ASSESSMENT OF THE CONDITION OF SOUTHERN CALIFORNIA DEPRESSIONAL WETLANDS

Application of Macroinvertebrate, Diatom and Overall Condition Indices for Assessing Southern California Depressional Wetlands

Jeffrey S. Brown[1], Eric D. Stein[1], Christopher Solek[1,2], and A. Elizabeth Fetscher[1,3]

[1]Southern California Coastal Water Research Project, Costa Mesa, CA
[2]Current address: Council for Watershed Health, Los Angeles, CA
[3]Current address: San Diego Regional Water Quality Control Board, San Diego, CA

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Southern California Coastal Water Research Project
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[1]*Current address: Council for Watershed Health, Los Angeles, CA*

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ACKNOWLEDGEMENTS

ACRONYMS

Term	Definition
CRAM	California Rapid Assessment Method
CSCI	California Stream Condition Index
DO	Dissolved Oxygen
IBI	Index of Biological Integrity
MI	Macroinvertebrates
SAFIT	Southwest Association of Freshwater Invertebrate Taxonomists
SPATT	Solid Phase Adsorption Toxin Tracking
STE	Standard Taxonomic Effort
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
USEPA	U.S. Environmental Protection Agency

EXECUTIVE SUMMARY

Depressional wetlands are the most abundant, yet most threatened wetland type in California. Despite their relatively ubiquitous nature, they are poorly characterized, and unlike streams, they are not subject to any systematic monitoring and assessment. Consequently, decisions regarding protection, restoration, and management are usually made without the benefit of any regional context of condition, knowledge of predominant stressors, or rigorous documentation of reference conditions.

Ambient monitoring of depressional wetlands can be a critical tool to inform management decisions. However, before such a program can be implemented, biological assessment tools must be evaluated, validated, and modified (if necessary). Once these steps are completed, preliminary assessments can begin to provide answers to critical questions regarding wetland condition and key stressors.

The goals of this study were 1) to adapt three readily available bioassessment tools for assessing depressional wetland condition in southern California. This included a statewide rapid assessment method that had been calibrated and validated for depressional wetlands (CRAM) but had no prior use in a regional assessment program, a macroinvertebrate index developed for depressional wetlands from a different geography (northern California), and a benthic diatom index which had been developed for a different habitat type (wadeable streams). 2) To evaluate the regional condition of depressional wetlands in southern California using the adapted tools. 3) To evaluate the relationship between condition and stress by sampling both local stressors (intensity of direct wetland use, water chemistry and sediment toxicity) and landscape stressors (adjacent land use, flow diversions, road density). Once achieved, these goals should establish the foundation for developing a robust and ongoing depressional wetland ambient monitoring program.

This study included perennial or seasonal depressional wetlands as defined by Brinson (1993) located within the boundaries of the Los Angeles, Santa Ana, and San Diego Regional Water Quality Control Boards. Wetlands were not considered for this study if they were concrete-lined, marine-influenced, treatment ponds, livestock wastewater ponds, riverine (i.e. dominated by riverine hydrology), dry (i.e. not seasonal), or on-military bases (due to access issues). Vernal pools were also excluded; while these are considered a subclass of depressional wetlands, they represent a distinct wetland habitat that is typically evaluated with specific methods. Furthermore, given their rarity and ecological sensitivity they are often assessed through focused studies.

Testing of the three available assessment tools revealed that with minor modification of field protocols and recalibration of reference thresholds, the tools can be reliably applied for ambient monitoring and assessment. Such assessments should use multiple indicators and assessment tools to provide a comprehensive evaluation of depressional wetland condition that accounts for the effect of multiple types of stressors across different spatial scales.

Fifty three wetlands were sampled in southern California during the spring or early summer of 2011, 2012 or 2013, including both perennial and seasonal wetlands. The proportion of wetlands in the region considered "likely intact" varied by indicator (Figure ES-1).

Figure ES-1. Proportion of wetlands considered "likely intact" according to the three indicators measured in this study.
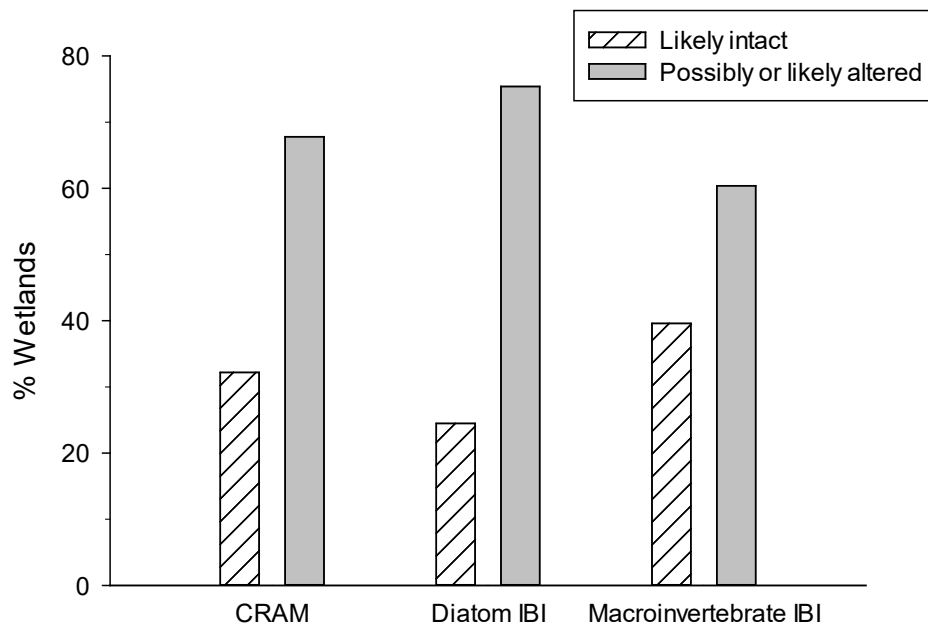
Approximately 40% of the sites were intact based on macroinvertebrate scores, 32% based on CRAM scores, and 25% based on diatom scores. When all indicators were integrated, 26% of wetlands were considered intact (based on high scores for ≥2 of the 3 indicators; Figure ES-2).

Excessive nutrients, variables related to ionic concentration, and direct habitat alteration were the dominant stressors affecting wetland condition, with different assessment indicators being sensitive to different stressors. CRAM scores were sensitive to the intensity of wetland use, interpreted from the wetland use-type categories, with the most common stressor being invasive plant species , lack of vegetation management to conserve natural resources, excessive human visitation, mowing/excessive herbivory, and flow diversions or unnatural inflows. Both diatom and macroinvertebrate indices were relatively insensitive to land use factors, but were sensitive to water quality factors. Diatom and macroinvertebrate assemblages were negatively correlated with alkalinity/conductivity/hardness, turbidity, phosphorous and nitrogen concentrations, with the relationships being stronger for diatoms than for macroinvertebrates. Between 60 and 80% of wetlands were considered eutrophic depending on the indicator used. Stressor relationships between biological indicators and landscape/water-quality data were largely the same between perennial and seasonal wetlands.

Figure ES-2. Locations of sampling sites and condition based on the number of indicators indicating intact wetlands.
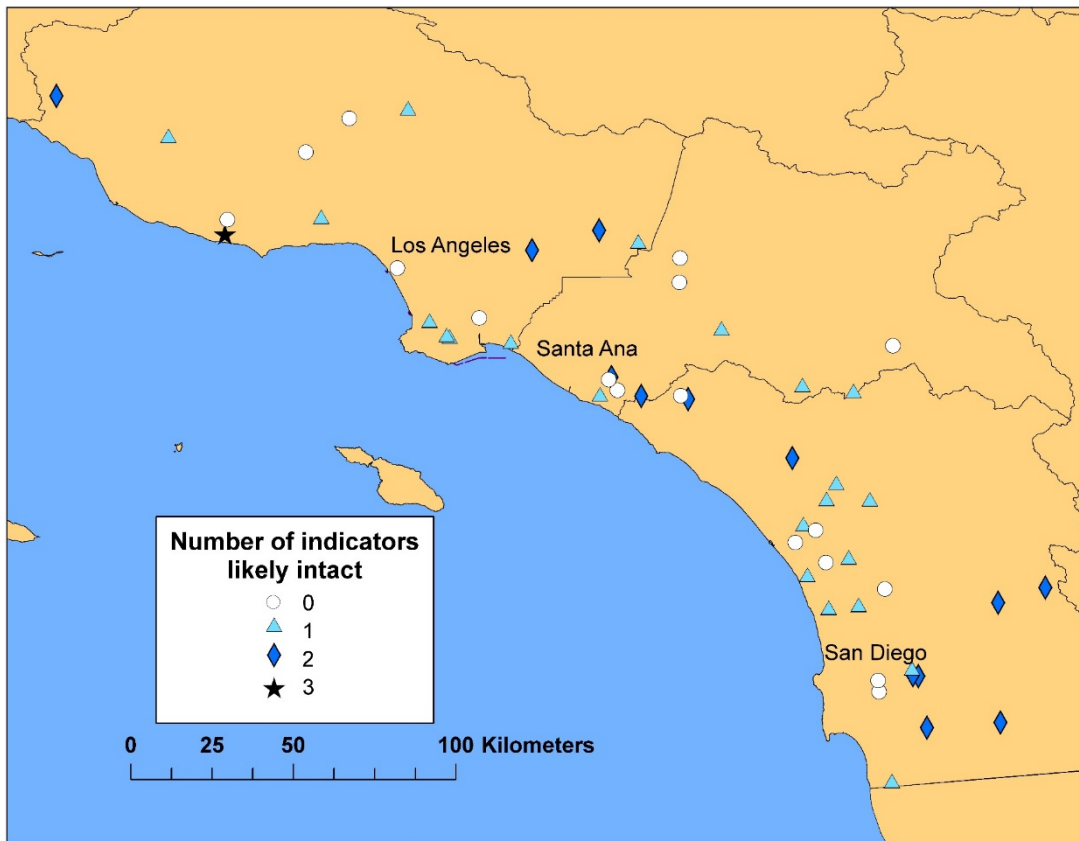
Based on the results of this assessment, we recommend that ambient assessment of depressional wetlands be expanded statewide to provide more comprehensive information on the condition of these ubiquitous, but highly threatened wetlands. We provide the following recommendations should there be expansion of the depressional assessment program to other areas:

- Reference thresholds need to be recalibrated for each new region assessed.
- Over the long term, a statewide predictive assessment index that provides site-specific reference expectations (similar to the current California Stream Condition Index) should be developed.
- The state's reference condition management program (RCMP) should be expanded to include depressional wetlands.
- Future assessment should include multiple indicators. Initially macroinvertebrates, algae, and CRAM. Ultimately assessment tools should expand to include higher trophic levels and evaluation of trophic interactions.
- Additional testing and method refinement/expansion is needed for highly seasonal wetlands. Ultimately, new tools or indicators may be necessary for wetlands with very short inundation periods.
- The wetland status and trends plots (should the state implement this program) should be used to provide a statewide sample frame for ambient assessment.

- Trend detection should be included in future ambient assessment programs. A portion of trend monitoring sites should be reference locations in order to capture short and long term natural variability in condition.
- Outreach activities should target application of existing (and future) assessment tools to a variety of programs, including wetland protection, stormwater management, timber harvest, agricultural runoff, and non-point source.

INTRODUCTION

Depressional wetlands are the most abundant wetland type in California (comprising approximately 45% of the State's 3.6 million acres of wetlands, Sutula et al. 2008). They tend to be widely distributed across the landscape, because they form in topographic lows where water can accumulate for sufficient duration to support wetland plant communities and allow hydric soil formation. Their relatively small size and distributed nature places them at substantial risk from contaminants in urban and agricultural runoff (e.g., Castro-Rao and Pinilla-Agudelo 2014, Riens et al. 2013), direct habitat loss (Dahl 1990, Holland et al. 1995), and colonization by invasive species (Brinson and Malvarez 2002). Despite these threats, they are seldom systematically monitored (Brown et al. 2010) due to lack of established assessment tools or monitoring programs.

Ambient monitoring of depressional wetlands can be a critical tool to inform management decisions, yet is complicated by several factors. First, assessment tools must be sensitive to a variety of different stressors. For example, ponds in urban settings are likely to receive runoff containing metal and petroleum-derived contaminants associated with transportation (Maltby et al. 1995a, Maltby et al. 1995b, Characklis and Wiesner 1997), while agriculture and golf course runoff may contain high levels of nutrients and pesticides (Glenn et al. 1999, Weston et al. 2004, King et al. 2007). Consequently, well-designed ambient programs must rely on indicators that differentiate effects from multiple stressors. Second, tools must be applicable to wetlands of various sizes. For example, Bird et al. (2013) suggested that developing indicators on smaller geographic scales, being cognizant of natural spatial heterogeneity, may improve the ability to detect human disturbance when natural environmental variability is high. The choice of appropriate scale has also been questioned by Wilcox et al (2002), who postulated that adapting bioassessment indicators from streams to wetlands can be problematic, due to differences in hydrologies between these habitat types, but that indices can be useful if they are calibrated to account for fluctuations in wetland hydrology. Third, assessment tools must be applicable across various hydrologic regimes from flooded to drying to accommodate the seasonal nature of many depressional wetlands (Batzer 2013, Lunde and Resh 2012).

Lunde and Resh (2012) have developed the only bioassessment index for depressional wetlands in California for the San Francisco Bay region. To date, there have been no biological assessment tools developed for depressional wetlands in southern California. In concept, biological assessment of depressional wetlands can utilize the same principles as for measuring condition in other aquatic habitats such as streams and lakes (Karr 1981, Burton et al. 1999, Simon et al. 2001, Blocksom et al. 2002, Fore and Grafe 2002). However, they must meet the three objectives stated above (ability to differentiate multiple stressors, applicability across various scales, and robustness to different hydrologic regimes). The goals of this study were 1) to adapt three readily available bioassessment tools for assessing depressional wetland condition in southern California. This included a statewide rapid assessment method that had been calibrated and validated for depressional wetlands but had no prior use in a regional assessment program (CRAM; CWMW 2013), a macroinvertebrate index developed for depressional wetlands from a different geography (northern California IBI, Lunde and Resh 2012), and a benthic diatom index which had been developed for a different habitat type (wadeable streams algae IBI, Fetscher et al. 2014). 2) To evaluate the regional condition of depressional wetlands in southern California using the adapted tools. 3) To evaluate the relationship between condition and stress by sampling both local stressors (intensity of direct wetland use, water chemistry and sediment toxicity) and landscape stressors (adjacent land use, flow diversions, road density). Once achieved, these goals should establish the foundation for developing a robust and ongoing depressional wetland ambient monitoring program.

METHODS

Sampling Approach

Fifty three wetlands were sampled in southern California during the spring or early summer of 2011, 2012 or 2013 (Figure 1). Sites were probabilistically selected from a candidate pool using the generalized random tessellation stratified sampling approach (Stevens and Olsen 2004). The sample draw was conducted from the southern California wetland maps produced by California State University, Northridge using the enhanced National Wetland Inventory (NWI) protocols and base imagery from year 2005 or newer. Sites were stratified by Regional Water Quality Control Board boundary (Regional Board). Allocation to Regional Board was guided by the amount of funding provided by each Regional Board, with a portion of funding from the Los Angeles and San Diego Regional Boards used to provide additional sampling locations in the Santa Ana Regional Board. Each wetland was visited once following initial reconnaissance. Both seasonal and perennial wetlands were sampled, and the wetland ponds represented a range of intensity of use-types (Table 1). Wetlands varied in size from $101 - 64,500$ m², and the level of urbanization within 500 m of the wetlands ranged from $0 - 90\%$. Wetlands were not considered for this study if they were concrete-lined, marine-influenced, treatment ponds, livestock wastewater ponds, riverine (i.e. dominated by riverine hydrology), dry (i.e. not seasonal), or on military bases (due to access issues). Vernal pools were also excluded; while these are considered a subclass of depressional wetlands, they represent a distinct wetland habitat that is typically evaluated with specific methods. Furthermore, given their rarity and ecological sensitivity they are often assessed through focused studies. Therefore, we excluded them from this analysis.

A multiple indicator approach was used to evaluate wetland condition and stressors. Indicators of condition included assemblages of macroinvertebrates (MI), benthic diatoms, and the California Rapid Assessment Method (CRAM), which is a visual assessment of the plants and physical habitat (CWMW 2013). Chemistry in the overlying water was measured as potential indicators of stress that could be affecting wetland condition (Table 2). Sediment grain size, total organic carbon (TOC) and sediment toxicity were also measured. General water quality constituents were analyzed during all three years, while nutrients and sediment parameters were only measured in 2012 and 2013, for a total of 37 of the 53 sites.
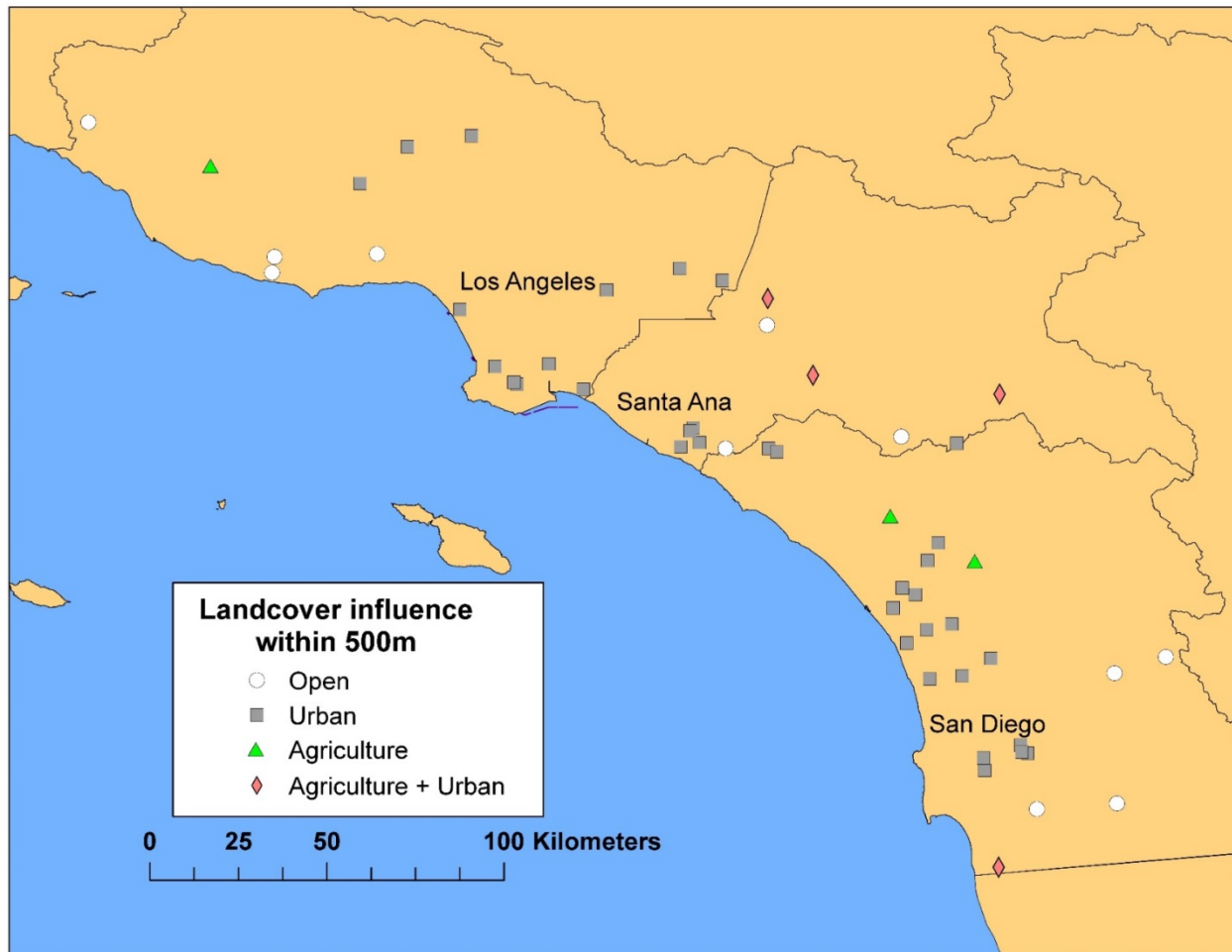
Figure 1. Distribution of depressional wetlands investigated. Categories are based on landcover within a 500m radius of the wetlands: >3% urban, >3% agriculture, >5% agriculture + urban, or open. Regional Water Quality Control Board boundaries are added for clarity.

**Table 1. Characteristics of the wetland locations. NA = not analyzed.**

StationID	Year Sampled	Water Regime	Wetland Use	Area (m²)	% Urbanization within 500 m	Specific Conductivity (µS/cm)
402DLG000	2011	Perennial	Abandoned stock pond	880	0	263
403MTVWGC	2012	Perennial	Golf course	483	1	1,557
403ROBRGC	2013	Perennial	Golf course	2,802	14	922
403VVALGC	2013	Perennial	Golf course	9,207	40	1,601
404BFM002	2011	Perennial	Habitat/stormwater	64,500	74	1,800
404CRX000	2011	Seasonal	Abandoned stock pond	490	0	1,014
404NFP000	2011	Perennial	Habitat/stormwater	3,600	0	282
404ZUN000	2011	Seasonal	Abandoned stock pond	600	0	421
405DPWCOV	2012	Perennial	Flood control	27,676	88	1,315
405LCSIMS	2013	Perennial	Habitat/stormwater	15,604	90	9,992
405PRMNGC	2012	Perennial	Golf course	1,155	45	867
408SMHLGC	2013	Perennial	Golf course	4,869	62	1,228
411HARBLK	2012	Perennial	Habitat/stormwater	1,216	57	445
411HRL000	2011	Perennial	Habitat/stormwater	3,900	65	504
411MAD000	2011	Seasonal	Habitat/stormwater	30,600	85	NA
412DGPLON	2012	Perennial	Flood control	33,456	75	946
481PLAKGC	2012	Perennial	Golf course	2,107	73	657
801BCCCGC	2013	Perennial	Golf course	5,348	60	1,794
801EAGNGC	2012	Perennial	Golf course	16,617	18	1,460
801EUCONT	2012	Seasonal	Stock pond	714	43	3,993
801IRWD05	2013	Perennial	Habitat/stormwater	27,911	34	3,131
801IRWDSJ	2012	Perennial	Habitat/stormwater	581	68	3,276
801ITR000	2011	Perennial	Flood control	2,000	61	1,312
801LAG001	2011	Perennial	Habitat/stormwater	24,000	0	886
801PRICHN	2012	Perennial	Habitat/stormwater	1,289	3	2,590
802HEMTGC	2012	Perennial	Golf course	4,579	24	1,801
802LSUMGC	2012	Seasonal	Golf course	3,980	0	4,067
802MUR000	2011	Seasonal	Habitat/stormwater	400	31	6,150
901CSDSGC	2013	Perennial	Golf course	1,496	38	4,348
901ONLRMG	2013	Perennial	Water Supply	3,203	14	933
902CRXFAL	2012	Perennial	Water Supply	23,960	0	508
903CCRVAL	2012	Perennial	Water Supply	1,648	0	1,897
903FOS000	2011	Seasonal	Habitat/stormwater	2,500	42	3,605
903GJL000	2011	Perennial	Habitat/stormwater	41,600	47	1,942
903OLVFAL	2012	Perennial	No active use	549	13	1,702
903TMBFAL	2012	Perennial	No active use	5,269	20	1,541
904AHC000	2011	Perennial	Abandoned stock pond	2,500	4	1,977
904BVPVST	2013	Perennial	Habitat/stormwater	4,760	59	1,727
904EMISGC	2012	Perennial	Golf course	1,363	69	521
904MANENC	2012	Seasonal	No active use	522	46	812
904PAR000	2011	Seasonal	Habitat/stormwater	180	60	4,730
905CHRRMN	2013	Seasonal	No active use	4,209	0	580
905FLCESC	2013	Perennial	No active use	1,628	35	1,767
905SDRSAN	2012	Perennial	Habitat/stormwater	715	42	10,500
907ADBKGC	2013	Perennial	Golf course	4,624	12	2,899
907CRRJLN	2013	Seasonal	No active use	358	0	475
907P11BAx	2013	Perennial	Recreation	56,530	79	1,356
907SDR000	2011	Perennial	Habitat/stormwater	1,170	62	2,006
907SL7SNT	2012	Perennial	Recreation	46,064	52	1,118
907SNTSAN	2013	Perennial	Habitat/stormwater	101	76	3,517
909SWASPV	2012	Seasonal	No active use	4,123	0	2,886
910LVRJAM	2012	Seasonal	No active use	931	0	774
911DMP000	2011	Perennial	Habitat/stormwater	26,250	39	893

Table 2. Constituent analytical methods and detection limits.

	Analysis Method	Method Detection Limit
Water Chemistry		
Turbidity	YSI 600 XL probe	
pH	YSI 600 XL probe	
Specific conductivity	YSI 600 XL probe	
Temperature	YSI 600 XL probe	
Dissolved oxygen	YSI 600 XL probe	
Alkalinity (field)	La Motte titration field kit	
Alkalinity (lab)	SM 2320B	1 mg/L
Hardness	SM 2340 B	0.1 mg/L
TKN	EPA 351.2	0.4 mg/L
Nitrate, nitrite	EPA 300.0	0.01 mg/L
Orthophosphate	EPA 300.0	0.0022 mg/L
Total phosphorus	SM 4500-P E	0.016 mg/L
Chlorophyll-a	EPA 445.0	0.01 µg/L
Sediment Chemistry		
TOC	SM 5310B	0.01%
Grain size	SM 2560	0.05%
Sediment Toxicity	USEPA 2000	
Diatoms	California Freshwater Algae Work Group Master Lists of Names for California	
Macroinvertebrate	Standard taxonomic effort (STE)	

Field Sampling

Samples for water quality, diatoms and MI were collected according to Fetscher et al. 2015. In brief, subsamples were collected from 10 evenly-spaced sampling nodes established around each pond (Figure 2). At each node there were 3 parallel transects, perpendicular to shore, one each for collecting water quality, sampling diatoms or MI. Each indicator type was collected at a specified distance from shore and depth. Water quality was collected at a spot up to 50% of the way to the wetland midpoint, but no deeper than 0.5 m. Diatoms and MI were collected at "near", "mid" or "far" distances from shore at consecutive sampling nodes. The near spot for diatoms was 0.5 m from shore, up to 0.25 m deep, while the mid spot was up to 50% of the wetland midpoint or 0.5 m deep, and the far spot was up to 80% of the wetland midpoint or 0.5 m deep. For the MI, the near spot was 1.5 m from shore up to 0.25 m deep, the mid spot was up to 50% of the wetland midpoint or 0.5 m deep, and the far spot was up to 80% of the wetland midpoint or 1 m deep (Figure 2).
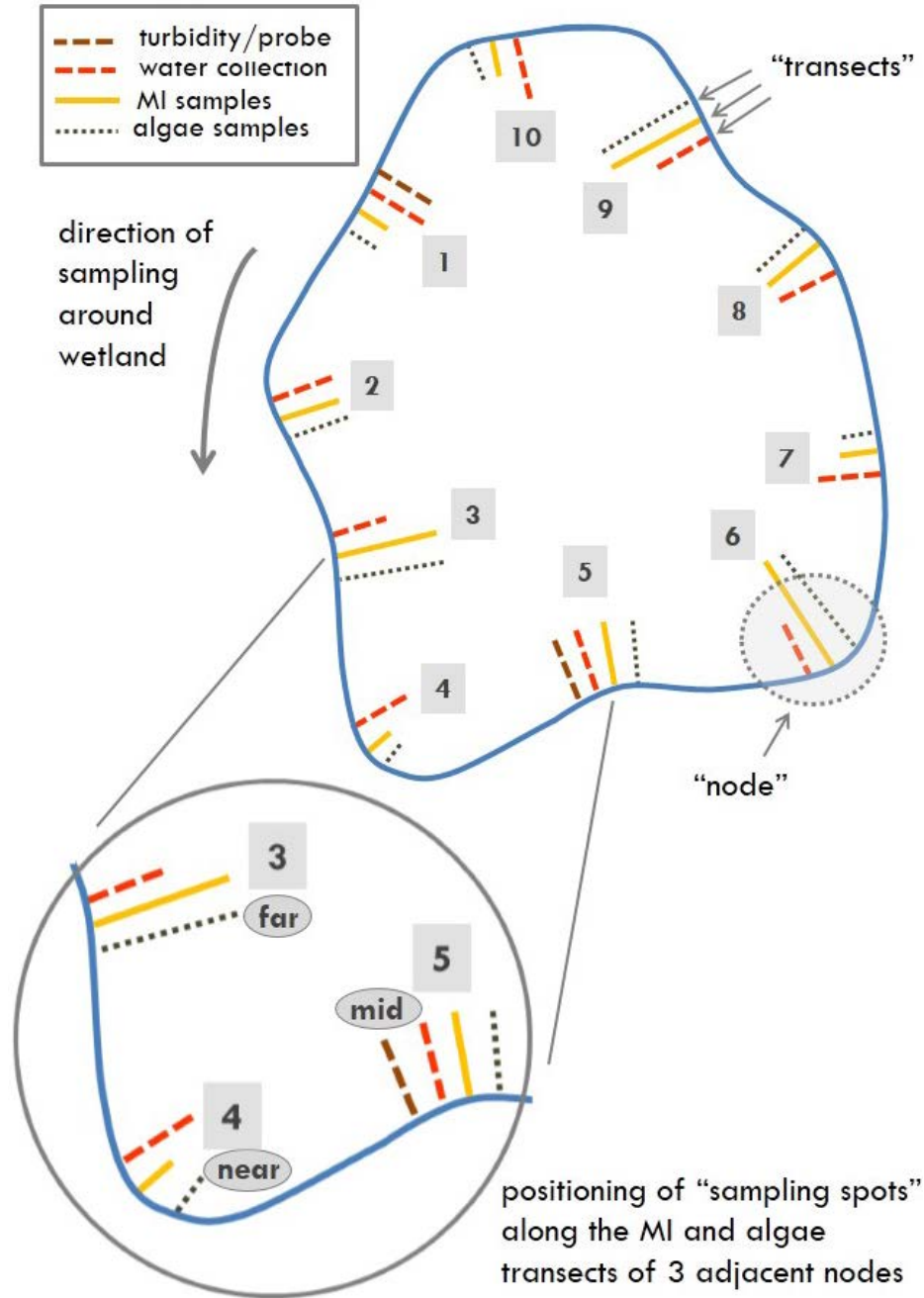
Figure 2. Placement of sampling transects (depicted as dashed or solid lines) for collection of water, MI, and diatom (algae) samples at each of the 10 nodes around the edge of the wetland's surface water, and collection of turbidity and probe (conductivity, temperature, pH, DO, etc.) data at nodes 1 and 5. Transects are nested within nodes, and the "far", "near", and "mid" sampling spots are located at the ends of the transects.

Water was collected 10 cm below the surface at each sampling node and composited into a 2 L glass bottle for water chemistry or a 1 L aluminum foil-covered bottle for chlorophyll-a. Subsamples for dissolved nutrients were passed through a 0.45 μm polytetrafluoroethylene tube (PTFE), then frozen in the field. Subsamples for total P were also frozen in the field, and subsamples for TKN were preserved in

the field with H_2SO_4. Chlorophyll-a samples were filtered within 4 h of collection using 0.7 µm glass fiber filters, and frozen. Cyanotoxin concentrations (including microcystin and saxitoxin) were also analyzed in the grab samples. For a subset of sites in the San Diego Region, continuously deployed passive samplers [solid phase adsorption toxin tracking (SPATT) bags] were also used for cyanotoxin sampling. These devices integrate cyanotoxin concentrations over longer periods of time, and results from the passive samplers were compared with the results from the grab samples.

MI were collected using a custom made 500 µm D-frame all-mesh net (Wildco[TM] 425-JD52-SPE). At each collection spot, the net was quickly lowered to the bottom with the opening of the net face down then pulled, gently rubbing the wetland bottom over a 1 m swath. The net was then quickly reversed and pushed in the opposite direction for a second sweep. In this manner, benthic, nekton (swimming), and neuston (floating) MI were captured. Samples were collected and composited from all 10 nodes, and large debris was discarded after inspecting for target organisms. The sample was then passed through a 500 µm mesh sieve and preserved in 95% ethanol.

Benthic diatoms were collected on sediments using a 60 mL syringe corer. This device was pushed into the sediment to a depth of >5 cm, then carefully withdrawn from the water. Sediment was slowly pushed out of the corer and discarded, in order to retain only the first 5 mL of sediment that went into the syringe (representing the top 2 cm of sediment). The sediment from each node was composited into a sample container. For hard substrates, a 60 mL syringe fitted with a white scrubber pad was brushed onto the substrate in order to obtain a sample. After collection, the scrubber pad was rinsed and wrung into the sample composite container. Composite samples were preserved to a final concentration of 2% formalin.

Sediment samples for toxicity testing were collected at areas within the wetlands that had fine grained sediments. The top 2 cm of sediment were collected using a pre-cleaned polyethylene scoop. Samples from multiple grabs were composited in the field for a total of 3 L, put into polyethylene bags and zipped tied. The bags were held on wet ice while transported to the toxicology lab.

CRAM was conducted according to the depressional wetlands field book (CWMW 2013). Four metric categories were scored in order to derive the final CRAM index, including buffer and landscape condition, hydrology, physical structure, and biotic structure.

Laboratory Analysis

All laboratory analyses were conducted using standard protocols and quality assurance measures. Duplicate samples for macroinvertebrates, diatoms and water quality were collected at 10% of the wetlands. Constituent analytical methods and detection limits can be found in Table 2.

Macroinvertebrates were identified using the naming conventions of the standard taxonomic effort (STE) list maintained by the Southwest Association of Freshwater Invertebrate Taxonomists (SAFIT). Taxa not already specified in this list (e.g. new taxa, or taxonomically ambiguous taxa) were reviewed and approved by SAFIT. Using the frequency distributions provided by the sorting laboratory, the MI Index of Biological Integrity (IBI) scores were derived using the methods of Lunde and Resh (2012). Eight metrics were scored in order to derive the MI IBI, including percent three dominant taxa, percent Tanypodinae/Chironomidae, percent Coleoptera, percent Ephemeroptera, Odonata and Trichoptera (EOT), scraper richness, EOT richness, Oligochaete richness, and predator richness.

For algae, identifications used the Master Lists of Names for California that are maintained by the California Freshwater Algae Work Group. Algae IBI scores were derived using the methods in Fetscher et al. (2014). Five metrics were used to derive the diatom IBI, including proportion halobiontic, proportion low total phosphorous indicators, proportion N heterotrophs, proportion requiring >50% DO saturation, and proportion sediment tolerant.

Sediment toxicity testing was conducted using the *Hyalella azteca* 10-day survival tests following procedures outlined by USEPA (2000). The tests were conducted in 1 l glass jars containing 2 cm of sediment (approximately 150 ml) and 800 ml of water. Five replicates were used for each sample and the control. Sediments were sieved through a 0.5 mm screen to remove resident organisms and debris. After a 24 h equilibration period, equilibration, 10 juvenile *H. azteca* (7-day to 10-day old) were added to each beaker to start the test. The exposures were conducted at 20°C. At the end of the exposure period, the sediment from the beakers was passed through a sieve to recover the animals, and the number of surviving animals counted. Control sediment exposures were conducted concurrently, using sediment obtained from a reference location in Yaquina Bay, Oregon, and washed with deionized (DI) water prior to use. A 96 h water-only copper reference toxicant test was conducted as a positive control. Survival data are expressed as a control-adjusted value, which is the percent survival in the sample divided by the survival in the control sediment.

Data Analysis

Setting Indicator Thresholds based on the Reference Condition

Candidate indices were adapted based on an evaluation of their initial performance relative to traditional index validation methods (Bockstaller and Girardin 2003). To be applicable for use in a regional survey, the candidate indices must be able to discern reference condition (relatively unaffected by anthropogenic activities) from non-reference condition, distinguish sites along a gradient of disturbance, and variability between condition classes must be substantially greater than that within a condition class (i.e. high signal:noise ratio, Dale and Beyeler 2001). Reference sites serve to set expectations for the condition of biotic communities with minimal disturbance (Stoddard et al. 2006). Potential reference sites were identified among the 53 wetlands in this study based on the amount of landscape disturbance within 500 m of the wetlands and the concentration of nutrients in the overlying water (Table 3). The landscape disturbance variables included % agriculture, % urban, % agriculture + urban, and road density and were derived from the 2001 National Land Cover Database. The nutrient variables included total N and total P. Because the original macroinvertebrate and algae indices were developed for different systems (San Francisco Bay area wetlands for macroinvertebrates and southern California streams for algae), we evaluated the relative ability of landscape disturbance vs. nutrients to differentiate reference from non-reference. Given the wide range of wetland sizes, we evaluated the sensitivity of the reference definition based on adjacent disturbances at scales ranging from 30 m to 3 km buffer widths around each wetland perimeter. Differences in indicator scores between reference and nonreference wetlands were analyzed using a Student's t-test, or a Welch's t-test when assumptions of equal variance (Levene's test) or normal distribution (Shapiro-Wilk test) were not met. The threshold approach (landscape-based or nutrient-based) that best separated the scores was used to identify reference sites for each indicator.

Table 3. Landscape and nutrient disturbance-class thresholds, taken from Fetscher et al. (2014). Because the area of influence for a depressional wetlands is anticipated to be smaller than for streams, a 500 m radius was used for the landscape spatial scale rather than the 1 km scale used for streams in Fetscher et al. (2014).

Parameter	Scale	Reference threshold
% Agriculture	500 m	3
% Urban	500 m	3
% Agriculture + Urban	500 m	5
Road density (km/km²)	500 m	2
Total N (mg/L)	Local	3
Total P (mg/L)	Local	0.5

Ambient Assessment

Regional wetland condition was evaluated in a two-step process, first by assessing the condition of each indicator, and then by examining the agreement of condition among indicators at each site. To assess the indicators, the 10th and 50th percentiles were calculated for each index among reference sites. These 10th and 50th percentile values were then compared with scores from all sites. Index scores below the 10th percentile were categorized as "likely altered", while scores between the 10th and 50th percentiles were categorized as "possibly altered", and scores above the 50th percentile were categorized as "likely intact". Agreement among indicator condition categories was then assessed. The proportion of sites that had 0, 1, 2 or all 3 indicators in agreement was evaluated for each category.

Stress factors that could potentially contribute to altered wetland conditions were assessed in a few ways. This included recording the occurrence of severe stressors at each site that were believed to have a substantial negative impact on the wetlands (e.g., pesticide application in the surrounding landscape, intensive agriculture, non-point discharges), correlation analysis between indicator scores and overlying water chemistry concentrations, and the relationship between index scores and the intensity of land-use in the adjacent landscape.

Results of cyanotoxins analyses were evaluated relative to existing statewide or international cyanotoxins action levels. The action levels for microcystin concentrations are 0.8 µg/L for human recreational uses (OEHHA, 2012).

Wetlands were also characterized for their trophic state by comparing chlorophyll-a and total P concentrations in the overlying water with the trophic thresholds from Chapra and Dobson (1981). According to the thresholds for chlorophyll-a, systems are considered oligotrophic at concentrations <2.9 µg/L, mesotrophic when chlorophyll-a is within the range of 2.9 – 5.6 µg/L, and eutrophic for systems >5.6 µg/L chlorophyll-a. For total P, systems are oligotrophic at <0.011 mg/L total P, mesotrophic in the range 0.011 – 0.0217 mg/L, and eutrophic for total P concentrations >0.0217 mg/L.

RESULTS

Evaluation and Adaptation of Assessment Tools

The disturbance criteria that best separated reference from non-reference sites varied by indicator (Figure 3). For CRAM, there was good separation in scores when reference was based on the landscape disturbance criteria, and poor separation in scores when reference was based on the nutrient criteria. Conversely, for diatoms and macroinvertebrate index scores, there was better separation between

reference and non-reference sites based on the nutrient criteria than the landscape criteria. Therefore, for this study, only the landscape disturbance criteria were used to define reference sites for CRAM, and only the nutrient criteria were applied to the diatom and MI data. Separation of reference from non-reference was not improved for any indicator when both the landscape and nutrients criteria were used together. The 10th and 50th percentile values of the reference site data appear in Table 4.

Table 4. Index threshold values developed from reference sites based on landscape (CRAM) or nutrient (diatom and macroinvertebrate) criteria. Thresholds for CRAM are based on disturbance within 500 m of the wetland. Reference thresholds from previous studies are shown for comparison.

Indicator	10th percentile of reference scores	50th percentile of reference scores	Reference thresholds from previous studies
CRAM	55.3	67.4	65.5[A]
Diatom	12.6	51.0	53[B]
Macroinvertebrate	19.9	39.4	41.8[C]

[A]Lower 95% prediction interval of CRAM index values from 24 reference depressional wetlands throughout California (CRAM depressional wetlands module development subcommittee, personal communication).

[B]Two standard deviations below the mean response among southern California reference stream sites, from Fetscher et al. (2014).

[C]Lower 95% prediction interval of data for 22 reference depressional wetlands in northern California identified in Lunde and Resh (2012).
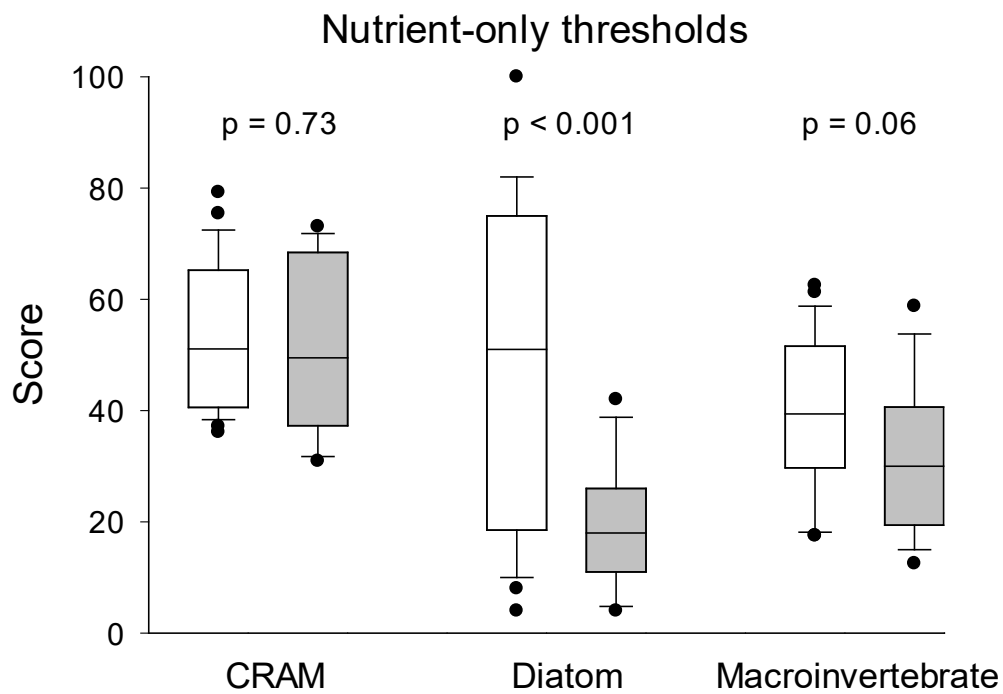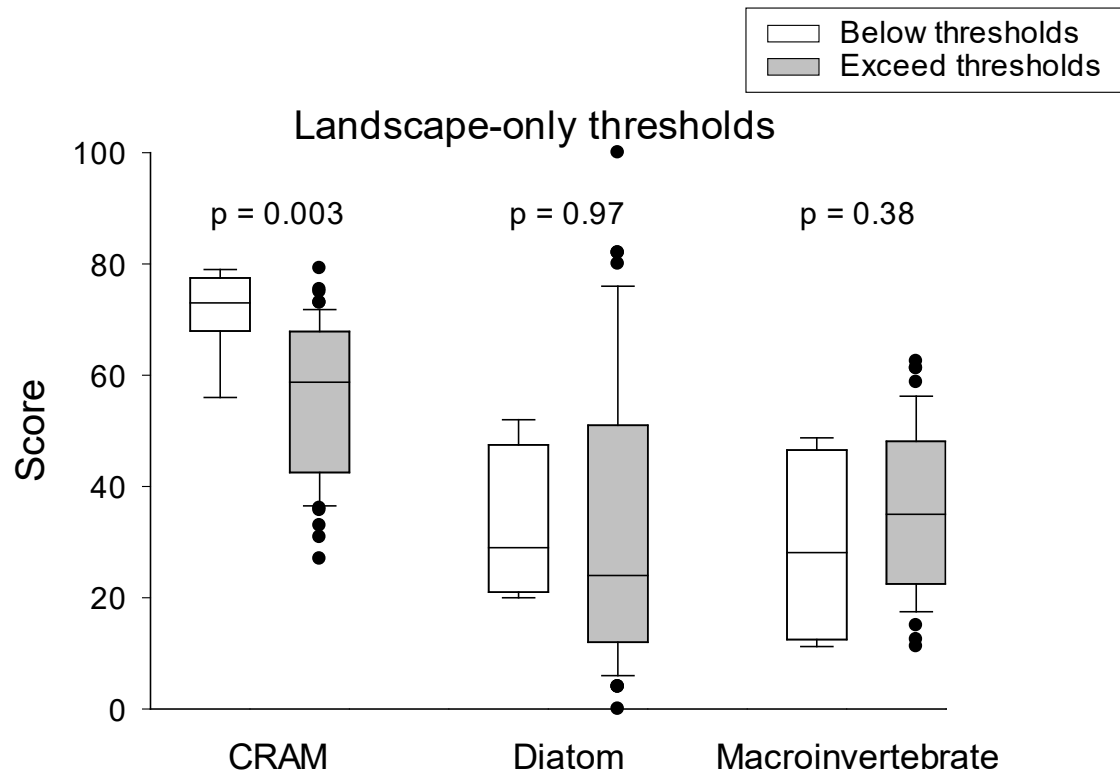
Figure 3. Comparison of the ranges in indicator response for sites that were above or below the landscape or nutrient thresholds in Table 3.

The 10th and 50th percentile thresholds calculated in this study were compared to thresholds obtained or derived from data taken from previous studies. For the CRAM threshold derived from previous work, the lower 95% prediction interval of scores was calculated for 24 reference depressional wetlands throughout California. These data were obtained from the CRAM depressional wetlands module development subcommittee (personal communication). For diatoms, the threshold was obtained from Fetscher et al. (2014), which represented two standard deviations below the mean response among southern California reference stream sites. The threshold for macroinvertebrates was calculated as the lower 95% prediction interval of scores from 22 reference depressional wetlands in northern California identified in Lunde and Resh (2012). Thresholds based on the 50th percentile of scores from reference sites (i.e. 50th percentile of the reference distribution) were comparable to thresholds derived from data taken from previous studies (Table 4). Superficially, the 50th percentile of reference CRAM, diatom and MI scores in southern California compare reasonably well to the statewide reference CRAM threshold (67.4 vs 65.5, respectively), the reference threshold for stream diatoms (51 vs 53), and the reference threshold for MI scores from northern California (39.4 vs 41.8). However, it is important to realize that thresholds from previous studies were based on the lower 95th prediction interval of reference scores for CRAM and macroinvertebrates, and two standard deviations below the mean reference score for diatoms. Thresholds based on the 10th percentile of the local reference distribution were substantially lower than the thresholds derived from previous studies.

The proportion of sites considered reference vs. non-reference varied based on differences in the buffer width used to calculate the adjacent land use disturbance; however, the proportion of sites considered reference vs. altered is relatively consistent at buffer distances of 500m and greater (Table 5). Predictably, the percent of wetlands considered as non-reference increased as the disturbance buffer increases from 30 to 3,000 m due to the increasing likelihood of encountering disturbed conditions such as roads, urbanization, and agriculture. The proportion of sites categorized as likely intact varied by a factor of 3 (13-43% of sites) over the range of buffer widths evaluated, while the proportion of likely altered sites varied by a factor of 5 (11-60% of sites). In most cases, variability in the percent of sites exceeding a reference threshold stabilized at around 500 m. Therefore, the remaining analyses are based on a 500 m threshold for landscape disturbances.

Table 5. Relationship between CRAM index thresholds and the proportion of wetlands with CRAM scores that are likely intact, possibly altered, or likely altered for different landscape disturbance scales. The proportion of wetlands in the likely-altered category is relatively consistent among disturbance scales ≥500m.

Disturbance scale	10th percentile of reference scores	50th percentile of reference scores	% Wetlands likely altered	% Wetlands possibly altered	% Wetlands likely intact
Landscape 30m	64.0	73.0	60.4	26.4	13.2
Landscape 150m	39.2	63.0	11.3	45.3	43.4
Landscape 500m	55.3	67.4	39.6	28.3	32.1
Landscape 1000m	56.5	69.4	43.4	34.0	22.6
Landscape 3000m	57.5	63.7	43.4	17.0	39.6

Ambient Assessment

The proportion of wetlands in the region considered "likely intact" varied by indicator (Figure 4). Approximately 40% of the sites had MI scores in the likely intact range, while 32% of the CRAM scores and 25% of the diatom scores were in this category. CRAM had the greatest proportion of scores in the "likely altered" category. Approximately 40% of the sites had CRAM scores that were likely altered, followed by diatoms (25%), and MI (19% of sites).
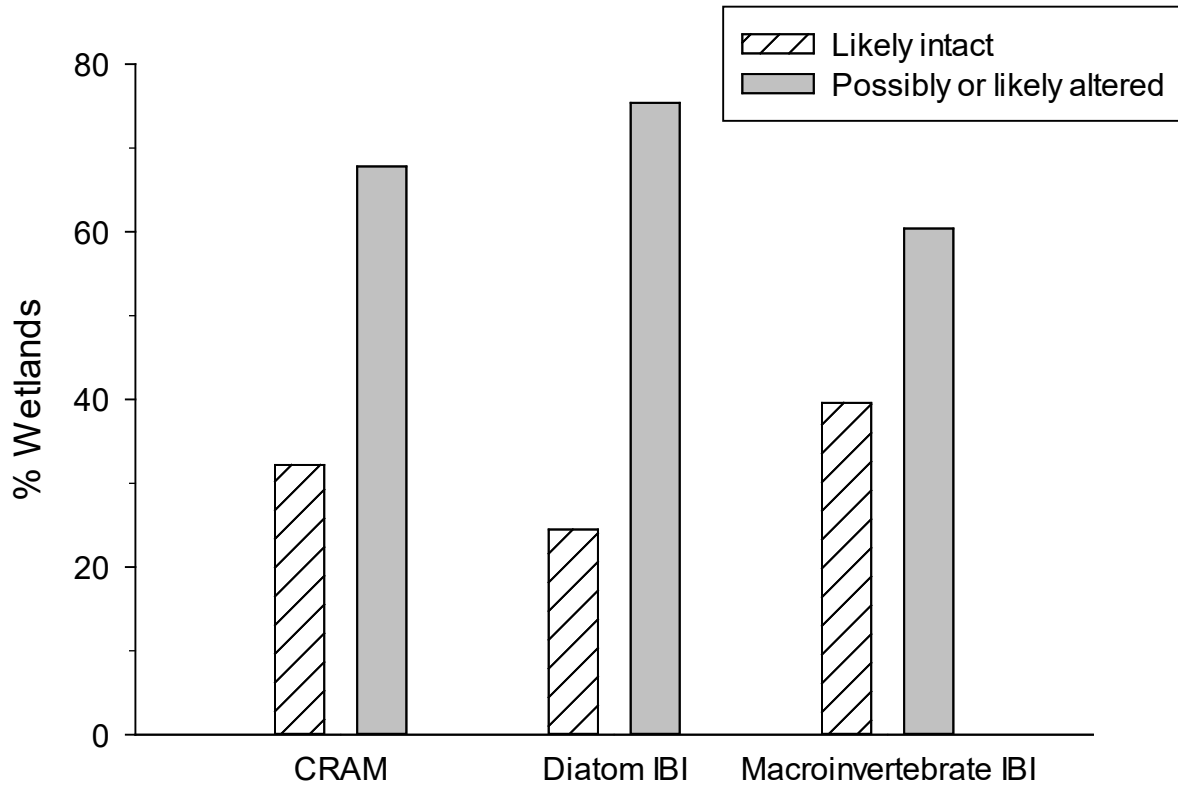
Figure 4. Proportion of wetlands considered "likely intact" according to the three indicators measured in this study.

Only one site was considered intact by all three indicators, and 25% of the sites were considered intact by two of the three indicators (Figure 5 and 6). In contrast, 32% of the sites were below the intact threshold for all 3 indicators (either possibly altered or likely altered). Only one site was considered likely altered by all three indicators, and 13% of the sites had two indicators in this category. Approximately 34% of the wetlands had no indicators in the likely altered category.
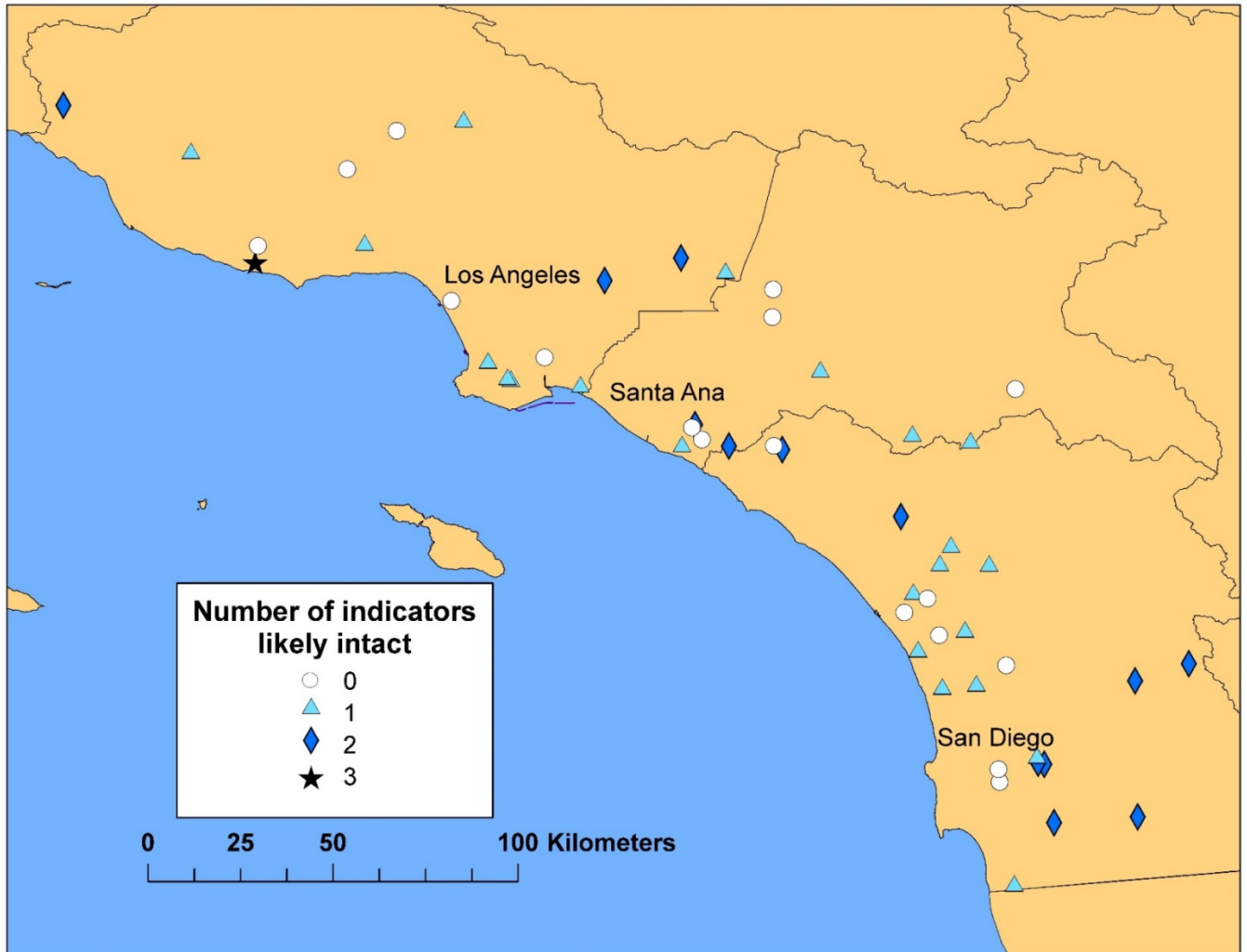
Figure 5. Map of study wetlands indicating their condition based on integrated information from biological indicators (CRAM, and assemblages of diatoms and macroinvertebrates)
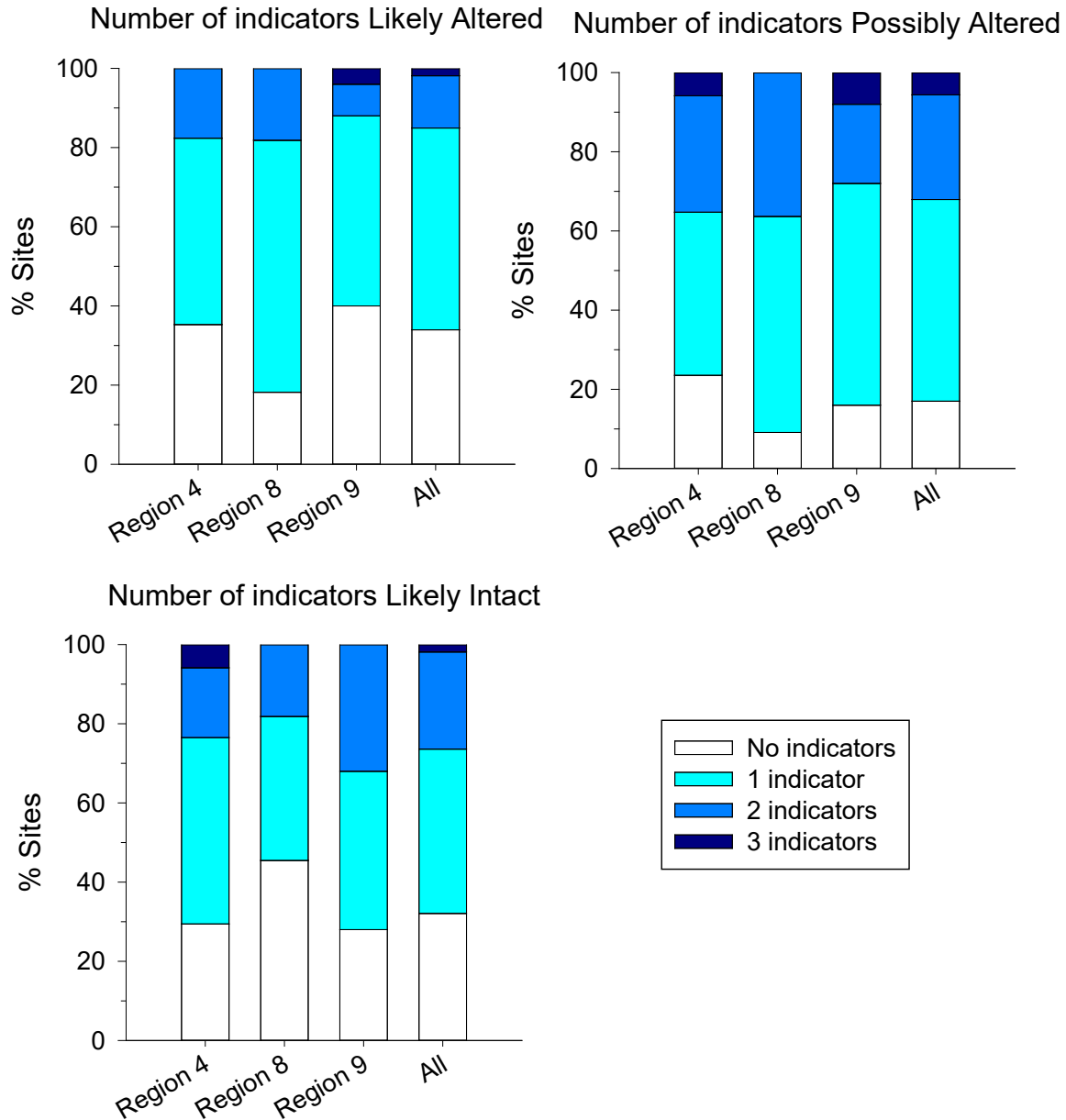
Figure 6. Proportion of wetlands with 0, 1, 2 or 3 indicators in the likely altered, possibly altered and likely intact categories.

The highest agreement among the indicators was between diatoms and MI (45% of the sites). The greatest agreement between these indicators was for the possibly altered category (23% of sites), while 15% of sites were considered to be likely intact by both diatoms and MI. CRAM and MI were in agreement at 26% of the sites. The least amount of agreement was between CRAM and diatoms (21% of the wetlands). Between these indicators, the highest agreement was in the possibly-altered category (11% of sites), followed by likely-altered (6% of sites).

Among direct use-type categories with at least four wetlands, the "no active use" category had the highest proportion of sites with at least two intact indicators (Figure 7). Half the wetlands in this use-type

category had two intact indicators. The one site considered intact by all three indicators was a habitat/stormwater wetland. Golf course ponds had the greatest proportion of sites with no intact indicators. Moreover, the only site in the study where all three indicators were likely altered was a golf course pond. Among the wetland use-type categories with three or fewer sites, water supply ponds had two intact indicators at 67% of the sites, while half of the recreation ponds were in this range, and 33% of the flood control sites had two intact indicators. None of the indicators in the only stock pond in this study were intact.

No active use (n = 8)
- 38%
- 12%
- 50%

Abandoned stock pond (n = 4)
- 25%
- 50%
- 25%

Habitat/stormwater (n = 19)
- 32%
- 47%
- 5%
- 16%

Golf course (n = 13)
- 46%
- 46%
- 8%

Flood control (n = 3)
- 67%
- 33%

Water supply (n = 3)
- 33%
- 67%

Recreation (n = 2)
- 50%
- 50%

Stock pond (n = 1)
- 100%

Legend:
- 0 indicators likely intact
- 1 indicator likely intact
- 2 indicators likely intact
- 3 indicators likely intact
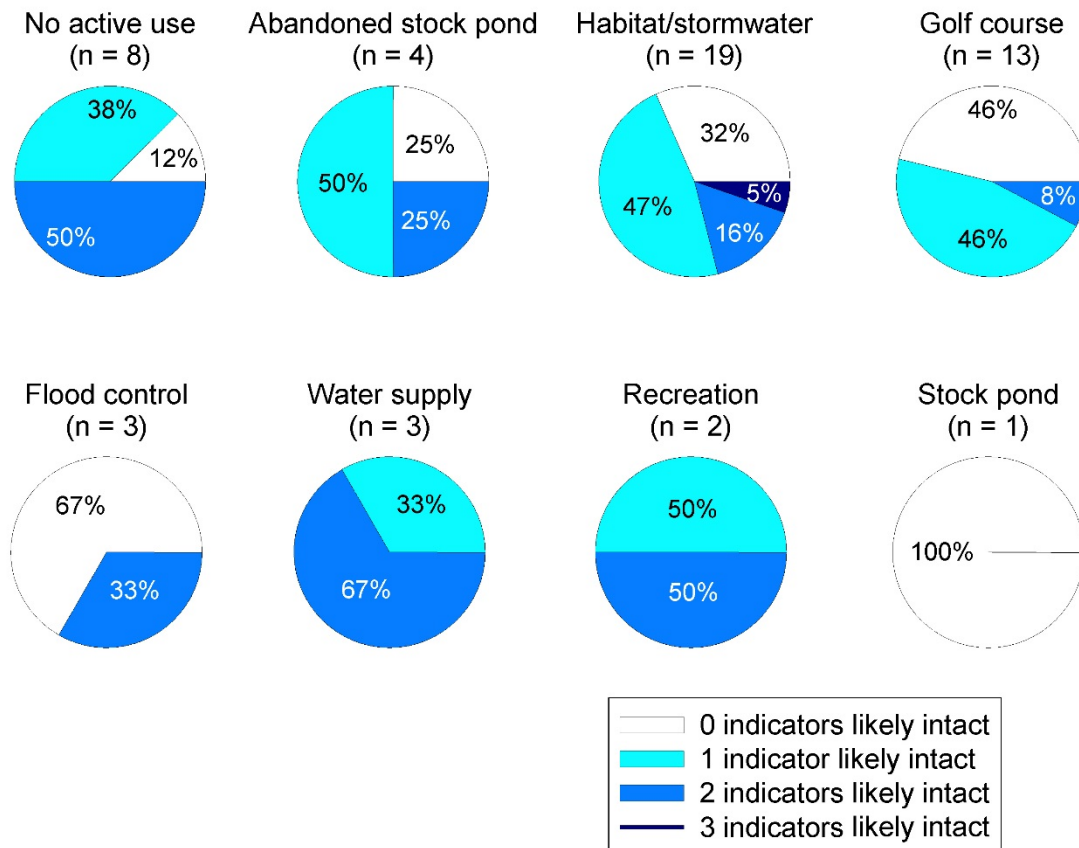
Figure 7. Proportion of sites within each wetland use-type category with 0, 1, 2 or 3 intact indicators. The categories are arranged left to right by increasing intensity of wetland use.

Among the Regional Water Quality Control Boards, Region 9 had the greatest proportion of sites with at least two intact indicators (32% of the wetlands in this Region), followed by Region 4 (24% of sites) and Region 8 (18% of wetlands in the Region) (Figure 6). The greatest proportion of sites with at least two likely altered indicators were in Regions 4 and 8 (18% of the sites in these Regions). Region 9 had the fewest sites with two likely altered indicators (12% of sites in this Region), although this was the only Region with a site considered to be likely altered by all three indicators.

Stressor Analysis

Indicator Relationship with Severe Stressors

CRAM scores decreased significantly with the total number of severe stressors present (r=-0.51, p<0.01), while diatom and MI scores appeared to be unrelated to the total number of severe stressors recorded (r=0.19, r=0.18, respectively). The most common stressor identified was invasive plant species in the area adjacent to the wetlands, which occurred at 42% of the sites (Table 6). Other frequently occurring severe stressors included lack of vegetation management to conserve natural resources (36% of sites), excessive human visitation (34% of sites), mowing/excessive herbivory (32% of sites), non-point discharges (32% of sites), and flow diversions or unnatural inflows (30% of sites).

Table 6. Stressor occurrence among sites. Values indicate the number of sites (out of 53) exhibiting a given stressor. Severe = stressor was considered to have a significantly negative effect.

Stressor	Severe	Present
Lack of treatment of invasive plant species adjacent to AA or buffer	22	37
Lack of vegetation management to conserve natural resources	19	28
Excessive human visitation	18	31
Mowing, grazing, excessive herbivory (within AA)	17	22
Non-point Source (Non-PS) Discharges (urban runoff, farm drainage)	17	38
Flow diversions or unnatural inflows	16	40
Grading/ compaction (N/A for restoration areas)	15	41
Sports fields and urban parklands (golf courses, soccer fields, etc.)	15	21
Transportation corridor	11	36
Actively managed hydrology	10	24
Dike/levees	10	24
Industrial/commercial	9	15
Flow obstructions (culverts, paved stream crossings)	8	19
Nutrient impaired (PS or Non-PS pollution)	7	29
Urban residential	6	37
Vegetation management	5	17
Bacteria and pathogens impaired (PS or Non-PS pollution)	4	12
Engineered channel (riprap, armored channel bank, bed)	4	16
Excessive runoff from watershed	4	17
Pesticides or trace organics impaired (PS or Non-PS pollution)	4	19
Plowing/Discing (N/A for restoration areas)	4	7
Active recreation (off-road vehicles, mountain biking, hunting, fishing)	3	8
Heavy metal impaired (PS or Non-PS pollution)	3	7
Filling or dumping of sediment or soils (N/A for restoration areas)	2	2
Orchards/nurseries	2	9
Predation and habitat destruction by non-native vertebrates	2	21
Ranching (enclosed livestock grazing or horse paddock or feedlot)	2	6
Trash or refuse	2	12
Excessive organic debris in matrix (for vernal pools)	1	3
Passive recreation (bird-watching, hiking, etc.)	1	19
Point Source (PS) Discharges (POTW, other non-stormwater discharge)	1	2
Ditches (borrow, agricultural drainage, mosquito control, etc.)	0	2
Dredged inlet/channel	0	3
Groundwater extraction	0	1
Intensive row-crop agriculture	0	5
Pesticide application or vector control	0	34
Rangeland (livestock rangeland also managed for native vegetation)	0	2
Removal of woody debris	0	3
Treatment of non-native and nuisance plant species	0	2
Tree cutting/sapling removal	0	6
Weir/drop structure, tide gates	0	5

CRAM

The factor that appeared to have the greatest effect on CRAM index scores was the intensity of wetland use, interpreted from the wetland use-type categories (Figure 8). The use-type categories ranged from those with little intensity of use (the ponds with no active use) to those used for active recreation, and the stock pond. As the intensity of use increased, the CRAM scores tended to decrease. Scores were consistently lower at wetland use-types with the greatest intensity of use (golf course, flood control, water supply, recreation, and stock pond). CRAM scores had a statistically significant negative correlation with the level of urbanization ($r = -0.30$, $p = 0.02$) and imperviousness ($r = -0.31$, $p = 0.02$) within 500 m of the wetlands, and with pH ($r = -0.42$, $p < 0.01$) and dissolved oxygen ($r = -0.58$, $p < 0.01$) (Table 7).
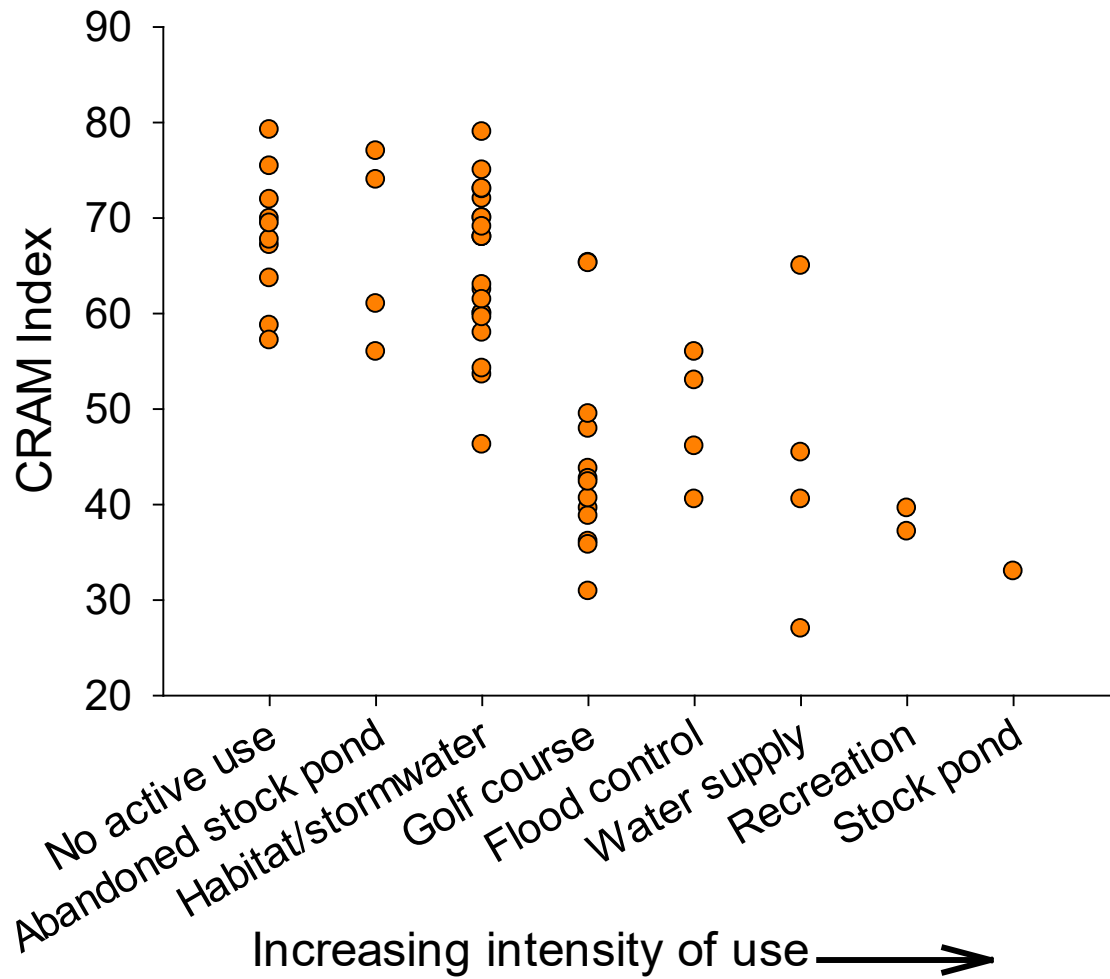

Figure 8. Relationship between CRAM and wetland use-type. The use-type categories are arranged by increasing intensity of use, from ponds with no active use, to the stock pond used to water cattle. CRAM scores decrease with increasing intensity of use.

Table 7. Relationships (correlation r-values) of condition indicators with landscape-level parameters and water-quality constituents. Values in bold indicate statistical significance (p ≤0.05).

Parameter	CRAM Index	Macroinvertebrate IBI	Diatom IBI
Landscape parameters			
Impervious, 150m	*-0.25*	*-0.21*	*-0.09*
Impervious, 500m	**-0.31**	*-0.10*	*0.01*
Impervious, 1000m	*-0.22*	*-0.08*	*-0.06*
Impervious, 3000m	*-0.17*	*-0.06*	*-0.13*
Road density, 150m	*-0.04*	*-0.10*	*0.003*
Road density, 500m	*-0.26*	*-0.15*	*0.04*
Road density, 1000m	*-0.22*	*-0.14*	*-0.05*
Road density, 3000m	*-0.16*	*-0.08*	*-0.11*
Urbanization, 150m	*-0.16*	*-0.20*	*-0.09*
Urbanization, 500m	**-0.30**	*-0.14*	*-0.01*
Urbanization, 1000m	*-0.23*	*-0.12*	*-0.09*
Urbanization, 3000m	*-0.18*	*-0.09*	*-0.14*
General			
Alkalinity	*0.05*	**-0.32**	**-0.45**
Turbidity	*0.04*	*-0.08*	**-0.41**
Salinity	*0.12*	*-0.25*	**-0.55**
Conductivity	*-0.11*	*-0.11*	**-0.44**
Hardness	*0.11*	**-0.33**	**-0.48**
pH	**-0.42**	*0.24*	*-0.08*
Dissolved Oxygen	**-0.58**	*0.11*	*0.13*
Chlorophyll-a	*0.001*	*0.04*	**-0.37**
Nutrients			
Orthophosphate	*0.15*	**-0.45**	**-0.39**
Total Phosphorus	*0.17*	*-0.26*	**-0.54**
Total Nitrogen	*-0.04*	*-0.09*	**-0.42**
Nitrate + Nitrite	*-0.10*	*-0.03*	*0.25*
TKN	*0.06*	**-0.36**	**-0.70**
Conditional indicators			
CRAM Index	—	*0.08*	*-0.21*
MI IBI	*0.08*	—	*0.18*
Diatom IBI	*-0.21*	*0.18*	—

Macroinvertebrate Assemblage

The MI assemblage was weakly related to the quality of the overlying water (Table 7, Figure 9). MI IBI scores tended to decrease with increasing levels of alkalinity ($r = -0.32$, $p = 0.02$), hardness ($r = -0.33$, $p = 0.04$), orthophosphate ($r = -0.45$, $p = 0.01$), and TKN ($r = -0.36$, $p = 0.03$). No significant relationships were observed between MI IBI scores and any of the landscape-level disturbances examined (Table 7), or with intensity of wetland use (not shown). MI IBI scores were not significantly different between perennial and seasonal ponds ($p = 0.74$).
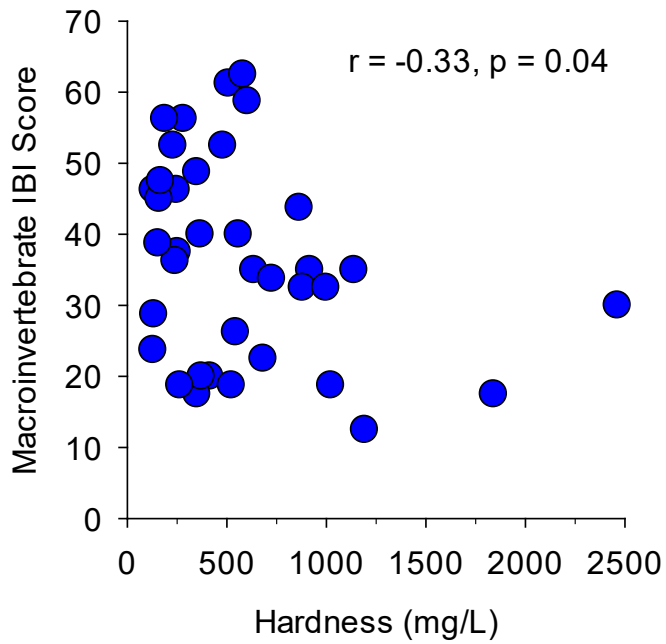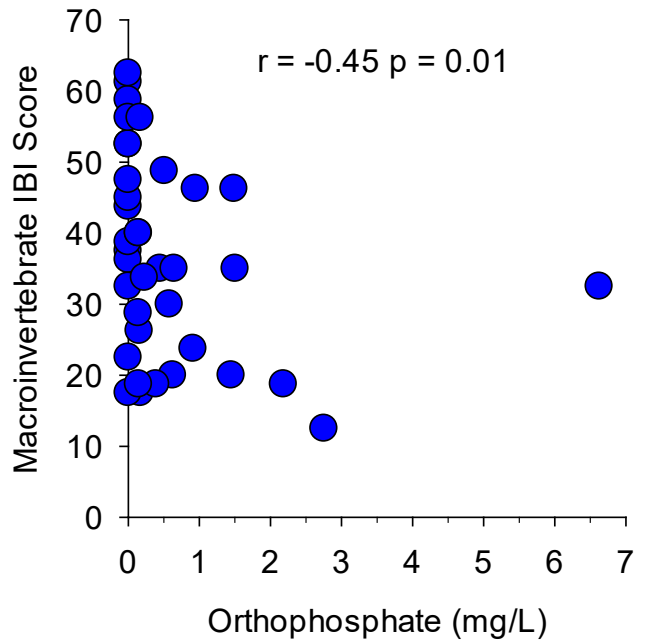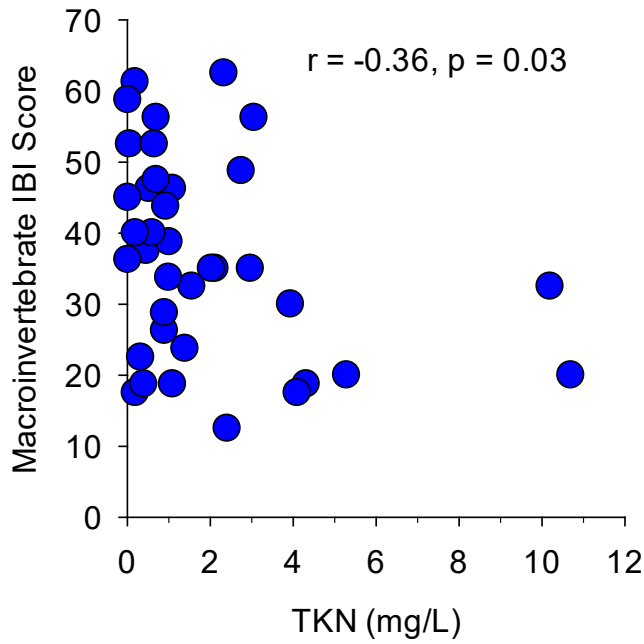
Figure 9. The relationship between macroinvertebrate IBI scores and water quality contaminant concentrations.

Diatom Assemblage

Diatom assemblages were negatively correlated with water column constituents (Table 7, Figure 10). The diatom IBI had a significant negative relationship with alkalinity ($r = -0.45$, $p < 0.01$), salinity ($r = -0.55$, $p < 0.01$), conductivity ($r = -0.44$, $p < 0.01$), hardness ($r = -0.48$, $p < 0.01$), turbidity ($r = -0.41$, $p < 0.01$), orthophosphate ($r = -0.39$, $p = 0.02$), total P ($r = -0.54$, $p < 0.01$), total N ($r = -0.42$, $p = 0.01$), TKN ($r = -0.70$, $p < 0.01$), and chlorophyll-a ($r = -0.37$, $p = 0.01$).
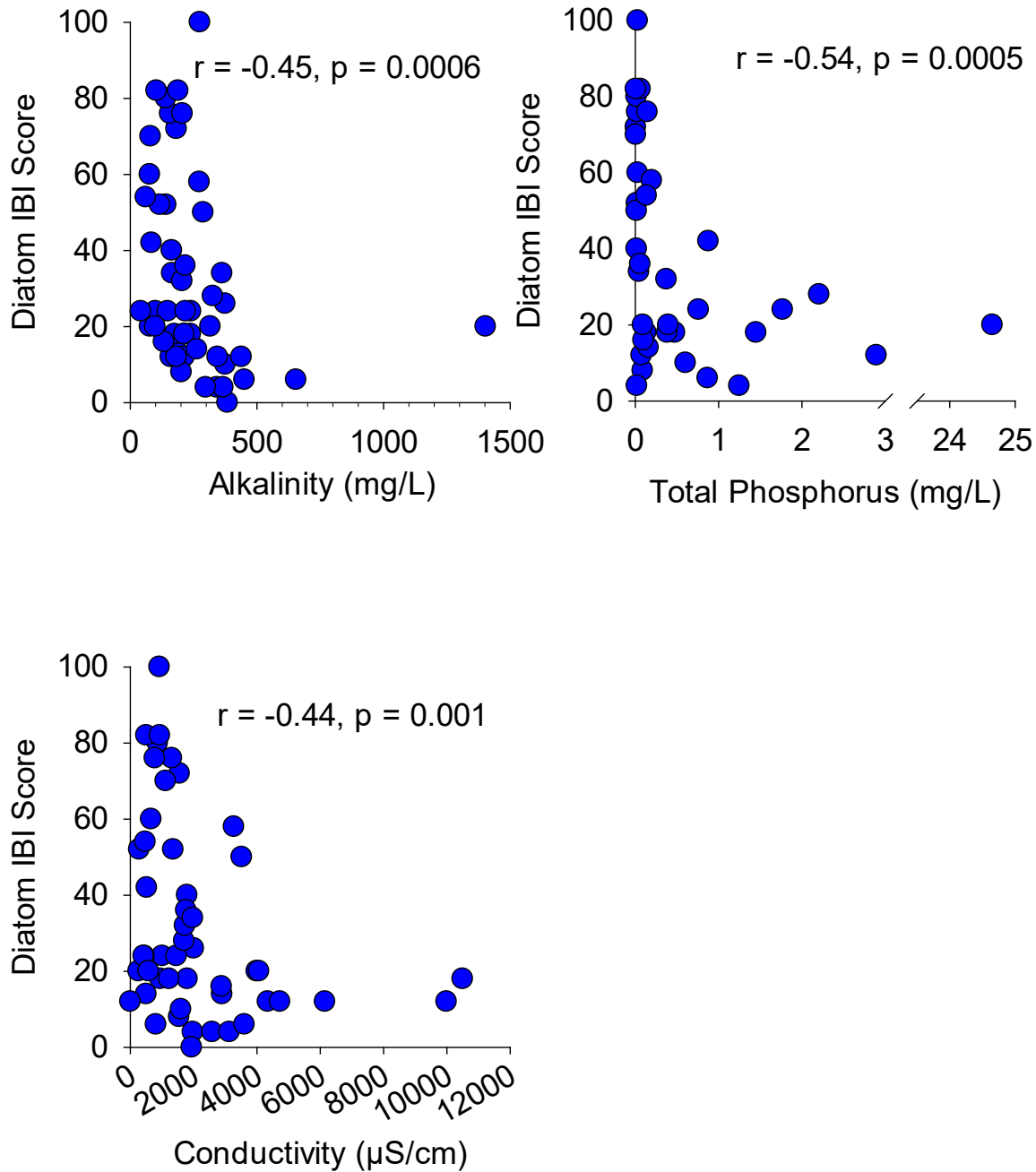
Figure 10. The relationship between diatom IBI scores and water quality contaminant concentrations.

There were no significant relationships between diatom IBI scores and landscape disturbances (Table 7, Figure 11). In addition, there was no significant difference in diatom IBI scores between perennial and seasonal wetlands ($p = 0.17$). However, there were significant negative correlations at seasonal wetlands with imperviousness, urbanization, and road density; diatom IBI scores tended to decrease with increasing imperviousness within 150 m ($r = -0.57$, $p = 0.04$) and 3000 m ($r = -0.61$, $p = 0.03$) of the seasonal wetlands, as well as with increasing urbanization within 150 m ($r = -0.57$, $p = 0.04$), 1000 m ($r = -0.56$, $p = 0.04$) and 3000 m ($r = -0.61$, $p = 0.03$) of the ponds, and also with increasing road density within 3000 m of the seasonal sites ($r = -0.57$, $p = 0.04$). Diatom IBI scores were not related with intensity of wetland use (not shown).
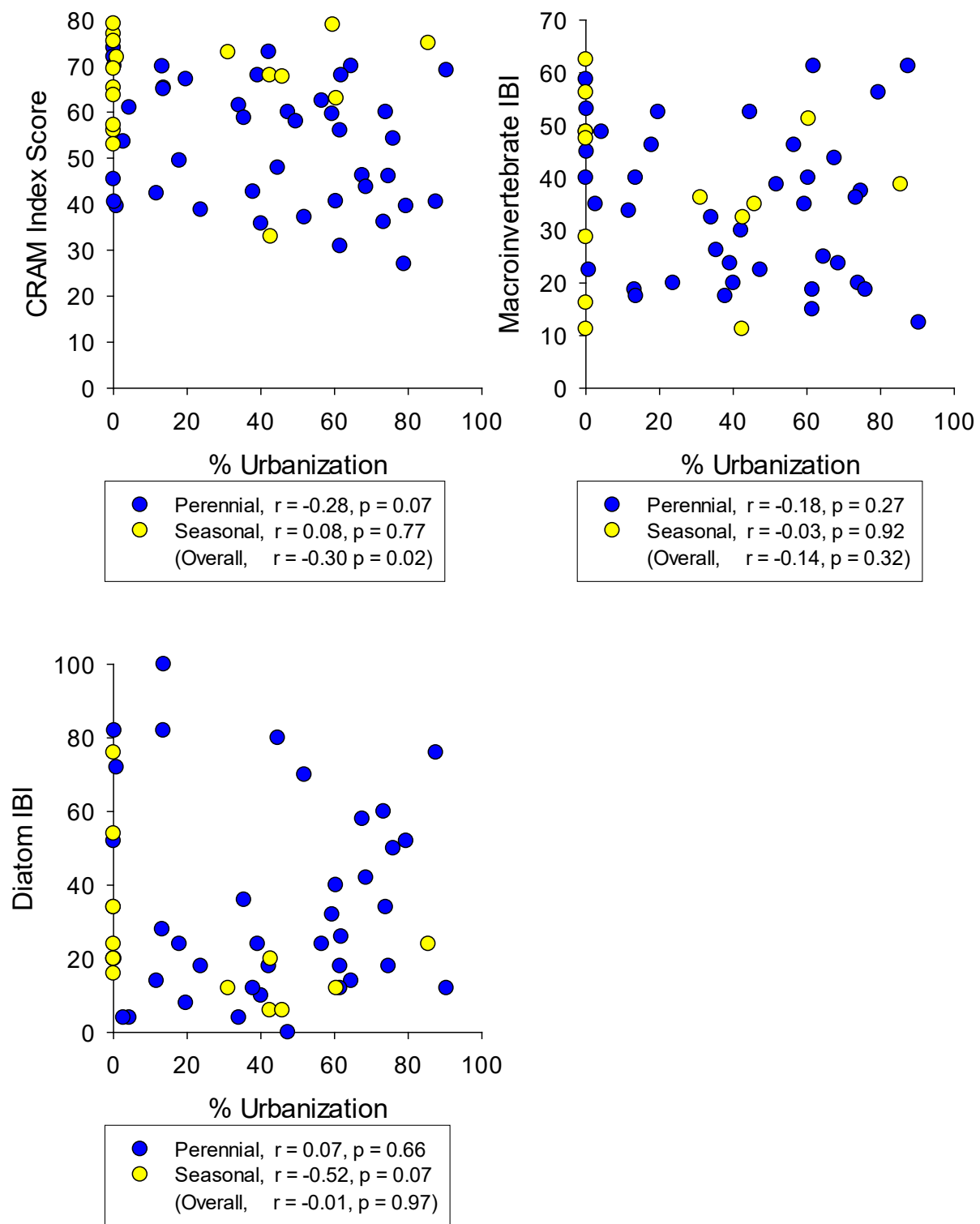
Figure 11. Relationship between urbanization within 500 m of the perimeter of study wetlands and CRAM index scores, macroinvertebrate IBI scores or diatom IBI scores for perennial vs. seasonal ponds.

Relationship between Landscape Disturbance and Water Quality

There were no significant relationships between water chemistry results and the amount of urbanization within 500 m of the wetlands when both seasonal and perennial sites were considered (conductivity r=0.20, p=0.15; hardness r=0.11, p=0.52; total N r=-0.07, p=0.66; total P r=-0.02, p=0.89). However, there were significant positive increases in certain contaminant concentrations with increased urbanization within 500 m of seasonal wetlands (hardness r=0.80, p=0.03; total N r=0.76, p=0.05; orthophosphate r=0.76, p=0.05; total P r=0.76, p=0.05). The number of seasonal sites that had water quality data, however, was relatively low (n=7). There was also a significant but weak relationship between wetland conductivity and the amount of urbanization within 150 m of the ponds when both seasonal and perennial wetlands were considered (r=0.38, p=0.01).

Trophic State

Most wetlands were eutrophic, according to the chlorophyll-a (60% of sites) and total P (78% of sites) trophic class concentration thresholds of Chapra and Dobson (1981) (Table 8). Water supply and flood control ponds were the only use-type categories for which at least half of the sites had chlorophyll-a concentrations below the eutrophic threshold. Recreation ponds were the only use-type class without the majority of sites in the eutrophic range for total P, although this analyte was not measured during the first year of the study, including at all four of the abandoned stock ponds.

Table 8. Summary of water quality and sediment parameters at southern California depressional wetlands.

Constituent	Min	Max	Median	Mean
General				
Alkalinity (mg/L)	42	1,404	214	255
pH	6.7	10.5	8.1	8.2
Salinity (g/kg)	0.2	6.2	0.8	1.2
Specific Conductivity (µS/cm)	263	10,500	1,557	2,164
Hardness (mg/L)	129	2,462	480	583
Turbidity (NTU)	0.4	262	9.1	24.9
Chlorophyll-a (µg/L)	0.6	1,238	13.3	46.3
Nutrients				
Orthophosphate (mg/L)	0.001	6.63	0.15	0.61
Total phosphorous (mg/L)	0.008	24.7	0.13	1.1
Total nitrogen (mg/L)	0.06	17.0	1.4	2.7
Nitrate+Nitrite (mg/L)	0.01	16.9	0.09	1.2
TKN (mg/L)	0.01	10.7	1.0	1.9
Sediment				
Total organic carbon (% dry wt)	0.4	9.8	3.1	3.8
Clay (%)	1.4	26.9	9.5	10.9
Silt (%)	8.8	71.2	41.7	40.0
Sand (%)	7.9	89.4	46.1	49.1

Sediment Toxicity

Only two of the wetlands had sediments that were toxic to amphipods. One of the toxic samples was from a golf course pond, with 2% control-adjusted survival, while the other was from a wetland with no active use and had 65% control-adjusted survival. Survival in the remaining samples ranged from 92-109% of the control-adjusted value.

Cyanotoxins

Microcystins were detected at 25% of sites, though the percentage of sites varied widely from year to year, as did the detectable concentrations. Microcystins were detected in all three years, ranging from 12.5% of all sites in 2011 and 2013 to 47% in 2012. The range of microcystin concentrations found varied from below detection to 2.5 µg L^{-1} in 2011, up to 0.45 µg L^{-1} in 2012, and up to 22 µg L^{-1} in 2013. The highest percentage of positive sites was in 2012; however, in no sites did the concentrations of microcystins exceed the California recreational action thresholds. In contrast, there were only 2 sites in the 2013 results where microcystins were detected; however, the concentrations at both sites exceeded the California recreation action thresholds. In contrast to microcystin, saxitoxin was detectable at very low concentrations (<0.4 µg L^{-1}) at only one site during 2012-2013 (Santee Lakes Recreation Preserve Lake). SPATT bags were deployed at the depressional wetlands sites sampled in the spring of 2012 in San Diego. During the summer and fall, microcystins were detected at only 29% of sites based on grab sample results, but at 83% of sites based on SPATT results.

DISCUSSION

Biological assessment of California depressional wetlands can be reasonably accomplished using existing assessment tools that readily fit with existing ambient monitoring frameworks. Availability of appropriate assessment tools should not be considered a barrier to broad implementation of bioassessment for both project-level and ambient monitoring. Such assessments should use multiple indicators and assessment tools to provide a comprehensive evaluation of depressional wetland condition that accounts for the effect

of multiple types of stressors across different spatial scales. However, the definition of reference thresholds needs to be evaluated and possibly adjusted for individual regions when applying the currently available assessment tools.

Indicator Adaptation

The three existing assessment tools we evaluated (i.e. CRAM, the southern California stream diatom IBI, and the Bay Area macroinvertebrate IBI) were able to differentiate intact from altered wetlands when the reference thresholds were "recalibrated" for southern California conditions. Minor adjustments to the field protocols were necessary to account for the seasonal nature of some wetlands in southern California's semi-arid climate (macroinvertebrate IBI) and to accommodate lentic vs. lotic water bodies (diatom IBI); however, substantial reconstruction or modification of the indices was not necessary.

Different reference definitions were needed for each assessment tool due to the different sensitivities of the indicators used by each tool. Nutrient concentrations were used as the primary reference screen for macroinvertebrates and algae because they have been shown to be an important factor in affecting the composition of these organisms in wetlands (Adamus et al. 2001). This differs from the common practice for streams, where intensity of catchment land use is often used as the primary reference screen (Ode et al. 2016, Yates and Bailey 2010). A key reason for this difference is that data on catchment land use is broadly available from sources such as the National Land Cover Data Set (Homer et al. 2015), whereas nutrient data is often restricted to relatively few sites, making its use as a reference screen impractical. When it is available, nutrient concentrations are believed to be appropriate reference screens for macroinvertebrates in streams (Herlihy et al. 2008). In our case, we had good coverage of nutrient data and it proved to have more discriminatory power in defining reference than land use factors. Furthermore, buffers as narrow as 30-40 m have been shown to largely mitigate the effects of adjacent land use on wetland condition (Hruby 2013, Sweeney and Newbold 2014), thus independently confirming its poor performance as a reference screen for these systems. The lack of any significant relationship between land use factors and water chemistry within the wetlands we studied suggests a similar decoupling in our study area. In contrast to macroinvertebrates and algae, CRAM has been calibrated statewide to be sensitive to changes in the intensity of surrounding land use (Sutula et al. 2006). Our analysis confirmed the utility of landscape disturbance as a reference screen for CRAM.

Regionalization of reference thresholds has been shown to be a useful approach for defining land use, physical or chemical reference criteria (Herlihy et al. 2008, Hawkins et al. 2010). Nevertheless, Hawkins et al. (2010) reported, based on a review of over 1,000 studies on establishing reference condition that these approaches still result in relatively coarse discriminatory ability and contribute more to unexplained variability in assessment results than differences in the assessment tools themselves. Hawkins et al (2010) strongly recommend that site-specific reference definitions using predictive models (as is done with the California Stream Condition Index (CSCI), Mazor et al. 2016) is ultimately the best approach for maximizing assessment accuracy. If state agencies decide to implement ongoing, statewide assessments of depressional wetlands, a predictive modeling approach should be used to develop a statewide scoring tool that provides site-specific reference expectations.

Need for Multiple Indicators

Our results confirm the well-established need for the use of multiple indicators to capture the complexity of ecological systems and to identify the contribution of different stressors on wetland condition (Dale and Beyeler 2001). Between 25 and 40% of wetlands were considered "intact" in our investigation depending on the indicator(s) used to judge condition. In general, the diatom indicators were most responsive to degradation of water chemistry (particularly nutrients), CRAM was most responsive to intensity of adjacent land use and macroinvertebrates were responsive to alkalinity, hardness, orthophosphate, and changes in hydrology; although macroinvertebrates were generally the least responsive of all indicators used. Changes in the assemblage of diatoms (and to a lesser degree

macroinvertebrates) resulting from elevated nutrients and alkalinity suggests that runoff from developed or agricultural land uses containing nutrients and salts may accumulate in depressional wetlands and affect the aquatic communities that live there (Whigham and Jordan 2003, Duffy and Kahara 2011). In contrast CRAM responds to general physical and biological characteristics of the wetland (CWMW 2013).

Previous investigations have also shown that using a single bioassessment indicator may not provide an accurate assessment of overall condition, and that it is prudent to select multiple indicators that complement one another by responding to different environmental stressors (Soininen and Könönen 2004, Johnson and Hering 2009, Purdy et al. 2012). For example, Soininen and Könönen (2004) and Beyene et al. (2009) both found that diatoms and macroinvertebrates responded differently to stressors on stream condition, with diatoms generally being more responsive. Diatom species distribution was most affected by conductivity and total phosphorous, while macroinvertebrates were responsive to physical habitat, conductivity and pH. They concluded that multiple pressures affecting the river ecosystems at different spatial and temporal scales should lead to choosing more than one biological monitoring method with clearly identifiable responses (Soininen and Könönen 2004).

Several past studies have suggested that measuring trophic interactions should be an integral component of wetland bioassessment and that observed responses in primary producer or consumer communities (i.e. algae and macroinvertebrates) are mediated by these interactions. In a review of 14 large-scale investigations of macroinvertebrate response to stressors in North American wetlands, Batzer (2013) found their utility as a reliable bioassessment tool to be equivocal. In general macroinvertebrates were more responsive to direct alterations of wetland hydrology, and less sensitive to changes in water chemistry or adjacent land use practices (Wilcox et al. 2002). In several studies, the presence of predators (e.g. fish or amphibians) was the factor that most accounted for differences in invertebrate communities between wetlands, regardless of other stressors. For example, Tangen et al. (2003) found that the only environmental variable affecting invertebrate communities in prairie potholes was presence or absence of fish, and it was concluded that invertebrates have minimal use for assessing impacts of land use on potholes. In contrast Hall et al. (2004) found that immediately adjacent land use did affect invertebrate species richness in Texas playas and Lunde and Resh (2012) found a significant relationship between their invertebrate IBI and percent urban development in the surrounding catchment. Finally, Hann and Goldsborough (1997) found that macroinvertebrate response to changes in nutrient concentrations were a secondary effect of changes in algal communities that they fed on, and that changes in micro or macro algal communities may be a more direct indicator of condition. As with other studies, they concluded that consideration of trophic interactions is important for accurate interpretation of wetland bioassessment results. Future assessments in California should consider incorporating measures of trophic interactions to better elucidate condition and effects of specific stressors.

The general patterns of wetland conditions based on the ambient assessment were strikingly similar to those observed for streams in the region (Mazor 2015). Approximately 25% of both streams and wetlands were considered intact in southern California based on integration of all the condition indicators. The proportion of wetland and streams considered intact was very similar based on diatom indices (25% vs. 27%) and CRAM (32% vs. 30%). However, macroinvertebrate indicators were less sensitive for wetlands (40% of wetlands intact) than they were for streams (29% of streams intact). This may be due to the fact that the macroinvertebrate IBI was calibrated based on sensitivity to the percent urban land use in the surrounding catchment, and is not particularly sensitive to nutrient concentrations or other water quality parameters (Lunde and Resh 2012). Bird et al. (2013) also found that natural environmental heterogeneity masked responses of macroinvertebrate communities to human disturbance factors and that unless disturbance gradients are severe the signal in macroinvertebrate indices may be hard to detect. Because many southern California wetlands are eutrophic, diatoms may be a more sensitive indicator than macroinvertebrates to the dominant stressor affecting wetland condition.

Stressors

As with streams, excessive nutrients, variables related to ionic concentration, and direct habitat alteration were the dominant stressors affecting wetland condition among those stressors that were tested for/recorded, whereas sediment toxicity was rarely identified. Results of the cyanotoxin analysis were somewhat inconclusive, but indicated that extended (passive) sampling of equilibrium concentrations may be a more appropriate way than single grab samples to assess the prevalence of this stressor, because grab samples sometime missed toxic events captured by the passive samplers. Furthermore, because depressional wetlands are usually hydrologically connected to nearby streams (Whigham and Jordan 2003, Nadeau and Rains 2007) they may serve to sequester nutrients and help protect adjacent stream water quality. The importance of direct habitat alteration as a wetland stressor also suggests that relatively straightforward management measures such as reducing competing uses (e.g. flood control), limiting active recreational uses, and reducing access to livestock can be important strategies for improving wetland health. For example, Jones et al. (2011) found that livestock grazing resulted in decreased richness of native plant communities. Lower plant community diversity can be associated with reduced invertebrate richness and diversity as reflected in bioassessment results, which can be reversed when grazing pressure decreases (Steinman et al. 2003).

Representativeness

Accurate interpretation of the results of a probabilistic assessment (such as the one done in this study) rely on the representativeness of the sampled wetlands. We believe that our samples were representative of the overall population despite the fact that 78% of the sites that passed office reconnaissance screening were eliminated, largely due to difficulties with site access. The representativeness of the sampled wetlands was assessed by comparing the wetland use-types of the ponds that were sampled with the use-type for sites that met the study physical criteria but permission to sample could not be acquired. For many of the wetland use-type categories, the number and proportion of sites was comparable between those that could be sampled and those where permission could not be acquired. The largest discrepancies were among the habitat/stormwater ponds (19 sites were sampled and 2 sites were inaccessible), as well as the sites on private residences used as landscape ponds (permission was acquired for 1 site, while 8 other sites were inaccessible). This may have slightly lowered the proportion of intact wetlands identified in our survey, but we do not believe it affected the overall conclusions of the assessment.

Recommendations

Ambient assessment of depressional wetlands should be expanded statewide to provide more comprehensive information on the condition of these ubiquitous, but highly threatened wetlands. Such assessments provide important context for evaluating proposed impacts, identifying high quality wetlands for protection, prioritizing management measures, and informing restoration practices. We provide the following recommendations should there be expansion of the depressional assessment program to other areas:

- Reference thresholds need to be recalibrated for each new region assessed. Over the long term, we recommend development of a statewide predictive assessment index that provides site-specific reference expectations. This would eliminate the need for reference calibration in each individual region. Development of a predictive scoring tool would likely require identification and assessment of additional references sites. This could be accomplished through expansion of the state's reference condition management program (RCMP) to include depressional wetlands.

- Assessment should include multiple indicators. Initially macroinvertebrates, algae, and CRAM should be used. Ultimately assessment tools should expand to include higher trophic levels and evaluation of trophic interactions.

- Additional testing and method refinement/expansion is needed for highly seasonal wetlands. Initially, there should be further evaluation of the assessment window to determine applicability of existing tools for the range of wetland hydroperiods. Ultimately new tools or indicators may be necessary for wetlands with very short inundation periods.

- The wetland status and trends plots should be used to provide a statewide sample frame for ambient assessment. Probabilistic assessment requires a comprehensive map of depressional wetlands, which currently does not exist in California. As an alternative, the probabilistic mapping plots produced by the wetland status and trends program can serve as a statewide sample frame (Stein and Lackey 2012).

- Trend detection should be included in future ambient assessment programs. Because of their lentic nature, depressional wetlands provide good sentinel sites for assessing long term patterns associated with climate changes, changes in land use practices, and watershed management actions. A portion of trend monitoring sites should be reference locations in order to capture short and long term natural variability in condition.

- Outreach activities should target application of existing (and future) assessment tools to a variety of programs, including wetland protection, stormwater management, timber harvest, agricultural runoff, and non-point source. Incorporation of tools in a variety of programs will help improve our understanding of the condition and management of depressional wetlands.

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Office and Field Reconnaissance Effort

The ability to sample depressional wetlands is dependent upon identifying sites that meet the physical criteria for the survey, and acquiring permission to sample the wetlands. This appendix summarizes the efforts to identify suitable sites.

A combination of office and field reconnaissance was used to identify sampling locations that met the requisites of the study. The initial list of depressional wetlands to be screened was obtained from depressional wetland maps provided by California State University, Northridge. Maps were based on 2005 or newer imagery and utilized the enhanced National Wetlands Inventory (NWI) mapping methodology. A probabilistic sample draw (Stevens and Olsen, 2004) was conducted on this sampling frame to identify sites for the study. In 2012, the wetlands were evaluated following the order in which they appeared in the sample draw, while in 2013 the order of the sample draw was used, but sites that were nearby were also included, regardless of their order.

Office reconnaissance

The office reconnaissance component was conducted first, to pre-screen sites for the more time-consuming field reconnaissance portion of the study. For the office reconnaissance, wetlands were evaluated for presence of characteristics incompatible with the goals of the survey, such as marine influence, concrete- or plastic-lined bottoms, those wetlands functioning as treatment ponds, or wetlands on military bases, and removed from the list accordingly. Google Earth[TM] imagery and Google Maps Street View[TM] were used for the office screening.

One thousand ninety five sites from the sample draw list were evaluated during the office reconnaissance (Table A1). Through the office screening process 710 sites (65%) were eliminated, mostly because they were consistently dry in Google Earth[TM] imagery (n=243), they appeared to be lined (n=113), or were not wetlands (n=114, which often included pond-shaped vegetation without surface water).

Table A1. Summary of office and field reconnaissance results.

	Region 4	Region 8	Region 9	Total
Planned number of sites to sample	17	11	25	53
Total number of sites evaluated in the office	470	145	480	1,095
Number of acceptable sites from office evaluation	140	55	190	385
Total number of sites evaluated in the field	75	38	146	259
Number of sites deemed acceptable from field evaluation and permission to sample was granted	17	11	27	55

Field reconnaissance

Sites were evaluated during field reconnaissance for 1) the ability of the field crews to safely sample the wetland, 2) the presence of water that was expected to be still present in May, 3) the presence of a wadeable littoral zone, 4) road access within 2 miles of the wetland, or within a 1.5 hour hike of the wetland, 5) the ability to legally gain access to the wetland, and 6) the absence of the exclusion criteria investigated during the office reconnaissance (e.g., marine influence, concrete-lined bottoms, etc.) that were not readily apparent in Google Earth[TM].

Site visits were attempted at 259 ponds during the field reconnaissance portion of the study (Table A2). Of these sites, 69 were inaccessible and could not be evaluated. Many of the inaccessible ponds were on private property. Of the 190 sites that could be evaluated (visualized) during field reconnaissance, 93 sites met the

physical criteria for this study. Permission to sample was acquired for 55 of these sites, including the 53 targeted number of sites, plus 2 backup wetlands. Of the 97 ponds that did not meet the physical criteria, the greatest proportion of sites were removed because they were dry (n=52), due in part to the extended drought (the cumulative rainfall for the 2011-2012 wet season was 39% of that of a typical year, while the total rainfall for 2012-2013 was 58% of normal). The other top reasons for removing sites during field reconnaissance were because they were lined (n=27), they were not wetlands (n=5), or they were unsafe to sample (n=3).

Table A2. Reasons for removing potential sampling sites during the office and field reconnaissance. Values represent the number of samples removed.

| Reason for removal	Office evaluation	Field evaluation
Dry	243	52
Not a wetland	114	5
Lined (concrete, plastic)	113	27
Military	96	1
No access (pond met physical requirements)	50	38
No access (could not evaluate pond)	0	67
Riverine	41	2
Livestock wastewater pond	22	1
Marine influenced	16	1
Treatment pond	13	2
Exceeds size limit	1	0
Unsafe to sample	0	3
Remote	0	2
Cows disturbing the pond	0	1
Dyed water	0	1
Vernal pool	0	1

Overall success rate

Combining the success rates during the office (35%) and field (22%) reconnaissance portions of the study, there was an overall success rate of 8% to identify candidate wetlands that met the physical criteria and acquire permission to sample.

Representativeness

The representativeness of the sampled ponds was assessed by comparing the wetland use-types of the ponds that were sampled (or permission was acquired but the pond was reserved as a backup) with the use-type for sites that met the study physical criteria but permission to sample could not be acquired.

While it was difficult to find ponds that met the physical criteria (especially as a result of the drought), among the ponds that met the criteria, permission to sample was acquired or not needed for over half of the sites (59%). For many of the wetland use-type categories, the number of sites was comparable between those that could be sampled and those where permission could not be acquired (Figure A1). The largest discrepancies were among the habitat/stormwater ponds (19 sites were sampled and 2 sites were inaccessible), as well as the sites on private residences used as landscape ponds (permission was acquired for 1 pond that was reserved as a backup site, while 8 other sites were inaccessible). Overall, the sites sampled in this study were representative of the types of wetlands that met the study physical criteria.
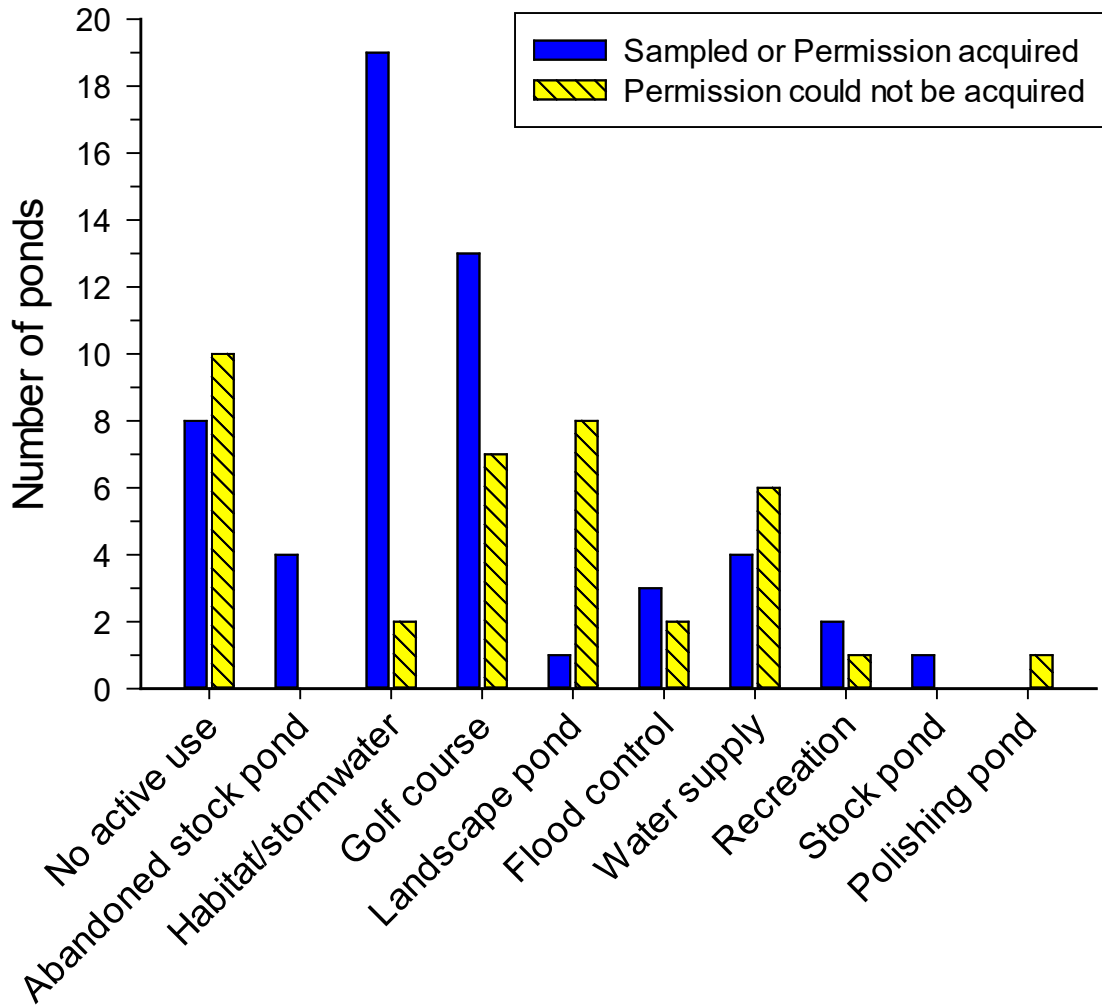
Figure A1. Comparison of the wetland use-types for ponds that permission could be acquired (or was not needed), and those that met the study physical criteria but permission to sample could not be acquired.