

**Final Report** February 2015

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

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**SWAMP-SOP-2015-0001**



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# TABLE OF CONTENTS

<b>Figures</b> .....	<b>3</b>
<b>Tables</b> .....	<b>4</b>
<b>Acknowledgements</b> .....	<b>4</b>
<b>List of Acronyms and Abbreviations</b> .....	<b>5</b>
<b>Preamble and Note about SWAMP Compatibility</b> .....	<b>6</b>
<b>Section 1. Introduction</b> .....	<b>7</b>
Field crew size and time estimates.....	8
<b>Section 2. Getting Started</b> .....	<b>9</b>
Sampling frame and site reconnaissance.....	9
Site access permits, permissions, and other considerations.....	11
When to sample.....	12
<b>Section 3. Site Delineation and Order of Data and Sample Collection</b> .....	<b>15</b>
Identification of sampling nodes .....	15
Order of data and sample collection .....	18
Photo documentation of site .....	18
<b>Section 4. Sample Collection and Processing</b> .....	<b>20</b>
Station information .....	20
Habitat observations.....	20
Water chemistry field measurements and water sample collection .....	22
Water chemistry field measurements.....	22
Water sample collection.....	23
Macroinvertebrate collection and habitat observations.....	30
Field elutriation and preservation of MI samples.....	32
Algae collection .....	33
Benthic diatom and quantitative soft-bodied algae sampling.....	33
Habitat observations associated with algae collection.....	37
Qualitative sampling of soft-bodied algae .....	40
Sediment sample collection for toxicity and chemistry .....	40
Collecting field duplicate samples .....	41
Water chemistry .....	41
Macroinvertebrates.....	42
Diatom/quantitative soft-bodied algae .....	42
Habitat observations.....	42

Post-field-collection decontamination of equipment.....	42
<b>Section 5. Sample Analysis.....</b>	<b>44</b>
<b>Section 6. Data Submission and Storage.....</b>	<b>45</b>
<b>References.....</b>	<b>46</b>
<b>Glossary .....</b>	<b>48</b>
<b>Appendix A: Sibbald Syringe Sampler (S3) .....</b>	<b>49</b>
<b>Appendix B: SOP for the use of formalin for fixing diatoms (adapted from Peck, et al. 2006) .....</b>	<b>53</b>
<b>Appendix C: SOP for the use of glutaraldehyde for the preservation of soft algae.....</b>	<b>55</b>

## FIGURES

<b>Figure 1.</b> Example of brush misidentified as a wetland in the mapping process .....	13
<b>Figure 2.</b> Example of a livestock waste pond.....	13
<b>Figure 3.</b> Example of a concrete-lined pond.....	14
<b>Figure 4.</b> Examples of treatment, and polishing, ponds.....	14
<b>Figure 5.</b> Placement of sampling transects.....	16
<b>Figure 6.</b> Label for chlorophyll <i>a</i> and cyanotoxin samples .....	26
<b>Figure 7.</b> Internal and external jar label .....	32
<b>Figure 8.</b> Label for algae taxonomy samples.....	38
<b>Figure 9.</b> Label for soft-bodied algae qualitative sample .....	40
<b>Figure 10.</b> Sample Label for sediment grain size and TOC .....	41

## TABLES

<b>Table 1.</b> Depressional wetland indicators of condition and stress .....	8
<b>Table 2.</b> Office evaluation and field reconnaissance criteria .....	10
<b>Table 3.</b> Field equipment and supplies.....	17
<b>Table 4.</b> Sample collection order .....	18
<b>Table 5.</b> Definitions of sampling spots for collection of water chemistry, MI, and algae samples.....	19
<b>Table 6.</b> Constituent containers, holding times, and storage conditions .....	27
<b>Table 7.</b> Recording Habitat Observations data when duplicate samples are collected .....	42

## ACKNOWLEDGEMENTS

The following individuals provided valuable input on the SOP (alphabetical): Jonathan Bloch, Meredith Howard, Billy Jakl, Willis Logsdon, Nathan Mack, Shawn McBride, Connor McIntee, Sean Mundell, Bill Patterson, Glenn Sibbald, Marco Sigala, Katrina Velasco, Wendy Willis, and Jennifer York. Mr. Sibbald also created, and provided the write up for, the syringe sampler device.

# LIST OF ACRONYMS AND ABBREVIATIONS

Term	Definition
<b>CEDEN</b>	<a href="#">California Environmental Data Exchange Network</a>
<b>CRAM</b>	<a href="#">California Rapid Assessment Method</a>
<b>DO</b>	Dissolved Oxygen
<b>EtOH</b>	Ethanol
<b>HDPE</b>	High density polyethylene
<b>IBI</b>	Index of Biological Integrity. A quantitative assessment tool that uses information about the community composition of one or more assemblages of organisms to make inferences about the condition of the environment they occupy (e.g., the assemblage of interest could be benthic diatoms or macroinvertebrates living in a wetland)
<b>MI</b>	Macroinvertebrate, including benthic (bottom dwelling), nekton (swimming), and neuston (surface)
<b>SAFIT</b>	<a href="#">Southwest Association of Freshwater Invertebrate Taxonomists</a>
<b>SOP</b>	Standard Operating Procedure
<b>STE</b>	Standard Taxonomic Effort
<b>SWAMP</b>	<a href="#">Surface Water Ambient Monitoring Program</a>
<b>TKN</b>	Total Kjeldahl Nitrogen
<b>TOC</b>	Total Organic Carbon

## PREAMBLE AND NOTE ABOUT SWAMP COMPARABILITY

This standard operating procedure (SOP) document is intended to provide standard data collection procedures that will ensure the highest possible data quality, minimize variability due to differences in data collection methods, and maximize data comparability to allow coordination and compilation of data from individual studies and sampling efforts. The California Surface Water Ambient Monitoring Program (SWAMP) Quality Assurance Program Plan (QAPrP) allows flexibility in sampling methods so that the most appropriate method(s) may be used in hypothesis testing and for project-specific objectives that differ from program objectives. Such situations may include, but are not necessarily limited to, special studies (e.g., evaluation of point-source discharges, before/after comparisons where statistical replication is needed), stressor-identification investigations, and long-term monitoring projects where consistent data comparability is desired and an alternative method is needed to achieve that comparability. In addition, in some rare cases where funding limitations would make it cost-prohibitive to complete a project in compliance with the protocols listed in this SOP, the project proponent may request a variance. Deviations from the protocols specified in this SOP may be granted by the Regional SWAMP Coordinator for non-SWAMP projects or the State Board SWAMP Coordinator for SWAMP-funded projects. Such deviations should be noted in the data records when submitted to SWAMP or the California Environmental Data Exchange Network (CEDEN).

SWAMP participants collecting water or sediment samples may reference the Marine Pollution Studies Laboratory SOP: [\*Collections of Water and Bed Sediment Samples with Associated Field Measurements and Physical Habitat in California. Version 1.1 updated March-2014.\*](#) This procedure may be used to collect samples for a number of analyses covered by the SWAMP QA program. Use of this procedure is a recommendation and not a requirement for SWAMP projects. Prior to sample collection, participants using this procedure shall check its requirements against the latest SWAMP [\*Quality Control and Sample Handling Guidelines.\*](#)

## SECTION 1. INTRODUCTION

Depressional wetlands are perennially or seasonally ponded systems up to 8 hectares (19.8 acres) in surface area and less than 2 m deep at their deepest, as defined by the US Fish and Wildlife Service Cowardin Classification (Cowardin et al., 1979) and the California Rapid Assessment Method (CWMW 2013). However, this protocol can be used in lentic habitats with depths greater than 2 meters as long as the wetland edge is safe to wade into. In small wetlands, approximately 50-100m<sup>2</sup> (0.05-0.01 hectares), this protocol can be applied if distances between sampling locations within nodes are slightly condensed in order to avoid overlapping nodes. Wetlands assessed can be natural or artificial in origin, and can be used for a variety of management purposes, including wildlife enhancement ponds, stock ponds, duck ponds, golf course water features, and stormwater treatment ponds. Examples of naturally occurring depressional wetlands include sag ponds and snow melt ponds, kettle-holes in moraines, cutoff (isolated) ox-bows on floodplains, and landslide impoundments. This SOP applies to all depressional wetlands that retain the ability to support wetland plants and aquatic organisms. The procedures described in this SOP apply to perennial and seasonal wetlands, provided that the seasonal wetlands retain water for a sufficient duration to support aquatic invertebrate communities (generally at least 3-4 months). Sampling for all indicators, except CRAM, requires the presence of ponded surface water. The SOP is not intended for use in ponds that are fully lined (concrete, plastic, etc., and lacking in a thick layer of sediment) 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 marine influence (e.g., those with elevation below 2-3m, high conductivity > 5000µS/cm, and/or saltwater plants present).

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 wetland types. 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. This SOP includes procedures for all indicators shown in Table 1. Note, however, that the indicators used for a particular effort may vary based on the objectives of the assessment and the questions being asked. The CORE condition and stressor indicators that are **recommended** for all depressional wetland assessments are shown in yellow and include macroinvertebrate and benthic diatom community composition to facilitate the calculation of biotic indices, the California Rapid Assessment Method (CRAM), basic water chemistry (conductivity, temperature, dissolved oxygen, turbidity, pH, alkalinity), and physical habitat. OPTIONAL indicators shown in blue are considered “second tier” and may be included based on specific program objectives or priorities. As of the time this SOP was prepared, field testing and evaluation had been completed for the core condition indicators (benthic macroinvertebrates, benthic diatoms, and CRAM). Evaluation of soft-bodied algae had not yet been completed and is therefore considered experimental for depressional wetlands assessment.

**Table 1. Depressional wetland indicators of condition and stress. Core indicators are highlighted yellow while optional indicators are highlighted blue.**

Effort Level	Condition Indicators
Core	California Rapid Assessment Method (CRAM)
	Benthic diatom community
	Macroinvertebrate community
Optional	Non-planktonic soft-bodied algae
	Stressor Indicators
Core	General water chemistry (e.g., DO, specific conductance, pH, temperature)
	Physical habitat
Optional	Water column nutrients
	Water column chlorophyll $a^1$
	Water column metals
	Sediment toxicity
	Sediment chemistry
	Cyanotoxins (e.g., microcystins)

### Field crew size and time estimates

These methods are designed to be completed by two teams each with two people [one team for collecting macroinvertebrates (MIs) and a separate team for collecting water and algae], or by one team of two people that collects both indicators in succession. 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. The sampling duration will vary by crew experience, site complexity, and whether CRAM is conducted during the same visit; however, the sampling and sample processing is likely to take a four-person crew approximately four hours, with an additional 1 hour for CRAM.

<sup>1</sup> And/or other pigments, if desired, such as phycocyanin.



## SECTION 2. GETTING STARTED

### Sampling 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 spatial balance and statistical validity of the sample set. Comprehensive wetland mappings of relatively recent vintage are not uniformly available in the State of California. Regional mapping is available for some parts of the state such as southern California ([Southern California Wetlands Mapping Project](#)), and the San Francisco Bay area [[Bay Area Aquatic Resource Inventory](#) (BAARI)]. As new mapping efforts are completed, they are usually appended into the California Aquatic Resources Inventory (CARI), which is accessible through the [SFEI website](#). It is unlikely that a comprehensive wetland map for the entire state will be available in the foreseeable future. To address this gap, a statewide probabilistic mapping program (the California Status and Trends Program, Lackey and Stein 2013) is currently under development. This program will provide a random subset of 4 km<sup>2</sup> plots that will be mapped, thus providing a sampling frame that can be used to support depressional wetlands assessment in all regions of the state.

Once candidate sampling sites are selected, an initial site evaluation (conducted in the office before visiting the site) and field reconnaissance are used to screen candidate sites prior to sampling. For the office evaluation, [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) (Table 2, 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, to determine if the wetland is accessible and can be sampled safely, or if any other exclusion criteria apply. All candidate sites for use in probabilistic surveys should be attributed with the results of both the office evaluation and field reconnaissance, indicating if they were able to be sampled, and if not, the reasons for them being excluded (Table 2). Site criteria information is best recorded in a table based on the master sample draw. This information is necessary to understand what proportion of the population the data represent (and it will also benefit the agency conducting the field reconnaissance), but it is not submitted to SWAMP or CEDEN. Past studies have shown that many sites are eliminated during the reconnaissance phase<sup>2</sup>; consequently a substantial “over draw” should be included when designing a probabilistic survey.

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<sup>2</sup> 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, and an overall success rate of 6% (combined office evaluation and field reconnaissance). Knowing this rate of success was useful during the second year of the study in order to estimate the approximate number of sites that would be needed for the field-reconnaissance effort.

**Table 2. Office evaluation and field reconnaissance criteria to determine when a wetland site is not suitable to be sampled with this protocol (non-target), or whether it was suitable (target) but was not sampled for acceptable reasons.**

Site "Target" Status	Site Evaluation (Sampling) Outcome	Comments	Site Status Detail Code
<b>Site is target, protocol is applicable</b>	Sampled	Wetland was suitable and sampled	T_S
	Permission denied	Wetland met physical sampleability criteria, but permission to sample was denied	T_NS_PD
	No permission Response	Wetland met physical sampleability criteria, but no response was received from landowner after two contact attempts (letters or phone calls)	T_NS_NR
	Site unsafe	Wetland met physical sampleability criteria, but conditions are unsafe to access the site or to sample the site (e.g., banks too steep to safely collect samples)	T_NS_IA
	Too remote	Wetland met physical sampleability criteria, but wetland cannot be hiked to, sampled, and hiked from in a single day (e.g., >2 miles from road)	T_NS_DIST
<b>Site is non-target, protocol is not applicable</b>	Not a wetland	Development has filled in the wetland, or feature never was a wetland	NT_NW
	Inappropriate size/depth	Wetland is too large (> 8 ha in surface area), too small (< 0.05 ha in surface area), too deep (> 3m average), or too shallow (< 30 cm average). Any of the above criteria is reason for exclusion.	NT_SI
	Riverine	Site is a riverine wetland and receives direct or indirect water from a river (or stream). However, note that sites on floodplains that are hydrologically isolated from streams should be retained.	NT_River
	Dry	Historical Google Earth™ imagery indicates wetland is usually dry during the spring sampling period, landowner confirms wetland does not support water during index period, or wetland was dry during field reconnaissance or attempted sampling	NT_Dry
	Concrete, gravel, or plastic lined	Feature is concrete, gravel, or plastic lined and lacks a thick layer of sediment	NT_Lined
	Marine influence	Low elevation (<2-3m) and proximity to saltwater, high conductivity (e.g. >5000 μS/cm), and/or saltwater vegetation present	NT_Tidal
	Livestock waste pond	Feature used as a livestock waste settling pond. Note this does not include stock ponds used as a water source.	NT_LWP

Site "Target" Status	Site Evaluation (Sampling) Outcome	Comments	Site Status Detail Code
<b>Site is non-target, protocol is not applicable</b>	Treatment pond	Wetland used as a human wastewater treatment wetland, or constitutes crop-based wastewater, or other water waste, but is not managed for wildlife. Note that some wastewater polishing ponds are designed for wildlife habitat, and this protocol would be suitable in that scenario as long as the wetland is safe to sample from a human health perspective.	NT_TP
	Other	Describe why this wetland is not suitable for biological assessment. For example, some wetlands are treated with algaecide to prevent algae growth. Additionally, some wetlands are built for groundwater recharge and use imported water.	NT_Other
	Suitability Unknown	Suitability of the site cannot be determined. It is unknown whether the protocol would apply to this site.	U

### Site access permits, permissions, and other considerations

Access permission must be acquired for all sites prior to sampling. Gaining the appropriate permits and permissions can be conducted concurrently with the office evaluation and field reconnaissance. Products such as [ParcelQuest™](#) and [Landvision™](#) can assist with determining ownership. For Water Board projects, statewide parcel data are available from State Board (contact the Division of Information Technology GIS Unit). When considering site access, take into account parking for vehicles, any required keys or lock combinations to open gates, and whether arrangements must be made in advance for an on-site escort to accompany the field crew. For any sampling on private and public land, be sure to acquire permission from the landowner and contact the on-site manager before sampling, as applicable.

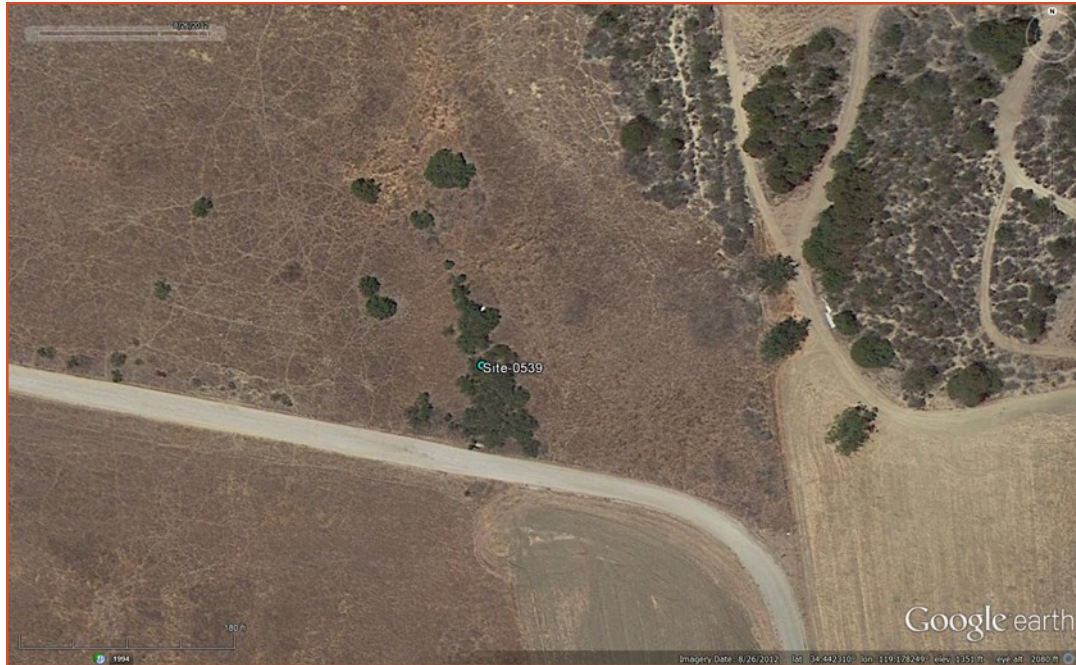
During sampling, the field lead should carry the name and contact information for private land owners and managers. A scientific collecting permit<sup>3</sup> is required for all sites, including private and public lands. For public lands, an access or additional biological sampling permit is also often 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<sup>4</sup>. Regardless of whether the site is on private or public lands, the appropriate property manager should be notified a few days in advance of when the intended sampling is to occur.

<sup>3</sup> Collecting permits should be obtained from the California Department of Fish and Wildlife

<sup>4</sup> 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.

## **When to sample**

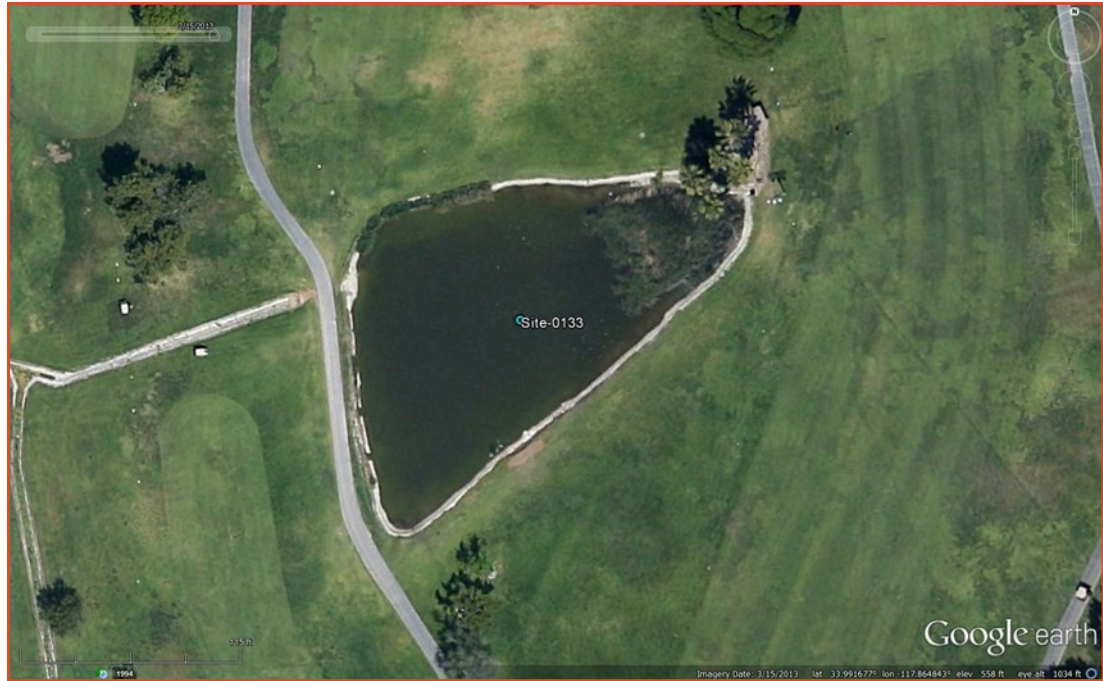
It is recommended that sampling be scheduled for periods immediately before larval invertebrate emergence. This is generally from May until mid-July, but the timing of emergence will be strongly influenced by weather and temperature. The timing of the sampling should also balance the availability of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



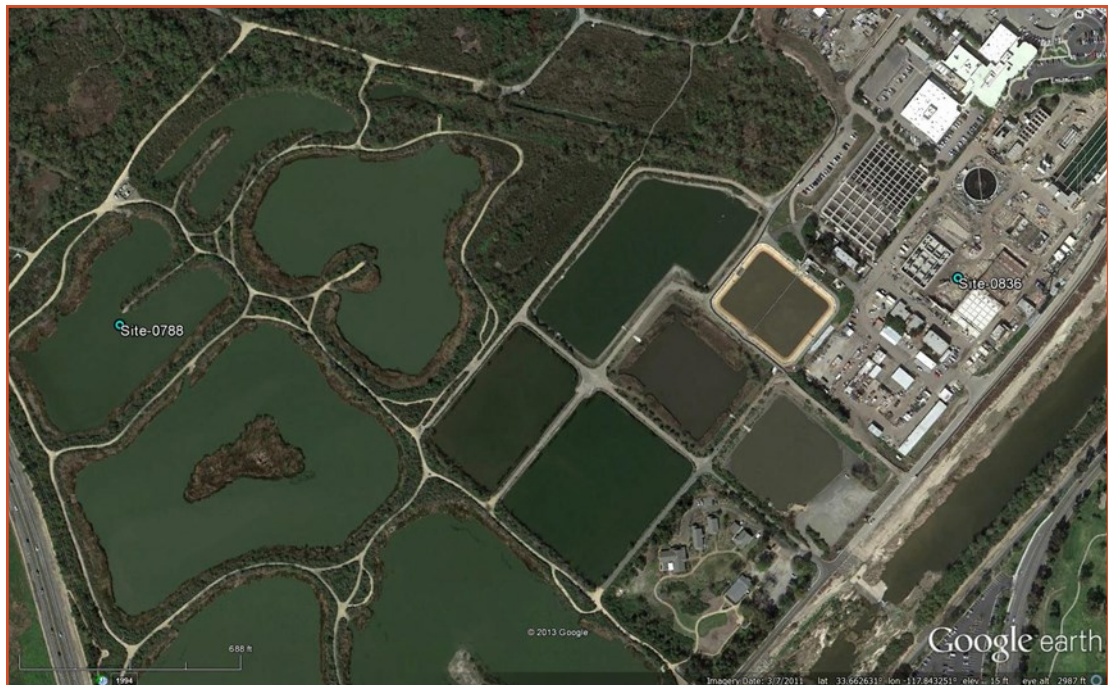
**Figure 1. Example of brush misidentified as a wetland in the mapping process. No wetland was observed in any of the Google Earth™ time line images. Vegetated areas like this probably have more moisture than the surrounding area, but do not have ponded water.**



**Figure 2. Example of a livestock waste pond (Table 2). The nearby rectangular metal roof structures that house animals 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 if it does not contain a deep layer of sediment.**



**Figure 4. Examples of treatment, 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 frequently included in depressional wetland surveys.**

## SECTION 3. SITE DELINEATION AND ORDER OF DATA AND SAMPLE COLLECTION

Depressional wetland sampling occurs at 10 sampling “nodes” evenly spaced around the entire wetland perimeter (Figure 5). Water, macroinvertebrate (MI), and diatom (and, if desired, soft algae) samples are collected at each node. The sampling location for each indicator is separated by a few paces along different “transects” perpendicular to the edge of the wetland. The steps to laying out the sampling nodes and transects are described below. Recommended field equipment and supplies are listed in Table 3.

### Identification of sampling nodes

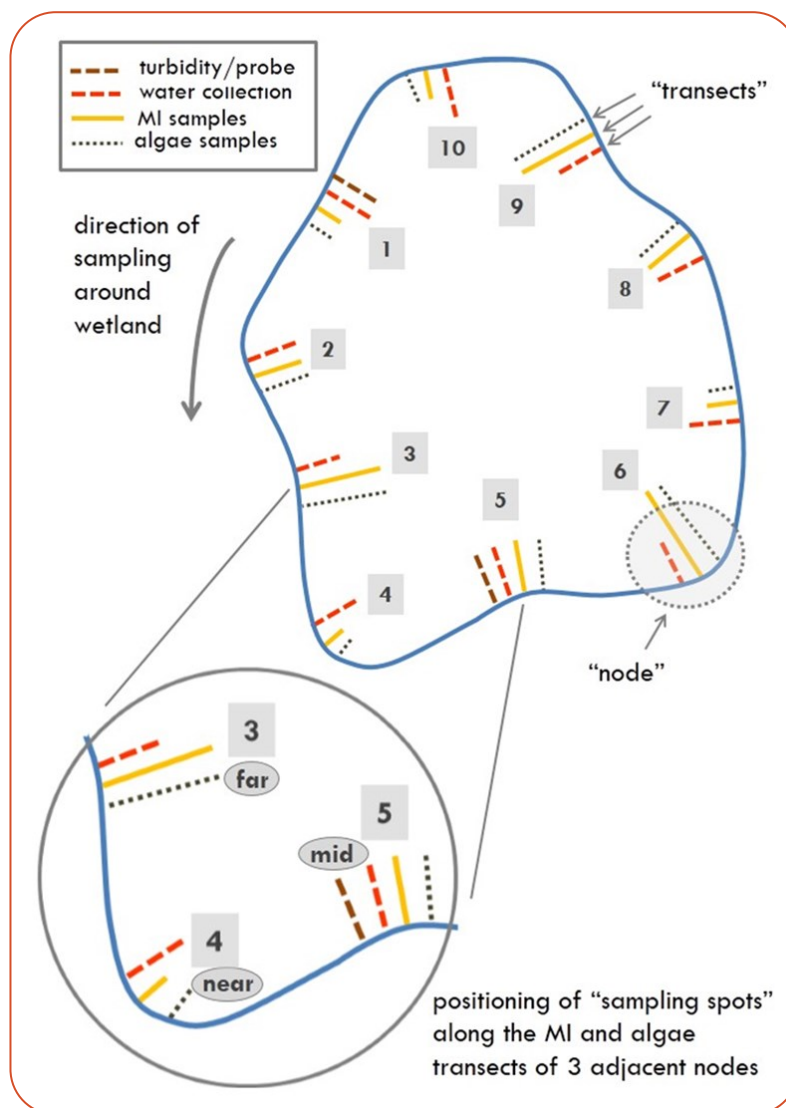
The distance between each node is based on the circumference of the wetland (Figure 5). Circumference data can either be collected before visiting the site by using remote photography, or it can be determined in the field. The steps for laying out the sampling nodes upon occupying the site are:

1. Identify a starting point (it could be where the wetland is first accessed) and walk along the periphery. Always maintain a constant, short distance from the edge of the surface water, and count how many evenly spaced paces are required to walk the entire perimeter. During this time, record on your [field sheet](#) observations of any species of birds or amphibians within, or in close proximity to, the wetland (see “Habitat observations” in Section 4, below).
2. Divide the total number of paces by 10 to yield the distance between adjacent sampling nodes along the wetland’s edge (Figure 5).
3. Pace the perimeter of the wetland again, using the same path as before, flagging a highly visible spot at each node where sampling transects will be located with orange transect flags or orange flagging tape. By convention, the flagging is done counter clockwise around the wetland. Water samples can be collected concurrently with placement of the orange flags (see the water chemistry portion of Section 4 below for indicators and methods).

Each of the 10 sampling nodes will have three parallel transects: one associated with water collection, one associated with MI sampling, and one associated with algae sampling (Figure 5). Each transect will be offset from its neighbors 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 be collected from transects located adjacent to the water collection transects associated with nodes 1 and 5). The MI samples will be collected from transects positioned 3 paces beyond (to the right of, when facing the water) each of the orange flags (as one walks counterclockwise around the perimeter of the wetland) at the appropriate near, mid, or far positions (see below). The MI

collector will place 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. Sediment will be collected in areas of the wetland where there is fine-grained sediment. The qualitative algae samples will be collected throughout the wetland, wherever representative species are located (see below).

For wetlands with thick vegetation that limit the access, divide the perimeter of the wetland that can be sampled into the 10 nodes. Document the reduced sampling area, and sketch the sampled and unsampled area of the wetland on the field sheet.



**Figure 5. Placement of sampling transects (depicted as dashed or solid lines) 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 the "far", "near", and "mid" sampling spots are located at the ends of the transects. Sediment is collected in appropriate spots where no other sampling has taken place, after all other samples have been collected (see below). Note diagram is not to scale (its purpose is to show the relative positions of the nodes; transects are truncated for clarity).**



Table 3. Field equipment and supplies

Physical habitat	Water Chemistry	Chlorophyll <i>a</i> and Cyanotoxins	Macroinvertebrate	Algae	Sediment
<ul style="list-style-type: none"> <li>• GPS unit</li> <li>• 10 orange transect flags</li> <li>• 10 yellow transect flags</li> <li>• meter stick (in cm)</li> <li>• transect tape</li> <li>• inclinometer/angle finder</li> <li>• <a href="#">field sheets</a> printed on Rite-in-the-Rain™</li> <li>• pencil/permanent markers</li> <li>• clipboard</li> <li>• rangefinder (optional)</li> <li>• decontamination solution for waders, nets, and equipment</li> <li>• orange and yellow flagging tape (if not using flags)</li> </ul>	<ul style="list-style-type: none"> <li>• “S3” water sampler (see Appendix A)</li> <li>• YSI™ or comparable probe(s), for dissolved oxygen, pH, temperature, and conductivity (with extra membranes and batteries)</li> <li>• field turbidimeter</li> <li>• field test kit for alkalinity (e.g. Hach™ AL-AP #2444301)</li> <li>• 2L bottle for integrated water sample</li> <li>• 3, 250 ml HDPE wide-mouth jars</li> <li>• 500 ml wide-mouth jar</li> <li>• 0.45 µm pore size, 22 mm diameter polytetrafluoroethylene (PTFE) filters for dissolved nutrients</li> </ul>	<ul style="list-style-type: none"> <li>• 1L HDPE bottle for integrated sample</li> <li>• “S3” water sampler (see Appendix A)</li> <li>• 250 mL, 500 mL, and 1 L plastic graduated cylinders</li> <li>• bottle brush</li> <li>• 250 and 500 mL capacity filtering apparatus</li> <li>• 0.7 µm pore size, 47 mm diameter glass fiber filters, (e.g. Whatman™ GF/F)</li> <li>• automatic electronic vacuum pump or hand-held vacuum pump with pressure gauge marked at 7 psi</li> <li>• deionized or distilled water</li> <li>• clean squirt bottle</li> <li>• flat-tipped filter forceps</li> <li>• 47 mm snapping Petri dishes</li> <li>• aluminum foil</li> <li>• 60 or 100 mL Whirl-Pak™ bags</li> <li>• sample labels</li> <li>• wet-ice cooler</li> <li>• clean plastic tarp</li> <li>• umbrella</li> <li>• dry-ice cooler or field freezer (if going to be in field all day)</li> </ul>	<ul style="list-style-type: none"> <li>• 500 µm D-frame custom all-mesh net with 52-inch handle (Wildco™ 425-JD52-SPE)</li> <li>• 500 µm sieve</li> <li>• 4 mm (4000 µm) sieve (optional)</li> <li>• 5-gallon bucket with lid</li> <li>• small aquarium net (approx. 2x2 inches with 1 mm mesh)</li> <li>• flexible and hard forceps</li> <li>• 3 dish tubs (approx. 3-gallon or 11-qt) for field elutriation</li> <li>• 1 L wide-mouth plastic bottles (assume 2 bottles per site but some may use more)</li> <li>• 500 mL polyethylene squirt bottle: one for water and one for ethanol)</li> <li>• 2 L of 95% (190 proof) ethyl-alcohol per site to preserve sample (but more required if using 3 or more jars)</li> <li>• plastic funnel (preferably with a ½-or 1-inch diameter spout)</li> <li>• Rite-in-the-Rain™ labels and pencil</li> </ul>	<ul style="list-style-type: none"> <li>• sediment corer (60 mL syringe)</li> <li>• silver or hot-pink permanent marker</li> <li>• syringe scrubber</li> <li>• spatula</li> <li>• shears</li> <li>• utility knife or pocket knife (for cutting sediment core and macroalgal mats)</li> <li>• white scrubber pads cut into circles for syringe scrubber</li> <li>• intact 60 mL syringes (to use for rinsing sample into container)</li> <li>• sample labels</li> <li>• 250 mL, 500 mL, and 1 L HDPE bottles (wide-mouth)</li> <li>• clear plastic tape</li> <li>• scissors</li> <li>• clean, soft-bristled toothbrushes</li> <li>• long, blunt-ended forceps for grabbing algal clumps out of sample bottle</li> <li>• 10% formalin</li> <li>• waterproof meter stick</li> <li>• meter tape</li> <li>• turkey baster</li> <li>• glutaraldehyde (only if sampling for soft-bodied algae, and kept in the laboratory, <b>not brought to the field</b>)</li> </ul>	<ul style="list-style-type: none"> <li>• HDPE scoop</li> <li>• homogenization container</li> <li>• 3 L glass jar for toxicity</li> <li>• 4 oz glass jar for grain size and TOC</li> <li>• 4 oz glass jar for metals and pyrethroids</li> </ul>

## Order of data and sample collection

Sampling should always be carried out in the following order: 1) habitat observations, 2) water chemistry measurements (turbidity, probe measurements) and water-column grabs for laboratory chemistry analyses, 3) macroinvertebrate samples, 4) quantitative algae samples, 5) qualitative algae sample, and 6) sediment for toxicity and chemistry (Table 4).

If CRAM is conducted during the same site visit, it should be carried out last. CRAM should be conducted by qualified practitioners following the procedures outlined in the depressional wetlands [CRAM](#) field book.

## 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.

**Table 4. Sample collection order. The appropriate sampling depths and distances from shore can be found in Table 5.**

Order	Indicator	Nodes	Location in relation to orange flags
1	Habitat observations	Recorded during the layout of the sampling nodes	
2	Turbidity and probe measurements	1 and 5	3 paces to the left of the orange flag
2	Water chemistry (e.g., nutrients); composited grabs for laboratory analysis	1 – 10, composited	Directly in front of orange flags
3	Macroinvertebrate samples	1 – 10, composited	3 paces to the right of the orange flags (at yellow flags)
4	Benthic diatom (and Quantitative soft-bodied algae, if applicable)	1 – 10, composited	6 paces to the right of the orange flags (3 paces to the right of yellow flags)
5	Qualitative soft-bodied algae (if applicable)	Throughout the wetland, in order to collect specimens of all different types of macroalgal filaments and mats, as well as microalgae	
6	Sediment toxicity and chemistry	At least 2 locations, away from areas disturbed by other sampling	

**Table 5. Definitions of sampling spots for collection of water chemistry, MI, and algae samples. Transect trajectory is always defined as perpendicular to the shore upon which the sample collector is standing.**

<b>Sampling spot location</b>	<b>Water collection/ turbidity/ probes</b>	<b>Macroinvertebrates</b>	<b>Algae</b>
<b>Near</b>	N/A	starting at a point 1.5 m from shore along transect trajectory, with the constraint of going no deeper than 0.25 m	starting at a point 0.5 m from shore along transect trajectory, with the constraint of going no deeper than 0.25 m
<b>Mid</b>	up to 50% of the way to wetland midpoint along transect trajectory, with the constraint of going no deeper than 0.5 m	up to 50% of the way to wetland midpoint along transect trajectory, with the constraint of going no deeper than 0.5 m	up to 50% of the way to wetland midpoint along transect trajectory, with the constraint of going no deeper than 0.5 m
<b>Far</b>	N/A	up to 80% of the way to wetland midpoint along transect trajectory, with the constraint of going no deeper than 1 m	up to 80% of the way to wetland midpoint along transect trajectory, with the constraint of going no deeper than 0.5 m

## SECTION 4. SAMPLE COLLECTION AND PROCESSING

Sampling procedures are presented here in the sequence in which they should be executed in the field. A list of sample holding times and conditions can be found in Table 6. Field sheets associated with this protocol are stored at the [SWAMP website](#).

### Station information

For SWAMP projects, the identification code chosen for each sampling site (“station”; called “StationID”) should follow [SWAMP guidelines](#). Station description should be useful in order to find the site from Google Earth™. The Protocol Code for this SOP is SWAMP\_2014\_DW. Sample Time is when the first sample is collected (typically this will be the first turbidity sample or YSI measurement). Project Code is a specific numeric identifier created for SWAMP projects. A SWAMP compliant code is recommended for non-SWAMP projects.

On the field sheets Location Assessment Area 1 should be circled for most projects. However, if a goal of your assessment is to understand one wetland in detail and the wetland is separated into multiple segments (each for individual assessment), then circle the Assessment Area number related to that specific segment.

### Habitat observations

During the initial wetland circumference pacing, data on physical characteristics of the wetland (that could serve as explanatory factors for the biotic community composition data) are collected at the level of the wetland as a whole. During biotic sampling, additional habitat observations are also recorded associated with each of the MI and algae sampling nodes. The latter data types are discussed in detail within the MI and algae sampling sections. All of the data under the “Habitat Observations” portion on the first page of the field sheets are meant to characterize the wetland as a whole. Some of the wetland characteristics should be determined beforehand during an office assessment, and then confirmed in the field. These include the wetland’s origin (natural or artificial), its age and function (if created), whether or not vector control activities currently occur at the wetland (and what kind), what the wetland’s hydroperiod is believed to be, and wetland area. Google Earth™ time series imagery and property managers are good sources for determining if the wetland is perennial or seasonal during typical years.

For our purposes, wetland trophic status is a general classification of three categories that are estimated based on observed algal and herbaceous plant percent-cover levels, from oligotrophic (little or no algae or plant biomass), mesotrophic (moderate algae and/or plant biomass), to eutrophic (very high algae and/or plant biomass). Other data to record include wetland length and width, which are assessed using a rangefinder, meter tape, or using Google Earth™/Bing™ imagery after knowing the current water surface elevation, and observations relating to weather conditions during the assessment period (e.g., precipitation, cloud cover, and wind conditions). The number of paces required to walk the perimeter of

the water are also recorded and translated into meters for the database, as are wetland maximum depth and average depth. Both depth assessments are estimates based on the topography of the wetland bottom. In a wetland that is 100% wadeable, the average depth will be close to the average of the near/mid/far sample depths (see below). However, in a system in which only a few meters of the littoral fringe is wadeable, the field crew will need to make a best-guess estimate of the depth. Depth information can also be obtained from landowners, when possible. Depth information can be measured in meters or centimeters in the field, and (as needed) is converted to centimeters for entry into the SWAMP database.

The percent cover of vegetation across the wetland, compartmentalized into categories for emergent, submerged, and surface (floating) vegetation, is recorded for the wetland as a whole. Within the latter two categories, separate percentages are given for algal vs. non-algal (“other”) plant material. This is analogous to the categories used for the MI and algae transect-specific habitat observations (see below). The percent of the wetland’s surface area that is wadeable (“% WADEABLE / LITTORAL” on the field sheet) is estimated (after the sampling crew has walked through portions of the wetland in the process of collecting MI and algae samples) and recorded. Likewise, the value of the “% SURFACE AREA OF MAX” is calculated by estimating the current surface area size as a fraction of the total surface area the wetland would have at “high water” stage (e.g., this would be 100% if the wetland happened to be assessed at high-water stage). High water marks around the wetland are observed and used to make this estimate. Perennial wetlands will tend to be closer to 100%, but non-perennial wetlands may be just a small fraction of their winter maximum size according to how much evaporation has occurred since the last rain event. Aerial photographs, in conjunction with ground-truthing, may also be useful for inferring maximum size of the wetland. “UPLAND SLOPE (%)” is measured at four representative locations above the water’s edge using an inclinometer, or survey equipment. All four measurements are recorded on the field sheet.

In addition to wetland physical characteristics, wildlife use is also noted by marking what types of animals are observed at the wetland during the assessment. These observations are best made when first approaching the wetland, before the actual transect laying and sampling begin, which may scare wildlife away. Any presence of amphibians should be recorded. An excellent amphibian resource for identification of eggs, larvae, and adults is [California Herps](#). For birds, the number of individuals falling into various guilds<sup>5</sup> (i.e., raptors, waterfowl, shorebirds, passerines) should also be recorded. Mention in the “COMMENTS, OBSERVATIONS” section any birds that you are unable to classify into the listed guilds.

Additional stressors are recorded on the field sheet. Note if any fish are present at the site, as they can alter MI communities. Most fish species found in wetlands will be invasive, warmwater fish, such as bass, sunfish, and mosquitofish. Fish information can be obtained by asking property owners, or individuals at the site (e.g., people who are fishing). Record the species of fish present if that information is known.

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<sup>5</sup> Raptors are birds-of-prey, such as hawks, kestrels, kites, terns, and osprey. Examples of waterfowl are ducks, geese, loons, and grebes. Shorebirds include herons and egrets, and passerines are songbirds, such as red-winged blackbirds and grackles.

Also note the presence of crayfish, which can alter both physical and vegetation conditions at wetlands and lakes. Early life stages of crayfish are likely to show up in the MI samples, or they may be detected onsite by looking for evidence of their burrows around the wet terrestrial margins of the wetland. “Recent grazing” is loosely defined as cattle activity within the past year. Note that grazing of seasonal wetlands may occur when the wetland is dry. In the field, look for active cow presence, cow patties from the current season (still somewhat moist or at least intact), muddy hoof prints around the wetland margin, or ask the landowners. If there is cow activity, but you are not certain if it is recent, still select YES. If the site was not grazed in the past year but was grazed within the past 2-10 years, note that in the comments section.

Sketch the wetland on the field data sheet where provided, showing the shape, placement of vegetation, and bank slope of the wetland.

## Water chemistry field measurements and water sample collection

### Water chemistry 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 are recorded at two nodes at roughly opposite ends of the wetland: Node 1 and Node 5 (Figure 5). *Probes should be calibrated prior to starting field work, as recommended by the SWAMP QAPrP (SWAMP 2013), and the calibration date added to the field data sheet.* Because of the influence of particulates, the order of measurements should be:

- turbidity (NTU) (either on YSI or using portable meter)
- water temperature (°C)
- specific conductivity (µS/cm)
- salinity (ppt)
- pH
- dissolved oxygen (mg/L and % saturation)

Let the YSI sonde stabilize for at least 3-5 minutes in the water, or according to the manufacturer’s instructions, before recording values. Do not let the sensors contact sediment or organic debris. If the oxygen sensor is consumptive (often will have a membrane cover in such circumstances), keep the sensors moving slightly during this process to prevent false low readings.

At each of the two nodes, the turbidity/probe measurements 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; Figure 5). For the purposes of this SOP, “mid” is always defined as 50% of the way to the midpoint of the wetland along the transect at hand, when sighting perpendicularly from the shore upon which the collector is standing (Table 5), or at a depth of approximately 0.5 m, whichever distance from the shore is shorter. *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 occur, 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. 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.

Probe measurements are made 10 cm below the water surface. For additional guidance on how water samples can be collected, consult the Marine Pollution Studies Laboratory's (MPSL) guidance on how to collect water and sediment samples (MPSL 2014). Alkalinity (mg/L) can be measured with a field test kit (e.g. Hach AL-AP #2444301) or in the laboratory using water collected from the composite water sample.

### Water sample collection

Water samples for laboratory analysis are collected and composited for the following core analytes:

- water-column chlorophyll *a*
- cyanotoxins (e.g., total microcystins)
- alkalinity (if not measured in the field)
- nutrients [e.g., total ammonia, nitrate, nitrite, orthophosphate, total nitrogen or total Kjeldahl nitrogen (TKN), and total phosphorus]
- dissolved organic carbon (DOC) (if desired)

The samples are collected at each of the 10 nodes 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 procedures 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, or introduction of surface scum, during the sampling process. The recommended approach is to use an extended syringe sampler device (see Appendix A) for collecting water at each node. When fully disassembled (Figure A5 in Appendix A), all pieces of the device fit into a 2-gallon bucket, for dilute bleach-solution cleaning. The plastic syringe is replaced after every site. Alternatively, using a hand-operated plastic syringe by holding the syringe at an arm's length can be effective in obtaining an undisturbed water sample. Syringe-based sampling is recommended for wetlands with loose benthic organic debris, surface or sub-surface macrophytes, and surface algae. In wetlands where stirring-up of sediments is not an issue, a bottle can be uncapped 10 cm below the surface, letting water slowly fill the container. The bottle is then recapped underwater to secure the sample.

At each point where there is an orange flag:

1. Wade into the wetland perpendicularly from the water's edge and deploy the sampling device to the "mid" sampling position<sup>6</sup> (Figure 5; Table 5).
2. Collect water from 10 cm below the water's surface.
3. Assuming a total volume of 2 L required for water samples, collect 200 mL from each node and add to the 2 L HDPE sample bottle for use in chemical analysis. Collect another at least 100 mL at each node to store in the 1 L aluminum foil-covered container used for chlorophyll *a* and cyanotoxin analysis. Alternatively, one large (3L+) foil-covered container could store water for both water chemistry and surface algae analyses.
4. Repeat this procedure at each of the remaining 9 orange flags, creating sample composites.
5. 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. To reduce the likelihood of pigment (chlorophyll *a*, etc.) degradation as much as possible during the sampling, 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 ensured during the entire filtering process. If no shade is available around the wetland, an umbrella can be used.

Subsamples for the lab-measured general constituents (i.e., alkalinity, 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 cyanotoxins, are discussed below.

### ***Preparation of dissolved and total nutrient samples***

The samples for dissolved nutrients (i.e., for nitrate, nitrite, and orthophosphate) are first field filtered using a 0.45 µm Polytetrafluoroethylene (PTFE) filter. The samples for analysis of total ammonia, TN, TP and TKN are not filtered (Table 6). 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, and multiple filters will probably be needed. Some field crews have successfully used larger diameter filters and vacuum pumps to obtain the necessary volumes. Project holding times, field preparation, bottle types, and recommended volumes for each water chemistry analyte can be found in Table 6. Consult the MPSL SOP (2014) for more detailed information on field sampling methods for nutrients.

### ***Additional water chemistry***

If additional water chemistry tests are desired, samples should be collected from the integrated water sample stored in the 2 L container (or stored in a different, appropriate type of container, if required, depending upon the specific analyte type). Bear in mind that more than 2 L of water may be required for

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<sup>6</sup> The water-chemistry samples are always collected at the "mid" position, regardless of the length of the neighboring transects at any given node.



all water chemistry analytes, especially when duplicate laboratory samples need to be collected. Consult the applicable analytical laboratory(ies) for information on sample volumes, storage containers, and holding times for any analytes not discussed in this SOP.

DO NOT use Sparquat™ 256, Quat™ 128, or any other ammonia-based 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 water column chlorophyll *a* and particulate cyanotoxin sample filters***

The chlorophyll-*a* and particulate 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 up to 250 mL of the well-mixed 1 L sample from the integrated sample bottle. A program coordinator may choose to collect a second filter with this method, which allows the option of testing for an additional pigment type (such as phycocyanin), if desired. Aim to filter the prescribed volumes for this sample type, but be sure to record the total filter volume on the sample labels, field sheets, and COCs for every sample. Higher filter volumes are always desirable, as they minimize sampling error, but sometimes the desired filter volume cannot be collected as a result of turbid water rapidly clogging the filter. For particulate cyanotoxin samples, filter up to 500 mL in the field. Record the volumes that were actually filtered on the field sheets and the sample labels.

Total cyanotoxins are sampled using unfiltered water. Pour 500 mL of the integrated water sample into a 500 mL amber plastic bottle. Store the sample on ice while in the field and freeze that afternoon when returning to the laboratory. Coordinate cyanotoxin collection and sample handling procedures with your lab as these steps depend on the analysis method.

Use 47 mm diameter, 0.7 µm pore size, glass fiber filters to obtain the samples for laboratory chlorophyll *a* and cyanotoxin analysis. During the filtering process, make sure that the pressure on the filter never exceeds 7 psi. An automatic electric pump is recommended, although a hand pump equipped with a gauge to ensure the 7 psi limit is observed can also be used. 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 or distilled 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 deionized or distilled water and wiped clean 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 the filter 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 preferably 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 laboratory 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.

Contract/Billing Code: _____		circle one: <b>chl a</b> <b>cyano</b>
Project: _____	Date: _____	Time: _____
Site Code: _____	Sample ID: _____	
Repl #: _____	Vol Filtered (mL): _____	
Wetland Name: _____		
County: _____	Collector: _____	

**Figure 6. Label for chlorophyll *a* and cyanotoxin samples.**

**Table 6. Constituent containers, holding times, and storage conditions. The majority of requirements below are based on the 2013 SWAMP Quality Control and Sample Handling Tables (SWAMP 2013); the elements that do not have SWAMP programmatic recommendations or requirements for the indicated quality control and/or sample handling aspect are denoted with an asterisk. Holding conditions of 4°C indicate samples that are held on ice during transport to the analytical labs. The chlorophyll-*a* and cyanotoxin samples are taken from the foil-covered 1 L bottle, while the other water chemistry samples are taken from the 2 L bottle.**

Data Type	Container Type <sup>7</sup>	Number of containers	Holding time	Holding condition
<b>Water Chemistry</b>				
Turbidity	Field measurement or plastic bottle	-	-	-
Alkalinity <sup>8</sup>	Plastic	1	14 days	Cool to ≤6°C
Hardness	Plastic	1	6 months	Cool to ≤6°C; HNO <sub>3</sub> or H <sub>2</sub> SO <sub>4</sub> to pH < 2
Chlorophyll- <i>a</i>	Glass fiber filter wrapped in aluminum foil within Petri dish	2	Samples must be frozen or analyzed within 4 hours of collection; filters can be stored frozen for 28 days	Centrifuge or filter as soon as possible after collection; if processing must be delayed, keep samples on ice or at ≤6°C; store in the dark
Particulate cyanotoxins*	Glass fiber filter within Petri dish*	1	6 months*	-20°C (-80°C preferred) *
Total cyanotoxins*	Plastic (amber)	1	4 months*	-20°C*
Orthophosphate	Plastic	1	48 hours	Filter with 0.45 µm PDPE filter within 15 min of collection; all portions cool to ≤6°C
Nitrate, nitrite	Plastic	1	48 hours	Cool to ≤6°C
Total ammonia, Total phosphorus, Total nitrogen (direct measurement)	Plastic	1	28 days	Cool to ≤6°C; H <sub>2</sub> SO <sub>4</sub> to pH < 2
Total Kjeldahl nitrogen (TKN)	Plastic	1	7 days; 28 days if acidified	Cool to ≤6°C; H <sub>2</sub> SO <sub>4</sub> to pH < 2
Dissolved organic carbon (DOC)	250 ml Glass	1	28 days	Filter and preserve to pH < 2 within 48 hours of collection; cool to ≤6°C

<sup>7</sup> Consult the analytical laboratory to determine whether they will supply the sample containers, or if any special treatment of the containers is needed prior to sampling.

<sup>8</sup> If not measured in the field.

Data Type	Container Type <sup>7</sup>	Number of containers	Holding time	Holding condition
<b>Sediment Chemistry</b>				
Total organic carbon (TOC)	Glass	1	28 days at $\leq 6^{\circ}\text{C}$ ; 1 year at $\leq -20^{\circ}\text{C}$	Cool to $\leq 6^{\circ}\text{C}$ or freeze to $\leq -20^{\circ}\text{C}$
Grain size	Glass	1	1 year	Wet ice to $\leq 6^{\circ}\text{C}$ in the field, then refrigerate at $\leq 6^{\circ}\text{C}$
Trace metals (except for methylmercury)	Glass	1	1 year; samples must be analyzed within 14 days of collection or thawing	Cool to $\leq 6^{\circ}\text{C}$ within 24 hours, then freeze to $\leq -20^{\circ}\text{C}$
Methylmercury	Glass	1	1 year	Freeze to $\leq -20^{\circ}\text{C}$ immediately
Pyrethroids	Glass	1	1 year at $\leq -20^{\circ}\text{C}$ in the dark; samples must be extracted within 14 days of collection or thawing and analyzed within 40 days of extraction	Short term storage: $\leq 6^{\circ}\text{C}$ in the dark; long-term storage, or storage of remaining sample: $\leq -20^{\circ}\text{C}$ in the dark
<b>Sediment Toxicity</b>				
	3 L glass or plastic jar	1	<14 days (recommended) or <8 weeks (required) @ 0 to 6 $^{\circ}\text{C}$ ; dark; do not freeze	Wet or blue ice in field; refrigerate at $\leq 6^{\circ}\text{C}$ ; keep in the dark at all times

\* At the time of publication of this SOP, SWAMP did not have programmatic recommendations or requirements for the indicated quality control and/or sample handling aspect

	Container Type <sup>7</sup>	Number of containers	Holding time	Holding condition
<b>Algae</b>				
Diatoms (preserved)	250 or 500 mL HDPE*	1	1 year*	Add 10% buffered formalin to a 2% final concentration immediately after collection; keep dark and away from heat
Soft-bodied algae (preserved)	250 or 500 mL HDPE*	1	1 year*	Keep unfixed samples in dark on wet (NOT DRY) ice; add glutaraldehyde ( <i>in a laboratory fume hood, wearing appropriate personal protective gear</i> ) to 2.5% final concentration as soon as possible, but not longer than 4 days post-collection; after fixing, keep dark and away from heat
Soft-bodied algae (qualitative)	250 mL HDPE*	1	2 weeks*	Keep unfixed samples in dark on wet (NOT DRY) ice
<b>Macroinvertebrate</b>				
	Wide mouth HDPE recommended*	Dependent on site conditions	Samples should be analyzed by laboratory as soon as possible. Minimum archive times: <ul style="list-style-type: none"> <li>• Vials of identified organisms: at least 5 years;</li> <li>• Sorted sample residue: at least 1 year;</li> <li>• Unsorted sample remainder: at least 2 years</li> </ul>	Preserve sample in field with EtOH, ≥70% final concentration

## Macroinvertebrate collection and habitat observations

Ideally, two people should work together to collect the MIs: one person to make the collections, and the other to carry equipment and record the required information on the field sheets. The MI sampling needs to be coordinated with the algae sampling. 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 MIs 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.

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, and facing the water) (Figure 5). The starting location for the MI collection is rotated from transect to transect through 3 positions relative to the water's edge: "near", "mid", and "far", starting with "near" at the first transect sampled. "Near" is defined as 1.5 m into the water from the water's edge. "Far" is defined as the closest distance from the edge at which the water is 1 m deep; 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 from the shore (Figure 5). "Mid" is defined as 50% of the way to the midpoint of the wetland along the transect in question. In deeper wetlands with a narrow littoral zone, the mid and far sampling spots may be based on depth. A summary of the definitions of near, mid, and far sampling spots is provided in Table 5.

Once the spot to be sampled is identified, the procedures for collecting the sample are as follows.

1. Approach the sampling spot slowly with your D-frame kick net (Table 3) in the air (not dragging underwater), in order to avoid frightening the MIs away, or accidentally collecting organisms, as you draw nearer.
2. 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.
3. Plunge the net into the water, quickly pulling it through the water column towards the wetland bottom.
4. Pull the net toward you, gently rubbing the wetland bottom in an undulating motion that covers a swath about a meter long. The idea is to catch MIs that are in the water column, clinging to vegetation, and along the sediment, all the while avoiding collecting a lot of sediment in the sample.
5. Once the 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, being careful not to collect too much sediment.
6. 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 capture 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 wetland bottom while trying to keep the net in forward motion to prevent escape of captured organisms.

7. 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.
8. Add the material collected to a 5-gallon bucket that has been filled approximately halfway with water from the wetland (a small aquarium net is useful to remove accidentally captured organisms from the wetland water, prior to adding samples collected with the D-frame kick net).
9. At each sampling area, establish an imaginary 1 m<sup>2</sup> area centered on the location where the MI sweep was made. Within the sampling area, record the percentages of each habitat type, based on vegetation, within the “Habitat Observations” section of the field sheets. Vegetation categories that can be used are “emergent”, “submerged”, “surface”, and “open” (which corresponds to open water without vegetation). Values are recorded as percent coverage of the 1m<sup>2</sup> area such that the total coverage for each sampling area equals 100%. The data collector will need to mentally “flatten”<sup>9</sup> the image of habitat types within the 1m<sup>2</sup> square area to determine what types of habitat are contained within it, and reflect this in the percentages. The submerged and surface vegetation categories are further divided into “algae” vs. “other” (where “other” corresponds to non-algal vegetation). Record the depth<sup>10</sup> (cm) of the water at the sampling area, the distance from the bank (m), and the distance of the water’s edge to high water mark (m). The latter is determined by measuring the distance from the wetland’s wetted edge to the seasonal high-water mark (e.g. bankfull), which is often marked by a clear change in vegetation type from terrestrial to aquatic. Note that the suite of habitat measurements for depressional wetlands is less comprehensive than what is specified in the SWAMP SOP for wadeable streams.
10. 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.
11. 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.
12. Once all 10 transects have been sampled for MIs, release any non-target organisms (e.g., larval amphibians and fish) that may have been captured during the sampling back to the wetland. A small aquarium net is useful to capture these organisms.

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<sup>9</sup> Periodic exercises in which different field crews assess the same area for percent cover values can be carried out in order to calibrate crews to one another and ensure consistency among practitioners.

<sup>10</sup> Graduated lines drawn on the pole of the net are useful for measuring depth.

## Field elutriation and preservation of MI samples

After all transects have been sampled for MIs and non-target organisms have been removed, prepare the MI samples for preservation:

1. Inspect the D-frame sampling net and use forceps to remove any organisms clinging to the net and place these in the bucket.
2. Prior to the addition of the sample, fill two large dish tubs (e.g., 11 qt) with wetland water and use an aquarium net to remove any macroinvertebrates from this water.
3. 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.
4. 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.
5. Leave the piece in the second tub for a few minutes before discarding back to the wetland. This step allows additional MIs to detach from debris.
6. Repeat this process for all large debris. When a majority of large debris has been removed, pour the tub's contents through a 500  $\mu\text{m}$  sieve held over an empty dish tub (to catch any sample that misses the sieve).
7. Also pour all of the remaining bucket material into the sieve<sup>11</sup>.
8. Transfer all of the material in the sieve (invertebrates and organic matter) into the sample jar.
9. Carefully inspect any gravel and debris remaining in the bottom of the bucket and tubs for any clams, snails, or other dense animals that might remain. Remove remaining animals by hand and place them in the sample jar.
10. Place a completed date/locality label (Figure 7) on the inside of the jar (use pencil only, as most "permanent" inks dissolve in ethanol) and completely fill with 95% ethanol.
11. Place a second label of the same type on the outside of the jar.
12. Add ethanol to the jar. Note that the target concentration of ethanol in the sample jar is 70% (test strips to confirm this are available), 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 to 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.
13. Record the total number of jars for a single sample on the external labels and field sheets.

Funding/Billing code: _____	MI_RWB Sample
Project _____	Date: / /
SWAMP ID: _____	Time: _____
Site Name: _____	Sample ID (lab) _____
Sample Type: Integrated	Method: MI_RWB_DW
County: _____	Jar # ___ of _____
Collector: _____	Replicate # _____

**Figure 7. Internal and external jar label printed on Rite-in-the-Rain™ or similar water-resistant paper and recorded in pencil.**

<sup>11</sup> A 4 mm sieve can be helpful in removing amphibian larvae first, if they are abundant at the site.



## Algae collection

Algae collection should occur after MI collection is complete. Subsamples for benthic algae are collected at each of the 10 sampling nodes and composited. Aliquots from the composite are used for analysis of benthic diatom community composition and, if called for by the project, benthic soft-bodied algae community composition as well. If soft-bodied algal data are to be collected, it is also necessary to collect a soft-bodied “qualitative” sample (described at the end of this section).

### Benthic diatom and quantitative soft-bodied algae sampling

Within each node, algae are sampled at transects 3 paces to the right (when facing the water) of each of the yellow flags (which indicate where MIs had been collected), when walking counterclockwise around the wetland (Figure 5). The procedure for identifying the spots for sampling algae is very similar to that for MIs. The major difference is that sampling for algae should never occur at a spot deeper than 0.5 m because these samples are collected by hand.

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. The dominant substratum is the one that covers the largest proportion of the general sampling area.

***IF soft-bodied algae are to be included in the sampling, it is necessary to take TWO adjacent grabs*** at each sampling node, although the samples from both grabs are added to the same sample composite (that is, collect two algae subsamples at each of the 10 nodes around the wetland for a total of 20 subsamples, all of which are 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. If only diatom, and no soft-bodied algae, analysis will be undertaken for the project, then collect only one grab from each node (which would result in only 10 subsamples, total, for the entire wetland).

Be careful never to collect a subsample from a spot that has already been sampled or otherwise disturbed. Furthermore, when collecting two subsamples at a node it is possible to collect from two different substratum types within a single sampling-spot area. For instance, if plants are relatively 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). If possible, avoid collecting *all* 20 of the samples for a wetland from live plants.

On the field sheet #3 under the “algae collection substrata” heading, record which substratum type(s) each subsample came from, for both replicate #1 and (if collecting duplicate algae samples at that site; see the “Collecting field duplicate samples” section below for more information) #2. For replicate 1 subsamples (of which there will be only one subsample if only diatoms are being analyzed, and 2 if both diatom and soft-bodied algae are being analyzed), write a “1” in the box corresponding to the substratum type from which the collection was made. If both subsamples for that replicate came from the same substratum type, record them in the same box as follows: “1,1”. If replicate samples are also

being collected at that site, use a “2” in the appropriate box(es) corresponding to the substratum type(s) from which the replicate subsamples were collected. In some (but not all) cases, all subsamples for both replicates may end up being collected from the same substratum type. In such circumstances, the following will be entered into the appropriate substratum box: “1,1,2,2”.

There are two different sampling devices for collecting algae. The most commonly employed one is a “sediment corer” (5.3 cm<sup>2</sup>), 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 wetland. The other device is a “syringe scrubber” (5.3 cm<sup>2</sup>), which is used for sampling hard substrata that cannot be removed from the wetland, and is identical to what is used for sampling underwater in wadeable streams.

The sediment corer 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 waterproof bright pink or silver marker) drawn around the perimeter of the barrel at 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 “2 mL” mark, then the line you draw around the barrel should be positioned at the “7 mL” mark. The open end of the corer must be flat and filed so as to make it as sharp as possible for cutting into sediment and macroalgal mats. Methods to construct the syringe scrubber are described in Fetscher et al. 2009.

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.

### ***Sampling on Soft Sediment Substratum***

If the sampling spot falls on an area of soft sediment, conduct the following steps.

1. Somewhere away from the targeted sampling spot, submerge the sediment corer in the wetland water to moisten it, and move the plunger up and down a few times to loosen it. Then adjust the plunger so that its pointed rubber tip is near the open end of the syringe barrel, but not protruding.
2. 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 > 5 cm. As contact is made between the device and the sediment, take care to move slowly and avoid creating a current of water, which could flush the top surface of the sediment away. Keep this surface intact, as it is where most of the algae will be.
3. 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.
4. Slowly push on the plunger to move the core back out of the syringe barrel.
5. 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. Cut the exposed end of the core off and discard it. What remains within the barrel (~5 mL of material) is the sample.

6. Hold this over a 500 mL<sup>12</sup> sample bottle and resume pushing on the plunger to release the core into the bottle.
7. Rinse the end of the plunger into the sample bottle, trying to capture all the sediment particles, but keeping the amount of water used to a minimum. An intact (uncut) 60 mL syringe filled with water from the wetland being sampled can be used to squirt the end of the corer to dislodge any residual sediment. Collect this rinsate into the sample container. Close the sample bottle between samples to prevent accidentally spilling the sample.

If the sediment is too loose to form a cohesive core inside the coring device, then discard what is in the barrel and take another grab from an undisturbed adjacent spot of the same substratum type, as follows:

1. Start with the seal ring of the plunger positioned at the mark you had drawn around the syringe barrel and insert the barrel down into the sediment, up to that mark.
2. Gently slide a spatula beneath the barrel, press them together to get a good seal, and pull the device out of the water with the spatula pressed against the barrel.
3. Remove excess sediment (outside of the syringe barrel) from the spatula. This can be done by gently swishing the device underwater, as long as the spatula remains firmly in place.
4. Dump the sampled material into the sample bottle.
5. Rinse any residual sediment off the corer and spatula into the sample container using a small amount of water from the wetland being sampled.

### ***Sampling on Plant Material Substratum***

If plant material (live or dead) is the substratum type encountered, use a soft-bristled toothbrush to ***gently*** remove biofilm coating from the plant within a 5.3 cm<sup>2</sup> area. Perform the following steps:

1. Clip the plant at the base (if rooted) with a shears or scissors.
2. Very slowly and gently remove it from the water in order to avoid shearing loosely attached algae from the plant as much as possible.
3. 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 to etch a circle of the area to brush.
4. After brushing within that area, rinse the area over the sample bottle to catch the rinsate and then discard the cleaned substratum.
5. 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.*** This entails use of a high-pressure rinse combined with rubbing the bristles between the fingers and rinsing with deionized or distilled water. Re-used brushes (and other sampling apparatus that touch the wetland) must also be thoroughly decontaminated between sites to prevent transfer of chytrid fungus and New Zealand mud snails (see Section entitled "Post-field collection decontamination of equipment").

<sup>12</sup> A 500-mL bottle will generally be of sufficient volume, however it is good to have a clean 1-L bottle on hand in case the sample volume exceeds 500 mL.

### ***Sampling on Macroalgae Mat***

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. Perform the following steps:

1. “Sandwich” the entire thickness of the macroalgal mat between the corer and a spatula underwater.
2. Using a utility knife, cut the algae around the perimeter of the corer.
3. Add the resulting “circle” of algae to the sample bottle.

This same general procedure can be used if the substratum type is a layer of dead leaves or other such organic debris resting on the wetland bottom. In this case:

1. Sandwich the leaf between the corer and a spatula underwater.
2. Pull the device out of the water, and use a utility knife to isolate a circle of the leaf matter.
3. 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.
4. 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.
5. Discard the cleaned leaf piece.

### ***Sampling on Hard Substratum***

If a hard substratum, such as a rock, is encountered at the sampling spot and it can be removed from the wetland 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 wetland (as would be the case with bedrock or consolidated sediment), use the syringe scrubber to collect the sample underwater.

To use the syringe scrubber:

1. Submerge it in the wetland (somewhere away from the targeted sampling spot) and pull/ depress the plunger a few times to loosen it.
2. Affix a fresh, white scrubbing pad circle onto the bottom of the plunger by attaching it to the Velcro™.
3. Press the plunger down so that the bottom of the scrubbing pad is flush with the bottom of the barrel.
4. 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.
5. 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.

6. 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.
7. 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.
8. Pull the instrument back up out of the water with the spatula still firmly sealed against the syringe-barrel bottom.
9. Hold the syringe scrubber over the sample container and then remove the spatula, allowing any water to fall into the container.
10. Carefully detach the pad from the plunger and hold the pad over the container.
11. Using rinse water sparingly, remove as much algal material from the pad as possible by rinsing it off with the intact syringe filled with water from the wetland, 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 node, even within the same wetland, and a new pad *must* be used whenever the old one becomes ineffective due to compaction and crimping. 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.

### **Habitat observations associated with algae collection**

Associated with each algae sampling spot is a suite of habitat observations recorded from an imaginary 1 m<sup>2</sup> quadrat centered around where the algae subsample grabs were made for each transect. The data recorder estimates the location of the boundaries of the 1m<sup>2</sup> quadrat. Most of the habitat observations are the same for both algae and MIs, but a few are associated strictly with one or the other. Record the percentages of habitat type, based on vegetation, within the appropriate data field using the categories of emergent, submerged, surface, and open (meaning open water, no vegetation). 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 m<sup>2</sup> quadrat to determine what types of habitat are contained within it, and reflect this in the percentages. As mentioned above, the submerged and surface vegetation categories are further divided into the algae vs. other (where “other” is non-algal vegetation). Record the depth (cm) of the water at the sampling point, as well as the distance from the bank (m) and relative distance category (near/mid/far). Also record the estimated % shading for benthic algae within the 1 m<sup>2</sup> quadrat, and the type of wetland substratum from which each sample was collected [soft sediment, macroalgae, plant (live/dead) including wood, or hard substrata (including rock, concrete, and consolidated sediment)].

### Fixed Algae Sample Preparation

Once all the subsamples have been collected and composited, the following steps are conducted, in the shade, to prepare samples for taxonomic analysis. Note that different procedures are needed depending on whether just diatoms, or diatoms as well as soft-bodied algae, are to be assessed for the monitoring program. Read through these steps completely before beginning the procedure to be sure to proceed correctly:

1. Cap and **very gently** agitate the sample container to mix the material well without breaking cells.
2. Pour the entire contents of the container into a 1 L graduated cylinder to measure the volume of the sample.
3. Wait ~5 minutes (in the shade) to allow the heavier sediment to settle to the bottom.
4. 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).
5. 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-bodied algae, if applicable) sample bottle(s).
6. Pour all of the material back into the sample container.
7. 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.*
8. Very gently mix the sample in the container until it is homogeneous.
9. **If both diatoms and soft-bodied algae are to be analyzed** for the monitoring program, use a graduated cylinder to pour **half** the sample (sediment included) into a 250 mL<sup>13</sup> (or 500 mL, if there is a lot of volume) sample bottle and label it for “diatoms”. Figure 8 shows a label for algae taxonomy samples. (**If only diatoms are to be analyzed for the project, then the full sample volume can be used as the diatom sample**, and Steps 10 and 11, below, can be skipped).
10. If soft-bodied algae are to be analyzed in addition to diatoms, pour the remaining half of the sample into another 250 (or 500) mL sample bottle and label it for “soft algae”.

Contract/ Billing Code: _____	<small>circle one:</small>
Project: _____ Date: _____ Time: _____	<b>diatoms</b> <b>soft</b>
Site Code: _____ Sample ID: _____	
Repl #: _____ Vol Aliquotted (mL): _____	
Composite Vol (mL): _____	
# of Grabs: Sediment Corer: <input type="checkbox"/> Syringe: <input type="checkbox"/>	
Fixative Added: _____	
Wetland Name: _____	
County: _____ Collector: _____	

**Figure 8. Label for algae taxonomy samples on Rite-in-the-Rain™ or similar water-resistant paper and recorded in pencil.**

<sup>13</sup> 250 mL sample bottles can be used for samples as long as the volume of fixative that will need to be added will not cause the final volume of sample to exceed 250 mL; otherwise, use 500 mL bottles.

11. 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 diatom and soft-bodied algae sample bottles. (***If only diatoms are being analyzed, the entire (uncut) clump of any macroalgae present is kept in the diatom sample bottle.***)
12. Once the soft-bodied algae sample has been prepared, label the bottle and place it in the wet-ice cooler. It must be kept very cold (but not frozen) and in the dark. This sample should be fixed with glutaraldehyde to a final concentration of 2.5% in a laboratory fume hood as soon as possible, but no more than 4 days after collection.
13. Diatoms are fixed with formalin while still in the field. Whenever using any kind of fixative, ***be sure to wear appropriate protective gear for eyes, body, and hands, and employ safe practices (such as ensuring adequate ventilation)***. Appendix C provides detailed guidance for this. The calculation used to determine the amount of fixative to be added to a sample is based on the volume of ***liquid composite*** for that sample. (i.e., excluding any sediment present), the fixative stock solution concentration, and the desired final concentration of fixative in the sample.

For the **diatom sample**, the following calculation is used to determine how much of a **10% formalin stock solution** should be added in order to realize a **final formalin concentration of 2%** in the sample:

$$\text{amount of 10\% formalin to add to sample} = 0.25 * \text{volume of } \mathbf{liquid} \text{ composite in the diatom sample bottle}$$

For example, if the total volume in the diatom sample bottle is 230 mL, but the ***liquid*** composite component of the 230 mL is only ***180 mL***, then 45 mL of 10% formalin needs to be added to the sample.

For the **soft-bodied algae sample** (if applicable), the following calculation is used for adding **glutaraldehyde** to the sample bottle (**assuming that a stock 25% stock solution of glutaraldehyde** is being used and that a **final concentration of 2.5%** glutaraldehyde in the sample is desired):

$$\text{amount of 25\% glutaraldehyde to add to sample} = 0.111 * \text{volume of } \mathbf{liquid} \text{ composite in the soft-bodied algae sample bottle}$$

**Work with glutaraldehyde only in a laboratory fume hood, wearing appropriate protective gear for eyes, body, and hands.** Consult Appendix D for safe use practices for glutaraldehyde.

### Qualitative sampling of soft-bodied algae

Qualitative algae sampling is conducted after the diatom/quantitative soft-bodied algae samples are processed. The purpose of the *qualitative* soft-bodied algae sample is to capture the taxonomic diversity of this assemblage and to aid in identification of ambiguous specimens collected in the quantitative sample. Therefore, it is only collected if a quantitative soft-bodied algae sample is taken. For the qualitative sample, soft-bodied algae are collected throughout the wetland (independently of node or transect), with the goal of representing specimens from all obviously different types of macroalgal filaments and mats that are observed during the course of working in the wetland. Also included are microalgae, which are collected by scraping from a few random spots of substratum scattered throughout the wetland using a utility knife (Table 3), and by suctioning the uppermost layer of sediment from a few random locations using a clean turkey baster. It is convenient to collect the soft-bodied algae specimens for the qualitative sample in conjunction with diatom collection activities, but macroalgal specimens that are noticed during other sampling activities. If you suspect something may be algae, but are not sure, it is always preferable to collect some of it; the laboratory will determine whether it qualifies for inclusion in the species list.

Contract/ Billing Code: _____	<b>qualitative (soft)</b>
Project: _____	Date: _____ Time: _____
Site Code: _____	Sample ID: _____
Site Name: _____	
Wetland Name: _____	
County: _____	Collector: _____

**Figure 9. Label for soft-bodied algae qualitative sample on Rite-in-the-Rain™ or similar water-resistant paper and recorded in pencil.**

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 attach the macroalgae to the substratum (if there are any attached macroalgae in the wetland), as these structures can be useful for taxonomic identification. Add all collected material to a labeled (Figure 9), 250-mL container. Unlike with the quantitative samples, **do not add glutaraldehyde or formalin (or any other fixative) to the qualitative sample**, but keep it on wet ice and in the dark while in the field, and refrigerate it immediately upon return to the lab. Be careful not to place the qualitative sample container right up against ice or frozen blue-ice bags, because this could cause the algae to freeze and thus destroy the sample. Because it is not preserved, the qualitative sample should be examined by a taxonomist as soon as possible (and within two weeks, at most), as it can decompose fairly rapidly.

### Sediment sample collection for toxicity and chemistry

An integrated sediment sample is collected within each wetland after all other sampling activities have been carried out, and prior to removal of the flags. The sediment samples will provide information about potential sources of toxicity to wetland biota. In addition, typical chemistry constituents to measure include:



- Total organic carbon (TOC)
- grain size
- metals
- Pyrethroids

Collect 2-cm deep subsamples of sediment from various, undisturbed spots dispersed throughout the wetland, wherever fine-grained sediment (silt and clay, fine sands) is available, and composite them into a single container. To the extent possible, try to avoid including dead plant matter in the sediment subsample. A total of 3 L of sediment is typically needed for toxicity assays, but consult with the laboratory to confirm the necessary volume (samples for chemistry are taken from the same sediment composite as that used for toxicity). Sample only from places that have not been trampled by previous sampling/data collection. If more information is desired, detailed sediment sampling guidance can be found in the MPSL-SOP (2014). Sediment for toxicity can typically be placed into a 3 L glass jar (but confirm the choice of container with the toxicology laboratory). Sediment for grain size and chemistry is placed into 4 oz glass jars (one jar for TOC and grain size, and another for metals and organics; Table 6). 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 is available. Otherwise write all of the necessary information on the bag with a pencil. 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 laboratories.

Funding/Billing code: _____	Sediment Chemistry Sample
Project _____	Date:    /    /                      Time: _____
SWAMP ID: _____	
Site Name: _____	
Sample Type: Integrated	Analyte: Grain size/TOC
Collector: _____	Replicate # _____

**Figure 10. Sample label for sediment grain size and TOC on Rite-in-the-Rain™ or similar water-resistant paper and recorded in pencil. A similar label can be modified for the sediment metals and organics container.**

## Collecting field duplicate samples

At 10% of the wetlands within a project, a field duplicate is 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.

## Water chemistry

In the case of water chemistry, duplicates are taken from within the same general sampling spot areas within their respective transects.

## Macroinvertebrates

The field duplicates are collected from adjacent sampling areas along the same transect. Collect field duplicates at the same time as the original samples. This requires coordination between the two MI collectors as they move concurrently through the near, mid, and far sampling spots. 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.

## Diatom/quantitative soft-bodied algae

For algae, collect one additional subsample from within the original sampling location, taking care to avoid resampling the same exact location as the original sample. Composite the additional subsamples into their own jar and label it as a field duplicate. If field crews are collecting both diatoms and soft-bodied algae, then collect a total of 4 subsamples per node, with 2 of them being deposited into the original sample bottle, and the other 2 deposited into the field duplicate bottle.

## Habitat observations

The vegetation cover, distance, and depth measures should be recorded separately for the two duplicate MI samples, because the imaginary 1 m<sup>2</sup> quadrat 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 m<sup>2</sup> quadrat at each transect, it is not necessary to record separate habitat data for the replicates (and the same information could be used to populate that component of the algae habitat 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 habitat data for duplicate samples of MIs and algae is summarized in Table 7.

**Table 7. Recording Habitat Observations data when duplicate samples are collected.**

Assemblage	Vegetation Cover	Depth/Dist. from Bank, Shading, etc.	Recording of Substrata Types
MIs	Distinct for Reps 1 & 2	Distinct for Reps 1 & 2	N/A
Algae	Same for Reps 1 & 2	Same for Reps 1 & 2	Distinct for Reps 1 & 2

## Post-field-collection 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 (USFS 2013).

A 5%<sup>14</sup> Quat™ 128 solution requires 30 seconds of soak time to kill chytrid fungus. However, the 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 1 pair of waders available for each crew member per site, until the waders are decontaminated. Be sure to keep any used waders in closed, heavy-duty garbage bags during transit, in order to avoid contaminating field vehicles and other field equipment. At appropriate intervals, all of the waders can be cleaned with a brush, rinsed, and treated with the appropriate concentration of bleach for the prescribed time ([USFS 2013](#)), dried, and then frozen. After each site visit, the syringe or 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, or a new syringe can be used at each site.

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<sup>14</sup> Test strips are available to test the concentration.

## SECTION 5. SAMPLE ANALYSIS

All environmental samples should be analyzed by qualified laboratories, according to the objectives in the project-specific Quality Assurance Plan, or for SWAMP work, according to the most recent SWAMP QAPrP.

Taxonomic identifications for macroinvertebrates should use 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) will need to be reviewed and approved by SAFIT before the dataset can be successfully uploaded into the SWAMP or CEDEN database. The recommended level of taxonomic resolution is SAFIT Level 2 with additional identifications of microcrustaceans (e.g. cladocerans, copepods) to genus. Lab processing guidelines for stream invertebrates was developed by Woodard et al. (2013). For algae, identifications should use the [Master Lists of Names for California](#) that are maintained by the California Algae and Diatom Taxonomic Working Group.

Methods to derive a score for the MI IBI using the frequency distribution provided by the sorting laboratory can be found in Lunde and Resh (2012). Methods to derive scores for the algae IBIs (developed for southern California wadeable streams) can be found in Fetscher et al. (2014). A reporting module developed for the SWAMP program can be used for calculating algae metrics and IBIs from algae data stored in the SWAMP database. For data not stored in the SWAMP database, an online calculator is available in the [Data Tools](#) section of the SCCWRP website.

## SECTION 6. DATA SUBMISSION AND STORAGE

Data for SWAMP-funded projects should be submitted to the [SWAMP database](#). Other projects should submit data to the California Environmental Data Exchange Network ([CEDEN](#)). CRAM data should be submitted to [eCRAM](#), since the SWAMP and CEDEN databases do not store CRAM data.

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# GLOSSARY

Term	Definition
Diatom	a unicellular alga that possesses a rigid, silicified (silica-based) cell wall in the form of a “pill box”
High water mark	The location of the edge surface water of the wetland during winter (“high-water”) conditions
Node	1 of 10 evenly spaced sampling regions around the wetland
Perennial	Systems that contain some surface ponding for the entire year during most years
Seasonal	Systems that support surface ponding for greater than 4 months but less than the entire year
Soft-bodied algae	Non-diatom algal taxa; for the purposes of this SOP, cyanobacteria are subsumed under this assemblage
Transect	Sampling lines perpendicular to shore, whose terminal ends correspond to the near, mid, and far positions for collection of subsamples for water chemistry and biota



## APPENDIX A, SIBBALD SYRINGE SAMPLER (S3)

This appendix describes the construction of a ponded-water-sampling device that fulfills the following criteria:

- Allows samples to be collected at a distance from personnel, in order to avoid contamination of the water sample by footwear or sediment stirred up as a result of wading
- Prevents contamination of the sample with water-surface film
- Poses a low threat of sample contamination from materials comprising the device
- Delivers a known volume of sample
- Can safely be sterilized/de-contaminated with bleach between sites

This device, the “Sibbald Syringe Sampler” (S3; Figure A1), was designed and built by Glenn Sibbald, a California Department of Fish and Wildlife Environmental Scientist at the Office of Spill Prevention and Response Aquatic Bioassessment Laboratory. It allows a field sampler to extend a clean, 60cc syringe roughly 1.5 m or more from his/her body and operate the syringe at that distance to collect a water sample from “undisturbed” water.

The S3 consists of a push/pull rod made of multiple sections of  $\frac{3}{4}$  inch Schedule 40 PVC tubing jointed together with slip couplings, that slides within multiple sections of  $1\frac{1}{4}$  inch Schedule 40 PVC tubing, also jointed with slip couplings. The  $1\frac{1}{4}$  inch outer tube is perforated to allow draining and reduce weight.

A syringe “cradle” made from  $1\frac{1}{2}$  inch Schedule 40 PVC attaches to one end of the S3 (Figure A2) and 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 A3, A4), 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 S3, a center-drilled PVC end cap is fitted on the  $1\frac{1}{4}$  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 A5), all pieces of the S3 fit into a 2 gallon bucket, for bleach-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 A1. S3 fully assembled.

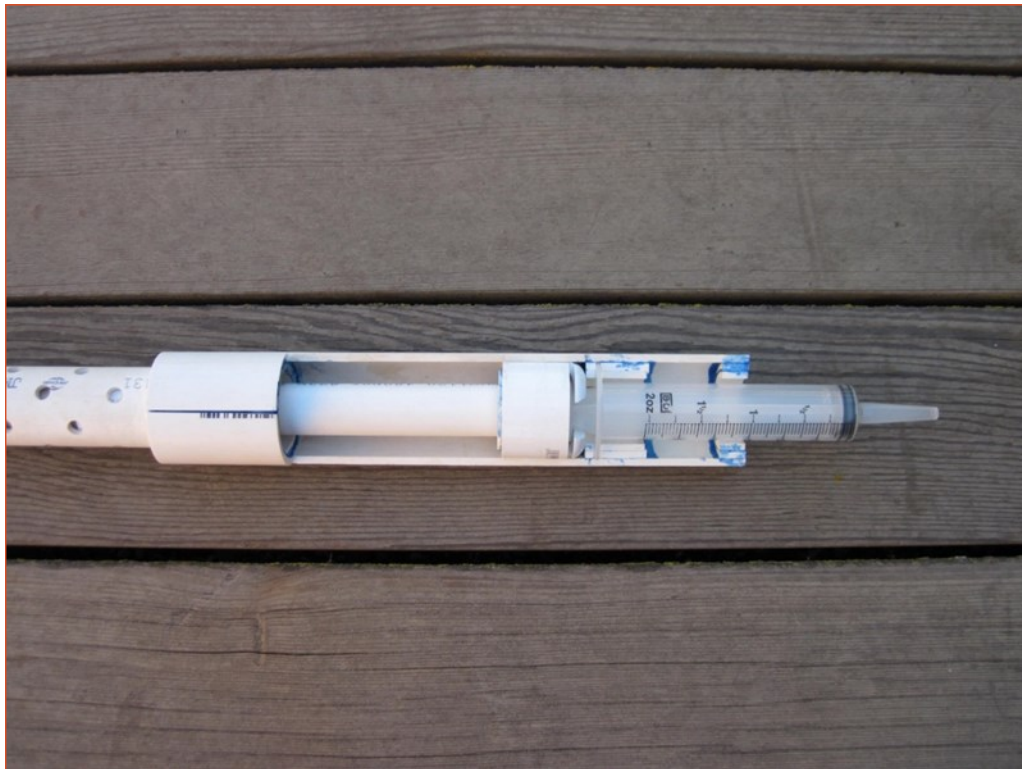


Figure A2. Syringe cradle.

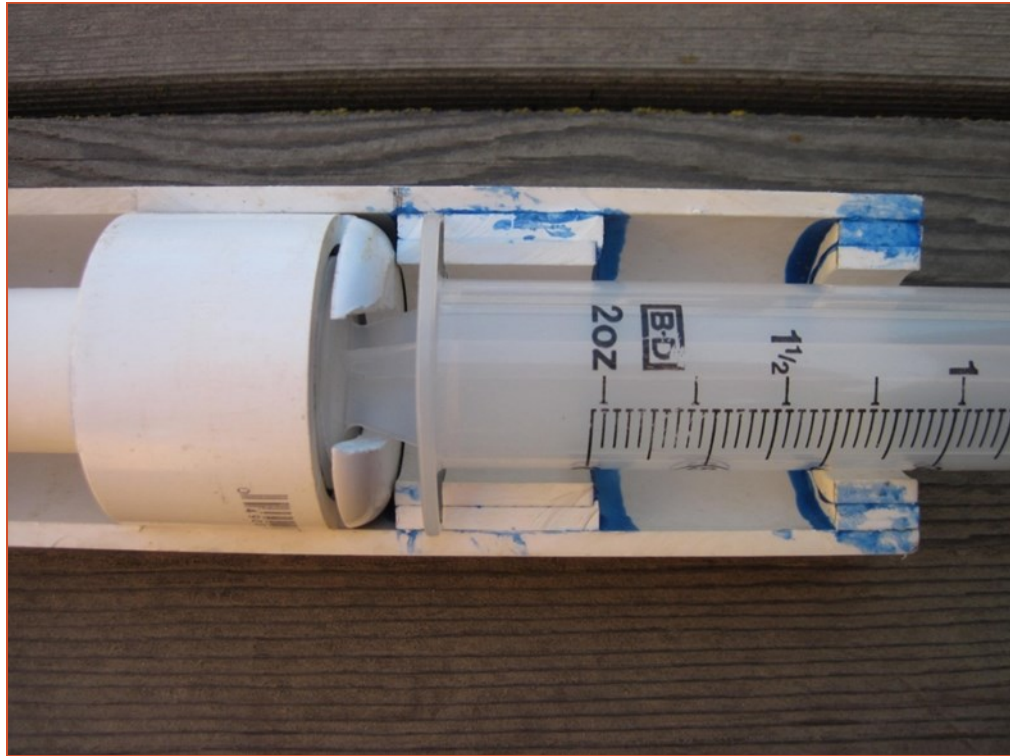


Figure A3. PVC coupler with syringe in place.



Figure A4. PVC coupler with syringe removed.

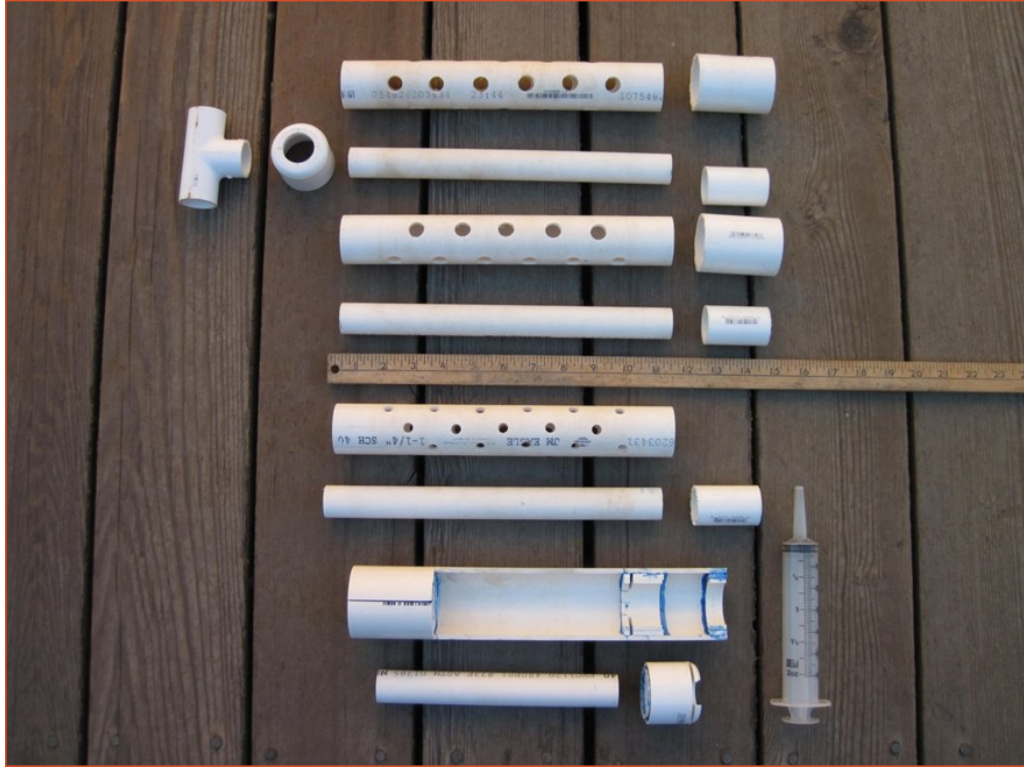


Figure A5. S3 fully disassembled.

## APPENDIX B: SOP FOR THE USE OF FORMALIN FOR FIXING DIATOMS (ADAPTED FROM PECK, ET AL. 2006)

APPENDIX C: SOP FOR THE USE OF FORMALIN FOR FIXING DIATOMS (ADAPTED FROM PECK, ET AL. 2006)

- Preparing a 1-L solution of 10-percent buffered Formalin (Moulton et al. 2002):
- Add 100 mL of formaldehyde (37-40%) to 900 mL of water in a chemically resistant, non-breakable bottle.
- Add about 3 g of borax to 10 mL of water and mix.
- Add dissolved borax solute, to buffer formalin solution.
- Tightly seal the bottle and mix by carefully inverting the bottle several times.
- Label the outside of the bottle with "10-percent buffered formalin," the date of preparation, and related hazardous chemical stickers.

**Note:** Formalin must only be handled by trained individuals who understand the safe handling and use of this chemical

Formaldehyde (or formalin) is highly allergenic, toxic, and dangerous to human health (potentially carcinogenic) if utilized improperly. Formalin vapors and solution are extremely caustic and may cause severe irritation on contact with skin, eyes, or mucous membranes. Formaldehyde is a potential carcinogen, and contact with it should be avoided. Wear gloves and safety glasses and always work in a well-ventilated area. In case of contact with skin or eyes, rinse immediate with large quantities of water. Store stock solution in sealed containers in a safety cabinet or cooler lined with vermiculite or other absorbent material. If possible, transport outside the passenger compartment of a vehicle.

During the course of field activities, a team may observe or be involved with an accidental spill or release of hazardous materials. In such cases, take the proper action and do not become exposed to something harmful. The following guidelines should be applied:

- First and foremost during any environmental incident, it is extremely important to protect the health and safety of all personnel. Take any necessary steps to avoid injury or exposure to hazardous materials. You should always err on the side of personal safety for yourself and your fellow field crew members.
- Never disturb, or even worse, retrieve improperly disposed hazardous materials from the field and bring them back to a facility for disposal. To do so may worsen the impact to the area of the incident, incur personal or organizational liability, cause personal injury, or cause unbudgeted expenditures of time and money for proper treatment and disposal of material. However, it is important not to ignore environmental incidents. You are required to notify the proper authorities of any incident of this type so they can take the necessary actions to respond properly to the incident.

Follow Department of Transportation (DOT) and the Occupational Safety and Health Administration (OSHA) regulations for handling, transporting, and shipping hazardous material such as formalin and ethanol. Regulations pertaining to formalin are in the Code of Federal Regulations (CFR, specifically 29 CFR 1910.1048). These requirements should be summarized for all hazardous materials being used for the project and provided to field personnel. Transport formalin and ethanol in appropriate containers with absorbent material. Dispose of all wastes in accordance with approved procedures (e.g., National Institute for Occupational Safety and Health 1981, US EPA 1986).

To dispense formalin in the field, wear formalin-safe gloves and safety goggles. Use a small syringe or bulb pipette to add 10 mL of 10% buffered formalin solution to 40 mL of the diatom sample in a 50 mL centrifuge tube. Alternatively, in order to avoid dispensing formalin solution in the field, clean 50 mL centrifuge tubes that will hold the diatom samples can also be pre-loaded with 10 mL of 10% buffered formalin in a laboratory fume hood prior to going into the field.

The preparation of the 10% buffered formalin stock solution should always be done by trained personnel under a laboratory fume hood while wearing protective gloves, clothing, and goggles.

# APPENDIX C: SOP FOR THE USE OF GLUTARALDEHYDE FOR THE PRESERVATION OF SOFT ALGAE

**Note:** Glutaraldehyde must only be handled by trained individuals who understand the safe handling and use of this chemical

## 1. Scope and Application

Glutaraldehyde is a colorless liquid with a pungent odor used as a preservative and sterilant. This SOP covers the use of Glutaraldehyde by Department of Fish and Game OSPR laboratories as a preservative for soft bodied algae.

## 2. Physical Hazards

The physical hazards associated with the use of Glutaraldehyde include;

- Incompatibility with strong oxidizing substances and bases
- Corrosive to metals
- Production of Carbon Monoxide and Carbon Dioxide during decomposition
- Discolors on exposure to air

## 3. Health Hazards

The health hazards associated with the use of Glutaraldehyde include;

### Inhalation

- Regulatory limit of 0.05 ppm as a ceiling level
- Chemical burns to the respiratory tract
- Asthma and shortness of breath
- Headache, dizziness, and nausea

### Skin

- Sensitization or allergic reactions, hives
- Irritations and burns
- Staining of the hands (brownish or tan)

### Eyes

- Irritation and burns. Eye contact causes moderate to severe irritation, experienced as discomfort or pain, excessive blinking and tear production
- May cause permanent visual impairment
- Conjunctivitis and corneal damage

### Ingestion

- Gastrointestinal tract burns; central nervous system depression, excitement
- nausea, vomiting
- Unconsciousness, coma, respiratory failure, death

*Note: Oral toxicity of Glutaraldehyde increases with dilution*

## 4. Engineering Controls

Strict engineering controls will be followed when using Glutaraldehyde. This chemical and processes using this chemical will only be used under a laboratory fume hood meeting the requirements of Title 8, CCR Section 5154.1. At no time will containers of Glutaraldehyde be opened outside of an operating fume hood.

Personnel using Glutaraldehyde will designate an area of the lab for its use. The area where it is used will be noticed with a sign reading:

CAUTION GLUTARALDEHYDE IN USE

Only trained personnel will be allowed to enter the designated area when using Glutaraldehyde.

## 5. Personal Protective Equipment

Personal Protective Equipment (PPE) is required to be worn at all times when working with Glutaraldehyde. This includes;

### Eye Protection

- Chemical splash goggles; or
- Safety glasses with face shield

### Hand Protection

- Nitrile or Polyvinyl Chloride (vinyl) gloves

### Body Protection

- Lab coat with polypropylene splash apron that cover the arms

Any PPE with noticeable contamination will be immediately removed and the affected area washed with water. Gloves and apron will be removed before leaving the designated area. Disposable PPE (gloves and aprons) will not be re-worn. Disposable PPE will be disposed of in a sealed waste receptacle approved for hazardous waste. Any non-disposable PPE (lab coats, chemical goggles) with noticeable contamination will be rinsed or cleaned as soon as practical, and secured in a manner that does not allow contamination of laboratory personnel.

Respiratory protection will not be required as long as strict engineering controls are followed.



## 6. Safety Shower and Eyewash

All employees using Glutaraldehyde must be aware of the location and use of the laboratory safety shower and eyewash, and must be able to reach it within 10 seconds from the time of contamination. At no time will processes using Glutaraldehyde be allowed that does not provide access to a safety shower and eyewash.

Employees who have skin or eye contact with Glutaraldehyde will immediately stop all processes and proceed to the safety shower and eyewash station. The employee will rinse the affected area for a minimum of 15 minutes. If eye contact has occurred, the upper and lower eyelids must be lifted to allow adequate flushing of the eyes.

## 7. Special Handling Procedures and Storage Requirements

Procedures will be followed that reduce exposure to Glutaraldehyde vapor to the lowest reasonable level. This includes;

- Ensure Glutaraldehyde is only used under a fume hood
- Use only enough Glutaraldehyde to perform the required procedure
- Every effort must be made to minimize splashing, spilling, and personnel exposure
- Once specimens are preserved, they will be capped or secured in a way that does not allow Glutaraldehyde vapor to escape into the lab
- At no time will open containers be removed from the fume hood
- All containers of Glutaraldehyde or solutions containing Glutaraldehyde will be appropriately marked with the chemical name, and hazard warning label at the end of the work day or whenever there is a personnel change
- Glutaraldehyde will be stored in tightly closed containers in a cool, secure, and properly marked location

## 8. Waste Disposal

Excess Glutaraldehyde and all waste material containing Glutaraldehyde must be placed in an unbreakable secondary container labeled with the following "HAZARDOUS WASTE GLUTARALDEHYDE." Wastes will be disposed of through the laboratory hazardous waste contract.

## 9. Spill and Accident Procedures

Drips and splashes will be wiped up immediately with a sponge, towel, or mop. Any material used to clean spills will be disposed of as hazardous waste. Large spills (Greater than 300 CC) require response by a local Hazmat team. The Hazmat team will be called by the laboratory supervisor. In the event of a large spill personnel will immediately leave the laboratory, and not re-enter until cleared by the laboratory supervisor.

## 10. Training

All personnel engaged in the use of Glutaraldehyde will be trained on the hazards associated with this chemical, before use. The training will include;

- OSPR's Hazard Communication Program and information contained in the chemical's Material Safety Data Sheet (MSDS)
- Health hazards and routes of exposure
- Specific procedures and techniques for use and handling
- Use of PPE and engineering controls
- The contents and requirements of this Standard Operating Procedure.