



Final Report

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# ASSESSMENT OF THE CONDITION OF SAN FRANCISCO BAY AREA DEPRESSIONAL WETLANDS

Eric D. Stein<sup>1</sup>, Kevin B. Lunde<sup>2</sup>, and Jeffrey S. Brown<sup>1</sup>

<sup>1</sup>Southern California Coastal Water Research Project <sup>2</sup>San Francisco Bay Regional Water Quality Control Board

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## **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 ambient monitoring and assessment in California. Consequently, decisions regarding protection, restoration, and management, such as issuance of Section 401 water quality certifications or prioritization of projects for wetland restoration grant funding, are usually made without the benefit of any regional context of condition, knowledge of predominant stressors, or rigorous documentation of reference conditions.

The goals of this study were to apply tools for ambient monitoring of depressional wetlands in the San Francisco Bay region to accomplish the following: 1) To evaluate the regional condition of depressional wetlands in this portion of northern California, and 2) To evaluate the relationship between condition and stress by sampling both local stressors (intensity of direct wetland use, water chemistry, and sediment chemistry) and landscape stressors (adjacent land use, flow diversions, and road density). Once achieved, these goals should establish the foundation for developing a robust ambient depressional wetland monitoring program.

This study included perennial (have surface water year round) and seasonal (lack surface water for part of the year) depressional wetlands as defined by Brinson (1993) located within the boundaries of the San Francisco Bay Regional Water Quality Control Board. Depressional wetlands in this study includes both natural water bodies such as sag ponds and small lakes, as well as created wetlands such as abandoned or active stock ponds, aesthetic ponds, and irrigation/treatment ponds. Wetlands were not considered for this study if they were concrete lined, marine influenced, wastewater treatment ponds, livestock wastewater ponds, riverine (i.e., dominated by riverine hydrology), dry, or vernal pools. It is likely that a majority of wetlands sampled in this study would be considered waters of the State of California; the field teams did not perform a regulatory-based delineation of a wetland as part of this study.

Thirty wetlands in the San Francisco Bay area were sampled during the spring of 2014, including both perennial and seasonal wetlands. The proportion of wetlands in the region considered "likely intact" (scores in the upper 50<sup>th</sup> percentile of the range found at reference sites) varied by indicator (Figure ES-1).

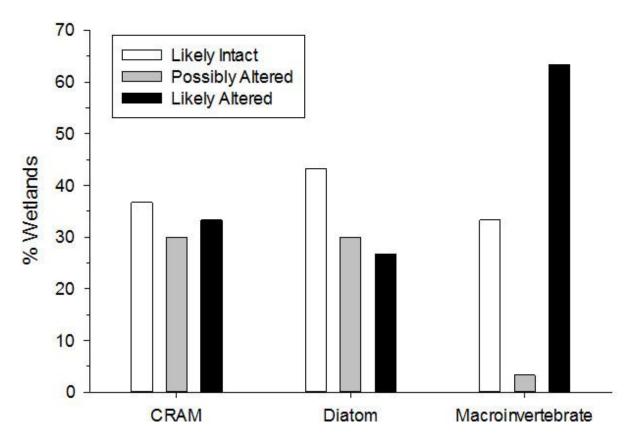


Figure ES-1. Proportion of wetlands considered "likely intact", "possibly altered" or "likely altered" according to the three indicators measured in this study.

Approximately 37% of the sites were likely intact based on CRAM scores, 43% based on diatom scores, and 33% based on macroinvertebrate scores. When all indicators were integrated, 30% of sites were likely intact based on at least two of the three indicators, with 17% of wetlands considered intact by all three indicators (Figure ES-2). Therefore, based on the concordance of at least two indicators and given that this study utilized a probabilistic sample draw for site selection, we can infer that approximately 30% of wetlands in the San Francisco Bay Area are likely intact. These findings are generally in good agreement with results from locally-derived macroinvertebrate indicator and the stream algae indicator developed in Southern California where approximately 40% of the sites were intact based on macroinvertebrate scores and 25% were intact based on diatom scores.

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 agriculture and urbanization. Both diatom and macroinvertebrate indices were relatively insensitive to surrounding land use factors, but were sensitive to water quality factors. Diatom assemblages were negatively correlated with alkalinity and conductivity and both indicators were sensitive to phosphorous- and nitrogen-containing nutrients. Stressor relationships between biological indicators and landscape/water-quality data were largely the same between perennial and seasonal wetlands. The most common stressors in the surrounding landscape were mowing/excessive herbivory, intensive row-crop agriculture, ranching, non-point source pollution, urbanization and rangeland.

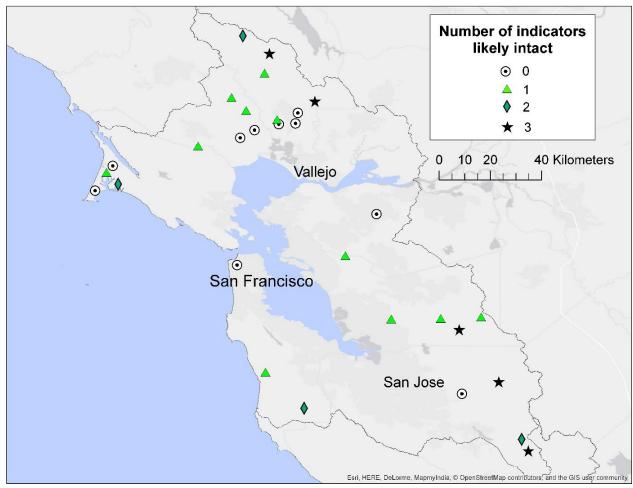


Figure ES-2. Locations of sampling sites and condition based on the number of indicators indicating intact wetlands.

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 for biological and CRAM indicators 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 Water Board's Surface Water Ambient Monitoring Program'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, such as amphibians or birds and evaluation of trophic interactions. The latter could be advanced through application of molecular methods which are being used in other places as a tool to assess food web complexity.
- Additional sampling procedures 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, such as 1-3 months.

- Integrate data from site-specific projects (e.g., 401s on wetland tracker) with ambient assessments where available.
- The wetland status and trends plots (should the state implement this program) should be used to provide a statewide sample frame for ambient assessment. This eliminates the need for comprehensive wetland mapping to support a probabilistic sampling design.
- In the more distant future, 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.

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Sediment and laboratory water chemistry measurements were performed by Moss Landing Marine Labs Marine Pollution Studies Laboratory (MPSL) and California Department of Fish and Wildlife Water Pollution Control Laboratory (WPCL), respectively. Macroinvertebrate sorting and identification was conducted by California Department of Fish and Wildlife Aquatic Bioassessment Laboratory. Diatom sorting and identification was conducted by EcoAnalysts. Data collected through this study is available through the California Environmental Data Exchange Network (CEDEN, <a href="http://ceden.org">http://ceden.org</a>), eCRAM (<a href="http://cramwetlands.org">http://cramwetlands.org</a>) and EcoAtlas (<a href="http://ecoatlas.org">http://ecoatlas.org</a>) websites.

# Acronyms

Term	Definition
CRAM	California Rapid Assessment Method
CSCI	California Stream Condition Index
DO	Dissolved Oxygen
IBI	Index of Biological Integrity
PEC	Probable Effect Concentration
SAFIT	Southwest Association of Freshwater Invertebrate Taxonomists
STE	Standard Taxonomic Effort
TKN	Total Kjeldahl Nitrogen

## 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-Roa 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. 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).

Fortunately, California has a growing set of assessment tools that have been shown to apply across wetland types in a variety of settings and are sensitive to different classes of indicators (Brown et al. 2016). The goals of this study were to apply these tools for ambient monitoring of depressional wetlands in the San Francisco Bay region to accomplish the following: 1) To evaluate the regional condition of depressional wetlands in this portion of northern California, and 2) To evaluate the relationship between condition and stress by sampling both local stressors (intensity of direct wetland use, water chemistry, and sediment chemistry) and landscape stressors (adjacent land use, flow diversions, and road density). Once achieved, these goals should establish the foundation for developing a robust ambient depressional wetland monitoring program.

### Methods

#### SAMPLING APPROACH

Thirty wetlands were sampled in the San Francisco Bay Area of northern California during May or June of 2014 (Figure 1). Depressional wetlands in this study included both created and natural water bodies, such as sag ponds, small lakes, abandoned or active stock ponds, farm ponds, and irrigation ponds. 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 using "open water" wetlands identified by Bay Area Aquatic Resources Inventory (BAARI) maps (<a href="http://www.sfei.org/baari">http://www.sfei.org/baari</a>). Each wetland was visited once following initial reconnaissance. Both perennial (surface water present year round) and seasonal (surface water not present year round) wetlands were sampled, and the wetland ponds represented a range of

intensity of use-types (Table 1). Treatment/irrigation ponds included agricultural runoff, stormwater runoff, winery wastewater, and ponds used to irrigate crops. Created stock ponds were classified as being actively grazed if cows or signs of cows were present, or abandoned if there was no active grazing evident at the site or based on information from the landowner. Aesthetic ponds were created ponds that supported water contact or noncontact water recreation uses. Ponds were classified as natural if field crews could see no evidence of artificial berms or landowners confirmed they were natural. Wetlands varied in size from 23 – 24,000 m², and the level of urbanization within



500 m of the wetlands ranged from 0-66%, while the level of agriculture ranged from 0-87%. Wetlands were not considered for this study if they were concrete lined, marine influenced, wastewater treatment ponds, livestock wastewater ponds, riverine (i.e., dominated by riverine hydrology), or dry at the time of sampling, per the detailed descriptions in the sampling protocol (Table 2; Fetscher et al. 2015). 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. Site reconnaissance resulted in some sites being rejected due to lack of access or permission to sample (see Appendix A); consequently, many of the sites actually sampled were on public lands. It is likely that a majority of wetlands sampled in this study would be considered waters of the State of California. The field teams did not perform a regulatory-based delineation of a wetland as part of this study and the inclusion of a site in this study does define it as waters of the State.

A multiple indicator approach was used to evaluate wetland condition and stressors. Indicators of condition included assemblages of macroinvertebrates, 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 and sediments were measured as potential indicators of stress that could be affecting wetland condition (Table 2).

#### FIELD SAMPLING

Samples for water quality, diatoms and macroinvertebrates 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 macroinvertebrate. 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 macroinvertebrates 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, 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).

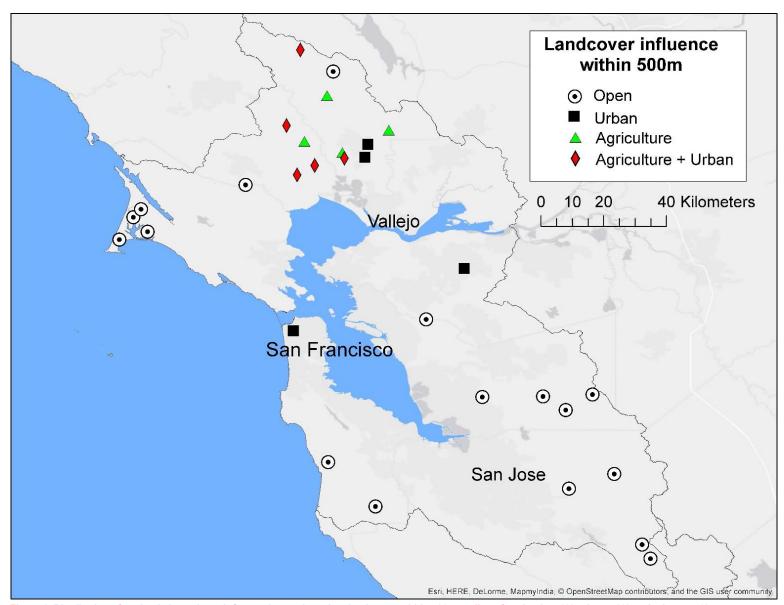


Figure 1. Distribution of wetlands investigated. Categories are based on land cover within 500 m radius of wetlands: >3% urban, >3% agriculture, >5% agriculture + urban, or open. Regional Water Quality Control Board boundaries are added for clarity

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

StationID	Water Regime	Wetland Use	Area (m²)	%Urbanization within 500m	% Agriculture within 500m	Specific Conductivity (µS/cm)
201DW0079	Perennial	Abandoned stock pond	750	0	0	276
201DW0143	Perennial	Abandoned stock pond	800	2	0	441
204DW0150	Seasonal	Abandoned stock pond	236	0	0	1,020
205DW0246	Perennial	Abandoned stock pond	500	0	0	91
201DW0207	Perennial	Active stock pond	600	0	0	787
201DW0271	Perennial	Active stock pond	188	0	0	1,165
204DW0039	Seasonal	Active stock pond	420	0	0	700
204DW0074	Perennial	Active stock pond	1,504	0	0	961
204DW0227	Seasonal	Active stock pond	96	0	0	620
205DW0011	Seasonal	Active stock pond	336	0	0	445
205DW0142	Seasonal	Active stock pond	336	0	0	839
206DW0016	Perennial	Active stock pond	875	2	0	309
206DW0068	Perennial	Active stock pond	215	6	7	142
206DW0123	Perennial	Active stock pond	23	0	0	1,000
206DW0228	Perennial	Active stock pond	471	4	47	384
202DW0050	Perennial	Aesthetics	4,335	32	0	509
202DW0194	Perennial	Aesthetics	2,880	0	0	573
206DW0100	Perennial	Aesthetics	6,075	36	0	790
206DW0212	Perennial	Aesthetics	800	4	19	289
206DW0239	Perennial	Aesthetics	400	20	66	632
206DW0244	Perennial	Aesthetics	636	55	0	455
207DW0017	Perennial	Aesthetics	13,980	0	10	143
204DW0202	Seasonal	Natural pond	375	0	0	409
205DW0151	Perennial	Natural pond	399	0	0	1,311
202DW0018	Seasonal	Treatment/Irrigation	480	0	0	128
206DW0132	Perennial	Treatment/Irrigation	1,320	0	45	104
206DW0171	Perennial	Treatment/Irrigation	300	15	25	342
206DW0180	Perennial	Treatment/Irrigation	24,000	2	16	321
206DW0235	Perennial	Treatment/Irrigation	400	2	87	2,760
207DW0167	Perennial	Treatment/Irrigation	2,079	66	0	220

Table 2. Constituent analytical methods, detection limits, and effects thresholds. dw = dry weight.

	Analysis Method		Effects Threshold	
Water Chemistry				
рН	Probe		<6.5, >8.5 mg/L	
Dissolved Oxygen	Probe		<5 mg/L	
Alkalinity	SM 2320B	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	SM 10200 H-1	4 μg/L		
Microcystin	ELISA (Envirologix QuantiPlate™ kit)	0.01 μg/L	0.8 µg/L¹	
Sediment Metals				
Arsenic	EPA 200.8	0.05 mg/kg dw	33 mg/kg dw <sup>2</sup>	
Cadmium	EPA 200.8	0.03 mg/kg dw	4.98 mg/kg dw <sup>2</sup>	
Chromium	EPA 200.8	0.05 mg/kg dw	111 mg/kg dw²	
Copper	EPA 200.8	0.05 mg/kg dw	149 mg/kg dw²	
Lead	EPA 200.8	0.05 mg/kg dw	128 mg/kg dw²	
Manganese	EPA 200.8	0.05 mg/kg dw		
Nickel	EPA 200.8	0.05 mg/kg dw	48.6 mg/kg dw <sup>2</sup>	
Selenium	EPA 200.8	0.27 mg/kg dw	4 mg/kg dw³	
Silver	EPA 200.8	0.08 mg/kg dw		
Zinc	EPA 200.8	0.05 mg/kg dw	459 mg/kg dw <sup>2</sup>	

Note: <sup>1</sup>Human recreational use action level (OEHHA 2012). <sup>2</sup>Probable effect concentrations (MacDonald et al. 2000). <sup>3</sup>Observed effects threshold (Van Derveer and Canton 1997).

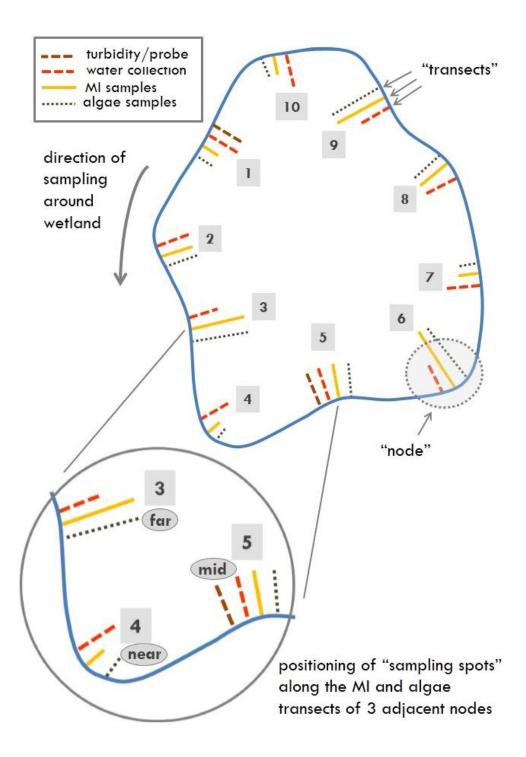


Figure 2. Placement of sampling transects (depicted as dashed or solid lines) for collection of water, macroinvertebrate (MI), and diatom (algae) samples at each of the 10 nodes around the edge of the wetland's surface water, and collection of turbidity and probe (conductivity, temperature, pH, DO, etc.) 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  $\mu$ m polytetrafluoroethylene (PTFE) filter then frozen in the field. Subsamples for total phosphorus (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  $\mu$ m glass fiber filters, and frozen. Microcystin concentrations were also analyzed in the grab samples.

Macroinvertebrates were collected using a custom made 500  $\mu$ m D-frame all-mesh net (Wildco<sup>TM</sup> 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) macroinvertebrates 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  $\mu$ m 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 chemistry 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. The containers were held on wet ice while transported to the analytical lab.

CRAM was conducted according to the depressional wetlands field book version 6.1 (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 SWAMP quality assurance program plan (SWAMP 2013). 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 macroinvertebrate 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 macroinvertebrate 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.

Samples for sediment trace metals were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICPMS) using U.S. Environmental Protection Agency Method 200.8 (1994).

#### DATA ANALYSIS

#### Identification of Reference Data in order to Derive Conditional Thresholds

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). For CRAM, potential reference sites were identified among the wetlands in this study based on the amount of landscape disturbance within 500 m of the wetlands. The landscape disturbance variables included % agriculture, % urban, % agriculture + urban, and road density and were derived from the 2001 National Land Cover Database. For diatoms, reference criteria were based on concentration of nutrients (total N and total P) in the overlying water. The landscape disturbance and nutrient criteria were taken from Fetscher et al. (2014).

For macroinvertebrates, reference data were based on 22 wetlands from northern California that lacked landscape disturbances and human uses in the surrounding area (Lunde and Resh 2012).

The reference datasets for each of the three indicators were used to derive thresholds to differentiate "likely intact", "possibly altered" and "likely altered" conditional categories used in the ambient assessment described below.

#### **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 10<sup>th</sup> and 50<sup>th</sup> percentiles (10<sup>th</sup> and 25<sup>th</sup> percentiles for macroinvertebrates) were calculated for each index among reference wetlands (Table 3). These 10<sup>th</sup> and 50<sup>th</sup> percentile values were then compared with scores from all sites. Index scores below the 10<sup>th</sup> percentile were categorized as "likely altered", while scores between the 10<sup>th</sup> and 50<sup>th</sup> percentiles (10<sup>th</sup> and 25<sup>th</sup> percentiles for macroinvertebrates) were categorized as "possibly altered", and scores above the 50<sup>th</sup> percentile (25<sup>th</sup> percentiles for macroinvertebrates) 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, and 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 microcystin analyses were evaluated relative to existing human recreational use action levels (0.8  $\mu$ g/L, OEHHA 2012).

Sediment metal concentrations were assessed by comparison to freshwater probable effect concentrations (PECs, Table 2) (MacDonald et al. 2000). The PEC thresholds are intended to identify contaminant concentrations above which harmful effects on sediment-dwelling organisms are expected to frequently occur. Selenium concentrations were evaluated by comparison to the observed effect concentration threshold by Van Derveer and Canton (1997).

Table 3. Index threshold values used to differentiate indicator condition. Index scores below the 10th percentile were categorized as "likely altered", while scores between the 10th and 50th percentiles (10th and 25th percentiles for macroinvertebrates) were categorized as "possibly altered", and scores above the 50th percentile (25th percentiles for macroinvertebrates) were categorized as "likely intact".

Indicator	10 <sup>th</sup> percentile of reference scores	25 <sup>th</sup> percentile of reference scores	50 <sup>th</sup> percentile of reference scores
CRAM Index Score	55		61
Diatom (D18)	28.8		52.0
Macroinvertebrate	46.6	52.5	

## Results

#### AMBIENT ASSESSMENT

The proportion of wetlands in the region considered "likely intact" varied by indicator (Figure 3). Approximately 37% of the sites had CRAM scores in the likely intact range, while 43% of the diatom scores and 33% of the macroinvertebrate scores were in this category. For CRAM, 30% of the sites were possibly altered and 33% of wetlands were in the likely altered category. Among diatom scores, 27% were likely altered and 30% of sites were possibly altered. Most macroinvertebrate scores were in the likely altered category (63% of wetlands), while 3% of sites were possibly altered.

Approximately 17% of the sites were considered likely intact by all three indicators, and 30% of the wetlands were likely intact based on at least two of the three indicators (Figure 4). One third of the wetlands had no indicator in the likely intact category, while 37% of sites had one likely intact indicator and 13% of sites had two likely intact indicators. Three wetlands (10% of the total) had all three indicators in the likely altered category. Natural ponds/abandoned stock ponds had the greatest proportion of wetlands with  $\geq$ 2 indicators that were likely intact (67% of sites,). "Aesthetics" ponds (including waterbodies in urban parks, ponds near houses, and a golf course pond) had the highest proportion of sites with no likely intact indicators (57%), followed by active stock ponds (45%). However, the differences in the number of likely intact indicators among wetland use-type categories were not statistically significant (ANOVA, p = 0.09; Figure 5). In contrast, there was a significant difference in the number of likely intact indicators among land cover influence categories (ANOVA, p=0.02; Figure 5). The number of intact indicators at wetlands with open land cover (mean = 1.5) was significantly greater than at urban wetlands (mean = 0.0).

There was generally good agreement between pairs of indicators (i.e., conclusions based on at least two of the three indicators agreed at approximately 50% of sites). The highest agreement among indicators was between diatoms and macroinvertebrates (50% of sites, correlation among scores r = 0.36, p = 0.05), followed by CRAM and diatoms (47% of sites, correlation among scores r = 0.29, p = 0.12). The least amount of agreement was between CRAM and macroinvertebrates (40% of sites, correlation among scores r = 0.41, p = 0.03). For those sites that had agreement between diatoms and macroinvertebrates, most agreement was among likely intact sites.

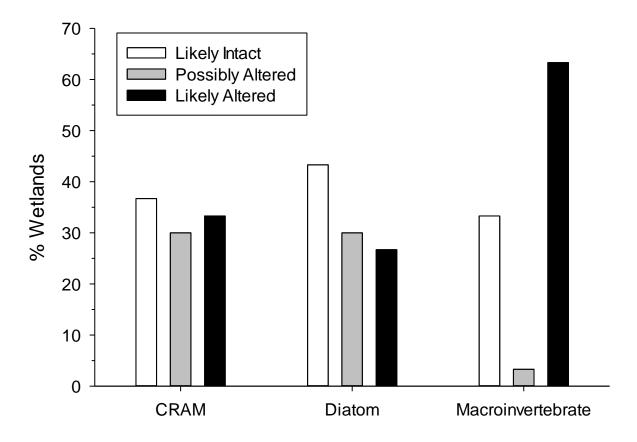


Figure 3. Proportion of wetlands considered "likely intact", "possibly altered" or "likely altered" according to the three indicators measured in this study.

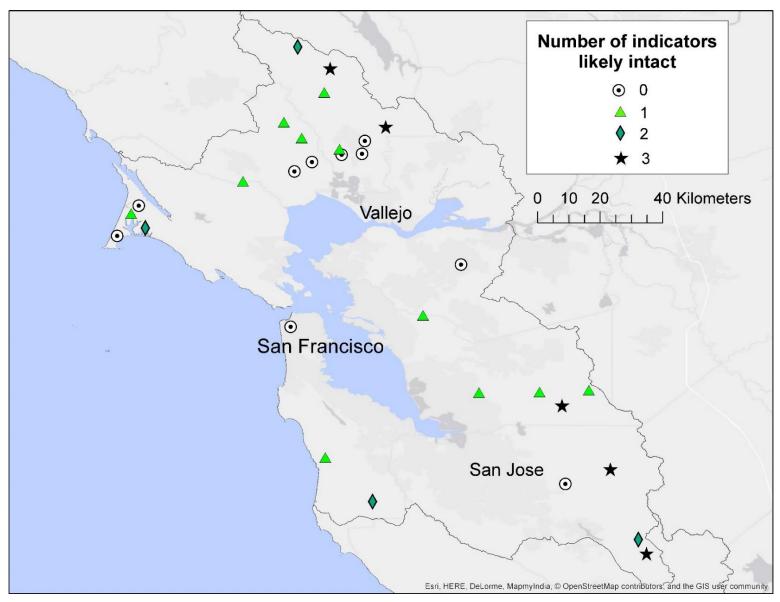
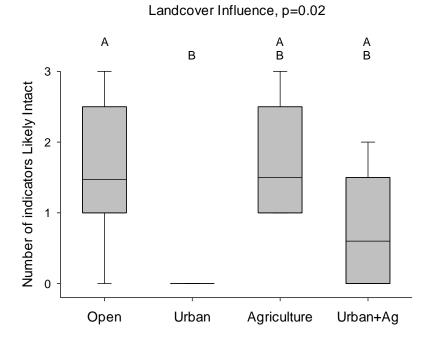


Figure 4. Map of study wetlands indicating their condition based on integrated information from biological indicators (CRAM, and assemblages of diatoms and macroinvertebrates).



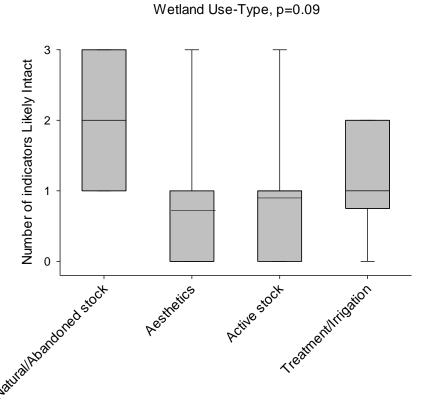
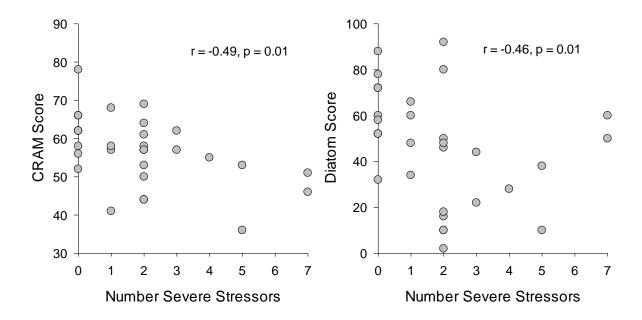


Figure 5. Number of indicators likely intact per site, by land cover influence and wetland use-type categories. Open land cover and urban sites had significantly different numbers of likely intact indicators, while the number of intact indicators was not significantly different among wetland use-type categories.

#### STRESSOR ANALYSIS

#### **Indicator Relationship with Severe Stressors**

CRAM, diatom, and macroinvertebrate scores all decreased significantly with the total number of severe stressors present (r=-0.49, p=0.01, r=-0.46, p=0.01, r=-0.44, p=0.01, respectively, Figure 6).



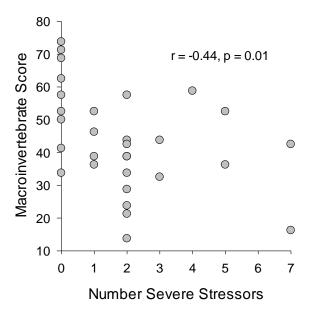


Figure 6. Relationship between indicator score and the number of severe stressors present in the landscape.

The most common stressor identified was mowing/grazing/excessive herbivory, which occurred at 40% of the sites (Table 4). Other frequently occurring severe stressors included intensive row-crop agriculture (20% of sites), ranching (17% of sites), non-point source discharge (13% of sites), urban residential (13% of sites), and livestock rangeland (13% of sites).

#### **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 7). CRAM scores had a statistically significant negative correlation with the level of agriculture (r = -0.41, p = 0.03) and agriculture+urbanization (r = -0.45, p = 0.01) within 500 m of the wetlands, as well as the level of water column turbidity (r = -0.37, p = 0.05) (Table 5). Scores were positively correlated with elevation (r = 0.39, p = 0.03). CRAM scores and the relationship with relevant stressors were not significantly different between perennial and seasonal wetlands (p = 0.22).

The distribution of CRAM scores (36-78) was narrower than for macroinvertebrates (14-74) or diatoms (2-92). However, after normalizing CRAM scores to the range that is possible with this indicator (25-100), CRAM scores were comparable with the range for macroinvertebrates (Figure 8). The range of possible diatom and macroinvertebrate IBI scores is from 0-100, so no normalization was necessary for these indicators.

#### **Macroinvertebrate Assemblage**

The macroinvertebrate assemblage data had a negative relationship with nutrient concentrations (Table 5, Figure 9). Macroinvertebrate IBI scores tended to decrease with increasing levels of ammonia (r = -0.56, p < 0.01), nitrate+nitrite (r = -0.38, p = 0.04), orthophosphate (r = -0.36, p = 0.05), TKN (r = -0.38, p = 0.04), and total P (r = -0.39, p = 0.03). No significant relationships were observed between macroinvertebrate IBI scores and sediment metal concentrations or any of the landscape-level disturbances examined (Table 5), or with intensity of wetland use (Figure 7). Scores were positively correlated with elevation (r = 0.48, p = 0.01). The distribution of macroinvertebrate IBI scores was not significantly different between perennial (mean = 42, standard deviation = 14.1) and seasonal ponds (mean = 50, standard deviation = 18.9) (p = 0.25) (Figure 10). Macroinvertebrates assemblages in Northern California differed slightly from those in Southern California according to an NMS ordination, although wetland disturbance was associated with similar shifts in community structure (Appendix B).

Table 4. Stressor occurrence among sites. Values indicate the number of sites (out of 30) exhibiting a given stressor as recorded during CRAM assessments. Severe = stressor was considered to have a significant negative effect.

Stressor	Severe	Present
Mowing, grazing, excessive herbivory (within AA)	12	3
Intensive row-crop agriculture	6	1
Ranching (enclosed livestock grazing or horse paddock or feedlot)	5	4
Non-point Source (Non-PS) Discharges (urban runoff, farm drainage)	4	10
Urban residential	4	4
Rangeland (livestock rangeland also managed for native vegetation)	4	1
Dike/levees	3	7
Excessive human visitation	3	3
Actively managed hydrology	2	9
Vegetation management	2	5
Point Source (PS) Discharges (POTW, other non-stormwater discharge)	2	4
Flow diversions or unnatural inflows	2	3
Industrial/commercial	2	2
Sports fields and urban parklands (golf courses, soccer fields, etc.)	2	1
Grading/ compaction (N/A for restoration areas)	1	2
Nutrient impaired (PS or Non-PS pollution)	1	2
Pesticide application or vector control	1	1
Weir/drop structure, tide gates	1	1
Predation and habitat destruction by non-native vertebrates (e.g., Virginia opossum and domestic predators, such as feral pets)	1	0
Dams (reservoirs, detention basins, recharge basins)	0	9
Passive recreation (bird-watching, hiking, etc.)	0	6
Trash or refuse	0	6
Bacteria and pathogens impaired (PS or Non-PS pollution)	0	5
Active recreation (off-road vehicles, mountain biking, hunting, fishing)	0	4
Engineered channel (riprap, armored channel bank, bed)	0	4
Transportation corridor	0	3
Lack of treatment of invasive plant species adjacent to AA or buffer	0	2
Plowing/Discing (N/A for restoration areas)	0	2
Dams (or other major flow regulation or disruption)	0	1
Biological resource extraction or stocking (fisheries, aquaculture)	0	1
Treatment of non-native and nuisance plant species	0	1

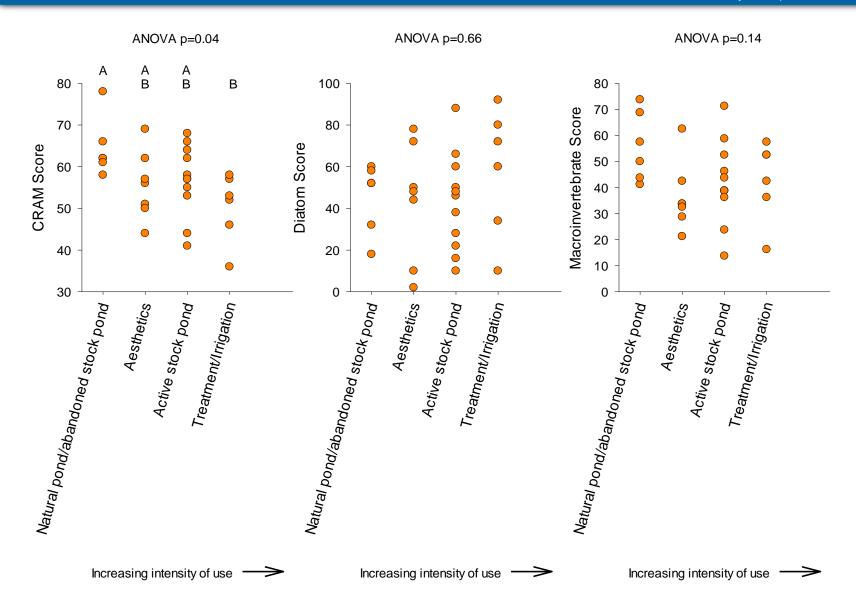
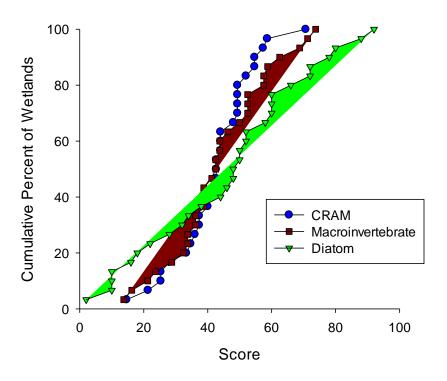


Figure 7. Relationship between indicator score and wetland use-type. The use-type categories are arranged by increasing intensity of use, from ponds with no active use, to those used as treatment or irrigation ponds. CRAM scores decrease with increasing intensity of use, with a significant difference between natural pond/abandoned stock ponds and treatment/irrigation ponds.

Table 5. 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			
Elevation	0.39	0.48	0.41
Agriculture, 500 m	-0.41	-0.15	0.01
Urbanization, 500 m	-0.35	-0.32	-0.35
Urban+Ag, 500 m	-0.45	0.34	-0.27
Road density, 500 m	-0.15	-0.28	-0.17
General water quality			
Alkalinity	-0.17	0.01	-0.46
Turbidity	-0.37	-0.30	-0.26
Conductivity	-0.01	0.03	-0.36
рН	-0.09	0.28	-0.12
Dissolved Oxygen	-0.14	0.29	-0.11
Chlorophyll-a	-0.58	-0.32	-0.25
Nutrients			
Orthophosphate	-0.32	-0.36	-0.29
Total Phosphorus	-0.29	-0.39	-0.55
Total Nitrogen	-0.18	-0.15	-0.50
Nitrate + Nitrite	-0.22	-0.38	-0.26
Ammonia	-0.09	-0.56	-0.51
TKN	-0.11	-0.38	-0.45
Sediment metals			
Arsenic	-0.06	0.11	0.07
Cadmium	0.07	-0.15	-0.04
Chromium	-0.14	0.17	-0.15
Copper	-0.29	0.14	-0.01
Lead	-0.07	-0.16	0.16
Manganese	-0.20	0.01	0.05
Nickel	-0.13	0.20	-0.25
Selenium	0.07	-0.03	0.03
Silver	0.00	0.13	0.32
Zinc	-0.19	0.13	0.06
Conditional indicators			
CRAM Index	_	0.41	0.29
Macroinvertebrate IBI	0.41	_	0.36
Diatom IBI	0.29	0.36	_



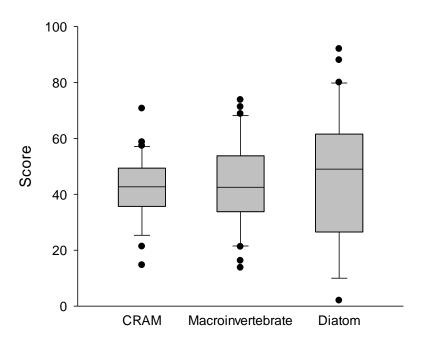


Figure 8. Cumulative frequency distributions and box plots of indicator scores. CRAM scores have been normalized from the range of scores possible (25-100) to be on the same scale as the IBI scores; the possible range of IBI scores for diatoms and macroinvertebrates is 0-100, so normalization was not needed.

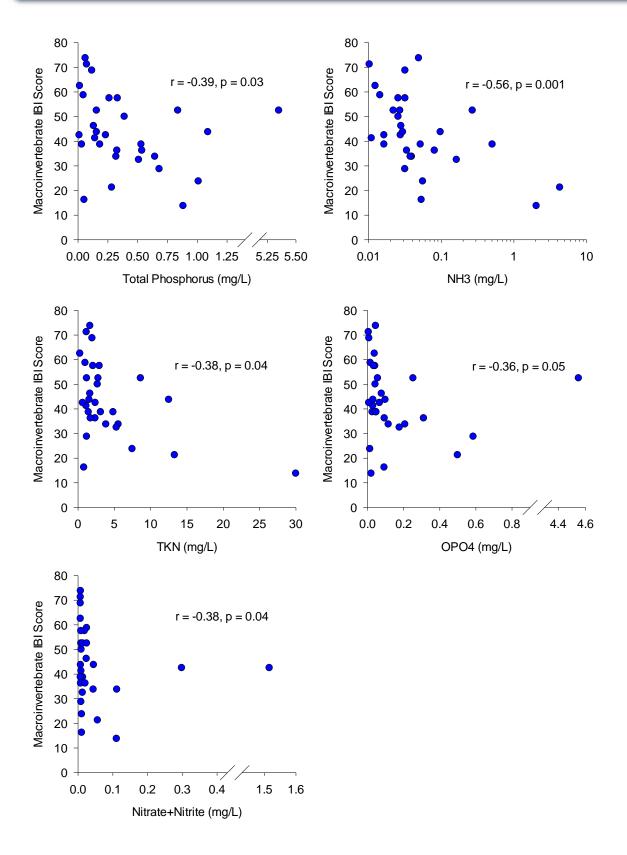


Figure 9. Relationship between macroinvertebrate IBI scores and water quality contaminant concentrations.

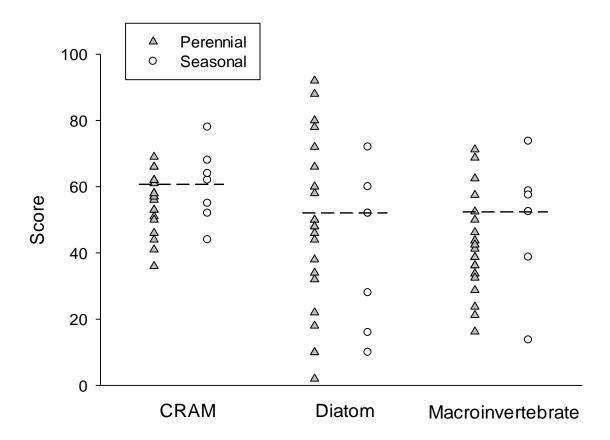


Figure 10. Distribution of indicator scores by water regime. The dashed horizontal line is the "likely intact" threshold for each indicator.

#### **Diatom Assemblage**

Diatom assemblages were negatively correlated with water column constituents (Table 5, Figure 11). The diatom IBI had a significant negative relationship with alkalinity (r = -0.46, p = 0.01), conductivity (r = -0.36, p = 0.05), ammonia (r = -0.51, p < 0.01), total P (r = -0.55, p < 0.01), total N (r = -0.50, p = 0.01), and TKN (r = -0.45, p = 0.01). Diatom scores were not correlated with sediment metal concentrations.

There were no significant relationships between diatom IBI scores and landscape disturbances (Table 5). However, scores were significantly correlated with elevation (r = 0.41, p = 0.02). Among perennial wetlands, there were significant negative correlations with urbanization

(r = -0.48, p = 0.02) and agriculture+urbanization (r = -0.41, p = 0.05). Diatom IBI scores were not related to the intensity of wetland use (not shown). There was no significant difference in diatom IBI scores between perennial and seasonal wetlands (p = 0.54) (Figure 10). Diatom assemblages in Northern California differed very slightly from those in Southern California according to an NMS ordination, and wetland disturbance was associated with similar shifts in community structure (Appendix B).

#### **Sediment Contamination**

Almost half of the wetlands (47%) had at least one metal with concentrations that exceeded a sediment probable effects threshold (Figure 12). Nickel was the metal with the greatest proportion of wetlands exceeding a sediment effects threshold (43% of wetlands), followed by chromium (23% of wetlands), selenium (17% of wetlands) and zinc (3% of wetlands). The greatest number of metals to exceed a threshold at any site was 3, which occurred at 13% of wetlands. The wetland use-type that had the greatest proportion of exceedances was the active stock ponds, with 64% of these wetlands exceeding at least one metal threshold; aesthetics ponds were determined to exceed at least one metal threshold at 43% of wetlands, while natural/abandoned stock ponds and treatment/irrigation ponds each had an exceedance rate of 33%.

#### **Water Quality**

Wetland pH measurements ranged from 6.4 - 9.4 (Table 6), with 20% of sites exceeding the upper pH threshold. Dissolved oxygen concentrations ranged from 0.6 - 17.2 mg/L, with 37% of sites below the minimum desired DO concentration of 5.0 mg/L.

Microcystin concentrations were below the reporting level for most of the wetlands (97%). The one wetland with measurable amounts of microcystin (0.03  $\mu$ g/L) was well below the OEHHA 2012 action level for human recreational uses (0.8  $\mu$ g/L).

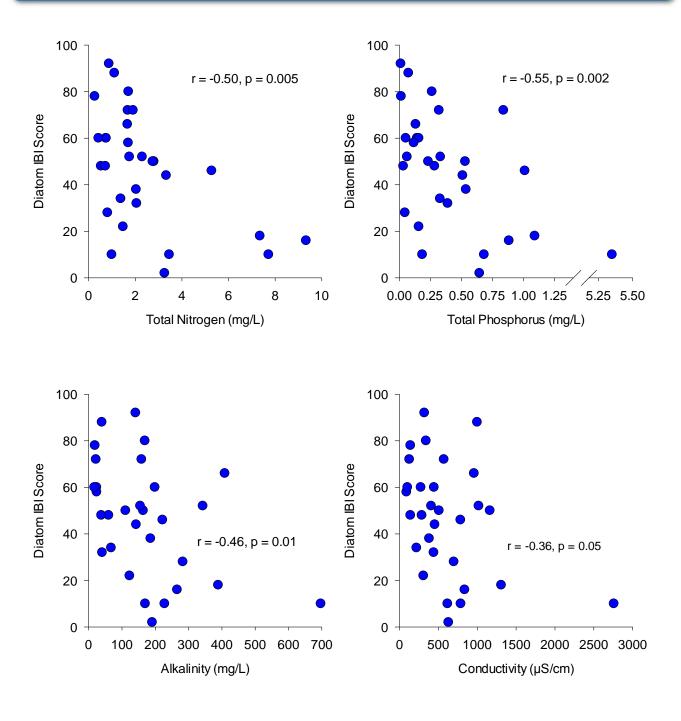


Figure 11. Relationship between diatom IBI scores and water quality contaminant concentrations.

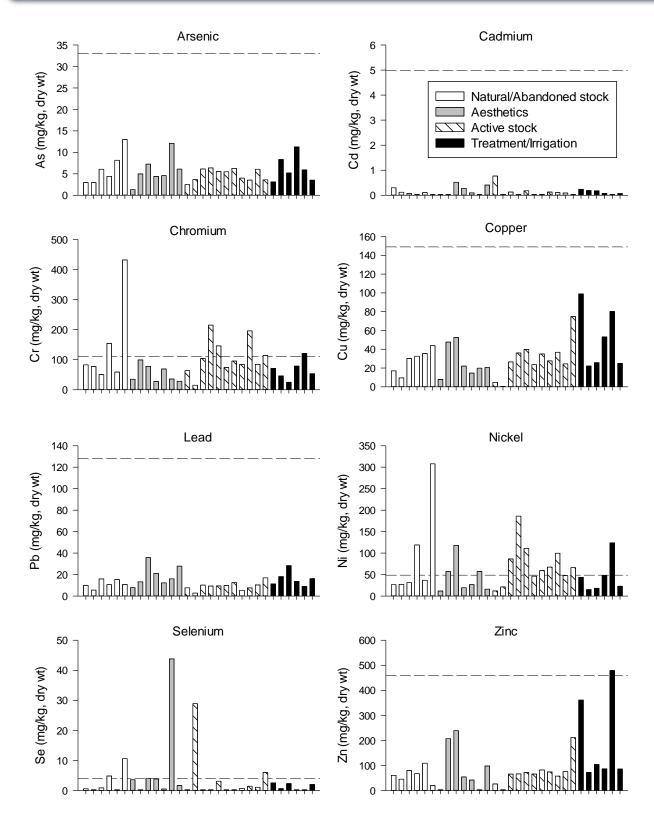


Figure 12. Metal concentrations measured in wetlands sediments. The sites are arranged by use-type category. The dashed horizontal lines indicate the probable effects thresholds (MacDonald et al. 2000 or Van Derveer and Canton 1997).

Table 6. Summary of water quality and sediment parameters at San Francisco Bay Area depressional wetlands.

Constituent	Min	Max	Median	Mean
General water quality				
Alkalinity (mg/L)	18	697	157	170
рН	6.4	9.4	7.8	7.8
Salinity (g/kg)	0.05	0.65	0.21	0.27
Specific Conductivity (µS/cm)	91	2760	450	606
Turbidity (NTU)	1.5	189	15.1	33.2
Dissolved oxygen (mg/L)	0.6	17.2	8.4	7.4
Chlorophyll-a (µg/L)	4	916	18.5	83.5
Nutrients (mg/L)				
Orthophosphate	0.006	4.55	0.05	0.25
Total phosphorous	0.01	5.4	0.27	0.51
Total nitrogen	0.3	9.3	1.7	2.4
Ammonia	0.01	4.31	0.03	0.27
Nitrate	0.005	1.48	0.005	0.07
Nitrite	0.002	0.106	0.004	0.012
TKN	0.27	30	2.2	4.2
Sediment metals (mg/kg)				
Arsenic	1.3	13.0	5.3	5.6
Cadmium	0.03	0.77	0.10	0.15
Chromium	15	432	78	94
Copper	0.5	99	27	33
Lead	2.7	35.8	11.0	13.4
Manganese	39	2528	430	558
Nickel	11.6	308	46.8	64.3
Selenium	0.27	43.8	0.99	4.19
Silver	0.08	1.28	0.08	0.16
Zinc	3.2	479	72	101

### Discussion

#### CONDITION OF BAY AREA WETLANDS

San Francisco Bay Area wetlands were in moderate condition with approximately 30% of wetlands in the San Francisco Bay Area being considered likely intact. Approximately 43% of wetlands were likely intact based on diatom scores, 37% based on CRAM scores, and 33% based on macroinvertebrate scores. When all indicators were integrated, 30% of sites were likely intact based on at least two of the three indicators, with 17% of wetlands considered intact by all three indicators. These findings are generally in good agreement with results from locally-derived macroinvertebrate indicator and the stream algae indicator developed in Southern California where approximately 40% of the sites were intact based on macroinvertebrate scores and 25% were intact based on diatom scores.

There was good agreement between the three indicators, which demonstrates that they were useful in determining condition but were not redundant. We did not expect an exact correlation between indictors because they each evaluate different communities or conditions of the wetland. Diatoms have been found to be useful indicators in other studies (Lane 2007, Rimet and Bouchez 2011). CRAM has been effectively used throughout CA to assess a wide range of wetland types (Stein et al. 2009, Solek et al. 2011). Macroinvertebrates are an informative indicator of biological condition in stream ecosystems (Resh et al 1996), which are now being used more broadly to evaluate wetlands (Batzer 2013). As monitoring programs in California sample more depressional wetlands with the standard SWAMP protocols (Fetscher et al. 2015), we can adapt the diatom and macroinvertebrate indicators. The diatom indicator performed well despite being developed in California streams.

#### **STRESSORS**



Excessive nutrients, variables related to ionic concentration, depressed DO concentrations, excess sediment metal concentrations, and direct habitat alteration were the dominant stressors affecting wetland condition among those stressors that were tested for/recorded. Because depressional wetlands are often 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 year round 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). A recent survey of wetlands in the western U.S. (which

included wet meadows, emergent marsh, fens, seeps, forested wetlands, and estuaries) concluded that physical alterations to wetland vegetation (vegetation removal at 61% of wetland area), biological stressors (non-native plants in 72% of wetland area), and hydrologic alterations [ditching at 76% of area, and surface hardening (e.g., pavement, soil compaction) at 70% of area] were the most prevalent stressors affecting wetland condition (USEPA 2016).

Water quality concentrations in wetlands of the San Francisco Bay area were within the range found for depressional wetlands in the National Wetland Condition Assessment (USEPA 2016) for most constituents (conductivity, pH, Chl-a, ammonia, TN, TP). The exception was for nitrate+nitrite; where the maximum concentration in the Bay Area (1.5 mg/L in Elk Glen Lake, Golden Gate Park) was almost twice the national maximum concentration (0.8 mg/L). Nutrient concentrations in Bay Area wetlands exceeded thresholds established for southern California wetlands at 93% of all sites sampled for total N and 77% of site for total P. Wetlands in the San Francisco Bay Area also had metal concentrations in excess of probable effects thresholds at a much higher rate than observed in the USEPA study of wetlands in the western U.S. (47% is S.F. Bay vs. 5% in the western U.S.). These results suggest that contaminant levels in Bay Area wetlands are generally higher than regional averages, perhaps due to the high density of urban effects and prevalence of legacy effects from past land use practices in the Bay Area.

#### **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 0 and 43% of wetlands were considered "intact" in our investigation depending on the indicator(s) used to judge condition. In general, the diatom and macroinvertebrate indicators were equally responsive to degradation of water chemistry (particularly nutrients), CRAM was most responsive to intensity of adjacent land use. Changes in the assemblage of diatoms and 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 stresses (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. 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). Results from the San Francisco Bay Area wetlands study also exhibited differences between diatoms and

macroinvertebrates in their sensitivities to constituents affecting water quality; both indicators were negatively correlated with nitrogen- and phosphorus-containing nutrients but only diatoms appeared to be sensitive to concentrations of alkalinity and conductivity.

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 conclude that consideration of trophic interactions is important for accurate interpretation of wetland bioassessment results. Future assessments in California should consider incorporating multiple trophic levels to better elucidate condition and effects of specific stressors.

#### 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:

- Develop a systematic, statewide monitoring program for depressional wetlands
- 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 Water Board's SWAMP reference condition management program (RCMP) 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, such as amphibians or birds. The latter could be advanced through application of molecular methods which are being used in other places as a tool to assess food web complexity.
- Additional testing and method refinement/expansion is needed for seasonal wetlands. The diatom and macroinvertebrate community change over the duration of a season, so establishing regional

- sampling index periods for seasonal wetlands will be necessary. Ultimately, new tools or indicators may be necessary for wetlands with very short inundation periods, such as 1-3 months.
- Integrate data from site specific projects (e.g., 401s on wetland tracker) with ambient assessments where available.
- The wetland status and trends plots as described by Stein and Lackey (2012) (should the state implement this program) should be used to provide a statewide sample frame for ambient assessment. This eliminates the need for comprehensive wetland mapping to support a probabilistic sampling design.
- In the more distant future, 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.

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# **Appendix A: Site Rejection Analysis**

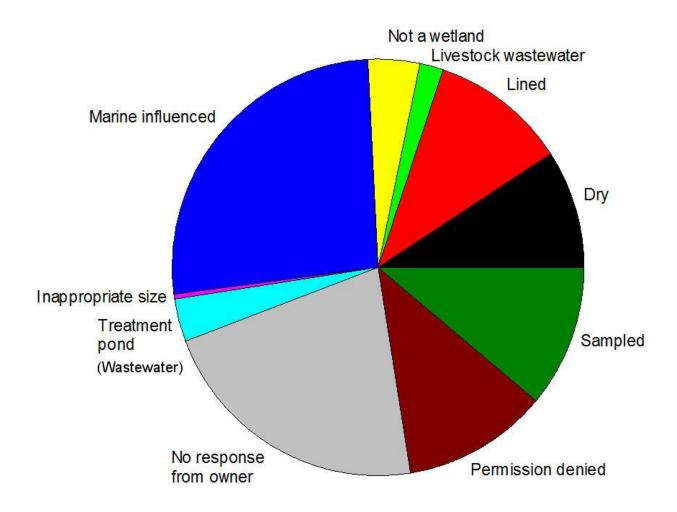


Figure A-1. Proportion of rejected and sampled sites identified in the sample draw.

# Appendix B: Comparison of San Francisco Bay Area and Southern California Diatom and Macroinvertebrate Species

## Diatom species differences between northern and southern California wetlands

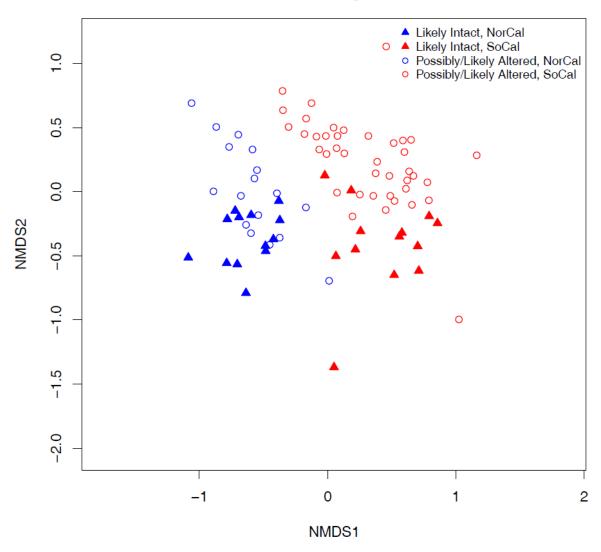


Figure B-1. Diatom species differences between San Francisco Bay Area and southern California. The x-axis appears to separate sites by geography, while the y-axis appears to separate wetlands by disturbance. Two-dimensional NMDS, stress = 0.22, Shepard plot non-metric fit R<sup>2</sup> = 0.95.

# Macroinvertebrate species differences between northern and southern California wetlands

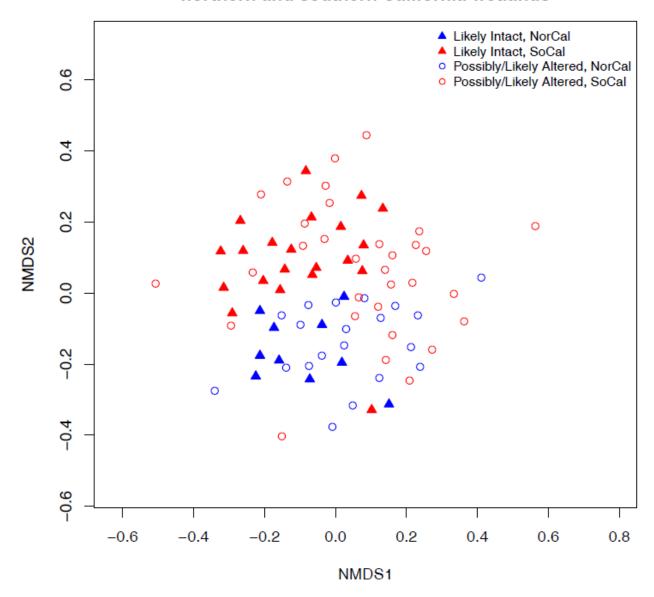


Figure B-2. Macroinvertebrate species differences between San Francisco Bay Area and Southern California. The x-axis appears to separate sites by disturbance, while the y-axis appears to separate wetlands by geography. Two-dimensional NMDS, stress = 0.30, Shepard plot non-metric fit R<sup>2</sup> = 0.92.