines of Evidence		Data From Outside the Case Lines of Evidence		
				Spatial Co-Occurrence	Stressor-Response From the Field	Reference Condition Comparison	Stressor Response From the Field	Stressor Response From the Lab
Elevated Conductivity	Increased Conductivity	Point Measurement of Conductivity (mmhos cm^{-1}) during biological sampling	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	Comparison of MLS to environmentally similar reference sites.	Relative risk calculation at stressor level observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	No data available
	Increased TDS	Mean of grab sample measurements of Dissolved Solids (mg L^{-1}) and Hardness (mg $CaC0_3$ L^{-1}) collected January - May	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	No data available	No data available
Increased Nutrients	Change in Food Source	Euclidean distance from MLS location in nMDS comparison of sites based upon the occurrence of course particulate organic matter, macrophyte, filamentous algae, woody debris, and fine sediments. Bray-Curtis similarity to MLS site based upon algal community structure.	NPDES Monitoring and Algal Community Special Study	Comparison of MLS to individual comparator sites in multivariate space	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among the comparator sites.	No data available	No data available	No data available
	Increase in Toxic Algal Compounds			No data available	No data available	No data available	No data available	No data available
	Increase in Algal Mats	Percent of reach with filamentous algae present at time of biological sampling. Mean microalgal mat thickness (mm) within the reach during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	Relative risk calculation at % of filamentous algae observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	No data available
	Increased Frequency of Hypoxia	Frequency of mild hypoxia (2-5 mg O_2 L^{-1}) observed in daytime point measurements during biological sampling. Frequency of hypoxia (<2 mg O_2 L^{-1}) observed in daytime point measures during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	No data available	No data available
	Increased Ammonia Concentration	Maximum observed value (mg L^{-1}) in grab samples from January - May	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	No data available	No data available

Candidate Cause/ Conceptual Diagram	Proximate Stressor	Data Available	Data Source	Data Within the Case Lines of Evidence		Data From Outside the Case Lines of Evidence		
				Spatial Co-Occurrence	Stressor-Response From the Field	Reference Condition Comparison	Stressor Response From the Field	Stressor Response From the Lab
Increased Pesticides	Increased Other Sediment Pesticides			No data available	No data available	No data available	No data available	No data available
	Increased Other Water Column Pesticides	Maximum observed value (mg L^{-1}) in grab samples from January - May for azinphos-methyl, bolstar, chlorpyrifos, danitol, total demeton, diazinon, dimethoate, disulfoton, ethyl parathion, malathion, merphos, methidathion, methyl parathion, mevinphos, phorate, phosmet, tetrachlorvinphos, and tokuthion. Frequency of all other pesticides observed above detection limit from January - May.	NPDES Monitoring	Comparison of individual compounds at MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance for individual compounds among MLS and the comparator sites.	No data available	No data available	Comparison of diazanon, chlorpyrifos, and malathion values observed at MLS to draft species sensitivity distribution (SSD) curves developed by US EPA.
	Increased Water Column Synthetic Pyrethroids	Maximum observed value (mg L^{-1}) in grab samples from January - May for allethrin, bifenthrin, total cyfluthrin, total l-cyhalothrin, esfenvalerate, fenvalerate, permethrin, and prallethrin. Frequency of all synthetic pyrethroids observed above detection limit from January - May.	NPDES Monitoring	Comparison of individual compounds at MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance for individual compounds among MLS and the comparator sites.	No data available	No data available	Comparison of bifenthrin values observed at MLS to species sensitivity distribution (SSD) curves developed by US EPA.
	Increased Other Sediment Pesticides			No data available	No data available	No data available	No data available	No data available
	Increased Sediment Pyrethroids	Observed value (ng g^{-1}) of allethrin, bifenthrin, Total cyfluthrin, total l-cyhalothrin, total cypermethrin, deltamethrin, esfenvalerate, fenvalerate, fluvalinate, permethrin, prallethrin, and resmethrin in sediment grab samples	Sediment Synthetic Pyrethroid special study	Comparison of individual compounds at MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance for individual compounds among MLS and the comparator sites.	No data available	No data available	No data available
	Increased Water Column Herbicides			No data available	No data available	No data available	No data available	No data available

Candidate Cause/ Conceptual Diagram	Proximate Stressor	Data Available	Data Source	Data Within the Case Lines of Evidence		Data From Outside the Case Lines of Evidence		
				Spatial Co-Occurrence	Stressor-Response From the Field	Reference Condition Comparison	Stressor Response From the Field	Stressor Response From the Lab
Habitat Alteration	Change in Food Source	Euclidean distance from MLS location in nMDS comparison of sites based upon the occurrence of course particulate organic matter, macrophyte, filamentous algae, woody debris, and fine sediments.	NPDES Monitoring	Comparison of MLS to individual comparator sites in multivariate space	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among the comparator sites.	No data available	No data available	No data available
	Increase in Channel Depth	Mean of thalweg depth (cm) measured at the transects and inter-transects of the reach during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	Relative risk calculation at stressor level observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	No data available
	Decrease in the extent of Riffle Habitat	Percent of reach with riffle habitat present during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	Relative risk calculation at stressor level observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	No data available
	Decrease in Woody Debris	Length of reach (m) with woody debris during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	Relative risk calculation at stressor level observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	No data available
	Increase in Sands and Fines	Percent of reach with sand or fine sediment substrate during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	Comparison of MLS to environmentally similar reference sites.	Relative risk calculation at stressor level observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	No data available
	Decrease in Substrate Complexity	Euclidean distance from MLS location in nMDS comparison of sites based upon the presence of different substrates (artificial, boulders, roots, woody debris, sands+fines, gravel, cobbles, or bedrock), filamentous algae, overhanging vegetation, undercut banks, large woody debris, and mean thalweg depth.	NPDES Monitoring	Comparison of MLS to individual comparator sites in multivariate space	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among the comparator sites.	No data available	No data available	No data available

Candidate Cause/ Conceptual Diagram	Proximate Stressor	Data Available	Data Source	Data Within the Case Lines of Evidence		Data From Outside the Case Lines of Evidence		
				Spatial Co-Occurrence	Stressor-Response From the Field	Reference Condition Comparison	Stressor Response From the Field	Stressor Response From the Lab
Habitat Alteration (cont.)	Decrease in Cobbles	Percent of reach with cobble substrate and mean percent of cobble embeddedness during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	No data available	No data available
	Increase in Water Temperature	Maximum observed value in point measures collected from January - May.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	No data available	No data available
	Decrease in Extent of Undercut Banks	Percent of reach with undercut banks during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	Relative risk calculation at stressor level observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	No data available
	Lower Dissolved Oxygen	Frequency of mild hypoxia (2-5 mg O_2 L^{-1}) observed in daytime point measurements during biological sampling. Frequency of hypoxia (<2 mg O_2 L^{-1}) observed in daytime point measures during biological sampling.	NPDES Monitoring	Comparison of MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance among MLS and the comparator sites.	No data available	No data available	No data available
Increased Metals	Increase in Dissolved Metals	Maximum observed values (mg L^{-1}) of Arsenic, Cadmium, Chromium, Copper, Nickel, Lead, Antimony, Selenium, and Zinc in grab samples collected from January - May.	NPDES Monitoring	Comparison of individual compounds at MLS to individual comparator sites.	Spearman's rank correlations with percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance for individual compounds among MLS and the comparator sites.	Box plots of available metal concentrations from similar sites with SDR sites imposed	Relative risk calculation at Copper, Lead, and Zinc levels observed at MLS for percent non-insect taxa, percent tolerant taxa, percent collector-gatherer abundance, and percent amphipod abundance using stressor and biological data from environmental similar sites to establish the expectation.	Comparison of Arsenic, Cadmium, Chromium, Copper, Nickel, Selenium, and Zinc values observed at MLS to species sensitivity distribution (SSD) curves developed by US EPA.
	Increase in Particulate Bound Metals			No data available	No data available	No data available	No data available	No data available
	Increased Concentration of Metals in Periphyton			No data available	No data available	No data available	No data available	No data available

Nearly all of the lower San Diego River where the test and comparator sites are located is generally low gradient (<1% slope) with a silty/muddy bottom. The CC site, however, was a higher elevation, higher gradient location, with more complex substrate. Despite being in a heavily urbanized area, there is some riparian vegetation along the length of the test and comparator sites. There was evidence of relatively regular connectivity between the river and the constrained riparian zone/flood plain during high flow events. The river had continuous, perennial flow, which is a function of regular discharges from the upstream El Capitan and Padre dams/reservoirs, miscellaneous point and non-point source urban discharges, and point source industrial discharges to the river. The entire length of the lower San Diego River, where the MLS site is located, is on the 2008-2010 US Environmental Protection Agency (EPA) 303(d) list as impaired for fecal coliform bacteria, *Enterococcus*, dissolved oxygen (organic matter/oxygen depletion), manganese, nitrogen, phosphorus, total dissolved solids (TDS), and toxicity.

The actual biological endpoints chosen as response variables in the assessment, in lieu of total IBI score, consisted of the relative abundance of amphipods and three submetrics of the Southern California IBI: 1.) % abundance of collector-gatherer taxa (e.g., *Baetis* spp); 2.) % of non-insect taxa (e.g., oligochaetes); and3.)% of tolerant taxa (e.g., *Physa* spp.).The three submetrics were chosen because they were of specific interest to the stakeholders and they allowed for greater differentiation among the target and comparator sites (Figure 2). Additionally, as components of the IBI, insights into the poor performance of these metrics should provide direct insights to the causes behind the low overall IBI scores. The relative abundance of amphipods (primarily *Hyalella* spp.and *Americorophium* spp.) was added as an additional biological response metric given their tolerant nature, their dominance at many of the sites, and their affinity for high conductivity conditions.

Macrobenthic community and stream physical habitat data were collected in May of 2010. Macrobenthic community sampling was done with kick-net samples composited from along a 150-m reach, encompassing approximately 1.0 m^2 of streambed. Water quality and water chemistry data were collected as grab or point samples in January, February, and May of 2010. All of the macrobenthic, physical habitat, water quality, and water chemistry data were collected, processed, and analyzed using California Surface Water Ambient Monitoring Program (SWAMP) protocols (Ode 2007, SWAMP Quality Assurance Team 2008, Woodard et al. 2012). The NPDES data were supplemented with algal community and sediment synthetic pyrethroid data. Algae and the sediment samples were also collected and processed using the relevant SWAMP guidelines (SWAMP Quality Assurance Team 2008, Fetscher et al. 2009).

With the exception of the CC site, all of the comparator sites had relatively similar macrobenthic community structure (Table 2) and IBI scores (Figure 2a) to MLS. The macrobenthic communities of the MLS and comparator sites were dominated by amphipods, dipterids, ostracods, and *Physa* spp. These patterns are indicative of degraded macrobenthic conditions.

The statewide perennial wadeable stream assessment data was used for casual assessment (Ode et al. in press). These data from outside the case were comprised of >600 reference sites and >1500 sites with varying level of stress. Streams selected for comparison to the MLS site were filtered for similar natural gradients: slope <1.5%; elevation <333 m. There were 32 samples from 22 reference sites and there were 540 samples from 515 stressed sites.

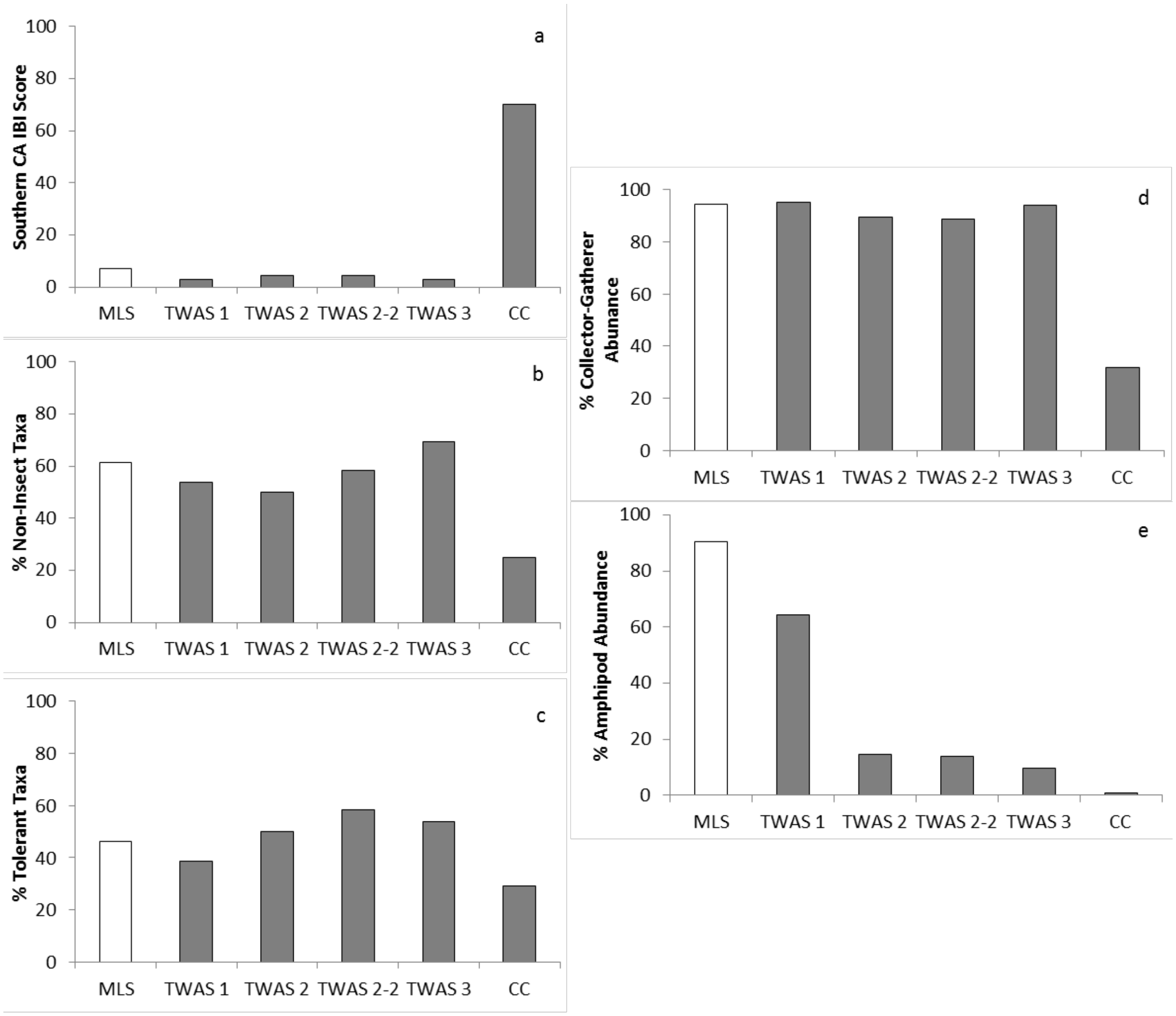

Figure 2. Southern California Index of Biotic Integrity (IBI) scores measured at the target and comparator sites in Spring 2010 (a), as well as the biological endpoints used in the stressor-response portions of the assessment (b – e).

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Table 2. Top 90+ % most abundant taxa at each of MLS and the comparator sites in May 2010.

Site	Order	Taxon	Relative Abundance
MLS	Amphipoda	*Americorophium* sp	59.6
	Amphipoda	*Hyalella* sp	30.9
	Pulmonata	*Physa* sp	2.9
TWAS-1	Amphipoda	*Hyalella* sp	64.4
	Diptera	*Simulium* sp	16.7
		Oligochaeta	5.1
		Ostracoda	2.2
	Pulmonata	*Ferrissia* sp	2.2
	Diptera	*Dicrotendipes* sp	1.0
TWAS-2 (1)		Ostracoda	23.1
	Diptera	*Tanytarsus* sp	15.4
	Amphipoda	*Hyalella* sp	14.7
	Diptera	*Simulium* sp	14.1
	Ephemeroptera	*Baetis adonis*	7.0
	Hemiptera	Corixidae	6.6
		Oligochaeta	3.3
	Diptera	*Micropsectra* sp	2.6
	Ephemeroptera	*Fallceon quilleri*	2.2
	Hypsogastropoda	Hydrobiidae	2.2
TWAS-2 (2)	Diptera	*Simulium* sp	20.7
	Amphipoda	*Hyalella* sp	13.9
	Ephemeroptera	*Baetis adonis*	13.9
		Ostracoda	12.4
		Oligochaeta	12.1
	Hypsogastropoda	Hydrobiidae	4.5
	Diptera	*Tanytarsus* sp	4.3
	Hemiptera	Corixidae	4.3
	Ephemeroptera	*Fallceon quilleri*	2.7
	Ephemeroptera	*Baetis* sp	2.2
TWAS-3	Diptera	*Simulium* sp	47.7
		Oligochaeta	9.9
	Amphipoda	*Hyalella* sp	9.3
		Ostracoda	8.8
	Diptera	*Dicrotendipes* sp	6.2
	Diptera	*Parachironomus* sp	4.9
	Pulmonata	*Physa* sp	3.5
	Diptera	*Chironomus* sp	1.9
Cedar Creek	Pulmonata	*Physa* sp	43.7
	Trichoptera	*Micrasema* sp	19.3
	Ephemeroptera	*Baetis adonis*	12.5
	Diptera	*Simulium* sp	7.1
	Ephemeroptera	*Serratella micheneri*	4.2
	Diptera	Rheotanytarsus	2.5
	Ephemeroptera	Ephemerellidae	2.3

CANDIDATE CAUSES

The following list of candidate causes was developed by the local regulated and regulatory stakeholders at workshop held February 2012. Stressors were proposed and eventually included/excluded for consideration based upon the local stakeholders' knowledge of the San Diego River watershed, the human activities therein, as well as its environmental, geological, and hydrological characteristics. Each candidate cause consisted of a series of proximate stressors, the stressors that are in direct contact with the in-stream biota.

Candidate Cause: Elevated Conductivity –Most freshwater streams have some degree of natural conductivity imparted by the underlying geology of the stream's watershed (i.e., CaO, MgO content). Alterations to that "natural" conductivity level can have adverse effects on macrobenthic community structure reducing the numbers of stenohaline taxa through outright toxicity or increased physiological/osmotic stress that consumes energy normally dedicated to growth and reproduction (Kinne 1971, Hassell et al. 2006). As noted in the conceptual model (Figure 3), there were two proximate stressors within the elevated conductivity candidate cause: 1.) Increased total dissolved solids (TDS); 2.) Increased conductivity

Candidate Cause: Habitat Alteration – Most wadeable streams have a high degree of physical habitat heterogeneity (e.g., riffles vs. pools, woody debris, undercut banks) at small spatial scales (10's of meters) that produce a multitude of different niches, which are in turn occupied by different macroinvertebrate species. This habitat heterogeneity increases the overall diversity of the macrobenthic community because individual taxa are often dependent on specific habitat characteristics (e.g., complex structure, fast moving water, or deep pools). Habitat alteration can have negative effects on the macrobenthic community. Habitat alteration ranges from direct modification of the stream bed and channel walls for flood or erosion control (concrete or rip rap walls), to modification of the riparian corridor, or development within the stream's upland watershed. Habitat alteration reduces habitat complexity and heterogeneity, acting as a barrier for certain taxa to recruit or survive in-stream. In the conceptual diagram (Figure 4), habitat alteration has 10 potential proximate stressors: 1.) Change in available food; 2.) Increase in channel deepening, 3.) decrease in the amount of riffle habitat, 4.) decrease in the amount of instream wood debris; 5.) increase in sands and fines; 6.) increase in water temperature; 7.) increase in the extent of undercut banks; 8.) increase in low dissolved oxygen; 9.) decrease in the number of cobbles; and 10.) decrease in overall substrate complexity.

Candidate Cause: Metals – While there are some natural sources of metals to streams due to the erosion of metal bearing soils in the underlying geology of a watershed (e.g., Aluminum or Iron), most metals observed in streams are related to anthropogenic activities. Most metals impact stream macroinvertebrates by causing cell wall failure, interference with ion transfer, and interference with respiratory function. Metals can be transferred to stream biota either through direct ingestion or absorption from the water column. Consequently, the conceptual model for increased metals (Figure 5) has three proximate stressors: 1.) increase in dissolved water column metals; 2.) increase in metal concentration of periphyton; and 3.) increase in particulate bound metals.

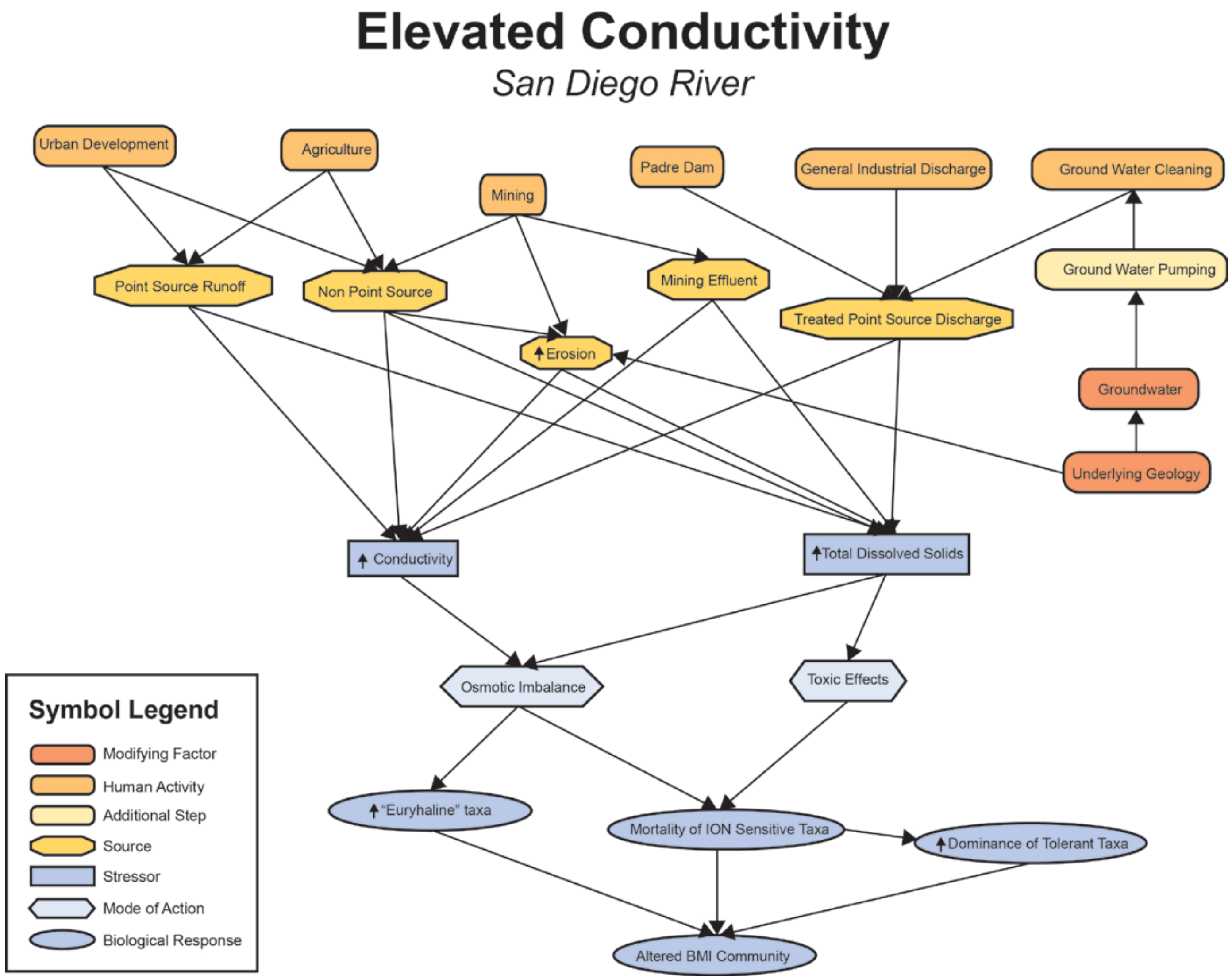

Figure 3. Elevated conductivity conceptual diagram detailing proximate stressors, potential sources, and potential modes of action to impacting the macrobenthic community.

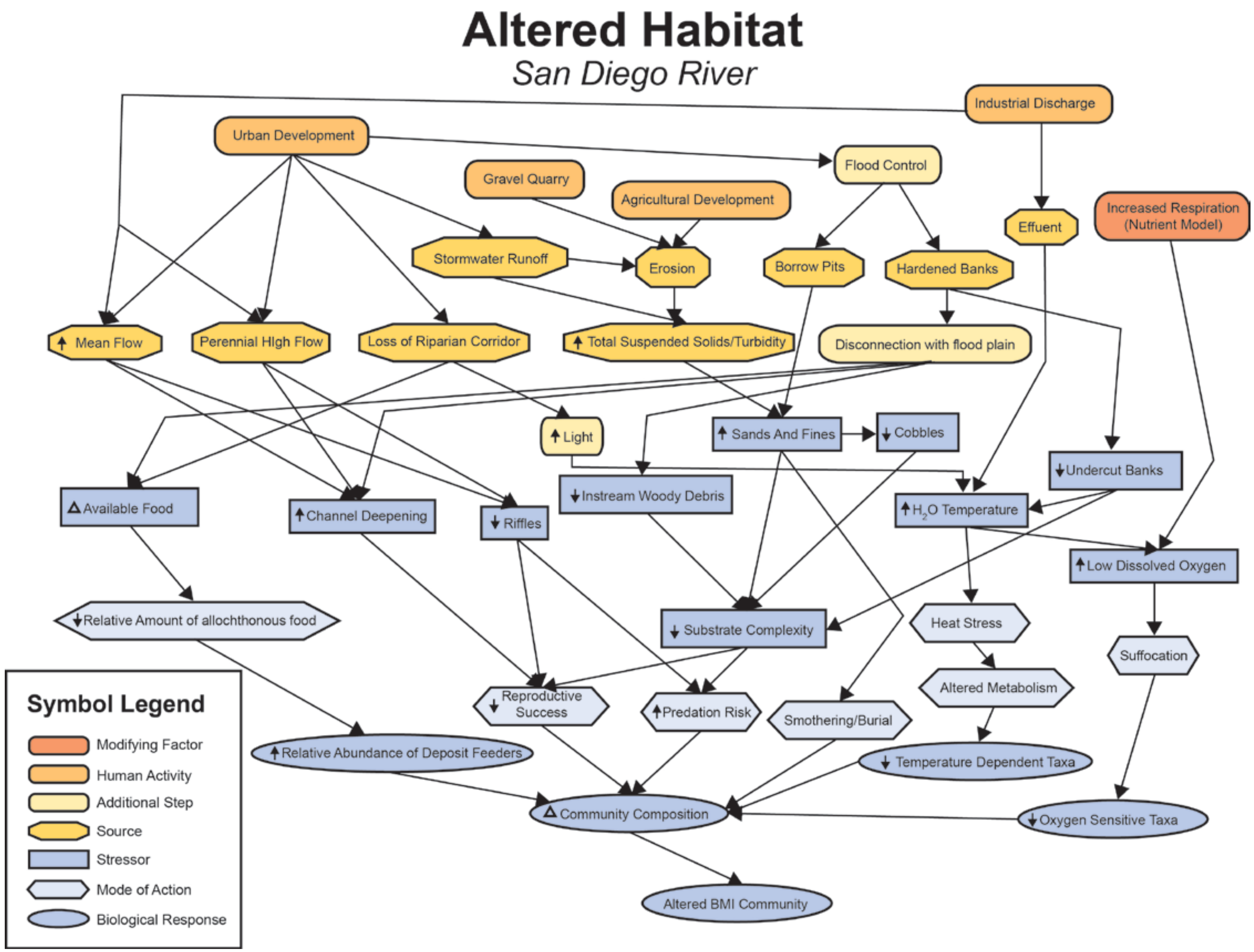

Figure 4. Pesticides conceptual diagram detailing proximate stressors, potential sources, and potential modes of action to impacting the macrobenthic community.

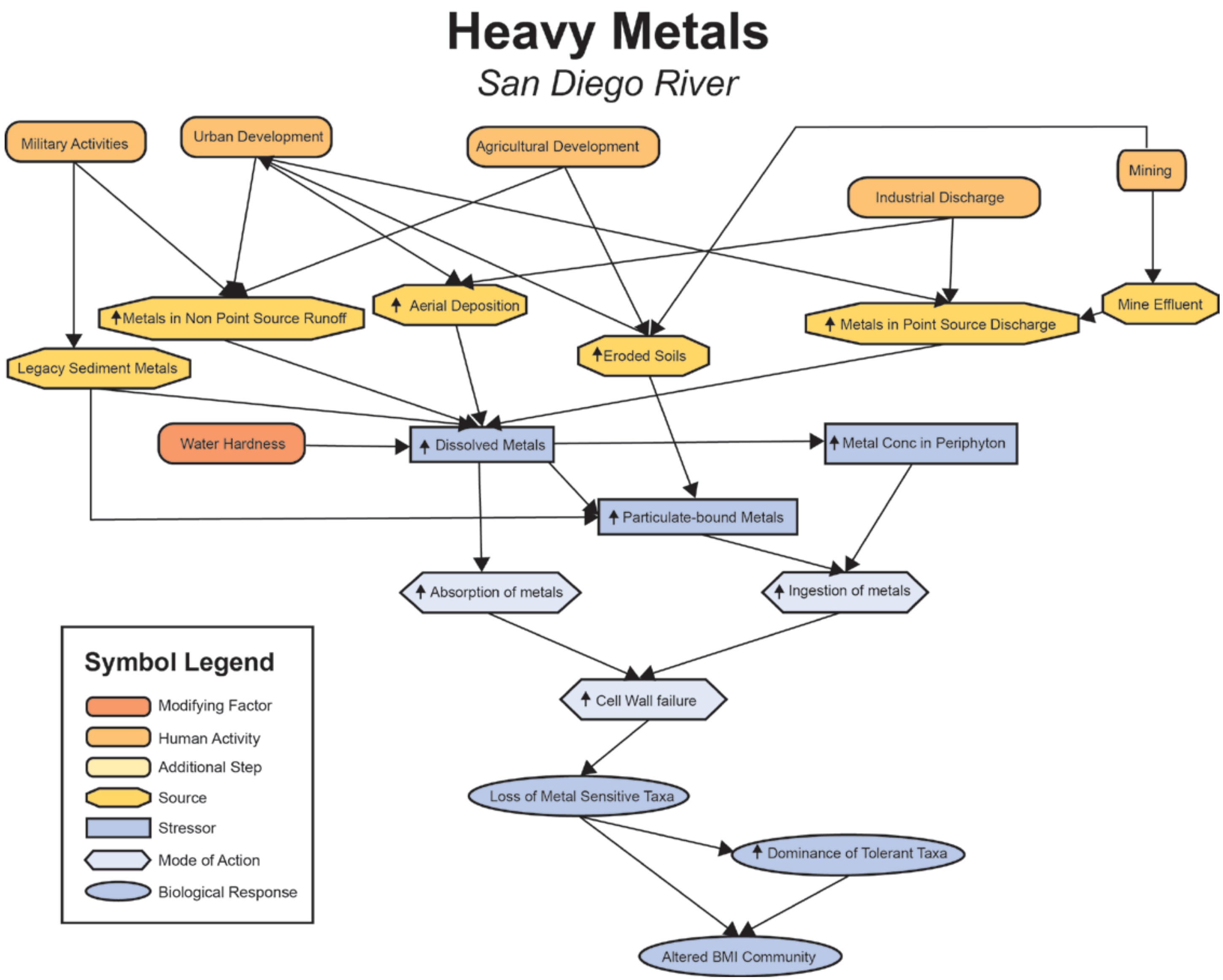

Figure 5. Metals conceptual diagram detailing proximate stressors, potential sources, and potential modes of action to impacting the macrobenthic community.

Candidate Cause: Increased Nutrients – Stream macroinvertebrates typically experience problems from increasing concentrations of the different species of nitrogen and phosphorus as indirect effects, where the increased nutrients influence autotrophic community structure and primary production rates. These effects can include clogging of micro-habitats by algal mats, changes in algal taxa and their palatability to grazers, increased dominance of cyanobacteria and other toxic algae, or night time hypoxia. Ammonia toxicity is the primary direct effect that increased nutrients can have on stream macrobenthic community structure, with certain taxa being more sensitive than others (Arthur et al. 1987, Hickey and Vickers 1994). In the conceptual diagram (Figure 6), increased nutrients is comprised of five proximate stressors: 1.) A change in algal community structure; 2.) An increase in toxic compounds; 3.) An increase in algal mat presence and thickness; 4.) An increase in the frequency of hypoxia; and 5.) An increase in ammonia concentration.

Candidate Cause: Pesticides – Much like metals, there is a large amount of evidence about the negative effects of pesticides on stream macroinvertebrates (e.g., Hickey and Clements 1998, Pollard and Yuan 2006). Pesticides, especially insecticides, have acute and chronic toxic effects on stream macroinvertebrates. This candidate cause includes current-use pesticides (synthetic pyrethroids) and legacy pesticides (diazinon, DDT), which can be dissolved in the water column or adsorbed to sediments. There were five proximate stressors in the conceptual model (Figure7): 1.) Increased water column synthetic pyrethroids; 2.) Increased sediment synthetic pyrethroids; 3.) Increased "other" water column pesticides; 4.) Increased "other" sediment pesticides; and 5.) Increased water column herbicides.

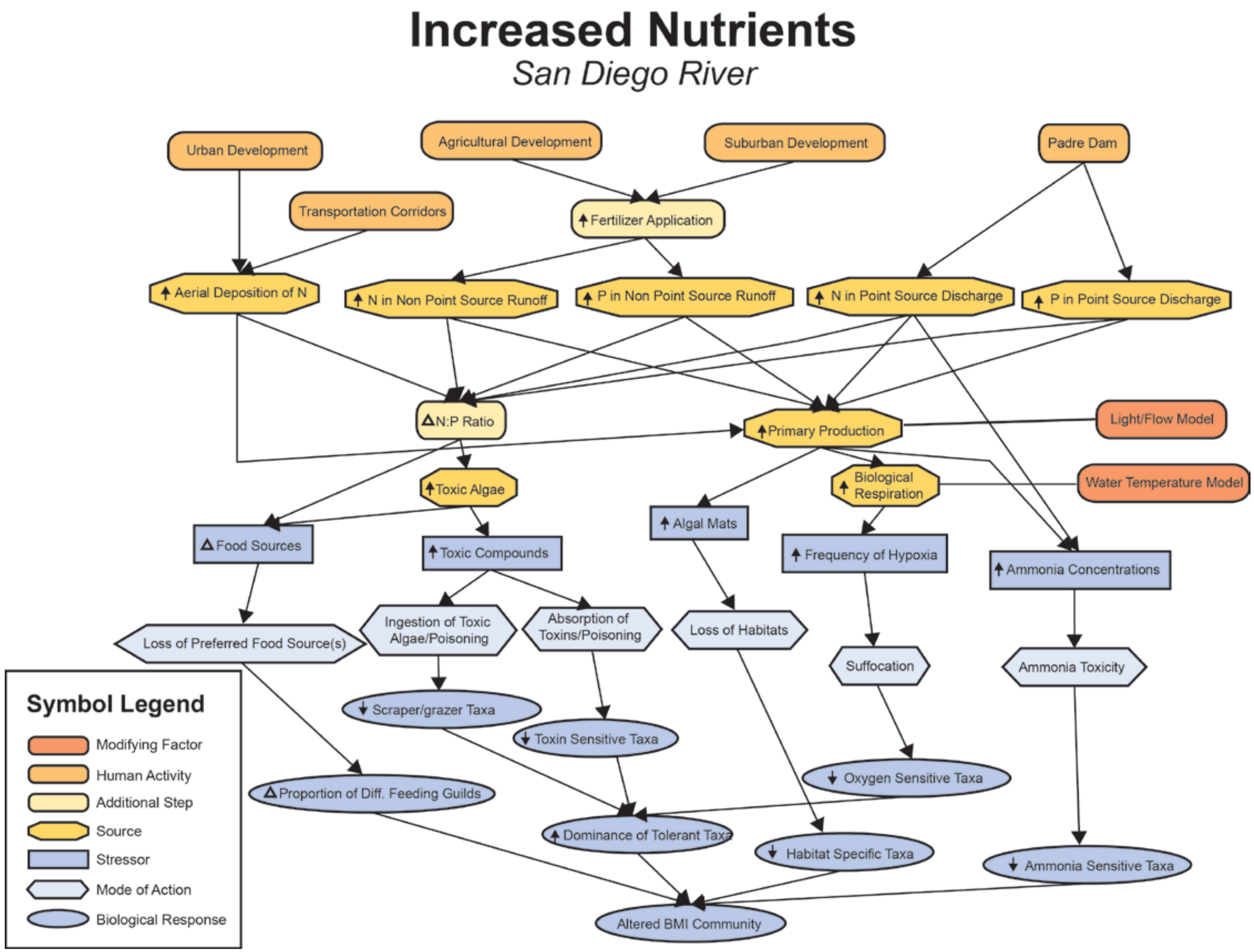

Figure 6. Altered habitat conceptual diagram detailing proximate stressors, potential sources, and potential modes of action to impacting the macrobenthic community.

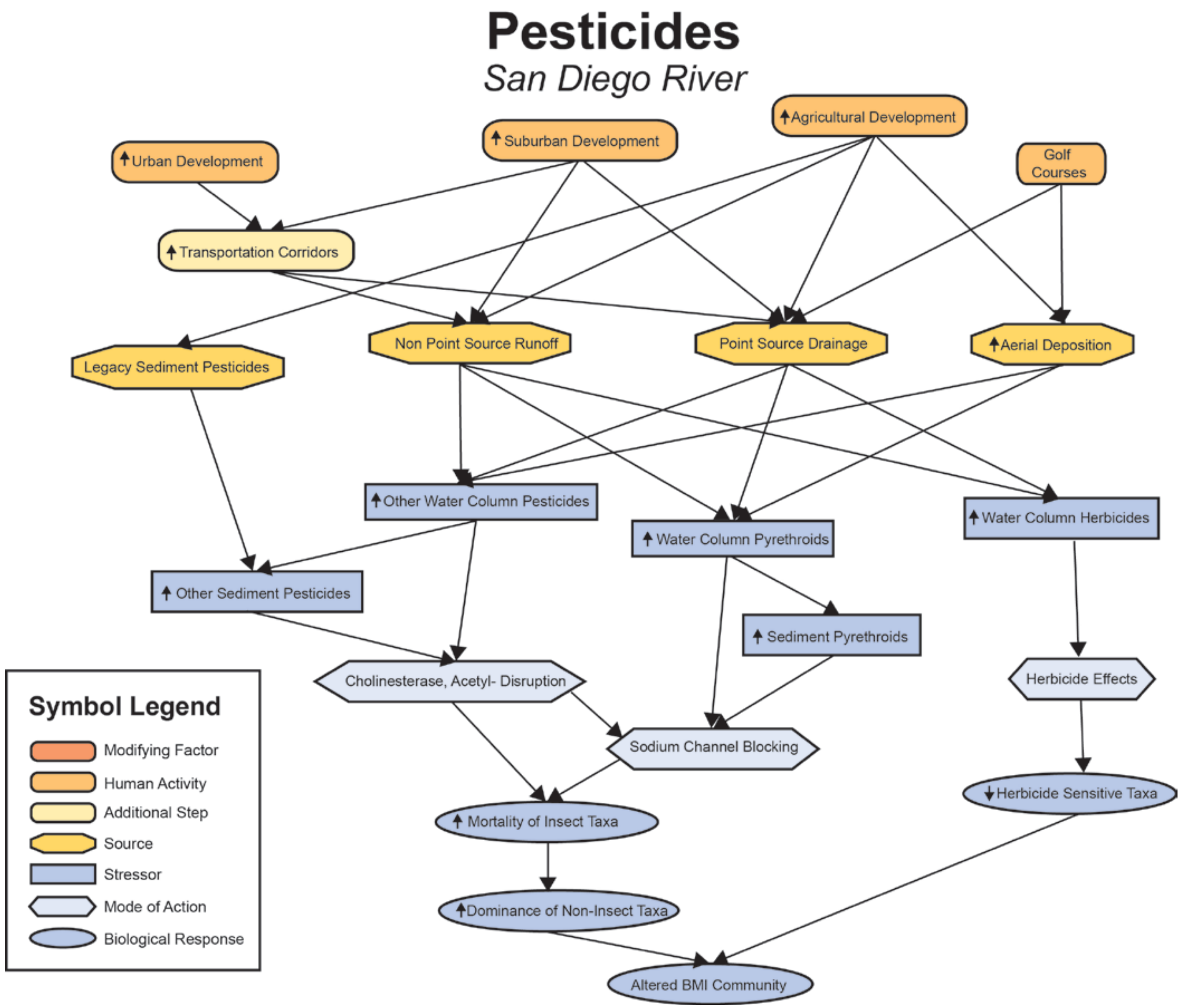

Figure 7. Increased nutrients conceptual diagram detailing proximate stressors, potential sources, and potential modes of action to impacting the macrobenthic community.

IDENTIFYING THE CAUSE

In this causal assessment for degraded biological condition observed at the MLS site in the lower San Diego River, five candidate causes were evaluated including conductivity, habitat alteration, metals, increased nutrients, and pesticides. The two most likely causes of low IBI scores at the MLS site in 2010 were elevated conductivity and pesticides based upon our review of the available data. Metals, specifically dissolved metals in the water column, were likely not a cause. Increased nutrients and habitat alteration were neither diagnosed nor refuted due to a lack of data and/or conflicting lines of evidence. The summary of all scores for all evidence types are presented in Table 3.

Elevated conductivity was diagnosed as a likely stressor based on three lines of evidence. First, there was a clear dose response between increasing conductivity and increased amphipods and other non-insect taxa (stressor-response from the field). Second, the levels of conductivity were high enough to potentially degrade levels of non-insect and tolerant taxa. Even though conductivity data were only available from a single point measurement at MLS and the comparator sites, there were measures of TDS and hardness across multiple months. Additionally, the benthic community at MLS was dominated by *Americorophium* and *Hyalella* amphipods, which are tolerant of saline conditions and are more typically observed in tidal freshwater estuarine systems than freshwater streams (especially *Americorophium*). These species are indicative of consistently elevated salinity conditions and generally represents degraded freshwater habitat (e.g., Arle and Wagner 2013). There was a consistent pattern of higher conductivity, total dissolved solids, and hardness at MLS compared to nearly all of the comparator sites and to environmentally similar reference sites from around the state.

Pesticides, specifically synthetic pyrethroids, was diagnosed as a cause of the degraded biological conditions at MLS was based on the presence of known toxic compounds in the water column and sediment (spatial-temporal co-occurrence), as well as the relationship between sediment synthetic pyrethroids and one of the biological endpoints (stressor-response from the field). Water column synthetic pyrethroids were consistently detected at MLS and the other comparator sites. Most synthetic pyrethroids are relatively hydrophobic compounds and their presence at detectable levels in the water column is likely indicative of a consistent input that could negatively impact the macrobenthic community. Conversely, there were few detectable measures of other non-pyrethroid pesticides, which weakens their evidence as a threat to the macrobenthic community at MLS. No data on chemicals known to be herbicides were collected, so no assessment of their impact on the biological condition of the MLS site was possible.

Table 3. Summary score sheets for MLS and each of the comparator sites in the San Diego River assessment. Each candidate cause score is the integration of the component proximate stressors scores, which are detailed in the supplemental material. The continuity line of evidence evaluates the continuity of each line of evidence for each of the four biological endpoints: collector abundance/non-insect taxa/tolerant taxa/amphipod abundance.

		MLS vs. Cedar Creek					MLS vs. TWAS 1					MLS vs. TWAS 2				
Candidate Cause		Elevated Conductivity	Habitat Alteration	Heavy Metals	Increased Nutrients	Pesticides	Elevated Conductivity	Habitat Alteration	Heavy Metals	Increased Nutrients	Pesticides	Elevated Conductivity	Habitat Alteration	Heavy Metals	Increased Nutrients	Pesticides
Spatial Co-Occurrence		+	+	NE	+	NE	+	---	+	---	+	+	0	+	+	+
Stressor Response	Collector Abundance	0	+	-	0	-	0	+	-	0	-	0	+	-	0	-
	Non-Insect Taxa	+	0	0	0	+	+	0	0	0	+	+	0	0	0	+
	Tolerant Taxa	-	0	-	0	0	-	0	-	0	0	-	0	-	0	0
	Amphipod Abundance	++	+	++	-	0	++	+	++	-	0	++	+	++	-	0
Reference Condition Comparison		+	+	NE	NE	NE	+	+	NE	NE	NE	+	+	NE	NE	NE
Stressor Response From Outside the Case	Collector Abundance	0	+	0	-	NE	0	+	0	-	NE	0	+	0	-	NE
	Non-Insect Taxa	+	0	0	0	NE	+	0	0	0	NE	+	0	0	0	NE
	Tolerant Taxa	+	0	0	0	NE	+	0	0	0	NE	+	0	0	0	NE
	Amphipod Abundance	0	0	0	0	NE	0	0	0	0	NE	0	0	0	0	NE
Stressor Response From Laboratory		NE	NE	--	NE	--	NE	NE	--	NE	--	NE	NE	--	NE	--
Continuity		+/+/-/+	+/+/+/+	+/0/+/-	-/0/0/-	+/-/0/0	+/+/-/+	-/-/-/-	-/-/-/-	+/+/+/+	-/-/-/-	+/+/-/+	+/0/0/0	-/-/-/-	-/0/0/-	-/+/-/-

		MLS vs. TWAS 2-2					MLS vs. TWAS 3				
Candidate Cause		Elevated Conductivity	Habitat Alteration	Heavy Metals	Increased Nutrients	Pesticides	Elevated Conductivity	Habitat Alteration	Heavy Metals	Increased Nutrients	Pesticides
Spatial Co-Occurrence		+	0	+	+	+	+	+	+	---	+
Stressor Response	Collector Abundance	0	+	-	0	-	0	+	-	0	-
	Non-Insect Taxa	+	0	0	0	+	+	0	0	0	+
	Tolerant Taxa	-	0	-	0	0	-	0	-	0	0
	Amphipod Abundance	++	+	++	-	0	++	+	++	-	0
Reference Condition Comparison		+	+	NE	NE	NE	+	+	NE	NE	NE
Stressor Response From Outside the Case	Collector Abundance	0	+	0	-	NE	0	+	0	-	NE
	Non-Insect Taxa	+	0	0	0	NE	+	0	0	0	NE
	Tolerant Taxa	+	0	0	0	NE	+	0	0	0	NE
	Amphipod Abundance	0	0	0	0	NE	0	0	0	0	NE
Stressor Response From Laboratory		NE	NE	--	NE	--	NE	NE	--	NE	--
Continuity		+/+/-/+	+/0/0/0	-/-/-/-	-/0/0/-	-/+/-/-	+/+/-/+	+/+/+/+	-/-/-/-	+/0/0/+	-/+/-/-

Metals, specifically dissolved metals in the water column, were not diagnosed as a potential cause for the observed biological degradation at MLS. Though there were a few dissolved metals that were higher at MLS than the comparator sites, there were no relationships with the different biological endpoints (stressor response from the field) and none of the concentrations observed at the MLS site were high enough to cause the major biological degradation at the site (stressor response from laboratory studies). This evaluation was based on water column dissolved metals. No data were available to evaluation the impact of sediment-bound or periphyton accumulated metals. However, some of the water column measurements were made during the winter-spring wet season when loadings and concentrations of metals from stormwater and other runoff would likely be at their highest (e.g., Lee et al. 2004, Soller et al. 2005) and still these concentrations were below levels expected to cause biological effects.

Increased nutrients could not be diagnosed or refuted from the available data. Aside from direct toxicity of high ammonia levels, most of the effects of increased nutrients on stream macroinvertebrates are indirect; translated through algal growth, primary production, and oxygen consumption. Some of the data used to measure these processes (e.g., algal mat thickness, % of reach with algae) are less precise than other kinds of data (e.g., ammonia concentrations, pesticides, or metals) used in the assessment. This could have, in part, produced the relatively similar observations at MLS and the comparator sites. Conversely, they could have truly been equivalent. Additionally, there were no correlations (supporting or weakening) of the different nutrient proximate stressors with the macrobenthic benthic invertebrate biological endpoints within the case. One of the more accurate and precise measurements – water column ammonia – were relatively low, near detections limits, at the test and comparator sites. As such, the increased ammonia stressor in the nutrient candidate cause could be eliminated. Other measurements such as dissolved oxygen (single point measures taken during the day) or the multivariate ordinations of some food types (created from only a partial list of food sources) were not ideal measures of their respective proximate stressors. The only data that were available from outside the case were the percent of the reach with filamentous algae. Given the imprecise nature of that particular measure, it was difficult to make any strong evaluations using the outside of the case lines of evidence. Taken as a whole, there was not enough compelling evidence based upon solid data in a consistent pattern indicating or excluding increased nutrients as the cause for the degraded biological condition at MLS. The one line of evidence that could not be evaluated was the influence of toxic algae, as there were no data available to use.

Similar to increased nutrients, altered physical habitat was undiagnosed based on conflicting lines of evidence inside the case, the uncertainty in many of the physical habitat measures, and the lack of data available for the outside of the case lines of evidence. The altered habitat conceptual model was the most complex in the assessment, encompassing different concepts or sub-models. Certain proximate stressors within the altered habitat candidate cause like the increase in sands and fine sediments did appear to be at problematic levels and correlated with poor biological condition. However, when evaluated as part of the whole altered habitat conceptual model, the influence of sands and fines on the biota was muted. In retrospect, future assessments may be better served by splitting up, and in turn simplifying, complex issues like habitat alteration into more distinct candidate causes. If this approach was followed, then aspects of altered physical habitat may have been more clearly indicated or excluded.

There were several lessons learned from this causal assessment. First, diagnosing candidate cause in this assessment was difficult because much of the lower San Diego River (i.e., the TWAS comparator sites) was highly degraded. With the exception of the CC site, there were marginal differences among the biological endpoints at MLS and comparator sites making traditional causal assessment approaches such as spatial temporal co-occurrence difficult. Second, since the use of within case evidence was hampered by widespread impacts at comparator sites, valuable evidence and a better perspective was gained by examining evidence from elsewhere. Additional data assessment tools should be developed to utilize the statewide data set for future causal assessments where nearby potential comparator sites may be as impacted as the test site. Furthermore, selection tools and criteria could be developed for identifying appropriate comparator sites free of the test site's impacts from the statewide dataset. Third, candidate causes comprised of a complex series of different proximate stressors such as habitat alteration might be better served by separation into simpler candidate causes. These simpler conceptual models could be centered around proximate stressors with similar modes of action in how they affect macrobenthic community structure.

DATA ANALYSIS FROM WITHIN THE CASE

Spatial Co-Occurrence

Spatial co-occurrence was one of the analyses with the most coverage of sites and proximate stressors for all of the different candidate causes in the San Diego River assessment. The analysis was set up as a comparison of the value of a potential stressor at the MLS site versus each of the comparator sites (TWAS 1, TWAS 2, TWAS2-2, TWAS 3, or CC). During the 2010 sampling events the TWAS 2 site was sampled twice (TWAS 2 vs. TWAS 2-2). The data between the duplicates was kept separate, allowing for five potential comparisons in these analyses, as well as in the stressor-response from inside the case analyses. In scoring these comparisons, a series of guidelines were created to assist in making consistent evaluations across the large dataset. Summarized in Figure 8 and assuming the presence of a variable was considered as having a negative impact on macrobenthic community structure: if the test site had a higher value than the comparator and that difference was greater than the detection limit of that variable, the data were scored "+"; if the test site had a higher value than the comparator site and the difference was less than the detection limit, the data were scored "0"; if the target and comparator sites had equal values, the data were scored "---"; if the test site had a lower value than the comparator site and the difference was less than the detection limit, the data were scored "---"; and if the test site had a lower value than the comparator site and that difference was greater than the detection limit, the data were scored as "---". If the variable was a positive variable, i.e., reducing its value would negatively affect macrobenthic community structure, the guidelines were reversed. An example score sheet for TWAS 1 with the detailed evaluation of each proximate stressor and its sub-components and the comments for each relationship are illustrated in Table 4. The summarized results from all of the comparator sites are found in Table 5.

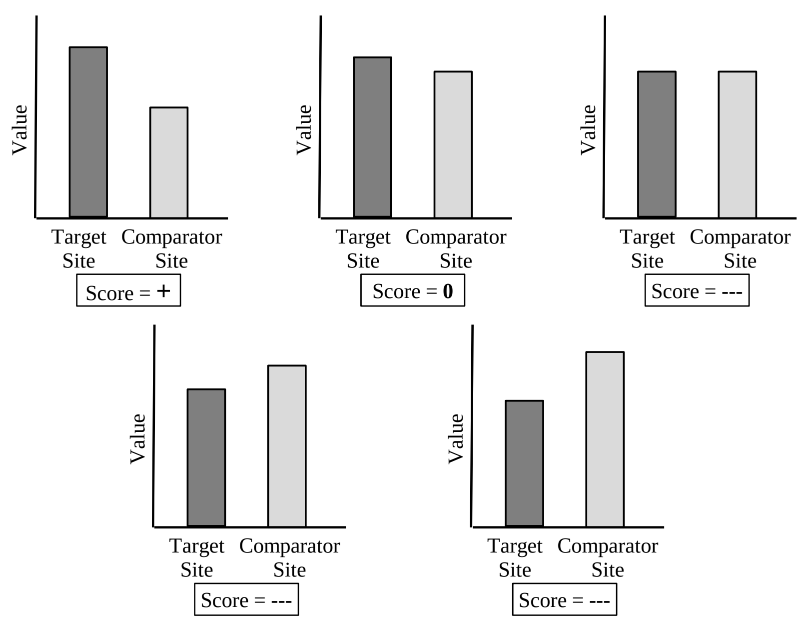

Figure 8. Illustration of scoring rules established during this case study for assessing spatial co-occurrence data assuming a negative variable. A + indicates supporting evidence, 0 indicates indeterminate evidence, and --- indicates contrary or weakening evidence.

Table 4 Example detailed spatial co-occurrence score sheet to illustrate the comparison of MLS versus TWAS 1 for each proximate stressor and the individual components therein. Data are scored + for supporting evidence, --- for strongly weakening evidence, 0 for indeterminate evidence, or NE for no evidence. bdl = below detection limit nd = no data n/a = not applicable.

Candidate Cause	Proximate Stressor	Measurement	Components	MLS	TWAS1	Difference	Score	Proximate Stressor Score	Comment
Elevated Conductivity	Increased Conductivity							+	
		Point Measurement	Conductivity (mmhos cm^{-1})	2420	2160	260.0	+		
	Increased TDS							+	
		Mean of Jan-May	Dissolved Solids (mg L^{-1})	1127.3	1117.7	9.7	+		
			Hardness (mg $CaCO_3$ L^{-1})	464.1	433.5	30.6	+		
Altered Habitat	Change in Available Food							---	
		nMDS Comparison of Sites Based Upon Food Type Availability	Euclidean Distance from MLS				---		
	Increase in Channel Depth							+	
		Mean Thalweg Depth	Depth (cm)	82.1	66.1	16.0	+		
	Decrease in Riffles							0	
		% of Reach with Riffle Habitat	Riffles (%)	0	12.5	-12.5	0		
	Decrease in Woody Debris							---	
		Length of Reach with Woody Debris	Small (< 0.3m) Wood (m)	17.7	8.2	9.5	---		
			Large (>0.3 m) Wood (m)	0.5	0.0	0.5	---		

Candidate Cause	Proximate Stressor	Measurement	Components	MLS	TWAS1	Difference	Score	Proximate Stressor Score	Comment
Altered habitat (cont.)	Increase in Sands and Fines							0	
		% of Reach with Sand+Fines Substrate	Sands and Fines (%)	81.7	81.6	0.2	0		
	Decrease in Cobbles							---	
		% of Reach with Cobble Substrate	Cobbles (%)	0	0	0	---		
		Mean % Embeddedness of Cobbles	Embeddedness (%)	36.8	nd	n/a	---		
	Decreased Substrate Complexity							---	
		nMDS Comparison of Sites Based on Habitat Types Present	Euclidean Distance from MLS				---		
	Decrease in Undercut Banks							---	
		% of Reach with Undercut Banks	Undercut Banks (%)	5.5	0	5.5	---		
	Increased Water Temperature							+	
		Maximum of Jan-May	Temperature (C)	21.25	21.10	0.15	+		
	Increase in Low Dissolved Oxygen							---	
		Frequency of Mild Hypoxia (2-5 mg L^{-1})	Frequency	1	1	0	---		Single point measurement taken during daytime
		Frequency of Hypoxia (<2 mg L^{-1})	Frequency	0	0	0	---		Single point measurement taken during daytime

Candidate Cause	Proximate Stressor	Measurement	Components	MLS	TWAS1	Difference	Score	Proximate Stressor	Comment
Heavy Metals	Increase in Dissolved Metals							+	
		Maximum of Jan-May	Arsenic (mg L^{-1})	0.0041	0.0032	0.001	+		Supporting evidence for Arsenic, Copper, Antimony, Selenium, and Zinc
			Cadmium (mg L^{-1})	bdl	bdl		---		
			Chromium (mg L^{-1})	0.0004	0.0002	0.000	0		
			Copper (mg L^{-1})	0.0035	0.0026	0.001	+		
			Nickel (mg L^{-1})	0.0028	0.0026	0.000	---		
			Lead (mg L^{-1})	0.00014	0.0001	0.000	0		
			Antimony (mg L^{-1})	0.0011	0.0006	0.001	+		
			Selenium (mg L^{-1})	0.0012	0.0006	0.001	+		
			Zinc (mg L^{-1})	0.0093	0.0046	0.005	+		
	Increase in Particulate Bound Metals				No Data Available			NE	
	Increase in Metals in Periphyton							NE	
Increased Nutrients					No Data Available				
	Change in Food Sources							0	
		nMDS Comparison of Sites Based Upon Food Type Availability	Euclidean Distance from MLS				---		
		nMDS Comparison of Sites Based Upon Algal Community Structure	Bray-Curtis Similarity to MLS				+		
	Increase in Toxic Algal Compounds				No Data Available			NE	

Candidate Cause	Proximate Stressor	Measurement	Components	MLS	TWAS1	Difference	Score	Proximate Stressor Score	Comment
Increased Nutrients (cont.)	Increase in Algal Mats							---	
		% of Reach with Filamentous Algae Present	Filamentous Algae (%)	1.4	11.8	-10.5	---		
		Mean Micro Algal Mat Thickness Within Reach	Mat Thickness (mm)	0.49	0.39	0.10	0		
	Increase in Low Dissolved Oxygen							---	
		Frequency of Mild Hypoxia (2-5 mg L^{-1})	Frequency	1	1	0	---		Single point measurement taken during daytime
		Frequency of Hypoxia (<2 mg L^{-1})	Frequency	0	0	0	---		Single point measurement taken during daytime
	Increase in Ammonia							---	
		Maximum of Jan-May	Ammonia (mg N L^{-1})	0.1	0.07167	-0.013	---		
Pesticides	Increased Water Column Pyrethroids							+	Support for Cyhalothrin-L and Fenvalerate, but weakening evidence for other compounds. Equivalent number of detections at both sites
		Maximum of Jan-May	Allethrin (µg L^{-1})	bdl	0.0468	-0.0468	---		
			Bifenthrin (µg L^{-1})	0.0321	0.0338	-0.0017	---		
			Cyfluthrin, total (µg L^{-1})	bdl	0.0151	-0.0151	---		
			Cyhalothrin-l, total (µg L^{-1})	0.0255	bdl	0.0255	+		
			Cypermethrin, total (µg L^{-1})	bdl	0.0399	-0.0399	---		
			Esfenvalerate (µg L^{-1})	0.0008	bdl	0.0008	0		
			Fenvalerate (µg L^{-1})	0.0066	bdl	0.0066	+		
			Permethrin (µg L^{-1})	bdl	bdl		---		
			Prallethrin (µg L^{-1})	bdl	0.0529	-0.0529	---		
		Frequency of Pyrethroid Observations Above Detection Limit	Observed/Measured	0.129	0.16129	-0.03225806	---		
	Increased Sediment Pyrethroids							+	
		Point Measurement	Bifenthrin (ng g^{-1})	8.4	0	8.400	+		

Candidate Cause	Proximate Stressor	Measurement	Components	MLS	TWAS1	Difference	Score	Proximate Stressor Score	Comment
Pesticides (cont.)	Increased Water Column Non-Pyrethroid Pesticides							---	
		Maximum of Jan-May	Azinphos Methyl (µg L^{-1})	bdl	bdl	n/a			
			Bolstar (µg L^{-1})	bdl	bdl	n/a			
			Chlorpyrifos (µg L^{-1})	bdl	bdl	n/a			
			Danitol (µg L^{-1})	0.001	0.0357	-0.0347	---		
			Total Demeton (µgL^{-1})	bdl	bdl	n/a			
			Diazinon (µg L^{-1})	bdl	bdl	n/a			
			Dimethoate (µg L^{-1})	bdl	bdl	n/a			
			Disulfoton (µg L^{-1})	bdl	bdl	n/a			
			Ethyl Parathion (µg L^{-1})	bdl	bdl	n/a			
			Malathion (µg L^{-1})	bdl	bdl		---		
			Merphos (µg L^{-1})	bdl	bdl	n/a			
			Methidathion (µg L^{-1})	bdl	bdl	n/a			
			Methyl parathion (µg L^{-1})	bdl	bdl	n/a			
			Mevinphos (µg L^{-1})	bdl	bdl	n/a			
			Phorate (µg L^{-1})	bdl	bdl	n/a			
			Phosmet (µg L^{-1})	bdl	bdl	n/a			
			Tetrachlorvinphos (µg L^{-1})	bdl	bdl	n/a			
			Tokuthion (µg L^{-1})	bdl	bdl	n/a			
		Frequency of Non-Pyrethroid Observations Above Detection Limit	Observed/Measured	0.026	0.02564	0	---		
	Increased Sediment Non-Pyrethroid Pesticides				No Data Available			NE	
	Increased Herbicides				No Data Available			NE	

Table 5. Sumary of spatial co-occurancecomparsions between MLS and each comparator site for the candidate causes and their component proximate stressors.

Candidate Cause	Proximate Stressor	TWAS 1 Proximate stressor Score	TWAS 1 Comment	TWAS 2 Proximate stressor Score	TWAS 2 Comment	TWAS 2-2 Proximate Stressor Score	TWAS 2-2 Comment	TWAS 3 Proximate Stressor Score	TWAS 3 Comment	Cedar Creek Proximate stressor Score	Cedar Creek Comment
Conductivity	Increased Conductivity	+		+		+		+		+	
	Increased TDS	+		+		+		---		NE	
Habitat	Change in Available Food	---		+		+		+		+	
	Increase in Channel Depth	+		---		+		+		+	
	Decrease in Riffles	---		---		---		---		---	
	Decrease in Woody Debris	---		---		---		+		---	
	Increase in Sands and Fines	0		0		0		+		+	
	Decrease in Cobbles	---		---		---		---		+	
	Decreased Substrate Complexity	---		0		0		+		+	
	Decrease in Undercut Banks	---		---		---		---		---	
	Increased Water Temperature	+		+		---		0		NE	
	Increase in Low Dissolved Oxygen	---		+	Only based on single field observation in	+	Only based on single field observation in May	---	Only based on single field observation in May	+	
Heavy Metals	Increase in Dissolved Metals	+	Supporting evidence for Arsenic, Copper, Antimony, Selenium, and Zinc	+	Supporting evidence for Arsenic and Selenium	+	Supporting evidence for Aresenic and Selenium, but weakening for other elements	+	Elevated levels of Arsenic, Copper, Antimony, and Zind, but weakening evidence for Cadmium	NE	
	Increase in Particulate Bound Metals	NE		NE		NE		NE		NE	
	Increase in Metals in Periphyton	NE		NE		NE		NE		NE	
Nutrients	Change in Food Sources	0		+		+		+		+	
	Increase in Toxic Algal Compounds	NE		NE		NE		NE		NE	
	Increase in Algal Mats	---		---		---		---		---	
	Increase in Low Dissolved Oxygen	---		+	Only based on single field observation in	+	Only based on single field observation in May	---	Only based on single field observation in May	+	
	Increaes in Ammonia	---		---		---		---		NE	
Pesticides	Increased Water Column Pyrethroids	+	Support for Cyhalothrin-λ and Fenvalerate, but weakening evidence for other compounds. Equivilent number of detections at both sites	+	Support for Cyhalothrin-λ and Fenvalerate, but weakening evidence for other compounds. Equivilent number of detections at both sites	+	Support for Cyhalothrin-λ and Fenvalerate, but weakening evidence for other compounds. Equivilent number of detections at both sites	+	Support for Cyhalothrin-λ and Fenvalerate, but weakening evidence for other compounds. Equivilent number of detections at both sites	NE	
	Increased Sediment Pyrethroids	+		+		+		+		NE	
	Increased Water Column Non-Pyrethroid Pesticides	---		---		---		---		NE	
	Increased Sediment Non-Pyrethroid Pesticides	NE		NE		NE		NE		NE	
	Increased Herbicides	NE		NE		NE		NE		NE	

Elevated Conductivity

The point measurement of conductivity taken during the midday at MLS (2420 μmhos cm^{-1}) was higher than at any of the comparator sites (2160 – 486 μmhos cm^{-1}) and all the evidence were scored as +. The maximum value measured in grab samples from January – May of dissolved solids (mg L^{-1}) and hardness (mg $CaCO_3$ L^{-1}) were higher at MLS than TWAS 1 TWAS 2 and TWAS 2-2, all of which were scored "+". Values of both were higher at TWAS 3 than at MLS, so they were scored as "---" and no measurements were made at the CC comparator site, scored as NE. The candidate cause score for all five comparator sites was "+", as conductivity was higher at MLS than the comparator sites and TDS and hardness were also higher at MLS than the comparator sites where they were measured; with the exception TWAS 3. The magnitude of the difference in conductivity between MLS and TWAS 3 (583 μmhos cm^{-1}) was deemed compelling enough to warrant a "+" score for the overall candidate cause score.

Habitat Alteration

The multivariate summary of available food at all of the comparator sites were different than MLS (scored as +), with the exception of TWAS 1, which was similar and scored as "---". Channel depth measured at the time of biological sampling was greater at MLS (82.1 cm) than all of the comparator sites (18 - 66 cm), which were therefore all scored "+". Decrease in riffle habitat, measured as the % of reach during biological sampling with riffles present, was scored as "+" for MLS (0 %) compared to the CC (46.5%) and TWAS 3 (25.5 %) sites. The TWAS 1, TWAS 2, and TWAS 2-2 sites had a greater amount of riffles (10 - 12.5%) than MLS, but at a magnitude within the perceived error of the measurement and were accordingly scored "0". The decrease in woody debris, measured as the length of the reach with small and large woody debris, was scored as "---" for all comparator sites, except TWAS 3 (scored +). The TWAS 3 site had more small woody debris present than MLS, which had more than the remaining comparator sites. The percentage of the reach with sands or fines was greater at MLS (81.7 %) than at CC (26.4%) or TWAS 3 (48.6%), so those sites were scored as "+". The percentage was only marginally greater at MLS (<2%) than the other comparator sites, so they were scored "0". Decrease in cobbles, measured as the percent of the reach with cobble substrate and the percent embeddedness of cobbles present, was scored "+" for MLS compared to CC, as there were more cobbles at CC and those at MLS were more embedded. There were equivalent amounts of cobbles and their embeddedness at MLS and the remaining comparator sites, so they were all scored as "---". The decrease in undercut banks, measured as the percent of the reach with undercut banks, was scored "---" for all sites, as there was a greater amount of undercut banks at MLS (5%) than the comparator sites (0 - 3.6%), but the difference was within the measurement error. The maximum observed temperature from January – May was greater at MLS than TWAS 1 and TWAS 2, so increased water temperature was scored as "+". It was scored "0" for TWAS 3, as MLS was slightly higher, but within the error of the measurement. No temperature data were collected for CC and it was scored NE. The increase in low dissolved oxygen, measured as the presence of mild hypoxia (2 - 5 mg O_2 L^{-1}) or hypoxia (<2 mg O_2 L^{-1}), was scored "+" in MLS versus all of the comparator sites except TWAS 3 as mild hypoxia was observed at MLS but not the comparator sites. Mild hypoxia was observed at TWAS 3, so it was scored as "---". It should be noted that all of these measurements were based upon a single midday point measure made during the biological sampling, which is not the ideal data from which to detect hypoxia. Multiple time point, diel measures of dissolved oxygen would have provided the best characterization of the dissolved oxygen conditions in the stream. The decrease in substrate complexity was evaluated as difference from MLS using a multivariate summary of multiple physical habitat characteristics (see Table 1 for details). There were large differences in CC and TWAS 3 from MLS (scored +), an intermediate difference for TWAS 2 and TWAS 2-2 (scored 0), and only a small difference for

TWAS 1 (scored "---"). The overall the Habitat Alteration candidate cause score was "+" for CC and TWAS 3 because most of the proximate stressors were "+", the candidate cause score was "0" for TWAS 2 and TWAS 2-2 because of the mixed similarities and differences of the proximate stressors, and "---" TWAS 1 due to the similarities to MLS among the proximate stressors.

Metals

Increased dissolved metals, measured as the maximum observed value from January to May, were scored "+" for all the comparator sites except CC (scored NE), where no data were collected. Data for a given comparator site were scored "+" if MLS had a greater concentration for any of the different elements measured: arsenic, cadmium, chromium, copper, nickel, lead, antimony, selenium, or zinc. As an example, arsenic, copper, antimony, selenium, and zinc were elevated at MLS compared to TWAS 1, while at compared to TWAS 2 only arsenic and selenium were elevated at MLS; yet, both were scored as "+". No data were collected for particulate bound metals or metals in periphyton, so all of the comparator sites were scored as NE for those proximate stressors. Based upon the available data, (i.e., the dissolved metals) the candidate case score for metals was "+" for all sites except CC, which was scored NE.

Increased Nutrients

Changes in food source, evaluated as similarity to the algal community at MLS and difference in overall food type availability at MLS (detailed in Table 1), were scored "+" at all the comparator sites except TWAS 1. The TWAS 1 site was scored "0" because the food type was dissimilar to MLS (scored "---"), but the algal community structure was similar (scored +). No data were available for evaluating the increase in toxic algal compounds, so all of the sites were scored as NE. Increase in algal mats was scored as "---" for all of the comparator sites because the MLS site had a smaller percentage of the reach (1.4%) with filamentous algae than the comparator sites (10.5 - 18%) (scored "---") and had similarly thick microalgal mats (scored as "0" or "---"-). The increase in low dissolved oxygen, measured as the presence of mild hypoxia (2 - 5 mg O_2 L^{-1}) or hypoxia (<2 mg O_2 L^{-1}), was scored "+" in MLS versus all of the comparator sites except TWAS 3 as mild hypoxia was observed at MLS but not the comparator sites. Mild hypoxia was observed at TWAS 3, so it was scored as "---". It should be noted that all of these measurements were based upon a single midday point measure made during the biological sampling, which is not the ideal data from which to detect hypoxia related to primary production night time oxygen depletion (multiple measures throughout the day would be best). Increases in ammonia, evaluated as the maximum ammonia concentration (N mg L^{-1}) observed from January to May, were scored "---" for TWAS 1, TWAS 2, and TWAS 3, which had equivalent concentrations to MLS (0.1 - 0.08). Ammonia was not measured at CC and was therefore scored NE.

Pesticides

Increased water column synthetic pyrethroids, evaluated as the maximum observed value from January to May and the frequency of compounds observed above machine detection limits, was scored "+" for all sites except CC, where no data were collected (scored NE). Data were scored "+" if MLS had a higher value than the comparator sites for any of the measured compounds: allethrin, bifenthrin, total cyfluthrin, total λ-cyhalothrin, total cypermethrin, esfenvalerate, fenvalerate, permethrin, or prallethrin. As an example, MLS had higher values of total λ-cyhalothrin and fenvalerate than TWAS 1 (both scored +), they had equivalent amounts of bifenthrin, esfenvalerate, and permethrin (scored "---", "0", and "---"), and TWAS 1 had higher amounts of allethrin, total cyfluthrin, total cypermethrin, and prallethrin (scored "---"). This pattern would have led to these data being scored "+". Increased sediment synthetic pyrethroids, measured from a single sediment grab, was scored "+" for all comparator sites except CC (scored NE. The MLS site had higher concentrations of bifenthrin in the sediment (8.4 ng g^{-1}) than TWAS 1, TWAS 2, TWAS 2-2, and TWAS 3 (BDL – 3.2 ng g^{-1}). Increased water column non-pyrethroid pesticides, measured as the maximum value observed from January to May, was scored "---" for all comparator sites, except CC (scored NE). The values of azinphos methyl, bolstar, chlorpyrifos, danitol, total demeton, diazinon, dimethoate, disulfoton, ethyl parathion, malathion, merphos, methidathion, methyl parathion, mevinphos, phorate, phosmet, tetrachlorvinphos, and tokuthion observed at MLS were lower or equivalent than those observed at the comparator sites, except CC where data were not collected. No data were available for evaluation of increased sediment non-pyrethroid pesticides and increased herbicides, so these were scored NE for all comparator sites. The overall pesticide candidate cause was scored as a "+" for TWAS 1, TWAS 2, TWAS 2-2, and TWAS 3 because of the observed water column and sediment synthetic pyrethroids at MLS compared to the comparator sites, with the exception of CC, which is scored NE due to lack of data to evaluate.

Stressor-Response from the Field

The stressor-response line of evidence had relatively good coverage across all of the candidate causes and nearly all of the proximate stressors could be evaluated. Stressor response relationships were evaluated by calculating Spearman's rank correlations between the different proximate stressors and the four biological response variables: % non-insect taxa, % tolerant taxa, % collector-gatherer abundance, and the % amphipod abundance measured at MLS and the five comparator sites. All of the biological endpoints are negative measures of community structure and habitat quality, where as a habitat is degraded these biological measures would be expected to increase. Data were scored based upon the rho (ρ) value of the correlation and the direction of the expected relationship between the biological endpoints and the different proximate stressors – a negative variable (e.g., % sands and fines) with negative biology would be a direct relationship, while a positive variable (% woody debris) with a negative biology would be an inverse relationship. As an example of an expected direct relationship: $\rho = -1$ to
-0.9 would be scored "--", $\rho < -0.9$ to -0.75 would be scored "-", $\rho < -0.75$ to < 0.75 would be scored "0", $\rho = 0.75$ to < 0.9 would be scored "+", and $\rho = 0.9$ to 1.0 would be scored "++". This pattern would be reversed for any expected inverse relationship. Any relationship scored "++" or "--" was investigated visually by plotting the proximate stressor and the biological endpoint and looking for spurious or less compelling relationships. If there was a question about the pattern of the correlation versus the ρ-value, the "++" or "--" was changed to "+" or "-". Additionally, if a chemical compound (i.e., metals or pesticides) was below detection limit at MLS, it was scored – (summarized in Table 6).

Table 6. Summary within the case stressor-response scores across the four biological endpoints for each proximate stressor in the candidate causes. Data are scored ++ for a strongly supporting response, + for a supporting response, 0 for ambivalent response, - for a weakening response, "--" for a strongly weakening response, and NE for no evidence.

Candidate Cause	Proximate Stressor	% Non Insect Taxa		% Tolerant Taxa		% CFCG Individuals		% Amphipod Individuals	
		Proximate Stessor Score	Comment	Proximate Stessor Score	Comment	Proximate Stessor Score	Comment	Proximate Stessor Score	Comment
Elevated Conductivity									
	Increased Conductivity	0		0		0		++	
	Increased TDS	+		-		0		+	
Altered Habitat									
	Change in Available Food	0		0		0		0	
	Increase in Channel Depth	0		0		0		++	
	Decrease in Riffles	0		0		0		+	
	Decrease in Woody Debris	-		-		0		0	
	Increase in Sands and Fines	0		0		+		++	
	Decrease in Cobbles	0		0		+		+	
	Decreased Substrate Complexity	0		0		0		+	
	Decrease in Undercut Banks	0		0		0		0	
	Increased Water Temperature	0		0		0		0	
	Increase in Low Dissolved Oxygen	NE		NE		NE		NE	
Heavy Metals									
	Increase in Dissolved Metals	0		-		-		++	
	Increase in Particulate Bound Metals	NE		NE		NE		NE	Supporting evidence for Arsenic and weakening evidence for Cadmium
	Increase in Metals in Periphyton	NE		NE		NE		NE	
Increased Nutrients									
	Change in Food Sources	0		+		0		0	
	Increase in Toxic Algal Compounds	NE		NE		NE		NE	
	Increase in Algal Mats	0		0		0		-	
	Increase in Low Dissolved Oxygen	NE		NE		NE		NE	
	Increase in Ammonia	0		0		0		-	
Pesticides									
	Increased Water Column Pyrethroids	0		0		-		0	
	Increased Sediment Pyrethroids	+		0		0		0	
	Increased Water Column Non-Pyrethroid Pesticides	0		0		-		0	
	Increased Sediment Non-Pyrethroid Pesticides	NE		NE		NE		NE	
	Increased Herbicides	NE		NE		NE		NE	

Elevated Conductivity

Increased conductivity was scored “0” for % non-insect taxa, % tolerant taxa, and % collector-gatherer abundance and scored “++” for % amphipod abundance. Increased TDS was scored as “+” for % non-insect taxa and % amphipods, scored “-” for % tolerant taxa, and scored “0” collector gatherer taxa. Overall, the elevated conductivity candidate cause score was: “++” for % amphipod abundance because of the consistent strong correlations across both of the proximate stressors; “+” for non-insect taxa because of the relationship seen with increased TDS, “-” for the % tolerant taxa because of the relationship seen with increased TDS; and “0” for % collector-gatherer abundance because of the “0” score evaluated in both proximate stressors.

Altered Habitat

The overall altered habitat candidate cause score for % non-insect taxa and % tolerant taxa was “0”. For both endpoints all of the proximate stressors were scored “0”, with exception of the length of the reach with small woody debris and increase in low dissolved oxygen, which were scored – and NE respectively. Low dissolved oxygen was scored NE because the data consisted only of a single measurement and the presence/absence nature of the measurement (i.e., the observation of mild hypoxia or hypoxia) were not appropriate for use in the correlation framework. The candidate cause score for % collector gather abundance was scored “+”. Increase in sands and fines and decrease in cobbles were both scored “+”, an increase in low dissolved oxygen was scored NE, and the remainder of the proximate stressors were scored “0”. The candidate cause score for % amphipod abundance was scored “+”. Increase in channel depth and increase in sands and fines scored “++”, while decrease in cobbles, decrease in riffles, and decreased substrate complexity were each scored “+”. The increase in low dissolved oxygen was scored NE and the remaining proximate stressors were scored “0”.

Metals

The candidate cause scores for all of the endpoints were driven by the increase in dissolved metals scores because the increase in particulate bound metals and increase in metals in periphyton were scored NE. The NE scores were due to a lack of data to evaluate. Increase in dissolved metals was scored “0” for % non-insect taxa, as all elements were scored “0” except for cadmium (--), which was below detection limit at all sites. The % tolerant taxa was scored “-”, based on the “-” relationship with arsenic, the “--” with cadmium (below detection limit), and scores of “0” with the remaining elements. The % collector-gatherer abundance was scored “-”, based on a “-” score for copper, a “--” for cadmium, and scores of “0” for the remaining elements. Dissolved metals were scored “++” for % amphipod abundance because of the “++” relationship with arsenic, “--” with cadmium, and scores of “0” for the remaining elements.

Increased Nutrients

The increased nutrients candidate cause score for % non-insect taxa % collector-gatherer abundance was “0”, as all of the component proximate stressors were scored as either “0” or NE (toxic algal compounds and low dissolved oxygen occurrence). The score for % tolerant taxa was “0” because most of the proximate stressors were scored as “0” or NE. The exception was change in food source, which was scored “+” due to the relationship of collector-gatherer abundance with algal community structure. The candidate cause score was evaluated as “0” in spite of this score because it was the only non-zero score and the indirect nature of the multivariate analysis and it was thought to not be as compelling as the other correlations. The candidate cause score for % amphipod abundance was scored “-”. This score was the product of the increase in algal mats and increase in ammonia concentration scoring “-”, changes in food source scoring “0”, and increased in toxic algal compounds and increase in low dissolved oxygen scoring NE.

Pesticides

The candidate cause for % non-insect taxa was “+”, which was driven by the “+” score associated with the sediment bound synthetic pyrethroids (bifenthrin). The other proximate stressors were either scored “0” (water column synthetic pyrethroids and water column non-pyrethroid pesticides) or NE (sediment bound non-pyrethroids and herbicides). The pesticide candidate cause score for % tolerant taxa and % amphipod abundance was “0”, as all of the proximate stressor scores were also either “0” or NE. The % collector-gatherer abundance was scored “-” because water column synthetic pyrethroids and non-pyrethroid pesticides proximate stressors were scored “-”, while sediment pyrethroids was scored “0”. As with the other biological endpoints, sediment bound non-pyrethroid pesticides and herbicides were scored NE due to lack of data to evaluate.

DATA ANALYSIS FROM OUTSIDE THE CASE

Reference Condition Comparison

The idea of this line of evidence was to provide context for the level of a given proximate stressor at the test site and to determine how different the observed value was from that seen in geographically similar reference sites. This comparison was made by characterizing the distribution of the proximate stressor values in the pool of reference sites, calculating the median, upper quartile, lower quartile, upper fence values (the upper quartile "+" 1.75X the interquartile range), and lower fence values. These types of data can be plotted in a schematic box and whisker plot (Tukey 1977) overlaid with the MLS value for ease of display and interpretation (Figure 9). Reference sites from the large bioassessment database available in California (see Ode et al in press for reference definition) were selected as similar based upon slope (<1.5%) and elevation (<333 m). Data from the MLS site were scored as follows (assuming a negative stressor): if the MLS value was less than the upper quartile, then score = -; if the MLS value was between the upper quartile and the upper fence, then score = 0; and if the MLS value was greater than the upper fence, then score = "+". If the proximate stressor was the loss of a positive variable, then the same rules would apply, but with reference to the lower quartile and fence values.

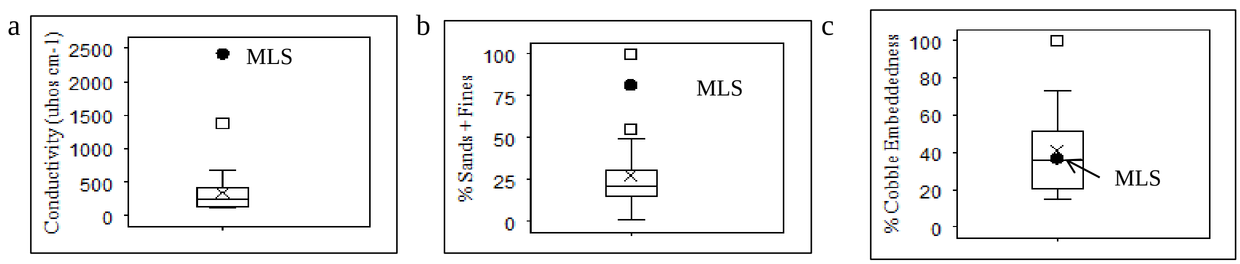

Figure 9. Examples of reference condition comparisons for outside of the case portions of the assessment. The box plot describes the reference site distribution of conductivity (a), % Sands+Fines (b), and % cobble embeddedness (c). The components of the plot are the solid line representing the median, the span of the box illustrating the upper and lower quartile, the whiskers are 1.75X the interquartile range, the cross representing the mean, and the hollow squares show outlier values. The dark circle overlaid represents the observed value at the test site.

Not every bioassessment program has collected the same data and after filtering from the larger pool of sites the only variables where there was enough data coverage were conductivity, % sands&fines, and % embeddedness of cobbles. Within the elevated conductivity candidate cause, the observed conductivity value at MLS (2420 μmhos cm^{-1}) was well above the upper fence value (683 μmhos cm^{-1}), scoring the proximate stressor "+". Consequently, the overall candidate cause score for elevated conductivity was also scored "+". Data were available to evaluate two proximate stressors within the habitat alteration candidate cause: % sands&fines and % cobble embeddedness. The observed % sands&fines (81.7) at MLS was above the upper fence (55.2%) and the data were scored "+". At the MLS site the % cobble embeddedness was 36.8, which was between the 1st and 3rd quartile value of the reference sites (20.3 – 51.7%), was scored "-". Taken together, the candidate cause scored "+", based largely upon the relative confidence in validity the sands&fines measure versus that of the cobble embeddedness. No data were available for the proximate stressors of the other candidate causes, so metals, nutrients, and pesticides were all scored NE.

Stressor-Response from Other Field Studies

A relative risk approach (Van Sickle et al. 2006, Agresti 2007) was used to characterize and provide context to the observed relationships between the different biological endpoints and different proximate stressors at MLS. These analyses were designed to assess whether the degraded biological condition captured in each biological endpoint could be the result of the observed level of the proximate stressor based upon patterns seen in other, environmentally similar sites within the State of California. Like the reference condition comparisons, sites were selected based upon slope (<1.5%) and elevation (<333 m) from the large bioassessment database available in California. An important difference however, was that both reference and non-reference sites (540 samples from 515 sites) were selected to span the range of potential biological and stressor conditions.

For these analyses semi-continuous relative risk values were calculated for all proximate stressors where enough data were available. The relative risk of observing degraded biology with 95% confidence intervals were calculated at 50+ increments of the proximate stressors observed across the environmentally similar sites from the state's biomonitoring database. Proximate stressor data from the MLS site were scored based upon the risk (+/- the 95% confidence interval) of the observed level of the stressor causing the degraded biological conditions (Figure 10). If the relative risk +/- CI was less than 1, then the data would be scored as "-". If relative risk +/- CI was greater than 1.2, then the data would be scored as "+". If the relative risk +/- CI was between 1 and 1.2 then the data would be scored "0" (Table 7).

Elevated conductivity candidate cause scores were based only upon conductivity scores, not TDS, which were scored "0" for % collector-gatherer abundance and % amphipod abundance and scored "+" for % non-insect taxa and tolerant taxa. Within the altered habitat candidate cause enough data were available for relative risk evaluation of an increase in channel depth, a decrease in riffles, a decrease in woody debris, an increase in sands&fines, and a decrease in undercut banks. Collector-gatherer abundance was scored "+" because increase in sands&fines was scored "+", decrease in undercut banks was scored "-", and the remaining proximate stressors except were scored "0". The % non-insect taxa was scored as "0" because all of the proximate stressors except decrease in undercut banks (scored -) were scored "0". The % tolerant taxa was scored "0", as decrease in riffles scored "+", decrease in undercut banks scored "-", and the remaining proximate stressors were scored "0". The % amphipod abundance was scored "0", as all of the proximate stressors scored "0". The metals candidate cause was scored "0" for all 4 biological endpoints because dissolved metals (Copper, Lead, and Zinc), the only proximate stressor with enough data, was scored "0". For the increased nutrients candidate cause, the only proximate stressor that could be scored was increase algal mats (increased filamentous algae sub component). For nutrients, % collector-gatherers were scored **–** and the remaining three endpoints were scored "0". No data were available to calculate relative risk values for pesticides, so all four biological endpoints were scored NE.

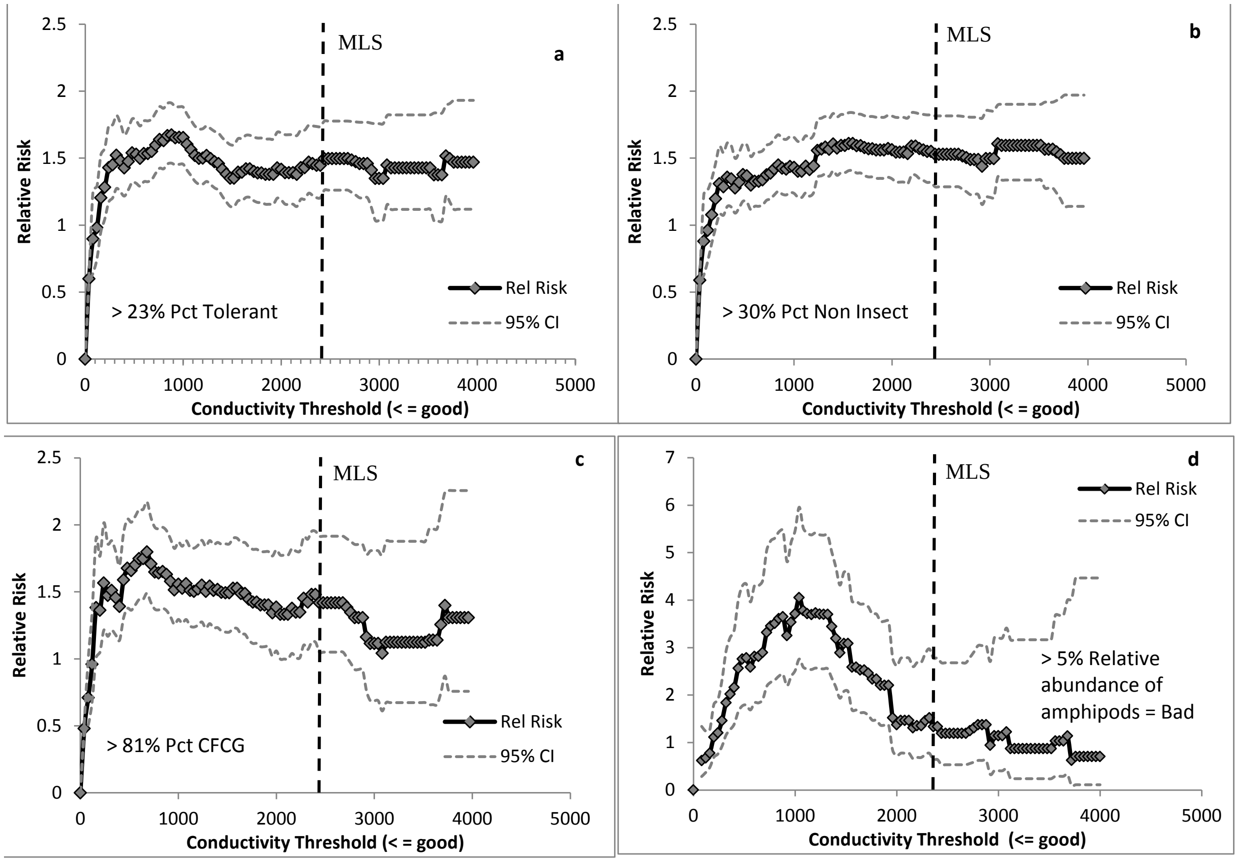

Figure 10. Examples of continuous relative risk plots using conductivity as the stressor and % tolerant taxa (a), % non-insect taxa (b), % abundance of collector-gatherers (c), % relative abundance (d) as biological endpoints. The solid dark line with grey diamonds represent the relative risk of observing biological impact at each respective value of the stressor, the dashed line represents the 95% confidence interval in that relative risk estimate, and the vertical dashed line represents the observed level of the stressor at the test site. Each panel describes the level of each biological endpoint above which was considered indicative of bad conditions.

Table 7. Summary of outside the case stressor-response from other field studies scores across the four biological endpoints for each proximate stressor in the candidate causes. Data are scored + for a supporting response, 0 for ambivalent response, - for a weakening response, and NE for no evidence.

Candidate Cause	Proximate Stressor	% Non Insect Taxa		% Tolerant Taxa		% CFCG Individuals		% Amphipods	
		Proximate Stressor Score	comment	Proximate Stressor Score	comment	Proximate Stressor Score	comment	Proximate Stressor Score	comment
Conductivity									
	Increased Conductivity	+		+		0		0	
	Increased TDS	NE		NE		NE		NE	
Habitat									
	Change in Available Food	NE		NE		NE		NE	
	Increase in Channel Depth	0		0		0		0	
	Decrease in Riffles	0		+		0		NE	
	Decrease in Woody Debris	0		0		0		0	
	Increase in Sands and Fines	0		0		+		0	
	Decrease in Cobbles	NE		NE		NE		NE	
	Decreased Substrate Complexity	NE		NE		NE		NE	
	Decrease in Undercut Banks	-		-		-		0	
	Increased Water Temperature	NE		NE		NE		NE	
	Increase in Low Dissolved Oxygen	NE		NE		NE		NE	
Heavy Metals									
	Increase in Dissolved Metals	0		0		0		0	
	Increase in Particulate Bound Metals	NE		NE		NE		NE	
	Increase in Metals in Periphyton	NE		NE		NE		NE	
Nutrients									
	Change in Food Sources	NE		NE		NE		NE	
	Increase in Toxic Algal Compounds	NE		NE		NE		NE	
	Increase in Algal Mats	0		0		-		0	
	Increase in Low Dissolved Oxygen	NE		NE		NE		NE	
	Increaeses in Ammonia	NE		NE		NE		NE	
Pesticides									
	Increased Water Column Pyrethroids	NE		NE		NE		NE	
	Increased Sediment Pyrethroids	NE		NE		NE		NE	
	Increased Water Column Non-Pyrethroid Pesticides	NE		NE		NE		NE	
	Increased Sediment Non-Pyrethroid Pesticides	NE		NE		NE		NE	
	Increased Herbicides	NE		NE		NE		NE	

Laboratory Data from Outside the Case

The laboratory data from outside the case line of evidence was evaluated by using species sensitivity distribution (SSD) curves to assess the relative toxicity of the observed metal and pesticide compounds measured at the MLS site. Species sensitivity distribution curves synthesize compound-specific laboratory toxicity tests, expressing the number of different taxa that show a toxic effect at different concentrations of that compound (e.g., Figure 11). Curves were available for Arsenic, Cadmium, Chromium, Copper, Nickel, Selenium, Zinc, bifenthrin, and malathion (Table 8). Data were scored "--" if the observed MLS concentration was below any observed toxic level, "-" if the concentration produced less than a 10% species loss, "0" if the concentration was equivalent to between 10 to 30% species loss, "+" if the concentration was between 30 to 60% species loss, and "++" if the concentration produced greater than 60% species loss.

All of the metal elements observed at MLS that had applicable SSD curves were scored "--", so dissolved metals were scored "--" and consequently, so was the metal candidate cause. The pesticides candidate cause was scored "--" based upon the scores of water column pyrethroids and non-pyrethroid pesticides. Increased water column synthetic pyrethroids were scored "-" based upon the bifenthrin score of "-". It should be noted that all of the SSD curves constructed for pesticides were still in draft form and have yet to undergo formal peer review (S. Hagerthey, *pers comm*). Specifically, the bifenthrin SSD curve was constructed from a relatively limited number of lab studies and therefore the conclusions drawn from the curves contained a large amount of variance. Increased water column non-pyrethroid pesticides were scored "--", with chlorpyrifos, diazinon, and malathion all scoring "--".

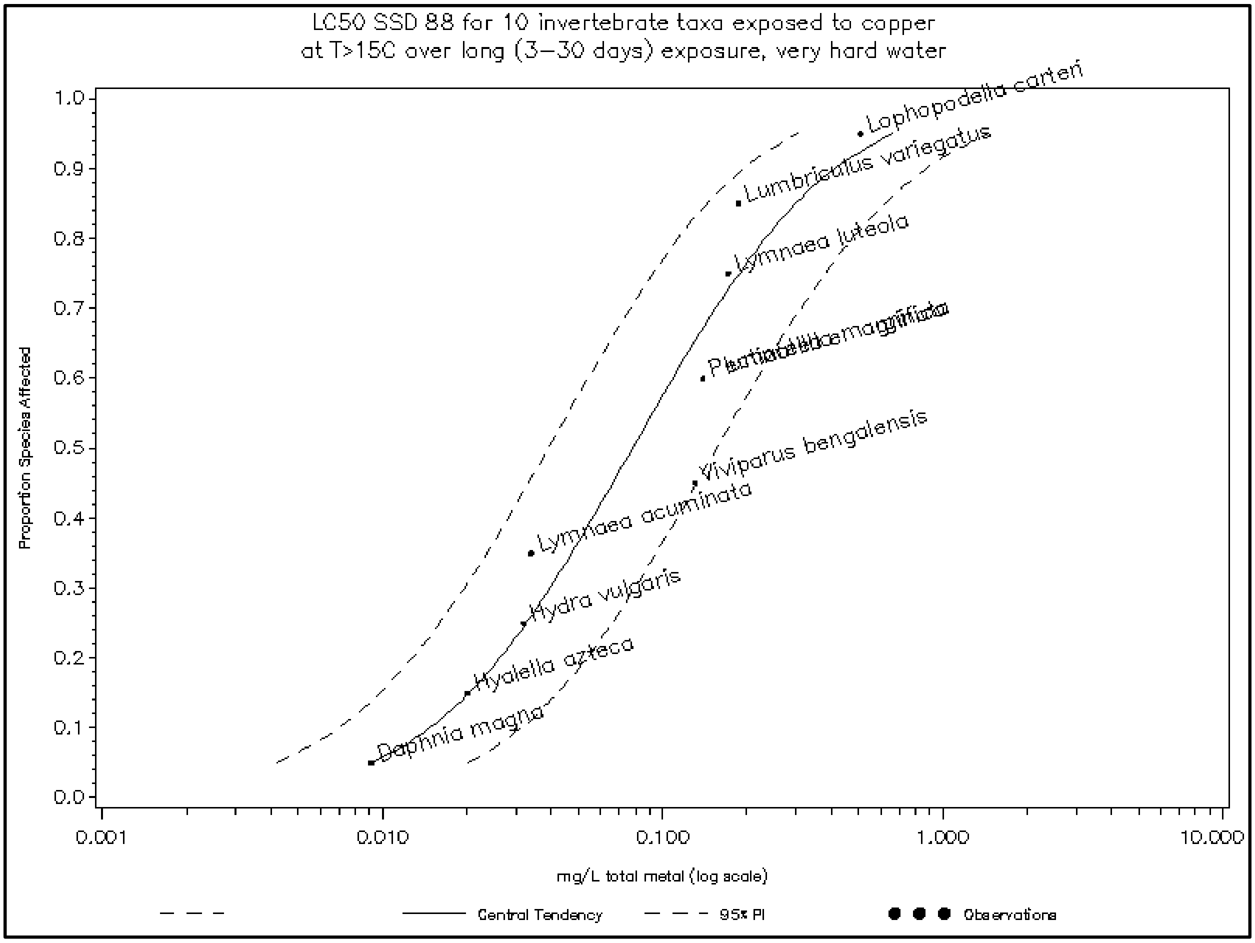

Figure 11. An example of a species sensitivity distribution curve (USEPA 2013) illustrating the different taxa where potential mortality would be expected from different concentrations of copper in water >15°C and >180 mg L^{-1} $CaCO_3$ (i.e., warm, very hard water). The dashed line represents the mean monthly observed concentration of copper at the MLS site (0.0035 mg L^{-1}).

Table 8. Scoring of the laboratory data from outside the case line of evidence. Data from the target site were compared to draft or published species sensitivity distribution curves. Data are scored ++ for moderately strong supporting evidence, + for strongly supporting evidence, -- for moderately weakening evidence, - for weakening evidence, 0 for indeterminate evidence, or NE for no evidence. bdl = below detection limit.

Candidate Cause	Proximate Stressor	Components	MLS Value	Component Score	Comment	Proximate Stressor Score
Heavy Metals						
	Increase in Dissolved Metals					--
		Arsenic (mg L^{-1})	0.0041	--	Not Hardness Corrected	
		Cadmium (mg L^{-1})	0	--		
		Chromium (mg L^{-1})	0.0004	-		
		Copper (mg L^{-1})	0.0035	-		
		Nickel (mg L^{-1})	0.0028	-		
		Lead (mg L^{-1})	0.00014	NE		
		Antimony (mg L^{-1})	0.0011	NE		
		Selenium (mg L^{-1})	0.0012	-	Not Hardness Corrected	
		Zinc (mg L^{-1})	0.0093	--		
Pesticides						
	Increased Water Column Pyrethroids					-
		Allethrin (μg L^{-1})	bdl	NE		
		Bifenthrin (μg L^{-1})	0.0321	-		
		Cyfluthrin, total (μg L^{-1})	bdl	NE		
		Cyhalothrin-l, total (μg L^{-1})	0.0255	NE		
		Cypermethrin, total (μg L^{-1}	bdl	NE		
		Esfenvalerate (μg L^{-1})	0.0008	NE		
		Fenvalerate (μg L^{-1})	0.0066	NE		
		Permethrin (μg L^{-1})	bdl	NE		
		Prallethrin (μg L^{-1})	bdl	NE		
	Increased Sediment Pyrethroids					NE
		Bifenthrin (ng g^{-1})	8.4	NE		
	Increased Water Column Non-Pyrethroid Pesticides					--
		Azinphos Methyl (μg L^{-1})	bdl	NE		
		Bolstar (μg L^{-1})	bdl	NE		
		Chlorpyrifos (μg L^{-1})	bdl	NE		
		Danitol (μg L^{-1})	0.001	NE		
		Total Demeton (μg L^{-1})	bdl	NE		
		Diazinon (μg L^{-1})	bdl	NE		
		Dimethoate (μg L^{-1})	bdl	NE		
		Disulfoton (μg L^{-1})	bdl	NE		
		Ethyl Parathion (μg L^{-1})	bdl	NE		
		Malathion (μg L^{-1})	bdl	--		
		Merphos (μg L^{-1})	bdl	NE		
		Methidathion (μg L^{-1})	bdl	NE		
		Methyl parathion (μg L^{-1})	bdl	NE		
		Mevinphos (μg L^{-1})	bdl	NE		
		Phorate (μg L^{-1})	bdl	NE		
		Phosmet (μg L^{-1})	bdl	NE		
		Tetrachlorvinphos (μg L^{-1})	bdl	NE		
		Tokuthion (mg L^{-1})	bdl	NE		

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