Standard Operating Procedures (SOP) for Collection of Macroinvertebrates, Algae, and Associated Physical Habitat Data in California Depressional Wetlands

DRAFT

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## List of abbreviations, acronyms and select terms

<table>
<thead>
<tr>
<th>TERM</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEDEN</td>
<td>California Environmental Data Exchange Network (<a href="http://www.ceden.org">www.ceden.org</a>)</td>
</tr>
<tr>
<td>CRAM</td>
<td>California Rapid Assessment Method (<a href="http://www.cramwetlands.org">www.cramwetlands.org</a>)</td>
</tr>
<tr>
<td>Diatom</td>
<td>a unicellular alga that possesses a rigid, silicified (silica-based) cell wall in the form of a “pill box”</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved Oxygen</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>HDPE</td>
<td>High density polyethylene</td>
</tr>
<tr>
<td>IBI</td>
<td>Index of Biological Integrity. A quantitative assessment tool that uses information about the composition of one or more assemblages of organisms to make inferences about condition the environment they occupy (e.g., the assemblage of interest could be diatoms or benthic macroinvertebrates living in a stream)</td>
</tr>
<tr>
<td>MI</td>
<td>Macroinvertebrate, including benthic (bottom dwelling), nekton (swimming), and neuston (surface)</td>
</tr>
<tr>
<td>Node</td>
<td>1 of 10 evenly spaced sampling points around the pond</td>
</tr>
<tr>
<td>Perennial</td>
<td>Systems that contain some surface ponding for at least 9 months during most years</td>
</tr>
<tr>
<td>PHab</td>
<td>Physical habitat data</td>
</tr>
<tr>
<td>Seasonal</td>
<td>Systems that support surface ponding for between 4 and 9 months of the year</td>
</tr>
<tr>
<td>SAFIT</td>
<td>Southwest Association of Freshwater Invertebrate Taxonomists</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>Soft-bodied algae</td>
<td>Non-diatom algal taxa; for the purposes of this SOP, cyanobacteria are subsumed under this assemblage</td>
</tr>
<tr>
<td>SWAMP</td>
<td>Surface Water Ambient Monitoring Program</td>
</tr>
<tr>
<td>TKN</td>
<td>Total kjeldahl nitrogen</td>
</tr>
<tr>
<td>TOC</td>
<td>Total Organic Carbon</td>
</tr>
<tr>
<td>Transect</td>
<td>Sampling lines perpendicular to shore, encompassing the near, mid, and far distances</td>
</tr>
</tbody>
</table>
Introduction

Depressional wetlands are perennially or seasonally ponded systems up to 8 Ha in size and less than 2 m deep, as defined by the US Fish and Wildlife Service Cowardin Classification (Cowardin et al., 1979) and the California Rapid Assessment Method (CWMW 2013). They can be natural or artificial in origin, and can be used for a variety of management purposes, including wildlife enhancement ponds, stock ponds, golf course water hazards, and stormwater treatment ponds. This SOP applies to all depressional wetlands that retain at least some ability to support wetland plants and aquatic organisms. The SOP is not intended for use in ponds that are fully lined (concrete, plastic, etc.) and maintained, livestock wastewater ponds (such as those associated with confined animal feeding operations), or wastewater treatment ponds not intended for use as habitat. In addition, this SOP is for use in freshwater systems only, and is not intended for use in wetlands that have a strong marine influence.

The methods described in this SOP have been designed to support programs aimed at assessing the condition of a variety of natural and artificial depressional wetlands. This document includes sections for selecting appropriate sites, site delineation, determining which indicators to include in the monitoring program, collecting and preserving samples, and laboratory analysis. Depressional wetland monitoring and assessment typically includes two types of indicators, those that evaluate condition and those that evaluate stress (or other factors) that affect condition (Table 1). The core condition indicators currently used by SWAMP-funded projects include benthic macroinvertebrates, algae, and the California Rapid Assessment Method (CRAM). Stressor/explanatory indicators can include physical habitat, water chemistry, and toxicity (Table 1). The exact complement of indicators may vary by location and program depending on specific priorities or objectives. This document includes procedures for sampling all condition and stress indicators listed in Table 1, except for hydrology which is beyond the scope of this SOP.

Table 1. Depressional wetland indicators of condition and effect.

<table>
<thead>
<tr>
<th>Condition Indicators</th>
<th>Stressor Indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>California Rapid Assessment Method (CRAM)</td>
<td>General water chemistry</td>
</tr>
<tr>
<td>Benthic diatom community</td>
<td>Water column nutrients</td>
</tr>
<tr>
<td>Macroinvertebrate community</td>
<td>Sediment toxicity</td>
</tr>
<tr>
<td>Soft-bodied algae</td>
<td>Sediment chemistry</td>
</tr>
<tr>
<td></td>
<td>Water column metals</td>
</tr>
<tr>
<td></td>
<td>Cyanotoxins</td>
</tr>
<tr>
<td></td>
<td>Physical habitat data (PHab)</td>
</tr>
<tr>
<td></td>
<td>Hydrology</td>
</tr>
</tbody>
</table>
Getting Started

Sample Frame and Site Reconnaissance

Depressional wetland sampling locations can be selected using either targeted or probabilistic approaches. Maps showing the location of depressional wetlands are necessary for either approach, but are most critical for probabilistic sampling where a representative base map is necessary to ensure the statistical validity of sample population. Comprehensive wetland mapping of relatively recent vintage is not uniformly available in the State of California. Regional mapping is available for some regions of the state such as southern California (Southern California Wetlands Mapping Project, http://www.socalwetlands.com/website/main.htm), and the San Francisco Bay area [Bay Area Aquatic Resource Inventory (BAARI), http://www.sfei.org/BAARI]. As new mapping efforts are completed they are usually appended into the California Aquatic Resources Inventory (CARI), which is accessible through http://www.sfei.org/it/gis/cari. A statewide probabilistic mapping program (the California Status and Trends Program) is currently under development and once completed will provide a sample frame that can be used to support depressional wetlands assessment in all regions of the state.

Once candidate sampling sites are selected, office and field reconnaissance is used to screen sites prior to sampling. For office reconnaissance, Google Earth imagery is helpful to identify and exclude sites that do not meet the study criteria (e.g., those that are concrete-lined, marine influenced, riverine, consistently dry, livestock wastewater ponds, brush/trees in the shape of a wetland without ponded water, sites clearly without access) (Figures 1–4). In addition, Google Earth time-series images can be used to help determine whether water is likely to be present during the planned sampling period. Public lands layers for Google Earth (kmz, kml files) are useful to help identify sites that are on State or Federal property, in order to apply for the necessary sampling permits well in advance of the sample collection.

Field reconnaissance is used to ensure water is present, determine if the wetland can be sampled safely, determine if exclusion criteria apply, and to gain access for those sites where the property owner could not be identified or contacted during the office screening. All sites should be attributed with the results of both office and reconnaissance, indicating if they were samplable, and if not, the reasons for them being excluded. This information will be valuable in future site selection efforts. Past studies have shown that many sites are eliminated during the reconnaissance phase; consequently a substantial “over draw” should be included when developing a probabilistic program. For example, the first year of the regional depressional wetland survey in southern California had a success rate of 17% during the field reconnaissance process for finding wetlands that met the study criteria and permission was granted to conduct the sampling. Knowing this rate of success was useful during the second year of the study to estimate the approximate number of sites that would be needed for the field reconnaissance effort.
Permits and permissions

Access to all sites will need to be acquired prior to sampling. Gaining the appropriate permits and permissions can be conducted concurrent with the office and field reconnaissance. In addition to gaining permission, this step is also used to obtain keys or combinations for locks, identify the best access route, determine where vehicles may be parked, etc. For private lands, permission should be acquired from the landowner and contact should also be made with the on-site manager (if it is different from the landowner). A name and contact number are often helpful to have handy in the field, in case of complications. A scientific collecting permit\(^1\) is required for both private and public lands. For public lands, an encroachment permit is also often needed. Both types of permits can take weeks or months to be issued, so plan accordingly. In some natural areas, it may be necessary to be aware of sensitive species issues, and potential restrictions due to breeding season, etc. Presence of state or federally listed threatened or endangered species may limit or preclude sampling at a site and/or may require state or federal “take” permits\(^2\). Regardless of whether the site is on private or public lands, the appropriate property manager will most likely need to be notified a few days in advance of when the intended sampling is to occur.

When to sample

It is recommended to schedule sampling for periods immediately after larval invertebrate emergence. This is generally from May until mid-July, but will be strongly influenced by weather and temperature. The timing of the sampling should also balance the availability of water in seasonal wetlands, and allow sufficient time since recent storm events. Collecting samples should occur at least 2 weeks after the most recent rain event that could perturb these systems (generally \(>0.1\)” rainfall).

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\(^1\) Collecting permits should be obtained from the California Department of Fish and Wildlife

\(^2\) U.S. Fish and Wildlife Service and the California Department of Fish and Wildlife should be contacted regarding restrictions or permit requirements associated with threatened or endangered species.
**Figure 1.** Example of brush misidentified as a wetland in the mapping process. No pond was observed in any of the Google Earth time line images. Vegetated areas like this probably have more moisture than the surrounding area, but do not have ponded water.

**Figure 2.** Example of a livestock wastewater pond (agriculture pond). The nearby rectangular metal roof structures are good identifiers of these systems. While these ponds should be excluded, abandoned stock ponds created to water livestock by damming seasonal streams in natural areas (not shown) are often included in depressional wetland surveys.
Figure 3. Example of a concrete lined pond. The white outline of the pond is a good indication that it is concrete lined and should be excluded.

Figure 4. Examples of treatment ponds and polishing ponds. The treatment ponds often appear as a series of rectangular structures (right) and should be excluded. Polishing ponds that appear later in the treatment process (left) often serve as wildlife habitat, and are often included in depressional wetland surveys.
**Site Delineation and Order of Sample Collection**

**Identification of sampling nodes**

Depressional wetland sampling occurs at 10 sampling “nodes” evenly spaced around the entire wetland perimeter; the distance between each node is based on the circumference of the wetland (Figure 5). Circumference data can either be collected before visiting the site by using remote photography, or it can be determined in the field. Upon occupying the site, identify a starting point (it could be where the wetland is first accessed). From this point, walk along the periphery, always maintaining a constant distance from the edge of the surface water, and count how many paces are required to walk the entire perimeter. During this time, take note of the wetland and make observations of any species of birds and amphibians and record them on your field sheet (see below). Divide the total number of paces by 10 to yield the distance between adjacent sampling nodes along the wetland’s edge (Figure 5). Gather 10 orange transect flags or a roll of orange flagging tape. Begin pacing the perimeter of the wetland again, using the same path as before, and stop to place a flag (or tie flagging tape) in a highly visible spot at each node where sampling transects will be located. Water samples can be collected concurrently with placement of the orange flags.

**Order of data collection**

Sampling should always be carried out in the following order: 1) water chemistry measurements (turbidity, probe measurements) and water-column grabs for laboratory chemistry analyses, 2) Macroinvertebrate (MI) samples, 3) quantitative algae sample, 4) qualitative algae sample, and 5) sediment (Table 2). Each of the 10 sampling “nodes” will have three parallel transects, one each associated with water collection, MI sampling, and algae sampling. Each transect will be offset by several paces in order to minimize disturbance associated with one element of sample collection affecting a subsequent sampling element. The water sample will have been collected corresponding directly to where each orange flag was placed (and turbidity and probe measurements will also be collected from transects located adjacent to the water collection transects associated with nodes 1 and 5). The MIs will be collected from transects positioned 3 paces beyond (to the right of) each of the orange flags (as one walks around the perimeter of the wetland) at the appropriate near, mid, or far positions (see below). The quantitative algae samples will be collected from transects positioned 6 paces beyond each of the orange flags. Sediment and qualitative algae samples will be collected throughout the wetland, where applicable (see below).

To facilitate placement of the sampling array, the MI collector will hang a yellow flag where the MI collection was made, so that the algae collector, who comes along subsequently, will know to collect 3 paces beyond that. In order to keep track of where each sample type is to be collected, the collectors should always walk around a given wetland in the same, counterclockwise, direction, and under no circumstances shall any sample be collected where the sediment/water column has been agitated by previous sampling activities.
The CRAM assessment does not have to be conducted during the same field visit as the sample collections, but ideally should be conducted during the same MI index period. If collected during a single site visit, CRAM should be done last. CRAM should be conducted by qualified practitioners following the procedures outlined in the depressional wetlands CRAM field book (www.cramwetlands.org).

**Table 2.** Sample collection order. The appropriate sampling depths and distances from shore can be found in Table 3.

<table>
<thead>
<tr>
<th>Order</th>
<th>Indicator</th>
<th>Nodes</th>
<th>Location in relation to orange flags</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Water chemistry, turbidity and probe measurements</td>
<td>1 and 5</td>
<td>Directly in front of orange flags</td>
</tr>
<tr>
<td>1</td>
<td>Water chemistry, grabs for lab analysis</td>
<td>1 - 10</td>
<td>Directly in front of orange flags</td>
</tr>
<tr>
<td>2</td>
<td>Macroinvertebrate samples</td>
<td>1 - 10</td>
<td>3 paces to the right of the orange flags</td>
</tr>
<tr>
<td>3</td>
<td>Quantitative algae</td>
<td>1 - 10</td>
<td>6 paces to the right of the orange flags</td>
</tr>
<tr>
<td>4</td>
<td>Qualitative algae</td>
<td></td>
<td>Where appropriate in the wetland in order to collect specimens of all different types of macroalgal filaments and mats, as well as microalgae</td>
</tr>
<tr>
<td>5</td>
<td>Sediment</td>
<td></td>
<td>At least 2 locations away from areas disturbed by other sampling</td>
</tr>
</tbody>
</table>

**Table 3.** Definitions of sampling spots for collection of water, MI, and algae samples. Transect trajectory is always defined as perpendicular to the shore upon which the sample collector is standing.

<table>
<thead>
<tr>
<th>Sampling spot location</th>
<th>Water collection/turbidity/probes</th>
<th>MI</th>
<th>Algae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Near</td>
<td>N/A</td>
<td>0.5 m from shore along transect trajectory, but no deeper than 0.5 m</td>
<td></td>
</tr>
<tr>
<td>Mid</td>
<td>¼ way across wetland along transect trajectory</td>
<td>¼ way across wetland along transect trajectory, but no deeper than 1 m</td>
<td>¼ way across wetland along transect trajectory, but no deeper than 0.5 m</td>
</tr>
<tr>
<td>Far</td>
<td>N/A</td>
<td>80% of the way to wetland midpoint along transect trajectory, but no deeper than 1 m</td>
<td>80% of the way to wetland midpoint along transect trajectory, but no deeper than 0.5 m</td>
</tr>
</tbody>
</table>
Figure 5. Placement of sampling transects for collection of water, MI, and 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 sampling spots are contained within transects. Sediment is collected in appropriate spots where no other sampling has taken place, after all other samples have been collected (see below).
Sample Collection and Processing

Sampling procedures are presented in the sequence that they should be collected in the field. All the typically measured parameters are included; however, each program will determine which parameters are actually collected based on their goals. A list of materials for the field measurements can be found in Appendix B, and a list of containers and sample holding conditions can be found in Table 4.

Water chemistry field measurements and water sample collection

Field measurements

Water chemistry field measurements and water samples are collected prior to any other sampling at each node. Before collecting water samples for laboratory chemistry analyses, turbidity and probe measurements [water temperature (°C), pH, specific conductivity (µS/cm), salinity (ppt), dissolved oxygen (mg/L and % saturation)] are recorded at two nodes at roughly opposite ends of the wetland: Node 1 and Node 5. Probes should be calibrated prior to starting field work, as recommended by the manufacturer. At each of these two nodes, the turbidity/probe measurement are collected at the “mid” sampling spot along transects placed 3 paces to the left of the orange flags associated with each node (i.e., they are placed on the opposite side of the orange flag from which the biotic samples will later be collected; Fig 1). For the purposes of this SOP, “mid” is always defined as ¼ of the way across the wetland along the transect at hand, when sighting perpendicularly from the shore upon which the collector is standing. Note, however, that under no circumstances shall sampling transects within a node, or entire nodes, be allowed to cross one another. If such a situation were to present itself, the samplers should be cognizant of this and should either shorten, or longitudinally shift, transects as needed in order to prevent them from overlapping.

Avoid kicking up sediments that will interfere with turbidity readings by paying close attention to the sediment plume resulting from wading into the wetland. Turbidity can be taken with a YSI multi-probe or a Hach 2100P Turbidimeter, or it can be measured in the laboratory. Alkalinity (mg/L) can be measured with a field test kit (e.g. Hach AL-AP #2444301) or in the laboratory. If using a portable meter, collect approximately 250 mL of water for turbidity measurements approximately 10 cm below the water surface and take two separate readings from subsamples of the same grab sample.

Once the turbidity sample has been collected at a given transect, the probe measurements [water temperature, pH, specific conductivity, salinity, dissolved oxygen (mg/L and % saturation)] can be taken. Measurements are made 10 cm below the water surface according to the MPSL-DFG SOP for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (http://swamp.mpsl.mlml.calstate.edu/wp-content/uploads/2009/04/swamp_sop_field_measures_water_sediment_collection_v1_0.pdf). Using a YSI or similar device, measure specific conductivity (set to 25°C; µS/cm), salinity (ppt), temperature (°C), dissolved oxygen (in % and mg/L), and pH.
Water sample collection

Water samples are collected for the following analytes: suspended chlorophyll a, cyanotoxins (including microcystin), turbidity, alkalinity, hardness, and nutrients [total Kjeldahl nitrogen (TKN), nitrate, nitrite, orthophosphate, total nitrogen, and total phosphorus]. Water samples for chemical analysis are collected at each of the 10 transects in order to create two composite samples; a 2 L composite for water chemistry analysis and a 1 L composite for chlorophyll a and cyanotoxin analysis. Two types of devices can be used to collect water samples at each node in a way that minimizes the introduction of excessive sediment from perturbation of bottom materials during the sampling process. The preferred approach is to use an extended syringe sampler device for collecting water at each node (see Appendix A). The advantages of the syringe device are that samples may be collected without disturbing the water column and the syringe device captures a more representative subsurface sample. As an alternative, a “grabber” can be devised by fixing a 300 mL plastic cup onto a length of PVC pipe and collected a subsample at each node. The subsamples from each node are then composited into a 2 L HDPE sample bottle for use in chemical analysis and a 1 L aluminum foil-covered container for chlorophyll a and cyanotoxin analysis.

At each point where there is an orange flag, wade into the wetland perpendicularly from the water’s edge and deploy the sampling device to the “mid” sampling spot for that transect. For the syringe sampler, draw the plunger to fill the chamber. For the alternative plastic cup device, dip the grabber’s cup face-down into the water slowly and gently in such a way that avoids disturbing the sediment on the wetland’s bottom, and that avoids allowing any surface scum that might be present on the water to enter the cup (use the air bubble inside the cup as a barrier to prevent this). Aim to collect water from 10 – 15 cm below the water’s surface. Once underwater, “burp” the cup to allow water to slowly enter. Then, maintaining the water grabber underwater, pull it toward you slowly and place a clean cover over the top of the cup. Lift it out of the water vertically, with the cup opening facing straight up and with the cover in place. Immediately return to the shore and discard all but 250 mL from the cup. Then pour the 100 mL of this into a clean, foil-covered 1 L bottle (for the chlorophyll a and cyanotoxins samples), and pour the remaining 150 mL into the other (2 L) bottle (for the laboratory measured turbidity, alkalinity, hardness and nutrients). Repeat this procedure at each of the remaining 9 flags. If possible, all water samples should be collected from the same spot for each transect. Once all 10 water collection transects have been sampled, mix each of the two integrated samples by capping the bottles and turning each upside-down and right-side-up for 5 cycles, slowly and gently. Subsamples for the lab measured general constituents (alkalinity, turbidity, hardness) are taken directly from the 2 L composite bottle. Subsampling of the 2 L container for nutrients, and the 1 L container for chlorophyll a and cyanotoxin are discussed below.
Preparation of dissolved nutrient samples

The sample for dissolved nutrients (i.e., for nitrate, nitrite, and orthophosphate analysis) is field-filtered using a 0.45 µm aqueous filter, whereas the sample for analysis of TN, TP and TKN are not filtered (TN and TP are directly frozen, while TKN is acidified, Table 4). Because the surface waters of depressional wetlands are usually more turbid than streams, the typical Luer-Lok tip syringe filters will often clog after a relatively small volume has been processed. Some field crews have successfully used larger diameter filters and vacuum pumps to obtain the necessary volumes. Field filtering for dissolved nutrients and freezing of nutrient samples are carried out in order to permit longer holding times. Project holding times, field preparation, preservation (if applicable), bottle types, and recommended volumes for each water chemistry analyte can be found in Table 4. Please consult the Marine Pollution Studies Laboratory – Department of Fish and Wildlife (MPSL-DFW) Standard Operating Procedures (SOPs) for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program (SWAMP) (http://swamp.mpsl.mlml.calstate.edu/wp-content/uploads/2009/04/swamp_sop_field_measures_water_sediment_collection_v1_0.pdf) for more information.

DO NOT use Sparquat 256, Quat 128, or any other related agent to decontaminate the water grabber for invasive species/pathogens, as this could chemically contaminate the grabber, thus affecting the quality of water chemistry data. Bleach may be used, instead (see decontamination section at the end of this document).

Preparation of suspended chlorophyll a and cyanotoxin sample filters

The chlorophyll-a and cyanotoxin samples are filtered in the field, stored temporarily on wet ice, and frozen within 4 hours of filtering (which may require that dry ice be brought to the field). To prepare the chlorophyll a sample, filter 250 mL of the well-mixed 1 L sample from the integrated sample bottle. Prepare duplicate filters for the chlorophyll a sample AT EVERY SITE. For the cyanotoxins sample, filter 500 mL. Aim to filter the prescribed volumes for each sample type, but always be sure to record the volumes that were actually filtered on the field sheets and the sample labels.

To reduce the likelihood of pigment (chlorophyll a, etc.) degradation as much as possible during the sampling, try to move rapidly from sampling node to sampling node when collecting water, and filter the samples as quickly as possible once sampling is complete. Do not leave the sample out in the sun or otherwise exposed to heat. Shading of the sample material should be

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3 It should be noted that freezing of nutrients constitutes a deviation from current procedures per the SWAMP QAPrP.
ensured during the entire filtering process. If no shade is available around the wetland, an umbrella can be used.

Use a 47 mm diameter, 0.7 μm pore size, glass fiber filters to obtain the samples for laboratory chlorophyll and cyanotoxin analysis. During the filtering process, make sure that the pressure on the filter never exceeds 7 psi. Use a hand pump equipped with a gauge to ensure this limit is observed. Pump slowly, if necessary, to regulate the amount of pressure on the filter. Once most of the sample has passed through the filter, rinse the sides of the filter reservoir with a few mL of deionized (DI) water, and continue filtering until all the visible surface water is drawn down. The filter should not be sucked dry, but rather left slightly moist, in order to avoid applying excessive pressure to the sample, which could cause algal cells to burst. Make sure the filter reservoir is thoroughly cleaned with DI water between sites in order to avoid contaminating samples with residual cyanotoxins, if present, from previous sites.

After isolating the algal material on the filter, fold it in half, with the side coated with material on the inside, and place it into a clean, snapping Petri dish. Then wrap the Petri dish in aluminum foil and place into a 60 or 100 mL Whirl-Pak bag along with a filled-out sample label (outward-facing) that has been printed on Rite-in-the-Rain paper and filled in with either a pencil or waterproof pen. Whirl the Whirl-Pak shut after pushing out as much air as possible, and then twist the wires together to seal shut. Do not store the filters in Ziploc® bags, as these will allow water to enter, if submerged in the wet ice chest.

In the field, store the bagged filters in a wet ice chest (or if possible, on dry ice), and keep them well submerged (not floating atop the water in the chest) in order to keep them as cold as possible. Once back at the lab each afternoon, place the filters in the freezer. They must be kept very cold from the time of collection onward, and must be frozen within 4 hours of collection. Figure 6 provides a label for the chlorophyll a and cyanotoxin samples.

Specific instructions for collecting other water chemistry samples are provided in a separate document.

![Sample label](image)

**Figure 6.** Sample label for chlorophyll a and cyanotoxin samples.
Table 4. Constituent containers, holding times and conditions. Holding conditions of 4°C indicate samples that are held on ice during transport to the analytical labs. The chlorophyll-a and microcystin samples are taken from the foil-covered 1 L sample composite, while the other water chemistry samples are taken from the 2 L sample composite.

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Container</th>
<th>Number of containers</th>
<th>Holding time</th>
<th>Holding condition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Water Chemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turbidity</td>
<td>Field measurement</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Alkalinity, hardness</td>
<td>250 mL HDPE</td>
<td>1</td>
<td>14 d</td>
<td>4 °C</td>
</tr>
<tr>
<td>TKN</td>
<td>500 mL HDPE</td>
<td>1</td>
<td>28 d</td>
<td>H₂SO₄</td>
</tr>
<tr>
<td>Nitrate, nitrite, ortho-phosphate</td>
<td>250 mL HDPE</td>
<td>1</td>
<td>28 d</td>
<td>Filtered, frozen (dry ice)</td>
</tr>
<tr>
<td>Total phosphorus, Total nitrogen (direct measurement)</td>
<td>250 mL HDPE</td>
<td>1</td>
<td>28 d</td>
<td>Frozen (dry ice)</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>Glass fiber filter wrapped in aluminum foil within petri dish</td>
<td>2</td>
<td>28 d</td>
<td>-20°C (-80°C preferred), dark</td>
</tr>
<tr>
<td>Microcystin</td>
<td>Glass fiber filter wrapped in aluminum foil within petri dish</td>
<td>1</td>
<td>Weeks</td>
<td>-80°C, dark</td>
</tr>
<tr>
<td><strong>Sediment Chemistry</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOC &amp; Grain size</td>
<td>4 oz glass</td>
<td>1</td>
<td>6 mo</td>
<td>4 °C</td>
</tr>
<tr>
<td>Metals, pyrethroids</td>
<td>4 oz glass</td>
<td>1</td>
<td>1 year</td>
<td>Frozen</td>
</tr>
<tr>
<td><strong>Sediment Toxicity</strong></td>
<td>Heavy duty plastic bag, double bagged &amp; zip tied, 3 L total</td>
<td>1</td>
<td>2 weeks</td>
<td>4 °C</td>
</tr>
<tr>
<td><strong>Algae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diatoms</td>
<td>250 or 500 ml HDPE</td>
<td>1</td>
<td>Months</td>
<td>Formalin, 2% final conc</td>
</tr>
<tr>
<td>Soft algae</td>
<td>250 or 500 ml HDPE</td>
<td>1</td>
<td>Months</td>
<td>Glutaraldehyde, 4° C</td>
</tr>
<tr>
<td><strong>Macroinvertebrate</strong></td>
<td>Wide mouth HDPE recommended</td>
<td>Dependent on site conditions</td>
<td>Months</td>
<td>EtOH, ≥70% final conc</td>
</tr>
</tbody>
</table>
Macroinvertebrate collection and PHab measurements

MI collection is conducted along a transect at a distance of 3 paces to the right of each orange flag (i.e., when walking counterclockwise around the perimeter of the wetland. The starting location for the MI collection should be rotated from transect to transect through 3 positions: “near”, “mid”, and “far”, starting with “near” at the first transect sampled. “Near” is defined as 1.5 m inward from the water’s edge. “Far” is defined as either the closest distance from the edge at which the water is 1 m deep, or if the entire transect is wadeable, “far” is defined as 80% of the distance to the midpoint between the edge of the wetland you are standing at and the opposite shore, as you fix your gaze perpendicularly outward from the shore (Figure 5). “Mid” is defined as one-quarter of the distance across the wetland along the transect in question. In deeper wetlands with a narrow littoral zone, the “mid” and “far” sampling spots may fall within the same distance from the shore. A summary of the definitions of near, mid, and far sampling spots is provided in Table 3.

Once the spot to be sampled is identified, the procedure for collecting the sample is as follows. Approach the sampling spot slowly with your D-frame kick net in the air (not dragging underwater), in order to avoid frightening the bugs away, or accidentally collecting organisms, as you draw nearer. When you have reached the spot, hold the net straight in front of you at arms’ length, with the opening of the net facing down. Plunge the net into the water, pulling it through the water column towards the wetland bottom. Then pull it toward you, gently rubbing the pond bottom in an undulating motion that covers a swath about a meter long. The idea is to catch bugs that are in the water column, clinging to vegetation, and along the sediment benthos, all the while avoiding collecting a lot of sediment in the sample. Once your net has nearly reached your feet, quickly swivel it around 180 degrees, and push it back away from you for a second sweep in the opposite direction, using a similar undulating motion. At the mid and far sampling spots (when in water deeper than the height of the net), pull the net back and forth a second time in the water column to sample swimming invertebrates and invertebrates associated with submerged vegetation. If sampling emergent vegetation, keep the sample area the same, but vigorously work the net against the vegetation and benthos while trying to keep the net in forward motion to prevent escape of captured organisms. Pull the net out of the water with its opening facing upward to keep the material inside, and then return to the shore by retracing the steps you took on your way in.

Add the material collected to a 5-gallon bucket that has been filled approximately halfway with pond water. Then carry out the same collection procedure at the appropriate spot (i.e., mid, far, near, mid…) at each of the remaining MI sampling transects around the wetland. Ideally, two people should work together to collect the MIIs: one person to make the collections, and the other to carry equipment and record the required information on the field sheets. For example, at each collection point the sampler will record the information on vegetation cover, which “sampling spot” was sampled at that transect (near, mid far), the depth at which the sample was collected, distance from bank (wetted edge), and distance from wetted edge to winter inundation line (bankfull) (field sheets appear in Appendix C). Note that this is an
abbreviated list of PHab measurements for depressional wetlands, compared to the list for streams.

At each sampling area, establish an imaginary 1 m² area centered around where the MI sweep was made for collection of a suite of habitat observations is recorded. Within each sampling area, the relative percentages of habitat type, based on vegetation, are recorded within the appropriate data field sheet. Vegetation categories that can be used are emergent, submerged, surface, and open. Values are recorded as relative percent coverage of the 1m² area, so that the total coverage for each sampling area totals 100%. The data collector will need to mentally “flatten” the image of habitat types within the 1m² square area to determine what types of habitat are contained within it, and reflect this in the relative percentages. The submerged and surface vegetation categories are further divided into the algae vs. other (where “other” is non-algal vegetation). The depth (cm) of the water at the sampling area, the distance from the bank (m), and the water line to bankfull (m), are also recorded. The water line to bankfull is determined by measuring the distance from the wetted edge to the seasonal high water mark, which is often marked by a clear change in vegetation type from terrestrial to aquatic.

After completing the MI collection, before moving on to the next transect, leave a yellow flag corresponding to the point where the wetland was entered for that MI sample. This will aid the algae collector in identifying where algae can be sampled.

Once all 10 transects have been sampled for MIs, release any larval amphibians or other non-target organisms (e.g., fish) that may have been captured during the sampling back to the wetland. A small aquarium net is useful to capture these organisms. It may be necessary to use one or more dish tubs as holding chambers to aid in the cleaning of debris from the sample.

**Field elutriation and preservation of MI samples**

Fill two large dishpan tubs (11qt) with surface pond water and use an aquarium net to remove any macroinvertebrates from this water. Inspect the D-frame sampling net and use forceps to remove any organisms clinging to the net and place these in the bucket. Take large clumps of debris (detritus, wood, live vegetation, macroalgal mats/filaments) that take up a lot of volume in the bucket and individually place them in the first tub. Gently rub the surfaces of the material to remove invertebrates, visually inspect the debris to make sure MIs were removed, and then place the piece into the second tub. Leave the piece in the second tub for a few minutes before discarding back to the wetland. Repeat this process for all large debris. When a majority of large debris has been removed, pour the tubs into a 500µm sieve held over an empty dish tub in case some of the sample misses the sieve. Then pour all of the remaining bucket material into the sieve. (A 4mm sieve can be helpful in trapping amphibian larvae first, if they are abundant at the site.) Transfer all of the material in the sieve (invertebrates and organic matter) into the sample jar. Carefully inspect the gravel and debris remaining in the bottom of the bucket and tubs for any cased caddisflies, clams, snails, or other dense animals that might remain. Remove any remaining animals by hand and place them in the sample jar.
Place a completed date/locality label (Fig 4) on the inside of the jar (use pencil only, as most “permanent” inks dissolve in ethanol) and completely fill with 95% ethanol. Place a second label of the same type on the outside of the jar. Note that the target concentration of ethanol in the sample jar is 70%, but 95% ethanol is used in the field to account for dilution from water in the sample. Do not add more than 50% debris or material into your 1L jar. If there is a lot of organic and inorganic material, multiple jars might be necessary to store a single composite MI sample. Record the total number of jars for a single sample on the external labels and field sheets.

![Figure 7. Internal and external jar label printed on Rite-in-the-Rain or similar water-resistant paper and recorded in pencil.](image)

**Algae collection**

Algae collection should occur after MI collection is complete. A benthic algae sample, soft-bodied quantitative sample, and soft-bodied qualitative sample are collected. There are two ways to coordinate MI and algae collection depending, in part, upon the number of people in the field crew. There can either be one team (ideally, of two people) for collecting MIs and a separate team (also ideally of two people) for collecting algae, or there can be a single team of two that collects both assemblages in succession. For the former option, the two teams can circle the wetland concurrently to collect subsamples, but the MI collectors should go first at each node, in order to limit the amount of activity in the general sampling area, thus reducing the likelihood that the bugs will be flushed. For the latter option, the team of two collects MIs at all 10 nodes first, and then goes around the wetland again to collect algae samples.

Within each node, algae are sampled at transects 3 paces to the right of each of the yellow flags (which indicates where MIs had been collected), when walking counterclockwise around the wetland. The procedure for identifying the spots for sampling algae is very similar to that for MIs. The major differences are that sampling for algae should never occur at a spot deeper than 0.5 m, and the “near” spot is approximately 0.5 m from the edge of the surface water. The “far” spot is either the closest place where the water is 0.5 m deep or, if the wetland does not get that deep along that transect, it is defined as 80% of the distance to the midpoint between the edge of the wetland you are standing at and the opposite shore, when sighting perpendicularly from the shore. “Mid” is defined as one-quarter of the distance across the wetland along the transect in question (and is also never deeper than 0.5 m). See Table 3.
Once the general area for the sampling spot is identified at each transect, the sample should be collected from the dominant substratum type in that area, and from a “representative” spot therein. ***Note that it is necessary to take **TWO adjacent grabs** for algae samples at each sampling node (that is, collect two algae subsamples at each of the 10 nodes around the wetland for a total of 20 subsamples composited into the sample container). This is to double the volume of material collected, thus facilitating the preparation of both a diatom sample and a soft-bodied algae sample. Be careful never to collect a sample from a spot that has already been sampled from or otherwise disturbed. Indicate on your field sheet what substratum type(s) you collected from at each transect (Appendix C). For instance, if you collected one of your 2 subsamples (grabs) from soft sediment and the other from a live cattail stem, then you would mark a “1” in the box corresponding to “soft sediment” and another “1” in the box corresponding to “plant (live)” for that transect. If both subsamples had been collected from soft sediment, then you would mark a “2” in the box corresponding to “soft sediment”. The other boxes would be populated with zeros. Only “0”, “1”, or “2” can be filled in any box for substratum type for a given replicate.

Unlike with MIIs, for which all collections are made using the same device, there are two different sampling devices for collecting algae. The most commonly employed one is a “sediment corer” (5.3 cm²), which is used both for soft sediments and for delineating a sampling area on plant material and hard substrata that can be removed from the pond. The other device is a “syringe scrubber” (5.3 cm²), which is used for sampling hard substrata that cannot be removed from the pond, and is identical to what is used for sampling underwater in wadeable streams. Methods to construct these devices are described in Fetscher et al. 2012. Keep a tally of the number of times each sampling device is used, and when sampling is complete, record this value on the field sheets and sample labels.

If the sampling spot encountered falls on an area of soft sediment, the following procedure applies. Use the “sediment corer”, which is a plastic 60 mL syringe with the tip of the syringe cut off near the base where the “0 mL” mark is, and with a line (preferably made with a bright pink or silver marker) drawn around the perimeter of the barrel for a distance corresponding to 5 mL up the barrel from the location of the cut. For example, if you cut the syringe barrel at the “5 mL”, then the line you draw around the barrel should be positioned at the “10 mL” mark. The open end of the corer must also be filed so as to make it as sharp as possible for cutting into sediment and macroalgal mats.

Submerge the coring device in the pond water to moisten it; then move the plunger up and down a few times to loosen it. Adjust the plunger so that its pointed rubber tip is near the open end of the syringe barrel, but not protruding. Submerge the corer in the water and slowly press the barrel vertically downward into the sediment at the sampling spot, at the same time pulling slowly on the plunger to aid insertion into the sediment to a depth of about 5 cm. Make sure as you make contact between the device and the sediment that you do not cause a current of water that will flush the top surface of the sediment away. You want to keep this intact, as it is where most of the algae will be.
After the core has been taken up into the device, carefully remove the device from underwater and hold it with the open end facing upward. Slowly push on the plunger to move the core out of the syringe barrel. Once the seal ring (the one closest to the end of the barrel) of the black rubber tip on the plunger reaches the mark you have drawn around the syringe barrel, stop pushing the plunger and cut the end of the core off and discard it. What remains within the barrel (~5 mL of material) is the sample. Hold this over the sample bottle and resume pushing on the plunger to release the core into the bottle. Then rinse the end of the plunger into the sample bottle, trying to capture all the sediment particles. An intact (uncut) 60 mL syringe filled with water from the pond being sampled can be used to squirt the end of the corer to dislodge any residual sediment and collect this rinsate into the sample container.

If the sediment is too loose to form a cohesive core inside the coring device, then take another grab from an undisturbed adjacent spot of the same substratum type. Start with the seal ring of the plunger positioned at the mark you had drawn around the syringe barrel, insert the barrel down into the sediment, up to that mark. Then gently slide a spatula beneath it, and pull the device out of the water with the spatula in place. Remove excess sediment (outside of the syringe barrel) from the spatula; then dump the sampled material into the sample bottle. Rinse any residual sediment off the corer and spatula into the sample container.

If plant material is the substratum type encountered, use a soft-bristled toothbrush to gently remove biofilm coating from the plant within a 5.3 cm² area. Do this by clipping the plant at the base (if rooted) with a shears or scissors, then very slowly and gently removing it from the water in order to avoid shearing loosely attached algae from the plant as much as possible. Collect material from the lower portion of the plant, near where it had been rooted. The sediment corer, with the plunger retracted, can be used as a guide for how much area to brush. After brushing within that area, rinse the area over the sample bottle to catch the rinsate and then discard the cleaned substrate. Also thoroughly rinse the brush into the sample container. **Before using the toothbrush at a given site, always make sure that it has been cleaned thoroughly and does not have any contaminating algae from a previous site.**

If a mat of macroalgae is the substratum type encountered, use the sediment corer to create a core of macroalgae (representing its full thickness) as the sample. This is done by “sandwiching” the entire thickness of the macroalgal mat between the corer and a spatula underwater and using a utility knife to cut the algae around the perimeter of the corer, then adding the resulting “circle” of algae to the sample bottle. This same general procedure can be used if the substrate type is a layer of dead leaves or other such organic debris resting on the pond bottom. In this case, sandwich the leaf between the corer and a spatula underwater, pull the device out of the water, and use a utility knife to isolate a circle of the leaf matter. Once the excess leaf material is cut away, hold the device over the sample bottle and pour all the water (which probably contains algae that had been loosely associated with the leaf) that is above the leaf inside the corer barrel, as well as the leaf piece, into the sample bottle. Then gently brush the algal biofilm off the leaf piece and rinse both the brushed part of the leaf and the brush itself into the bottle. Discard the cleaned leaf piece.
If a hard substratum is encountered at the sampling spot and it can be removed from the pond bottom, very slowly pull it out of the water, delineate the sample area using the sediment corer, and use a toothbrush and rinse water as was described above for sampling from plants. If the substratum cannot be removed from the pond, then use the syringe scrubber to collect the sample underwater. Refer to Fetscher et al. (2010) for instructions for constructing a syringe scrubber.

To use the syringe scrubber, submerge it in the pond and work the plunger a few times to loosen it up. Then affix a fresh, white scrubbing pad circle onto the bottom of the plunger by attaching it to the Velcro®. Press the plunger down so that the bottom of the scrubbing pad is flush with the bottom of the barrel. Then submerge the instrument, press the syringe firmly against a flat area of the substratum, and rotate the syringe scrubber 3 times in order to collect the biofilm from the substratum surface onto the scrubbing pad. If the surface of the substratum where your sampling spot fell is not flat enough to allow for a tight seal with the syringe barrel, objectively choose whatever sufficiently flat area on the exposed face of the substratum is closest to where the original spot fell, and sample there. After sampling, and before removing the syringe scrubber from the substratum, gently retract the plunger just slightly, so it is not up against the substratum anymore, but not so much that it pulls a lot of water into the barrel. Carefully slide the spatula under syringe barrel (which should be pulled just slightly away from the substratum on one side to allow the spatula to slide under), trying not to allow too much water to rush into the barrel. Then pull the instrument back up out of the water with the spatula still firmly sealed against the syringe-barrel bottom.

Hold the syringe scrubber over the sample container and then remove the spatula, allowing any water to fall into the container. Carefully detach the pad from the plunger and hold the pad over the container. Using rinse water sparingly, remove as much algal material from the pad as possible by rinsing it off with the intact syringe filled with pond water, and wringing it into the sample container before discarding the used pad. Start this process by rinsing from the backside of the pad (the side that had been affixed to the plunger) to “push” the collected algae forward out of the front surface of the pad. It is recommended that a fresh (new) pad be used each time a sample is collected, even within the same pond. Under no circumstances should the same pad be used at more than one site.

Whenever rinsing substrata and sampling devices into the sample bottle, try to be sparing enough with the water to keep the final sample volume under 500 mL, if possible, and definitely under 1 L.

Note that, because two subsamples of algae will be collected from each algae transect (for a total of 20 across the wetland), it is possible to collect from two different substratum types within a single sampling-spot area. For instance, if plants are abundant within a given sampling spot area, one of the subsamples should be collected from the plant material, and another should be collected from sediment or whatever the dominant bed material is within that spot (i.e., organic debris, wood, rock). No more than half of the 20 samples for a wetland should be collected from live plants.
Once all the subsamples have been collected, cap and very gently agitate the sample container to mix the material well without breaking cells. Pour the entire contents of the container into a 1 L graduated cylinder to measure the volume of the sample. Wait awhile (in the shade) to allow the heavier sediment to settle to the bottom. Once a clear boundary between sediment and liquid is apparent, determine the approximate volume of the liquid portion of the sample as well as the volume of the whole sample (including sediment). Record the liquid portion as “composite volume” on your field sheet (but make note of the volume of the entire sample, including sediment, for later, as you will use this information to determine how much sample to pour into the diatom and soft-algae sample bottles). Pour all of the material back into the sample container. Gently pour back and forth between the graduated cylinder and the sample container a few times, if necessary, in order to get residual material out of the graduated cylinder. Do not use new water for the rinsing, as this would add volume, requiring that you record a new composite volume.

Very gently mix the sample in the container until it is homogeneous. Using a graduated cylinder, pour half the sample (sediment included) into a 500 mL plastic sample bottle and label it for “diatoms”, and pour the remaining half of the sample into another 500 mL sample bottle and label it for “soft algae”. Figure 8 shows a label for algae taxonomy samples. Note that if the total volume of material collected was well under 500 mL, then 250 mL sample bottles could be used for the diatom and soft-bodied algae samples, as long as the volume of fixative that will need to be added to the bottles will not cause the final volumes to exceed 250 mL each.

If any macroalgal clumps are in the sample, use a pair of long forceps to “fish” the material out of the sample bottle. Cut each distinct clump in half, one-by-one, with shears or scissors, and add the resulting halves to each of the sample bottles (diatom and soft-algae).

Once the soft algae sample has been prepared, place the bottle in the wet ice cooler. It must be kept very cold (but not frozen) and in the dark. This sample should be fixed in the laboratory with glutaraldehyde to a final concentration of 2.5% as soon as possible, but no more than 4 days after collection. Diatoms are fixed while still in the field, using enough 10% formalin to yield a final concentration of 2%. Be sure the wear appropriate protective gear and employ safe practices when handling this fixative. Fetscher et al. (2010) provides detailed guidance for this. The calculations used to determine the amount of fixative needed for both the diatom and soft samples can be based on the sample volumes for each being equivalent to ½ the measured composite volume (i.e., the liquid portion of the sample, excluding the sediment).
Figure 8. Label for algae taxonomy samples.

Just as with MIs, associated with each sampling spot, a suite of habitat observations are recorded from an imaginary 1 m² area centered around where the algae subsample grabs were made for each transect. The data recorder estimates the location of the boundaries of the 1m² area. Most of the habitat observations are the same for both algae and MIs, but a few are associated strictly with one or the other. At each algae sampling spot, the relative percentages of habitat type, based on vegetation, are recorded within the appropriate data field, just as with MIs, again using the categories of emergent, submerged, surface, and open. For each row, the values in the cells should add up to 100%. The data collector will need to mentally “flatten” the image of habitat types within the 1 m² square area to determine what types of habitat are contained within it, and reflect this in the relative percentages. As mentioned above, the submerged and surface vegetation categories are further divided into the algae vs. other (where “other” is non-algal vegetation). The depth (cm) of the water at the sampling point, as well as the distance from the bank (m) is recorded. For algae, the estimated % shading within the 1 m² area is also recorded. In addition, the type of pond substratum from which each sample was collected (soft sediment, plant (live/dead) including wood, or rock (including concrete and consolidated sediment)) is also recorded.

Qualitative sampling of macroalgae

The purpose of the qualitative soft-algal sample is to capture the taxonomic diversity of this assemblage in the wetland and to aid in identification of ambiguous specimens collected in the quantitative sample. For qualitative soft-bodied algae, samples are collected throughout the wetland (independent of node or transect) in order to collect specimens of all obviously different types of macroalgal filaments and mats, as well as microalgae (in the forms of scrapings using a utility knife), and depositional samples (suctioned from along the surface of sediments using a clean turkey baster). If you suspect something may be algae, but are not sure, it is always preferable to collect some of it for the qualitative sample. The laboratory will determine whether it qualifies for inclusion in the species list. Collect from as many distinct locations as possible throughout the wetland so as to capture as much of the apparent diversity as you can. Also, when possible, try to grab part of the holdfast structures that attached the
macroalgae to the substrate (if there are any attached algae in the wetland) as these structures can be useful for taxonomic identification. An attempt should be made to be as exhaustive as possible in sampling the various taxa present. Add all material collected to a labeled (Fig. 6), 250-mL container. Keep the bottle in the dark on wet ice while in the field. **Upon returning to the laboratory, fix the material to a final concentration of 2.5% glutaraldehyde and store in the refrigerator in the dark (Note: this is a deviation from the stream protocol, in which the qualitative sample is not fixed, but kept fresh and delivered to the lab as soon as possible for analysis.)**

![Figure 9. Label for soft-algae qualitative sample.](image)

**Sediment sample collection**

An integrated sediment sample is collected within each wetland after all other sampling has been carried out, and prior to removal of the flags. The sediment samples will provide information about potential sources of toxicity. Collect 2-cm deep subsamples of sediment from various spots dispersed throughout the wetland, wherever fine-grained sediment is available. A total of 3 L of sediment are needed. Sample only from places that have not been trampled by previous sampling/data collection, and which appear to be reasonably representative of the overall wetland. Detailed sediment sampling instructions can be found in MPSL-DFG SOP for Conducting Field Measurements and Field Collections of Water and Bed Sediment Samples in the Surface Water Ambient Monitoring Program ([http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/standard-operating-procedures](http://swamp.mpsl.mlml.calstate.edu/resources-and-downloads/standard-operating-procedures)). Sediment for toxicity can be put into heavy duty plastic bags and zip tied. Sediment for chemistry is put into 4 oz glass jars (Table 4). An example label for sediment grain size and TOC analysis can be found in Figure 10. For the sediment toxicity bags, use an adhesive label if a suitable one can be found, otherwise write all of the necessary information on the bag with a Sharpie. After the sediment samples have been collected, all of the transect flags can be retrieved from the field. Sediment samples are held on ice for transport to the toxicity and chemistry analytical labs.

The *Hyalella azteca* 10 day survival test has been used most often in the southern California depressional wetland survey.
Collecting field duplicate samples

At 10% of the wetlands (selected at random), a field duplicate should be collected for each of the sample types: water chemistry, MI, algae, and sediment. Ideally, duplicates of all sample types are collected at the same set of sites. In the case of both the water chemistry and the algae samples, duplicates can be taken from within the same general sampling spot areas within their respective transects. In other words, for algae, instead of collecting 2 subsamples within the sample spot area, 4 are taken, with 2 of them being deposited into one sample bottle, and the other 2 deposited into a second bottle. (The collector must be careful never to resample the same exact spot.) For the MIs, it is not possible to use the same sampling area because of the wide swath the net makes in the course of capturing the MIs. Instead, the duplicate samples should be taken concurrently, from adjacent sampling areas along the same transect (i.e., with two collectors standing next to each other, both positioned at the near, mid, or far sampling spot, depending upon which spot is being sampled from the transect in question). The two MI collectors should be working concurrently, side-by-side, rather than in succession, so that neither is likely to scare MIs away from the other’s sampling area.

The vegetation cover, distance, and depth measures should be recorded separately for the two duplicate MI samples, because the imaginary 1 m² areas associated with the net sweeps to collect the duplicates are distinct. Conversely, for the algae, because the replicate samples can all be collected within the same 1 m² area at each transect, it is not necessary to record separate PHab data for the replicates (and the same information could be used to populate that component of the algae PHab data for both replicates 1 and 2 in the database). However, with respect to recording substratum type associated with the algal replicate subsamples, this must be done separately for replicates 1 and 2. Information on recording PHab data for duplicate samples of MIs and algae is summarized in Table 5.

Figure 10. Sample label for sediment grain size and TOC. A similar label can be modified for the sediment metals and organics container.
Table 5. Recording PHab data when duplicate samples are collected.

<table>
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<tr>
<th>Assemblage</th>
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<th>PHab Data Type</th>
<th>Recording of Substrata Types</th>
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<tr>
<td>Algae</td>
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<td>Same for Reps 1 &amp; 2</td>
<td>Distinct for Reps 1 &amp; 2</td>
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</table>

Photo documentation of site

At least one photo should be taken of each wetland sampled. If it is a small wetland, a single photo may be sufficient, as long as it captures the wetland area in its entirety. For large wetlands, several photos will be necessary. Take the photos from whatever vantage points best allow the wetland to be captured photographically. Record the photo numbers on the field sheet.

Decontamination of equipment

In depressional wetlands, the primary concern for aquatic invasive species (AIS) and disease is chytrid fungus, which has been decimating amphibian populations worldwide, including causing declines in mountain yellow-legged frogs in the Sierra Nevada. Consult the decontamination SOP from USFS Region 4, which has an excellent summary table of viable methods for multiple types of AIS [http://www.fs.fed.us/r4/resources/aquatic/guidelines/2011techguidelines_fire_AIS.pdf]. A 5% Quat 128 solution requires 30 seconds of soak time to kill chytrid fungus. However, New Zealand mud snail (NZMS), although unlikely to be found in wetlands, could nonetheless be present, and requires 10 minutes of soak time at the same concentration. Because lakes and reservoirs may have NZMS and be sampled with this protocol, a 10 min soak time for all gear in 5% Quat 128 solution or similar Quat related product should prevent movement of all potential invasive species, including aquatic diseases. If a non-chemical solution is preferred, waders can be fully cleaned of mud and debris, then exposed to sun for three hours and allowed to rest completely dry for 48 hrs. Please refer to the USFS guidance for specifics. **Note: freezing gear alone will not kill chytrid fungus, so this is not an acceptable method of decontamination for wetland sampling.**

An alternative method for decontaminating gear for chytrid fungus is via the use of a bleach solution, along with freezing to kill any NZMS that may be present. In consideration of the difficult logistics of field decontamination, a potential approach would be to have 5 pairs of waders available for each crew member, who will use a different pair of waders each day (one site/day). At the end of the week, all of the waders can be cleaned with a brush, rinsed, and treated with the appropriate concentration of bleach for the prescribed time [http://www.fs.fed.us/r4/resources/aquatic/guidelines/2011techguidelines_fire_AIS.pdf], dried, and then frozen. After each site visit, the water grabber can be thoroughly scrubbed and
then treated with bleach (as described above for waders), rinsed well, and allowed to dry to promote evaporation of any residual bleach.

**Analysis**

All environmental samples should be analyzed by qualified laboratories, according to the objectives in the project-specific Quality Assurance Plan.

Taxonomic identifications should be compared to the Depressional Wetland Master Taxa List. Taxa not already specifically listed in the Depressional Wetland Master Taxa List (e.g. new taxa, or taxonomically ambiguous taxa) will need to be reviewed and approved before that dataset can be successfully uploaded into the SWAMP or CEDEN database.

Methods to derive the macroinvertebrate IBI using the frequency distribution provided by the sorting lab can be found within Lunde and Resh 2012. Methods for the diatom IBI can be found in Fetscher et al. 2014.

**Data Submission and Storage**

Data for SWAMP-funded projects should be submitted to the SWAMP database (http://checker.swamp.mpsl.mlml.calstate.edu/SWAMP_Checker/SWAMPUpload.php). Other projects should submit data to the California Environmental Data Exchange Network (CEDEN, http://www.ceden.org). In either case, the CRAM data should be submitted to eCRAM (http://www.cramwetlands.org/dataentry), since the SWAMP and CEDEN databases do not accept CRAM data.
References


Appendix A, Sibbald Syringe Sampler (S3)

A water sampling device was required to collect near-surface water samples as part of SWAMP depressional wetlands bioassessments. As such a device was not readily available to the field crews immediately prior to the start of field sampling, it was decided to attempt to design and build an acceptable device.

The parameters for the device were:

- It should allow samples to be taken at a distance from wading personnel to avoid contamination from waders and sediment disturbed by wading
- It should prevent surface film contamination of the sample
- It should pose low threat of sample contamination from its own materials
- It should deliver a known volume of sample
- It should be able to be sterilized/de-contaminated between sites

Such a device (Figure 1) was designed and built by Glenn Sibbald, CDFW Environmental Scientist, working for the OSPR Aquatic Bioassessment Laboratory. The device allows a field sampler to extend a sterile 60cc syringe roughly 1.5m or more from his/her body and operate the syringe at that distance to collect a water sample from “undisturbed” water.

The device consists of a push/pull rod made up of multiple sections of ¾ inch Schd 40 PVC tubing, jointed together with slip couplings, that slides within multiple sections of 1 ¼ inch Schd 40 PVC tubing, also jointed with slip couplings. The 1 ¼ inch outer tube is perforated to allow draining and lighten weight.

A syringe “cradle” made from 1 ½ inch Schd 40 PVC attaches to one end of the device (Figure 2). It holds the syringe in place during operation. A specially designed “coupler” attaches to the end of the push/pull rod that extends into the cradle (Figure 3, 4), allowing the end cap of the syringe piston to be attached to the push/pull rod, so that the syringe can be operated by the push/pull rod.

At the other end of the device, a center-drilled PVC end cap is fitted on the 1 ¼ inch exterior tube. The push/pull rod extends through the cap, which keeps it aligned for easy operation. A PVC T-joint is fitted to the end of the push/pull rod for better grip and ease of operation.

When fully disassembled (Figure 5), all pieces of the device fit into a 2 gallon bucket, for chlorox-solution cleaning. The syringe is replaced after every site.

The length of the S3 can be increased or decreased by the addition or subtraction of push/pull rod and outer tube segments.
Figure 1. S3 fully assembled

Figure 2. Syringe Cradle
Figure 3. PVC coupler with syringe in place

Figure 4. PVC coupler with syringe removed
Figure 5. S3 fully disassembled
Appendix B, Equipment Lists

**Equipment List – MI sampling and PHab data collection**

- 500 µm D-frame custom all mesh net with 52-inch handle (Wildco 425-JD52-SPE)
- 500 µm sieve
- 4 mm (4000 µm) sieve (optional)
- two 500 ml polyethylene squirt bottles (one for water and one for ethanol)
- flexible and hard forceps
- 1 L Nalgene bottles (assume 1-2 bottles per site)
- 5 gallon bucket with lid
- 3 dish pans (approx 3 gallon or 11 qt) for field elutriation
- plastic funnel (best if 1/2 or 1 inch diameter spout)
- 2 L of 95% (190 proof) ethyl-alcohol per site to preserve sample
- test strips to examine % alcohol in samples
- small aquarium net (approx 2x2 inches with 1 mm mesh)
- MI Rite-in-the-Rain labels and pencil
- transect tape
- meter stick (in cm)
- “angle finder”
- 10 yellow flags
- Yellow flagging tape

**Equipment List – Algae sampling and PHab data collection**

*Chlorophyll a and cyanotoxin sampling*

- either 1, 1L bottle or 1, 2L bottle for integrated sample
- water grabber
- 250 mL, 500 mL, and 1 L plastic graduated cylinders
- bottle brush
- 250 and 500 mL capacity filtering appariati
- glass fiber filters (47 mm diameter, 0.7 µm pore size)
- hand-held vacuum pump with pressure gauge marked at 7 psi or equivalent
- deionized (DI) water
- clean squirt bottle
- flat-tipped filter forceps
- 47 mm snapping Petri dishes
- aluminum foil
- 60 or 100 mL Whirl-Pak bags
- sample labels
- wet ice cooler
• umbrella
• dry ice cooler or field freezer (if going to be in field all day)

Algae specimen sampling

• sediment corer
• silver or hot-pink marker
• syringe scrubber
• spatula
• shears
• utility knife or pocket knife (for cutting sediment core and macroalgal mats)
• white scrubber pads cut into circles for syringe scrubber
• intact 60 mL syringes (to use for rinsing sample into container)
• sample labels
• 250 mL, 500 mL, and 1 L HDPE bottles (wide mouth)
• clear plastic tape
• scissors
• clean, soft-bristled toothbrushes
• long, blunt-ended forceps for grabbing algal clumps out of sample bottle
• 10% formalin
• 25% glutaraldehyde (for use in the laboratory; only if sampling soft-bodied algae)
• waterproof meter stick
• meter tape
• turkey baster

Algae PHab and misc field supplies

• pencil/permanent markers
• clipboard
• field sheets printed on Rite-in-the-Rain
• rangefinder
• GPS unit
• clean plastic tarp
• orange and yellow transect flags and flagging tape

Decontamination for waders and equipment

• Sparquat 256 or Quat 1284, or bleach
• test strips to confirm appropriate Quat concentration

4 DO NOT use Sparquat 256 or Quat 128 on ANY gear that could potentially result in contamination of water chemistry sampling equipment (e.g., the water grabber/pole). See SOP text for alternative methods.
• scrub brushes
• large washing bin

Water chemistry data and sample collection

• 1, 2L bottle for integrated water sample
• YSI or comparable probe
• Field turbidimeter
• Field test kit for alkalinity (e.g. Hach AL-AP #2444301)
• 3 x 250 ml HDPE wide mouth jars
• 1 x 500 ml wide mouth jar
• 0.45μm filters for dissolved nutrients MCE (mixed cellulose esters) (e.g., Fisher Scientific Cat No 09-719B and 60 mL Luer-Lok tip syringes (e.g., Fisher scientific Cat No 13-689-8)
• 10 orange flags
• Orange flagging tape

Sediment toxicity samples

• 1 x Heavy duty plastic bag, double bagged & zip tied, 3 L total

Sediment chemistry samples

• 1 x 4 oz glass jar for grain size & TOC
• 1 x 4 oz glass jar for metals and pyrethroids
Appendix C, Field Collection Sheets
Ambient Water Quality Measurements

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<th>Calib Date</th>
<th>Water</th>
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COMMENTS, OBSERVATIONS:
### SWAMP Field Data Sheet - Depressional Wetlands - EventType = BA

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<th>Emergent Veg</th>
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<th>Surface Veg</th>
<th>Open / No Veg</th>
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<th>Sampling Spot (Near, Mid, Far)</th>
<th>Station Water Depth (cm)</th>
<th>Dist Sample from Wetted Edge (m)</th>
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<th># Jars</th>
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**Habitat Observations (CollectionMethod = Habitat_Generic)**

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* This section represents whole samples collected

Coll Dev: 1 = D-Frame Kick net (500 micron net, 0.3048 m²)

**Algae Transect (reps 1 & 2)**

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<th>Emergent Veg</th>
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</table>

**ALGAE QUALITATIVE SAMPLE COLLECTED:** Yes / No

**COMMENTS, OBSERVATIONS:**
## SWAMP Field Data Sheet - Depressional Wetlands - Event Type = BA

**Habitat Observations (Collection Method = Habitat_Generic)**

<table>
<thead>
<tr>
<th>Mi Transect (rep 2)</th>
<th>Emergent</th>
<th>Submerged</th>
<th>Surface</th>
<th>Open</th>
<th>Diatom Vol (mL)</th>
<th>Soft Algae Vol (mL)</th>
<th>Dist Sample from Wetted Edge (m)</th>
<th>Sampling Spot (Near, Mid, Far)</th>
<th>Sample Depth (cm)</th>
<th>Dist Wetted Edge to Bankfull (m)</th>
</tr>
</thead>
<tbody>
<tr>
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</tbody>
</table>

### Algae Collection

<table>
<thead>
<tr>
<th>Replicate</th>
<th># Trans Sampled</th>
<th>Coll Dev</th>
<th>Composite Vol (mL)</th>
<th>Diatom Vol (mL)</th>
<th>Soft Algae Vol (mL)</th>
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</thead>
<tbody>
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</tbody>
</table>

* This section represents whole samples collected (20 sub-samples total);
  Coll Dev: 1 = Sediment Corer (5.3 cm²); 2 = Syringe Scrubber (5.3 cm²)

**Location:** Assessment Area 1 2 3 4

**MAP OF POND:**