SWAMP
Surface Water Ambient Monitoring Program

APPENDIX A
Supplemental Guidance for SWAMP Bioassessments

SWAMP SOP-SB-2025-0001

California Water Boards

www.waterboards.ca.gov/swamp

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A1. General Guidance for Bioassessment Sampling

Recommended labor division for efficiency in the field

Sampling is most efficient if tasks are divided among teams or individuals, each focusing on different parts of the protocol. For example, one team may collect water samples, deploy water quality probes, and measure discharge while another team lays out transects. The first team can then begin collecting benthic macroinvertebrates and algae, while the second team conducts physical habitat assessments. Other divisions of labor may be practical, as long as both teams take care to avoid interfering with collection of water or bioassessment samples. With appropriate divisions of labor and adequately sized and trained teams, sampling time should rarely exceed 4 hours with an experienced crew.

Recommendations for preparing for fieldwork

- Use the equipment checklist provided below (A2) to make sure all necessary supplies are brought along.
- Check with analytical laboratory(ies) for information about sampling containers, pre-combusted glass-fiber filters, addition of glutaraldehyde to soft-bodied algae quantitative samples, and storage and shipping of samples.
- Prepare and double check site dossiers to make sure they are complete with maps/directions to sites and scaled aerial photo(s). Note that before heading out to the field, it is convenient to add a 150 m (or 250 m) line to the map adjacent the stream to be sampled in order to get an idea about the anticipated approximate upstream and downstream boundaries of the monitoring reach. Bring along smartphone and/or county maps, atlases, and Thomas Guides to further aid location of sites.
- Other considerations when planning fieldwork include whether site access permits, passes, and/or gate keys are needed. Furthermore, some landowners require notice prior to each site visit, or that an on-site escort accompany the field crews during sampling, such that pre-visit coordination with the landowner is necessary.
- At the site, make sure the vehicle is parked in a safe spot and there are no “No Parking” signs. Stick a business card with a cell phone number in the driver’s window. Be sure to display the brown administrative pass placard if you are on National Forest land (or the letter of permission that is in your site dossier, if applicable).

Avoiding the introduction of invasive species and pathogens

Field crews must be aware of regional species of concern and take appropriate precautions to avoid transfer of these species. Crews should make every attempt to keep apprised of the most up- to-date information regarding the emergence of new species of concern, as well as new advances in approaches to hygiene and decontamination to prevent the spread of all such organisms.

Current organisms of concern in California include:

- Eurasian watermilfoil (*Myriophyllum spicatum*)
- Golden mussel (*Limnoperna fortune*)
- New Zealand mud snail (*Potamopyrgus antipodarum*)
- Zebra or quagga mussel (*Dreissena* spp.),
- *Myxobolus cerebralis* (the sporozoan parasite that causes salmonid whirling disease),
- *Batrachochytrium dendrobatidis* (a chytrid fungus that threatens amphibian populations)

General information about freshwater invasive species is available from the U.S. Geological Survey Nonindigenous Aquatic Species website (http://nas.er.usgs.gov) and the California Department of Fish and Wildlife's Invasive Species website https://wildlife.ca.gov/Conservation/Invasives) should also be consulted regularly for updates.

Decontamination procedures:
The California Department of Fish and Wildlife offer guidance for cleaning and disinfecting gear between sampling events (Hosea and Finlayson 2005, and California Invasive Species Program). The California DFW aquatic invasive species decontamination protocols appendix covers multiple treatment methods and their effectiveness against different invasives (CDFW Aquatic Invasive Species Decontamination Protocol 2022).

A pamphlet for prevention of the spread of New Zealand Mudsnail prepared by SeaGrant Oregon State University can be accessed here: NZ_Mudsnails_10-page.pdf

The following procedures for decontamination of equipment to prevent the spread of chytrid fungus are taken from the SWAMP depressional wetlands SOP (Fetscher et al. 2014): Chytrid fungus has been decimating amphibian populations worldwide, including causing declines in mountain yellow-legged frogs in the Sierra Nevada. Consult the decontamination SOP from the National Park Service (Chellman and McKenny 2018; https://www.fws.gov/sites/default/files/documents/MYLF%20Attachment%204_Decon%20Protocol.pdf).

A 5% Quat 128 solution requires 30 seconds of soak time to kill chytrid fungus. However, New Zealand mud snail (NZMS), although unlikely to be found in wetlands, could nonetheless be present, and requires 10 minutes of soak time at the same concentration. Because lakes and reservoirs may have NZMS and be sampled with this protocol, a 10 min soak time for all gear in 5% Quat 128 solution or similar Quat-related product should prevent movement of all potential invasive species, including aquatic diseases. If a non-chemical solution is preferred, waders can be fully cleaned of mud and debris, then exposed to sun for three hours and allowed to rest completely dry for 48 hrs. 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. 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 (https://www.fws.gov/sites/default/files/documents/MYLF%20Attachment%204_Decon%20Protocol.pdf) 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.

A2. List of Supplies

Table 1. List of Supplies. The first column indicates the task(s) in the SOP the item is needed for: "G" = general; "W" = water quality measurements; "P" = PHab data collection; "B" = BMI sampling; "D" = diatom sampling; "S" = soft-bodied algae sampling; "M" = molecular algae sampling, "C" = chlorophyll a sampling; "A" = AFDM sampling, "PCN" = particulate carbon: nitrogen sampling

Element	Item	Quantity per site	Specifications/Comments
G	Sampling SOP (this document)	1	
G	Equipment decontamination supplies		
G	Hip or chest waders, or wading boots/shoes (not felt-soled)	At least 1 pair/person	Felt-soled boots cannot be cleaned to prevent the spread of invasive species and must not be used. Rubber-soled wading boots with or without studs are recommended in lieu of felt
G	Full set of datasheets printed on waterproof paper (e.g., Rite-in-the-Rain™)	1 full set (and spare set recommended)	
G	Fine-tipped and thick-tipped waterproof/alcohol-proof pens and markers	2 to 3, each	
G	Pencils	2 to 3, each	
G	Clipboard	2 to 3	
G	Site dossier containing site maps, aerials, etc.	1	
G	Regional maps, topographic maps	as needed	
G	First aid kit	1	
W	Centigrade thermometer	1	
W	pH meter	1	
W	DO meter and spare membrane (if applicable)	1	
W	Conductivity meter	1	
W	Turbidimeter and vial(s) (optional)	1	
W	Field alkalinity meter or test kit (e.g., Hach)	1	
W	Water and sediment chemistry containers	as needed	
W	Calibration standards	1 set	
W	Spare batteries, user's manuals, and spare parts for meters	as needed	
P	Digital camera & spare batteries	1	

Element	Item	Quantity per site	Specifications/Comments
P	GPS receiver & spare batteries	1	
P	Measuring tape; 150 m (and 250 m, optional)	1	
P	Lengths of rope (7.5 m and 12.5 m, optional)	1 each	For measuring distance between main and inter-transects in delineating the monitoring reach
P	Digital watch/stopwatch & spare batteries	1	For timing duration of float for NBO stream velocity measure; also can be used to generate random number for selecting locations to place net for TRC sampling
P	Stadia rod (metric)	1	
P	Waterproof meter stick	1	With mm and cm graduations to measure water depth and substrate size
P	Clinometer	1	
P	Autolevel and tripod	1	For slopes <1%
P	Hand level (optional)	1	
P	Current velocity meter & top-setting rod	1	Examples: Swoffer Instruments propeller-type flow meter; Marsh-McBirney inductive probe flow meter; check battery and calibration as needed
P	Biodegradable Flagging tape	1 roll	To determine direction of stream flow for proper angling of the current velocity meter probe
P	Convex spherical densiometer	1	Taped to expose only 17 intersections of the grid
P	Transect flags; or biodegradable flagging tape	21 total flags, 1 roll each color flagging tape	Two colors; label with main transect (11) and inter-transect (10) names
P	Rangefinder & spare batteries (optional)	1	For measuring transects in open streams
P	Fresh orange peel OR plastic film canister partially full of water OR ice cube (when velocity meter not used)	1	Use as neutrally buoyant object
P, S	Algae viewing bucket (optional)	1	
B	D-frame kick net (fitted with 500-micron net)	1	

Element	Item	Quantity per site	Specifications/Comments
B	Wide mouth 500-mL or 1000-mL plastic jars	several	
B	White sorting pan (enamel or plastic; optional)	1	
B	95% EtOH	1 gallon	
B	Fine-tipped forceps or soft forceps	1	
B	Waterproof paper and tape for attaching labels	as needed	
B	Large spill tray	1	Transfer from net to the sample jar in order to reduce loss of sample material
B	Preprinted waterproof labels (e.g., on Rite-in-the-Rain™ paper)	As needed	It is recommended that the label be printed on a laser printer using alcohol-proof ink
B	Disposable gloves/elbow length insulated gloves (optional)		
D, S, C, A, M, PCN	White dish tub, rectangular, plastic, 11.5 qt, OR white plastic 5-gallon bucket with lid, 5L	1	Must be white, to avoid potential interference of pigmented shards from the tub or bucket in the chlorophyll a analysis
D, S, C, A, , M, PCN	Scrubbing brush or scouring pad to clean dish tub or bucket	1	
D, S, C, A, M, PCN	Composite sample receiving bottle (wide-mouth dark colored HDPE jar with cap, 1 L)	1	Fisher 05-719-239
D, S, C, A, M, PCN	Graduated cylinder, 1L, 500 mL, 100 mL, and 25 mL, plastic	1 each	e.g., Fisher 03-007-42 & 03-007-39
D, S, C, A, M, PCN	Bottle brush to clean graduated cylinders, etc.	1 sm, 1 lg	
D, S, C, A, M, PCN	PVC delimiter, 12.6 cm2 area	1	
D, S, C, A, M, PCN	Masonry trowel (flat, pointed, with a surface area > 12.6 cm2)	1	
D, S, C, A, M, PCN	Rubber delimiter, 12.6 cm2 area	1	
D, S, C, A, M, PCN	Toothbrush, firm-bristled	1	
D, S, C, A, M, PCN	Syringe scrubber, 60 mL syringe, 5.3 cm2 area	1	
D, S, C, A, M, PCN	White (non-pigmented) scrubbing-pad circles	11 per replicate	

Element	Item	Quantity per site	Specifications/Comments
D, S, C, A, M, PCN	Tally meter (optional)	1	Ben Meadows 9JB-102385
D, S, C, A, M, PCN	Scissors	1	
D, S, C, A, M, PCN	Wash bottles or Luer Lock Syringe	2	Label bottles with "stream water" and "DI water"
D, S, C, A, M, PCN	Exacto™ , Swiss-army-style knife, scissors	1	
D, S, C, A, M, PCN	Sample labels (printed on waterproof paper)	4 per replicate	
D, S, C, A, M, PCN	Clear plastic tape, 5 cm wide	Length of ~20 cm per replicate	
D, S, C, A, M, PCN	Ice chest with wet ice	1 (2 preferred for multiple sites)	
D, S, C, A, M, PCN	Tarp, plastic, clean (optional)	1	To cover the ground at the algae processing station
D, C, A, M, PCN	Wide-mouthed measuring cup with a broad pouring spout	1	For pouring homogenate sample into the diatom sample vial, and for preparation of biomass filters
D, S	Centrifuge tubes, 50 mL, plastic	2 per replicate	Cole Parmer 06344-27
D, S	Rack for 50 mL centrifuge tubes	1	
D, S	Lugol's (10% potassium iodide, 5% iodine) diluted 50%	1-5 mL per replicate	Carolina #872795
D	Safety goggles or face shield	1	
D	Small syringe or bulb pipette	1	
S	Small Ziploc bag	As needed	
S, C, A, M, PCN	Whirl-Pak bag, 100 mL	3 per replicate	Cole Parmer 06498-00
C, A, PCN	Filter forceps	1	Fisher 0975350
C, A, PCN	Pointed forceps	1	Fisher 08-900
C, A	Filtering chamber/tower, 47 mm, plastic	1	Hach 2254400
C, A	Hand vacuum pump	1	Fisher 13-874-612B
C, A	Deionized water	500 mL	
C, A	Dry ice (if not returning to lab immediately following the day's fieldwork)	10 lbs	
C, A, PCN	Snapping Petri dish, 47 mm	2 per replicate	Fisher 08-757-105
C	Glass fiber filter, 47 mm, 0.7 pore size	1 per replicate	Fisher 09804142H
C	Aluminum foil	~100 cm2 per replicate	

Element	Item	Quantity per site	Specifications/Comments
A	Glass fiber filter, 47 mm, 0.7m pore size, pre-combusted	1 per replicate	Check with analytical laboratory ahead of time; they should be able to supply these
PCN	Glass fiber filter, 25 mm preferred, 47 mm possible, 0.7m pore size, pre-combusted	1 per replicate	Check with analytical laboratory ahead of time; they should be able to supply these
PCN, M	Swinnex-style filtration unit (for 25 mm filters) or filter funnel	1	
M	0.45mm mixed cellulose esters filter or nitrate cellulose filter	1 per replicate	Millipore #HAWP04700; Thermo Scientific #1450045
M	2ml screwcap tube	1 per replicate	Fishersci #NC9044906
M	Preservation solution (Bead solution)	1 per replicate	Qiagen #12955-4-BS
M	Latex/Nitrile gloves	1 pair per site	
M	MilliQ water (100ml)	1 per day	
M	DNA-Away or 10% bleach	1	ThermoFisher #7010
M	500ml or 1000ml bottle	1	

A3. Determining Bankfull Height in Streams

Bankfull height: What is it and how to locate it?

Bankfull height is the elevation of a stream channel above which water just begins to flow onto the adjacent floodplain. Several procedures in this manual require you to locate what is known as the "bankfull channel edge," "bankfull height" or simply "bankfull." This is an important concept in understanding the workings of a stream.

Streams create their own channels by moving sediment from the surrounding hillslopes and by eroding the stream channel itself. Major episodes of such movement occur during floods called "channel forming events." These events determine the size of the channel needed to convey the water. In a period of relatively stable climate and landcover, a stream system will develop an equilibrium between its flows and the size of the channel, whereby the channel is large enough to contain the stream under most flow conditions. When flows are greater than this capacity, the stream overflows its banks and flooding occurs.

In such streams, the channel is usually big enough to contain a high-flow event that recurs on an average of every 1 to 2 years (also called the "1.5-year flood"). Such a frequency of inundation is frequent enough that perennial vegetation can't grow there, either because its roots are too wet or its seedlings get swept away. Water may briefly overtop the banks during flooding conditions. Usually, the cross-section of a stream channel is a sort of "bowl" that contains the stream most of the time, inside which no perennial vegetation grows, and a place over the top of this bowl where the water can flow during a high-water event greater than a 1.5-year flood. This "floodplain" may be on one or both banks, depending on the reach.

Indicators of bankfull height

Most stream systems are in a continual cycle of change, and every site is unique; thus, no single indicator of bankfull can always get you the "right answer." There are several indicators which can help to identify the bankfull channel edge, and you should consider all that are present at a given site:

A. **Bank slope:** In stream channels with natural (undiked) riparian areas and a low, flat floodplain, the bankfull edge is located at the edge of this plain. Often, the banks will slope down gradually and then steepen abruptly. This abrupt change in bank slope is usually a good indicator. However, you may find such an abrupt change on only one bank, or none at all, for instance if the channel has cut down into the streambed. The slope-break may be impossible to find on a bank that is slumping or undercut or highly erodable.

B. **Vegetation:** The bankfull edge is often indicated by a demarcation line between lower areas that are either bare or have aquatic and annual vegetation, and higher areas with perennial upland vegetation such as ferns, shrubs, and trees. Keep in mind that the vegetation line is always in transition, retreating during wetter periods and advancing during dryer ones. One particular confusion arises from willow or alder trees growing within the bankfull channel, because the channel has migrated into them, or they fell into the stream and managed to reestablish themselves. Therefore, when you look at vegetation, you should also look at soils.

C. **Soils:** Look for a transition as you move up the bank, from cobble/gravel to sand/silt to soil. Above bankfull level, you should find old leaf litter forming into soil with organic matter. (Beware: this may be covered by flood deposits, so you may have to dig down.)

D. **Point bars and bank undercuts:** Often on the inside of meander bends, the stream will build up a bar of sediment from the eddy current created by the bend; the top of such a bar is the minimum height of bankfull. Similarly, on the outside of such bends, the stream will often undercut the bank and expose root mats. If you reach up beneath this mat, you can estimate the upper extent of the undercut. This is also the minimum bankfull height.

E. **Lines on boulders/bedrock:** If you're in a steep channel with no clear floodplain, look for the highest mineral-stain line or the lowest line of lichen or moss on stable rock.

F. **Adjacent indicators:** If the indicators are unclear where you're looking, try looking up or downstream to see if there is a clear bankfull line from which you can extrapolate.

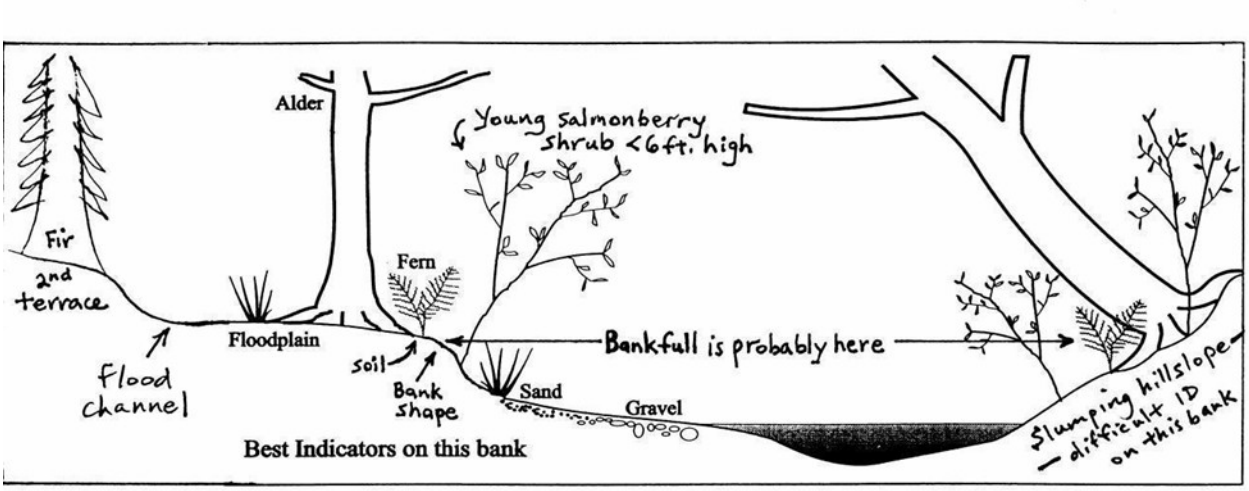

Figure 1. Typical bankfull ID situation, adapted from Pleus and Schuett-Hames, 1998.

Additional tips for identifying bankfull height

Bankfull indicators may not be obvious in certain portions of a reach. Therefore, it can help to look upstream and downstream for corroborating indicators if they are hard to discern in the location you are assessing.

Bankfull height is the same on both the left and right banks. Therefore, it can help to look for corroborating indicators on both banks.

Newly deposited gravel bars can make it difficult to discern bankfull height. Vegetative indicators may be unreliable because riparian vegetation is often disturbed during large storm events and revegetation of bars with perennial vegetation may take many years. In these cases, examine the margins of the channel for perennial riparian vegetation and extend a horizontal line across the channel to determine if the bar tops are above or below the bankfull level. If you are still in doubt after doing this, include the area within the bankfull channel in your search. In other cases, physical obstructions such as debris jams, undercut banks, or a general lack of indicators may make determination or measurement of bankfull dimensions impossible at the transect position (see SOP section 6.1, MODULE C). In these cases, take the measurement at the nearest place where it is feasible.

A4. Benthic Macroinvertebrate Sampling: Targeted riffle composite procedure (TRC)

The reachwide benthos (RWB) method is preferred for BMI collection in all stream types. However, the Targeted Riffle Composite (TRC) method was historically used by SWAMP and other monitoring programs and may be used for long-term data comparability. The TRC is designed for sampling BMIs in wadeable streams that contain fast-water (riffle/run) habitats and may not be suitable in reaches that lack these habitats. Riffles are often used for collecting biological samples (e.g., the old CSBP methods) because they often have the highest BMI diversity in wadeable streams. This method expands the definition to include other fast water habitats, however care should be taken when attempting to apply this method in low gradient streams. Note: Since all streams (even low gradient streams) have variation in flow habitats within the channel, this guidance should not be interpreted as including areas within low gradient streams that are only marginally faster than the surrounding habitats.

The TRC was developed by the Western Center for Monitoring and Assessment of Freshwater Ecosystems (www.cnr.usu.edu/wmc) in Logan, Utah (Hawkins et al. 2003) and slightly modified by the EPA program (Peck et al. 2004). The TRC has been widely used in California by the US Forest Service (USFS) and the EMAP Western Pilot, and in the interest of methodological consistency between state and federal water resource agencies, has been adopted as the standard riffle protocol for bioassessment in California. The version described here is the EMAP modification, which distributes the sampling effort throughout the reach.

Sampling Locations – Acceptable Habitat Types

Riffles are the preferred habitat for TRC sampling, but other fast water habitats are acceptable for sampling if riffles are sparse. Common flow-defined habitat types are listed in the table below in decreasing order of energy (Table 2). Most streams contain some or all of the following fast water habitat types: 1) cascades/falls, 2) rapids, 3) riffles, 4) runs. All of these are acceptable for TRC sampling if riffles are not available. Note: Because the common habitat types are arranged on a continuum of high to low energy environments, the categories grade into each other continuously and are not discrete. Thus, determination of habitat types requires somewhat subjective decision-making.

Table 2. Common habitat types in stream channels, arranged in decreasing order of energy

Type	Description
Cascade/ Falls	Short, high slope drops in stream bed elevation often accompanied by boulders and considerable turbulence. In high-slope streams, cascades and falls are often associated with step-pools. To qualify for this category, water must drop > 0.5 m in height within a short longitudinal distance (< 0.5 m).
Rapid	Sections of stream with deep (>0.5 m), swiftly flowing (>0.3 m/s) water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles.
Riffle	"Shallow/fast" (< 0.5 m deep, > 0.3 m/s); riffles are shallow sections where the water flows over stream bed particles that create mild to moderate surface turbulence.
Runs/ Step-Runs	"Deep/fast" (> 0.5 m deep, > 0.3 m/s); long, relatively straight, low-slope sections without flow obstructions. The streambed is typically even and the water flows faster than it does in a pool. Unlike rapids, runs have little surface turbulence.
Glide	"Shallow/slow" (< 0.5 m deep, < 0.3 m/s); sections of stream with little or no turbulence. Includes still or slow-moving shallow backwaters and shallow margins of pools.
Pool	"Deep/slow" (> 0.5 m deep, < 0.3 m/s); a reach of stream that is characterized by deep, low-velocity water and a smooth surface.
Dry	Any surface area within the channel's wetted width that is completely dry. Do not count mid-channel point bars or tops of emergent rocks and boulders as dry habitat.

Sampling Locations – Selecting Habitat Units

A TRC sample is a composite of eight individual kick samples of 1 ft^2 (0.09 m^2) of substrate each. During your initial survey of the reach, take a mental note of the number and position of the main riffles in a reach (and other fast water habitats if needed). Randomly distribute the eight sub-samples among the fast water habitats in the reach, giving preference to riffles where possible (Figure 2). Unless you are sampling in small streams, try to avoid very small riffle units (i.e., <5 ft^2). If fewer than eight riffles are present in a reach, more than one sample may be taken from a single riffle, especially if the riffles are large.

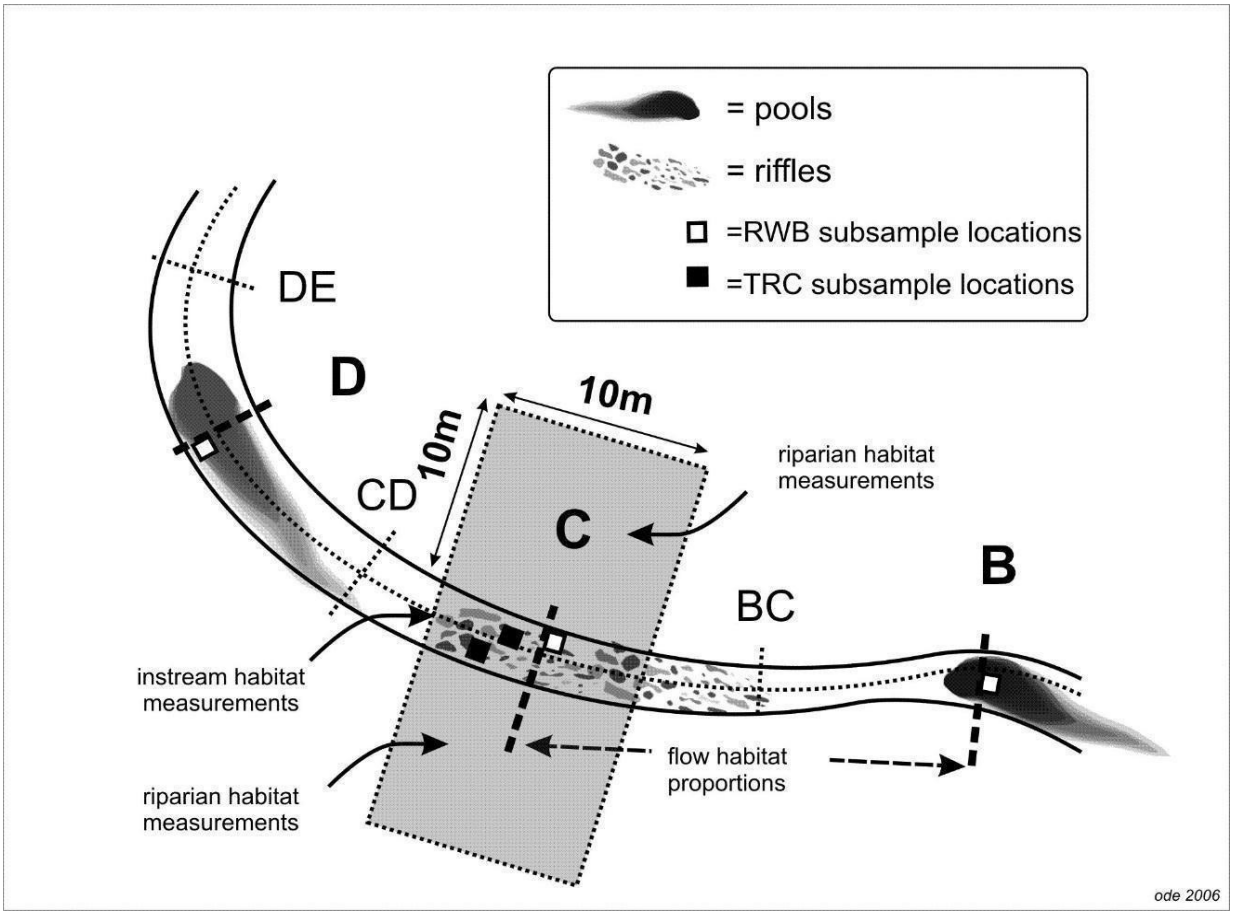

Figure 2. Section of the standard reach expanded from illustration in the SOP showing the appropriate positions for collecting benthic macroinvertebrate samples, instream and riparian habitat measurements and flow habitat proportion measurements.

Sampling Procedure

Begin sampling at the downstream end of the reach at the first randomly selected riffle and work your way upstream.

TRC-Step 1. Determine net placement within each habitat unit by generating a pair of random numbers between 0 and 9. Examples of convenient random number generators include the hundredths place on the stopwatch feature of a digital watch, a 10-sided die and a random number chart. The first number in each pair (multiplied by 10) represents the percent upstream along the habitat unit's length. The second number in each pair represents the percent of the riffle width from right bank. For example, if the two generated random numbers are 4 and 7, you will walk upstream 40% of the distance of the riffle and then go 70% of the distance across the riffle. This position is the center of the 1 ft^2 (0.09 m^2) sampling quadrat for that riffle. If you are unable to sample this location because it is too deep or it is occupied by a large boulder, select a new pair of random numbers and pick a new spot.

TRC-Step 2. Position a 500-µ D-net (with the net opening perpendicular to the flow and facing upstream) quickly and securely on the stream bottom to eliminate gaps under the frame. Avoid, and if necessary remove, large rocks that prevent the sampler from seating properly on the stream bottom.

TRC-Step 3. Holding the net in position on the substrate, visually define a square quadrat that is one net width wide and one net width long upstream of the net opening. Since D-nets are 12 inches wide, the area within this quadrat is $1ft^2$ ($0.09\ m^2$). Restrict your sampling to within that area. If desired, a wire frame of the correct dimensions can be placed in front of the net to help delineate the quadrat to be sampled, but it is often sufficient to use the net dimensions to keep the sampling area consistent.

TRC-Step 4. Working backward from the upstream edge of the sampling plot, check the quadrat for heavy organisms such as mussels and snails. Remove these organisms from the substrate by hand and place them into the net. Carefully pick up and rub stones directly in front of the net to remove attached animals. Remove and clean all of the rocks larger than a golf ball within your sampling quadrat such that all the organisms attached to them are washed downstream into your net. Set these rocks outside your sampling quadrat after you have cleaned them. If the substrate is consolidated or comprised of large, heavy rocks, use your feet to kick and dislodge the substrate to displace BMIs into the net. If you cannot remove a rock from the stream bottom, rub it (concentrating on cracks or indentations) thereby loosening any attached insects1. As you are disturbing the plot, let the water current carry all loosened material into the net2.

TRC-Step 5. Once the coarser substrates have been removed from the quadrat, dig your fingers through the remaining underlying material to a depth of about 10 cm (this material is often comprised of gravels and finer particles). Thoroughly manipulate the substrates in the quadrat. *Note: the sampler may spend as much time as necessary to inspect and clean larger substrates but should take a standard time of 30 seconds to perform Step 5.*

TRC-Step 6. Let the water run clear of any insects or organic material before carefully lifting the net. Immerse the net in the stream several times to remove fine sediments and to concentrate organisms at the end of the net but be careful to avoid having any water or foreign material enter the mouth of the net during this operation.

[1] Brushes are sometimes used to help loosen organisms, but in the interest of standardizing collections, do not use a brush when following this protocol.
[2] In sandy-bottomed streams, kicking within run habitats can quickly fill the sampling net with sand. In these situations, follow the standard procedures but use care to disturb the substrate gently and avoid kicking.

TRC-Step 7. Move upstream to the next randomly selected habitat unit and repeat steps one through six, taking care to keep the net wet but uncontaminated by foreign material when moving the net from riffle to riffle. Sometimes, the net will become so full of material from the streambed that it is no longer effective at capturing BMIs. In these cases, the net should be emptied into sample jars as frequently as necessary, following guidelines described below in the "Preparation of BMI Sample Jars" section. Continue until you have sampled eight $1ft^2$ ($0.09\ m^2$) areas of benthos.

TRC-Step 8. PROCEED to Chapter 5 – Processing Biotic Samples (in main SOP).

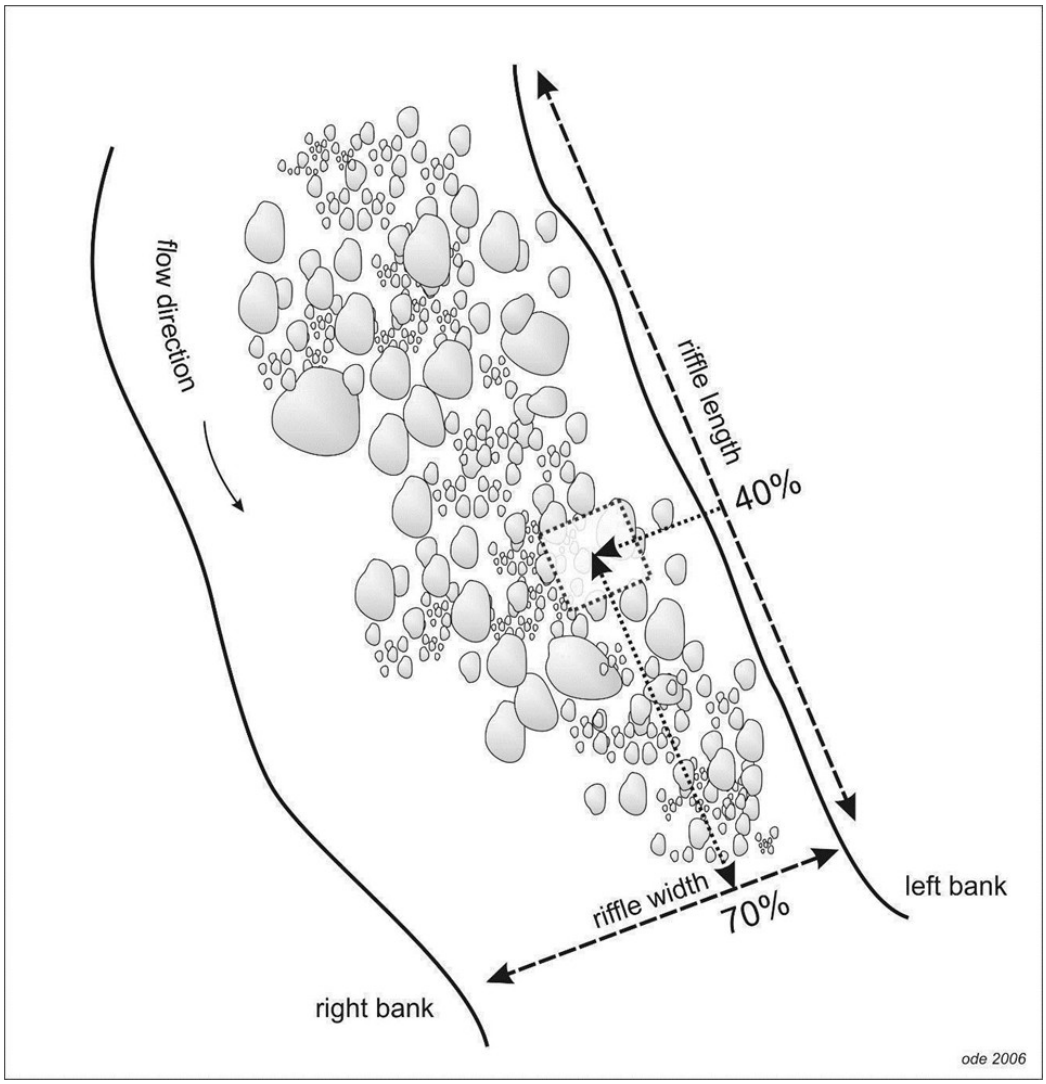

Figure 3. Example showing the method for selecting a subsampling position within a selected riffle under the TRC method. In this example, the random numbers 4 and 7 were selected.

Some additional suggestions:

- If TRC samples will be collected, while scouting the reach, also identify all riffle habitats suitable for sampling and note their positions so that a subset can be identified for sampling.

- Both reachwide benthos (RWB) and TRC methods for BMI sampling use 500-µm mesh D-frame nets. The two samples can be collected at the same time by carrying two D-nets and compositing the material from the two samples in their respective nets. If a two-person collecting team is responsible for both the physical habitat data and benthic invertebrate samples, it is generally best to collect the benthos at each transect, then immediately record the physical habitat data before moving to the next transect. Obviously, this requires especially careful handling of the D-nets during the course of sampling to avoid loss or contamination of the samples. It can be helpful to clearly label the two D nets as RWB and TRC. Larger field crews may choose to split the sampling between biotic team and a physical habitat team and have the biotic team go through the reach first. The positions of both the TRC and RWB subsampling locations are illustrated in Figure 2.

A5. Instructions for Constructing Algal Sampling Devices

Three essential algae sampling devices are not available for purchase and must be assembled from readily available materials. Most of the materials required for construction of the algae sampling tools may be purchased in a hardware or drug store. The rubber delimiter requires some supplies that can be purchased either at bicycle shop or sporting goods store, and the syringe scrubber requires a purchase from a laboratory supply company. We have suggested particular product brand names (and in some cases part numbers) because these materials proved hassle-free during fabrication and withstood field operations without noticeable wear-and-tear but are not endorsements.

Rubber Delimiter

For use on large "erosional" / hard surfaces, such as cobbles and wood that can be removed from the stream, the rubber delimiter is made from a cut bicycle inner tube with a hole cut in the center. A rubber washer is affixed to the inner tube to reinforce the hole. Fabrication of this device involves steps similar to patching a bicycle inner tube. Recommend two per field crew.

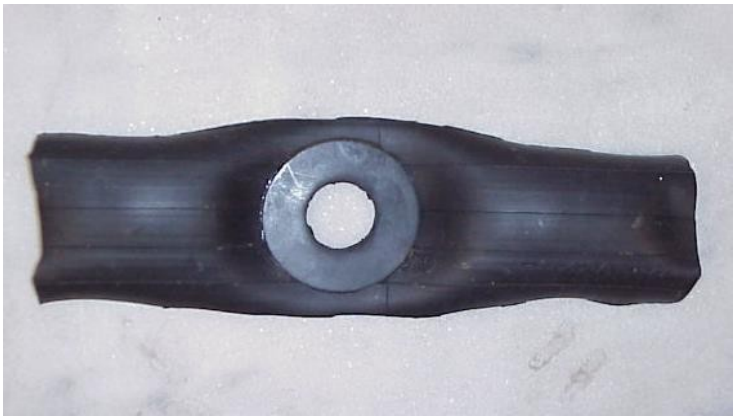

Figure 4. Rubber delimiter

Required supplies (makes 2):

- 1 x 26 inch diameter (mountain bike) inner tube
- 66 mm outer diameter rubber washer. The washer will be found at a home improvement store in the plumbing section. It is made for sealing the flange at the bottom of a tub or shower drain and is exactly 66mm outer diameter (OD), 41mm inner diameter (ID) and 3mm thick.
- Rubber cement or a rubber vulcanizing compound (e.g., from a bike tire repair kit or Liquid Nails LN-20). Make sure the adhesive you use is designed to fuse rubber to rubber; super glue won't work.
- 4 cm diameter circle template (Burt's Beeswax Lip Balm Tin (.30 oz) works well)
- Razor blade or X-acto™ knife (must be very sharp)
- Coarse (40-80) grit sand paper

- Acetone or rubbing alcohol (90%)
- Coin or bicycle tire iron

Instructions:

1. Cut the inner tube
    a. Discard the valve stem and cut the tube in half.
    b. Slice along one seam of the tube (the long way) to create a flat strap.
    c. Cut the hole in the center of the strap with a sharp blade, using the 4 cm template (e.g., lip balm) as a guide. Note: as long as the knife is very sharp this produces a much cleaner cut than tracing the hole with a pen first.[3]
2. Affix the rubber washer to the hole
    a. Sand both surfaces to be glued together until visibly scuffed. Clean all sanded surfaces with the acetone or alcohol. Allow to dry a couple of minutes.
    b. Apply cement to both clean flat sanded surfaces. Allow to dry just until the rubber cement feels tacky. DO NOT allow to dry completely.
    c. Affix the washer to the inner tube around the hole. *Note: The inner diameter of the washer will be ~ 1 mm too large for the edge of the hole.* Press the washer firmly into place and then gently rub the edge of coin or a bicycle tire iron from the inner to the outer edge of the washer, all the way around. Clamp or apply weight to the assembly and leave to dry overnight
    d. Next morning, run a bead of cement around both the inner and outer edge of the washer and allow to dry completely for at least 24 hours.

ABS Delimiter

The ABS delimiter used for "depositional"/soft substrates like sand, small gravel, and silt is made from a 1½" ABS sewer cleanout fitting, which can be found at a home-improvement or plumbing supply store. The hole in the bottom of the cleanout is 4 cm in diameter. The bottom edge of the cleanout is filed to make it sharp, to ease insertion into silt/sand. To facilitate consistent sampling, it is useful to paint a bright line indicating a depth of 1 cm around the outer surface of the bottom of the sampling device. Recommend two per field crew. This tool does not float!

The paint line indicates the depth to which to insert the delimiter into the substrate when sampling. Masking this line with tape before marking / painting helps to make the line clean and consistent.

[3] The area sampled must be correct to calculate biomass accurately and consistently, so the size of this hole is critical to your measurements.

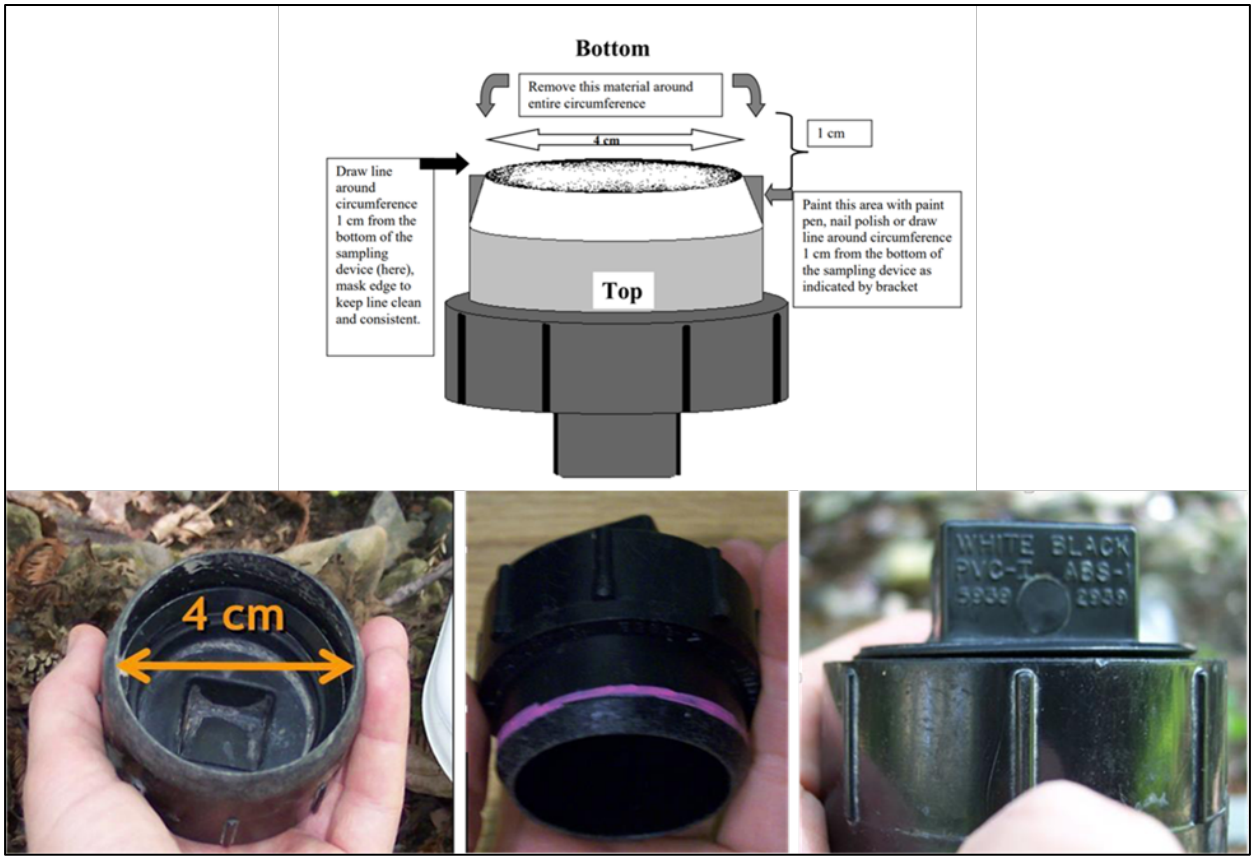

Figure 5. The ABS delimiter used for depositional substrates. *Note that the black line in the image is incorrect – it should point to the bottom of the conical section (1 cm from the opening)*

Required supplies (makes 2):

- (2) 1½ inch ABS sewer cleanout fittings[4]
- (2) 1½ inch ABS cleanout fitting caps[5]
- Paint pen in bright color
- Fine point permanent marker
- Acetone or rubbing alcohol (90%)
- Masking or plain Scotch™ tape
- Ruler with cm increments
- Electric sander / large coarse file
- C-clamp / Bench vise
- Eye protection

[4] The sewer cleanout has an inner diameter of 4 cm.

[5] The cleanout cap screws into the threaded end of the sewer cleanout and has a square top. It makes a convenient handle for the delimiter.

Instructions:

1. Sharpen (bevel) the edge.
    a. Using the fine point permanent marker, mark a line around the outer edge of the cleanout's smooth end (not threaded end), 1cm up from the bottom edge.
    b. Screw the lid into the cleanout. Clamp the square end of the lid tightly into the C-clamp or bench vise.
    c. Use a file or sander to sharpen (bevel) the bottom outer diameter of the cleanout, below the 1cm line. Carefully remove the outer material, leaving the inner material of the inner diameter intact. Work at a shallow angle so that the bevel begins at the line marked 1cm up from the bottom edge and ends at the bottom edge. This will eventually leave the bottom inner edge sharp.
2. Clean the delimiter with acetone or rubbing alcohol (90%) and allow to dry.
3. Redraw the line.
    a. Mask around the fitting 1 cm up from the sharpened bottom edge with tape. This should be where your bevel ends. Make sure to account for the angle now present. Do not measure flat against the bevel or "hypotenuse." The line needs to be 1cm from the edge in the plane parallel to the fitting.
    b. Use the paint pen to draw a bright line around the delimiter's outer circumference, 1 cm up from the bottom edge.

Syringe Scrubber

The syringe scrubber is for use on hard substrates that cannot be picked up out of the stream, like submerged bedrock and concrete channel bottoms. It is made from a 60 mL syringe barrel with the end cut off and its plunger fitted with Velcro-type material. Disposable, white (non-pigmented) scrubbing pads circles are then affixed to the end of the plunger and used to scrub the algae from the substrate. Recommend two per field crew.

Required supplies:

- (2) 60-mL plastic syringe (Cole Parmer EW-07940-30)
- Velcro pads (¾ inch squares)
- Package of white scrubber pads
- Rubber cement or similar adhesive
- Acetone or rubbing alcohol (90%)
- Super fine point permanent marker
- Coarse (80-100) grit sandpaper
- Sharp knife
- Cutting board
- Ruler with mm increments

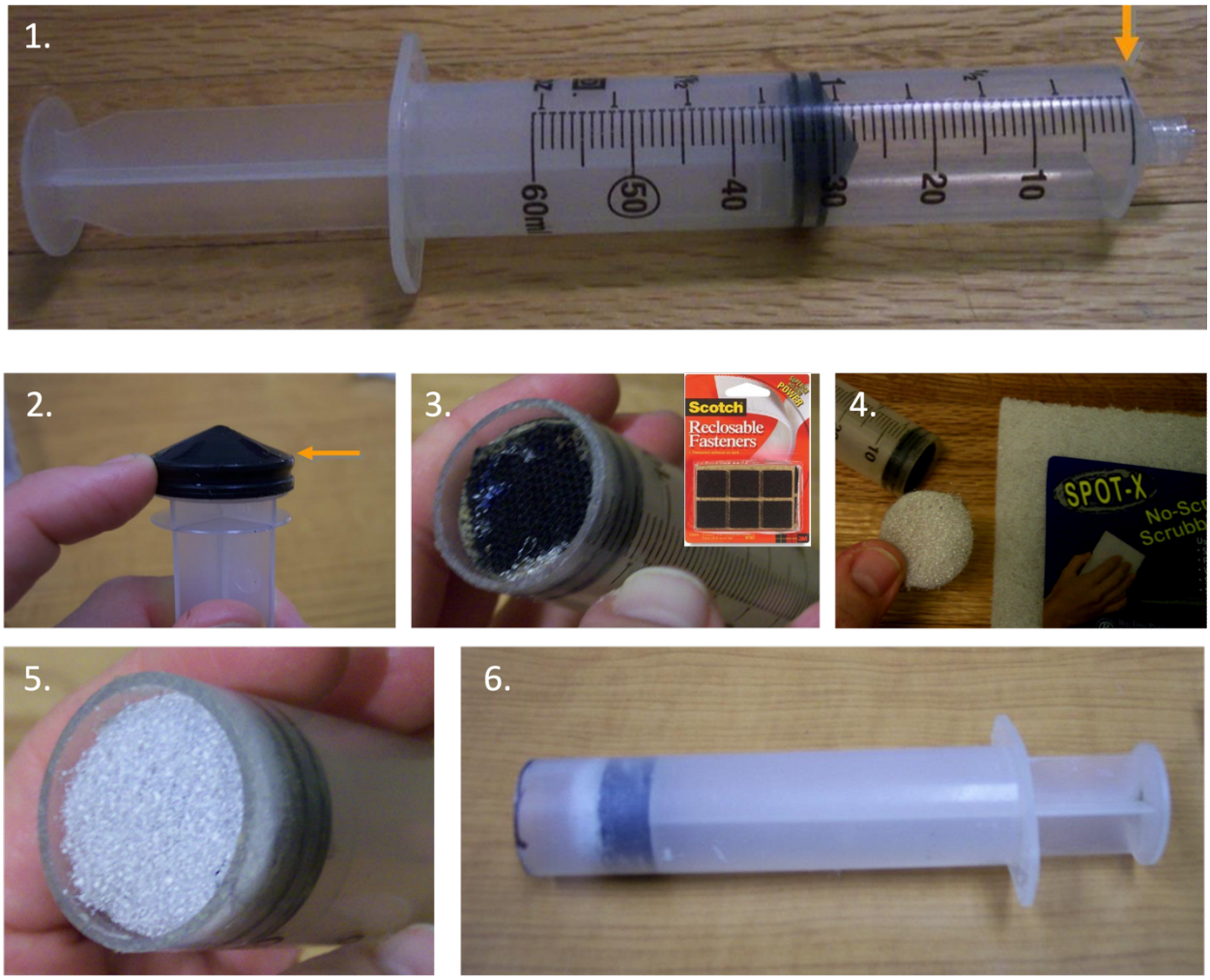

Figure 6. Construction details of the syringe scrubber used for hard substrates. *Note yellow arrows in images 1 and 2 showing cutting position of cutting points.*

Instructions:

1. Cut off the outlet end of the syringe
    a. Fully depress the plunger. Note the position of the bottom end of the plunger and draw or score a circle around the outside of the end of the syringe at this point. *Note: You will be cutting below the 10ml line.*
    b. Remove the plunger.
    c. Roll the syringe on the cutting board against the blade of the knife, scoring around the template/ guideline. DO NOT just saw through the end of the syringe – gently score the template line first, then follow that score around several times by rolling the syringe on the cutting board with the edge of your knife until the end comes off.

d. Sand the cut end of the syringe with sandpaper laid on a flat hard surface. Sand until the cut you have made is even and flat all the way around, so that when the end of the syringe is set on a flat surface it sets squarely and there are no gaps.
e. Measure and note the inner diameter of the syringe.

2. Firmly affix the rubber tip to the plastic plunger. When you are sampling with this device you must be able to rotate the scrubbing pad to "scrub" the sample surface. If the plunger spins in the rubber tip, your scrubbing pad patch will not rotate and you will not get a usable sample.
    a. Remove the rubber tip from the plunger.
    b. Scuff the surfaces to be glued together (the end of the plunger and the inside of the rubber tip). Clean the plunger and inside of the rubber tip with acetone or rubbing alcohol (90%). Allow to dry for a couple minutes.
    c. Use cement to glue the rubber tip to the plunger. Allow to dry for an hour or more.
3. Affix the Velcro pads to the plunger.
    a. Use the hook side of the Velcro, not the fuzzy side. Cut all four corners of the square pieces so that it just covers the nose of the plunger.
    b. Use sandpaper to roughen and flatten the nose of the plunger. If the plunger is made of rubber, you can cut off the pointy tip with a razor blade or sharp utility knife before you sand. If you do need to trim off the tip of the rubber plunger, be sure it is even and flat. Make sure to sand over the whole area to be glued, including the rubber.
    c. Clean the plunger with acetone or rubbing alcohol (90%). Allow to dry.
    d. Evenly spread glue over the nose of the plunger. Firmly press trimmed Velcro pad into place. Allow to dry.
    e. Cut white scrub pad circles for use in the field. Make at least two pads per site.[6]
    f. Use the cut end of the syringe as a template to draw circles in the scrubber pad. When you cut out the circle you MUST NOT leave any ink on the finished circle, this can interfere with the chlorophyll α analysis. Use a waterproof adhesive to affix the Velcro circle to the end of the plunger.

Note: The area sampled by the device must be correct to calculate biomass accurately and consistently. The SWAMP data sheets assume the syringe scrubber samples an area of 5.3 cm^2, which assumes the inner diameter of the syringe barrel is 26 mm. If the diameter of your syringe is different, you must correct the area on the data sheets (area = πr^2, r = ½ d).

[6] Do not reuse pads from site to site; this would result in sample contamination.

Viewing bucket (optional)[7]:

Figure 7. Plexiglas viewing bucket for viewing submerged algae.

A viewing bucket can be useful for visualizing submerged algae (for finding good qualitative algal samples), particularly in instances of a turbulent stream surface that obscures the stream bottom. A viewing bucket can be constructed from a cylinder of clear Plexiglas (approximately 8 inches in diameter) whose bottom is fitted with a circle of thick glass and sealed with silicone. If desired, one or two handles can also be fashioned out of Plexiglas and attached to the side(s) of the cylinder.

[7] SWAMP's Freshwater Harmful Algal Bloom program uses a bathyscope to conduct visual assessments of submerged algae, and this bathyscope may be used in the same fashion as a viewing bucket.

A6. Processing Quantitative Benthic Algal Samples for Soft Algae Taxonomy

Taxonomic analysis of soft-bodied algae is an optional component of the bioassessment SOP. The following is a description of how to proceed when the optional microscopy soft-bodied algae taxonomic ID sample is to be prepared AND macroalgal clump(s) are present in the sample in the dish tub (or other post-sampling processing container).

Figure 10 below is meant to replace the instructions in Section 5.2 in the main SOP. It is recommended that this flowchart be printed in color, laminated (if possible) or printed on water-proof paper, and brought along to the field for a quick reference on handling macroalgal clumps in the composite sample.

Note: It is unlikely that the ¼ macroalgal clump will occupy all the space in the soft-bodied algae quantitative sample tube, but if it does, a second tube will be needed in order to accommodate all the sample material plus liquid. If such an action is taken, it should be noted in the Comments section of the field sheets and the tubes should be clearly identified as belonging to the same sample, for record-keeping purposes. Do not fill either tube so full that there will not be enough room for the fixative.

The steps to generate the composite sample are as follows:

Step 1. Any pieces of macrophyte (i.e., vascular plants, not algae), twigs, or dead leaves that had been collected with the ABS delimiter should be massaged thoroughly between the fingers and rinsed into the tub in order to remove the algae coating them. These vascular plant fragments can then be discarded. If there are any clumps of macroalgae in the dish tub, there is a special step required for processing them. The procedure is described in detail below (see Figure 12).

Step 2. Systematically massage all the sand and/or silt in the dish tub between the fingers to dislodge clinging microalgae. For pieces of gravel, use a toothbrush to remove algal material from surfaces. Rinse toothbrush and brushed gravel into the tub. Rinse the sediment thoroughly (but as sparingly as possible) with stream water so as to create a suspension of the dislodged microalgae (i.e., the sample).

The final volume of liquid in the dish tub will be measured before the algal taxonomic and biomass samples are prepared. To do this, the liquid in the tub will be separated from the rinsed sediment such that the volume measured does not include sediment (see below). After the liquid sample has been measured, discard the rinsed sediment into the stream.

Step 3. If one or more macroalgal clumps are present in the dish tub, first remove them from the dish tub, wring them out gently into the tub, and roll them into cylinder shapes that are relatively even in thickness along their length. If there appears to be more than one type of macroalgae (i.e., obviously different species based on color/texture) in the sample, separate cylinders should be made for each one.

Step 4. Measure the length of the cylinder(s) with a ruler and cut a quarter off of each one, lengthwise, with scissors. Place all the quarter pieces together into the (still empty) soft-bodied algae ID sample (50 mL centrifuge) tube. Push the clump of combined macroalgal specimens down into the sample tube and flatten the top so that the volume of the clump can be estimated using the graduations on the tube. The estimated volume of this clump will be used in a later calculation (see Figure 8, Figure 10 and Figure 11).

Step 5. Place the remaining three-quarters length of the cylinder(s) in a Whirl-Pak™ bag. Seal and label the bag and store it in the wet ice cooler.

Step 6. Once algal specimens have been removed from all the substrates (sand, gravel, cobble, wood, leaves) in the dish tub, according to the procedure described in Steps 1 and 2 at the beginning of Section 5.2, gently agitate the dish tub to suspend the microalgae in the liquid, and then start pouring this suspension into a clean graduated cylinder to measure the volume of the liquid. Try to leave all sediment (silt, sand) behind. Transfer the measured liquid into a clean 1L plastic bottle. Rinse the sediment once or twice until it appears that little to no additional suspended material (microalgae) is coming off because the rinsate is clear (or nearly clear). Add this rinsate to the graduated cylinder to measure it also. If necessary, repeat this process (regularly agitating the dish tub) until all the liquid has been measured and transferred to the sample bottle. Note: use water sparingly, because the total sample volume plus rinsate should be no more than about 400-500 mL. Because as much of the silt and sand as possible is being left behind, the final volume should ideally reflect only the liquid component of the sample. On the field sheet, under the Algae Samples section, record the total volume of all the liquid that had been in the dish tub, plus the water used for rinsing the substrates and sampling devices. This is the “composite volume”. Record this value on all algae sample labels (biomass and taxonomic samples).

Step 7. Pour freshly-agitated liquid composite sample from the 1 L bottle into the soft-bodied algae sample tube (on top of the clump of macroalgae, if present) up to the 45 mL mark. If no macroalgal clumps had been collected during sampling, simply pour the liquid sample into the empty soft-algae sample tube to the 45 mL mark. Midway through pouring, swirl the composite sample some more (first clockwise, then counter-clockwise) to ensure that the microalgae are still fully suspended. Cap the tube tightly. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Place the tube in the wet ice chest to keep it in the dark and as cold as possible, but make sure it is never allowed to freeze.

Lugol's is necessary for fixing soft-bodied algae samples. Fixatives such as Lugol's should be used with great care. Appropriate safety equipment, including gloves and eye protection, must be worn when handling fixatives. Lugol's stock solution should only be handled in a fume hood. To fix the soft-bodied algae sample: working under a fume hood or in a well-ventilated area in the field, add 5 mL of Lugol's (10% potassium iodide, 5% iodine) to 45 mL of sample. Distribute the Lugol's throughout the sample by inverting the tightly closed tube repeatedly. Once the samples are fixed, they must be stored in the dark in a refrigerator. Wrap the tubes in foil if necessary to maintain darkness.

Step 8. Volume adjustment. In the field, after the (unfixed) soft-bodied algal sample has been prepared, and before preparing the diatom sample (and biomass samples, which will be discussed in the next steps), if a macroalgal clump was present in the dish tub, then the volume of the remaining composite liquid must be reduced to equal ¾ of the original volume. This is necessary because ¼ of the macroalgal clump was taken out of the composite sample but a full ¼ was not removed from the liquid portion. As such, the original ratio between liquid and macroalgae must be restored before further sample preparation.

The following procedure is used to reduce the volume of liquid composite to ¾ of the original. As always, whenever pouring off aliquots, be sure to agitate the composite liquid adequately in order to resuspend any settled microalgae before pouring off the calculated volume. For convenience, the following formula (Equation 1, Figure 9) can be used to calculate how many mL to pour off and discard from the composite:

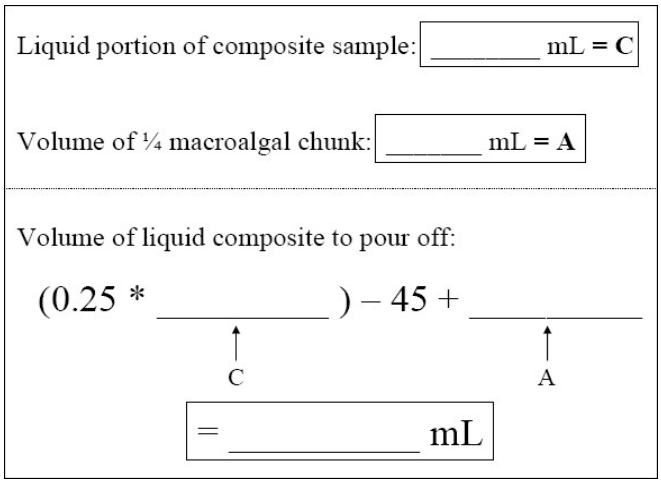

Figure 9. Worksheet for restoring the sample concentration. Where "C" is the original composite volume and "A" is the approximate volume of the clump(s) of macroalgae placed in the soft-bodied algae sample tube (tamped down and flattened). Note: Be sure to multiply before you add and subtract.

Step 9. Once the required amount of composite liquid has been discarded, the remaining ¾ of the macroalgal cylinder (from the bag in the wet ice cooler) is cut with scissors into fine pieces (resulting in strands that are no more than ~3 mm long), and these are added to the reduced-volume composite liquid. The pieces should be chopped small enough so that they practically "blend" into the liquid such that distinct fragments of macroalgae are not easily discernible, because the goal is to "homogenize" the macroalgae into the liquid as much as possible. If a macroalgal clump was present in the dish tub, but no sample is to be prepared for analysis of the soft-bodied algal community, then ALL of the macroalgal clump should be finely chopped into the full volume of measured composite liquid. In this case, there would be no need to discard ¼ of the composite volume before introducing the chopped macroalgal into the liquid.

Step 10. After introducing the finely chopped macroalgae into the composite liquid, cap the composite bottle and agitate sufficiently to homogenize the tiny bits of algae into the liquid as much as possible, while not agitating so hard as to risk busting cells and releasing chl a.

Step 11. Aliquot 45 mL of freshly agitated sample homogenate into the diatom ID sample tube, swirling the composite sample bottle again midway through pouring to keep the algae suspended. Add 1 mL of 50% Lugol's to the sample to preserve. *Note: see guidance above on safety precautions when using Lugol's.*

Step 12. Cap the tube tightly and invert it several times to mix the Lugol's into the sample. Fill out a sample label and affix it to the sample tube. Cover the label completely with clear plastic tape to prevent the writing on the label from smearing. Keep the fixed diatom samples in the dark and away from heat. The remaining composite sample homogenate can be used to prepare the chl-a, AFDM, and molecular (DNA) filters as described below.

Step 13. Now the molecular and/or biomass samples can be prepared. The procedure to filter chl a samples should be carried out quickly, and in the shade as much as possible, to minimize exposure of the sample to light/heat, thus minimizing chl a degradation. Use clean filter forceps to center a glass fiber filter (47 mm, 0.7 µm pore size) onto the mesh platform of a clean filtering apparatus and rinse the filter a little with DI water to seat it well into the mesh before attaching the filter chamber on top. Never touch the filters with anything other than clean forceps. Agitate the sample homogenate to resuspend all the macroalgal fragments and microalgal material. Measure 25 mL using a small, clean graduated cylinder. Midway through pouring the 25 mL, swirl the homogenate again to ensure that the material is still fully suspended. Pour the remainder of the 25 mL into the filter chamber. Once empty, rinse the graduated cylinder with a few mL of DI water, and add this to the filter chamber.

Step 14. To filter the sample, create a gentle vacuum with the hand pump. Be sure to proceed very slowly, and pump only one stroke at a time until all of the liquid in the sample is passed through the filter. Pressure on the sample should never exceed 7 psi, as this could cause cells to burst and release contents, including chl a, into the filtrate and be lost. If it becomes impossible to filter a whole 25 mL of the sample and remove the water efficiently, discard the filter and try again with a smaller volume (e.g., 10 mL). It is not necessary to collect on multiple filters to try to achieve a total volume of 25 mL. Simply filter as much as possible on a single filter, up to 25 mL, and then use that filter as the sample. Be sure to record the volume of the composite sample that was actually filtered, both on the datasheet, and on the sample label.

Rinse the sides of the filter chamber with a few mL of DI water and continue filtering until the 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. After all the liquid has passed through, check the filter to see if there are any bits of non-algal plant matter (like tiny seedlings or bits of leaves). If so, remove them with clean, pointed forceps, being careful not to remove any algae in the process. Remove the filter from the filtering device. Always thoroughly rinse the sides of the filter chamber and the interface between the mesh filter seating and the screw-on part of the apparatus with DI water between samples.

Step 15. Fold the filter in half (with the sample material on the inside, like a taco) using the forceps, and place it inside a clean, snap-top Petri dish. Envelope the Petri dish completely within a small sheet of aluminum foil in order to prevent any light from reaching the filter. Place the covered Petri dish and its corresponding, filled- out sample label (face outward) into a 100 mL Whirl-Pak™ bag, purge as much of the air out of the bag as possible, "whirl" it shut, and seal it tightly by twisting its wire tabs together, so that water in the cooler will not be able to enter the bag. Shove the sample packet down into the ice in the cooler to make sure it stays submerged and does not float to the top. This may be achieved by sealing the sample bags in a large Ziploc™ bag with a rock in it. Keep chlorophyll a filters as cold as possible: place them in the freezer (-80º, if available) or on dry ice within four hours of collection; the analytical holding time for the chl a filters is 28 days from collection, when kept frozen.

Step 16. For the AFDM samples, use glass-fiber filters (47 mm, 0.7 µm pore size) that have been pre-combusted. Never touch the filters with hands or anything other than clean forceps. Follow the same process as that used for chl-a sample filtering. Record the volume filtered for the AFDM sample. Keep AFDM filters as cold as possible until the samples can be frozen back at the laboratory that evening, or place on dry ice until they can be stored in the laboratory freezer. The analytical holding time for the AFDM samples is 28 days from collection, when kept frozen.

Step 17. For the molecular (DNA) filters, use a sterile cellulose nitrate or mixed cellulose ester filter with a pore size of <0.45um. Filter 25ml of composite material; if the filter becomes clogged before the 25 ml is completed, record the total volume filtered. Remove the filter using sterile forceps and fold the filter at least twice. Place the filter in a 2ml Eppendorf tube (or equivalent screw-cap tube) pre-filled with preservation solution. Invert the tube five times to mix preservation solution and submerge the filter. Store tubes in a -20ºC or -80ºC freezer. Filters in preservation solution are stable at this temperature for one year. See additional guidance in algae molecular SOP below.

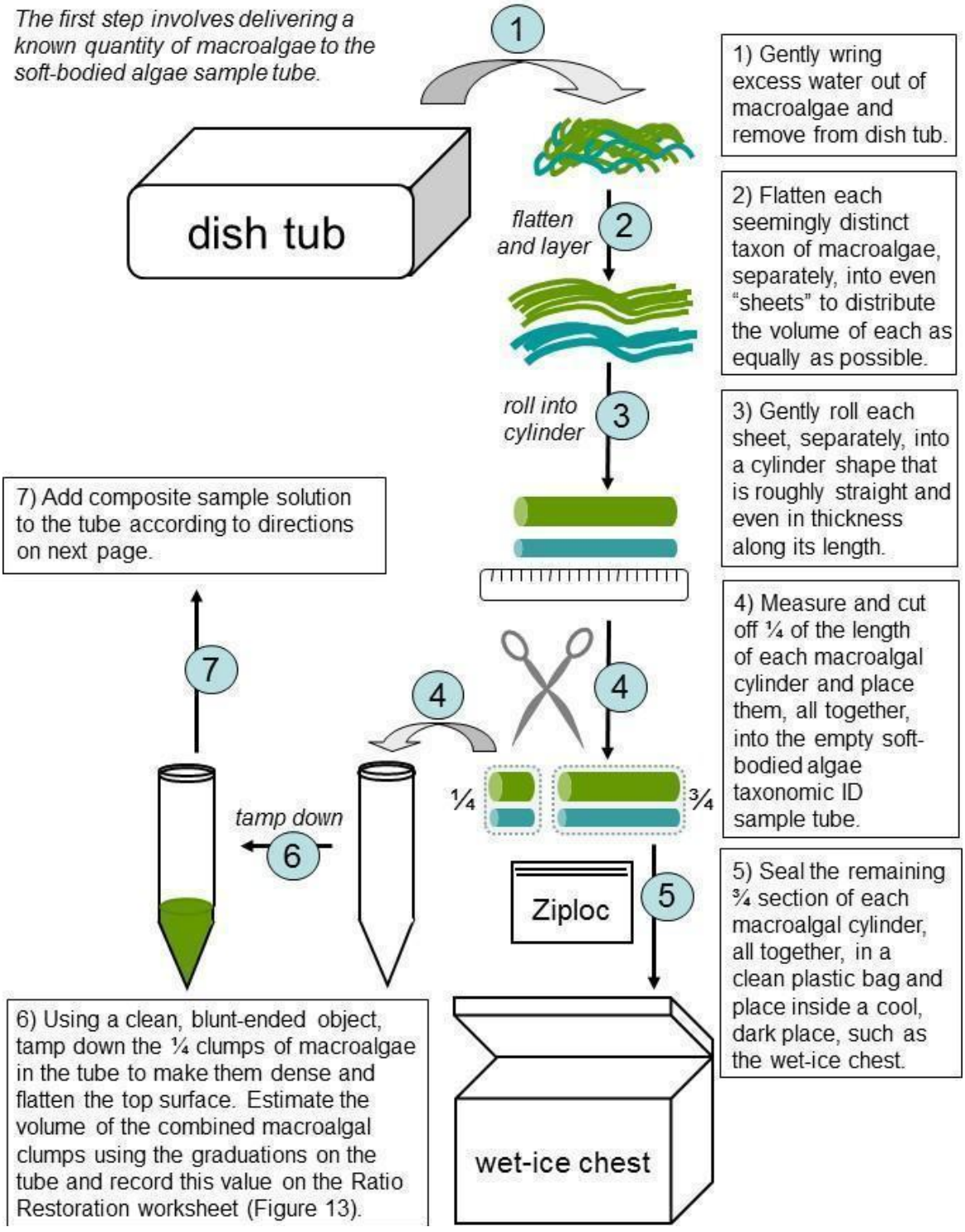

Figure 10. Instructions for processing quantitative benthic algal samples for soft algae taxonomy (steps 1-7). *Note that Steps 1-3 in this figure correspond to only Step 3 in the text.*

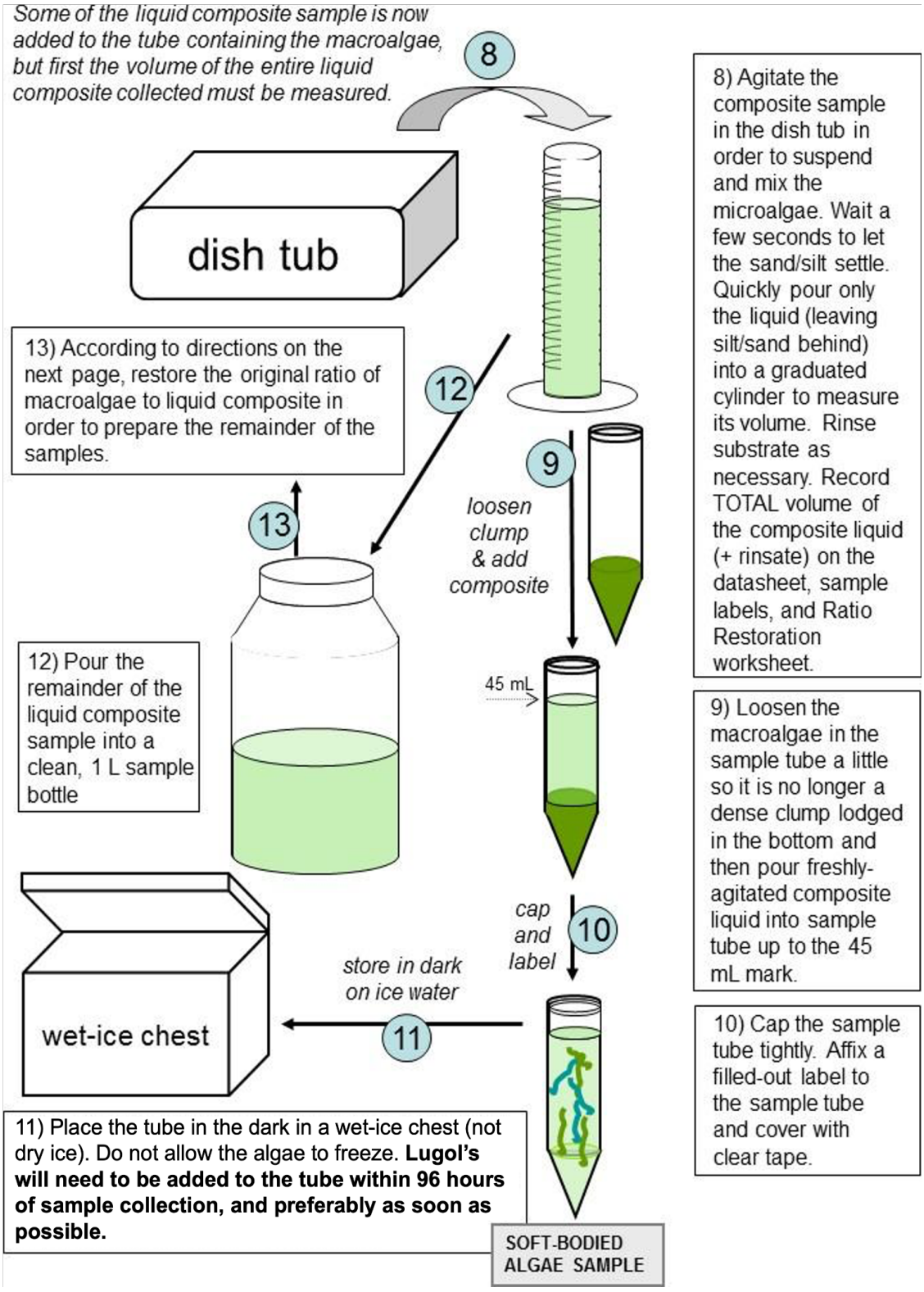

Figure 11. Instructions for processing quantitative benthic algal samples for soft algae taxonomy (Steps 8-13).

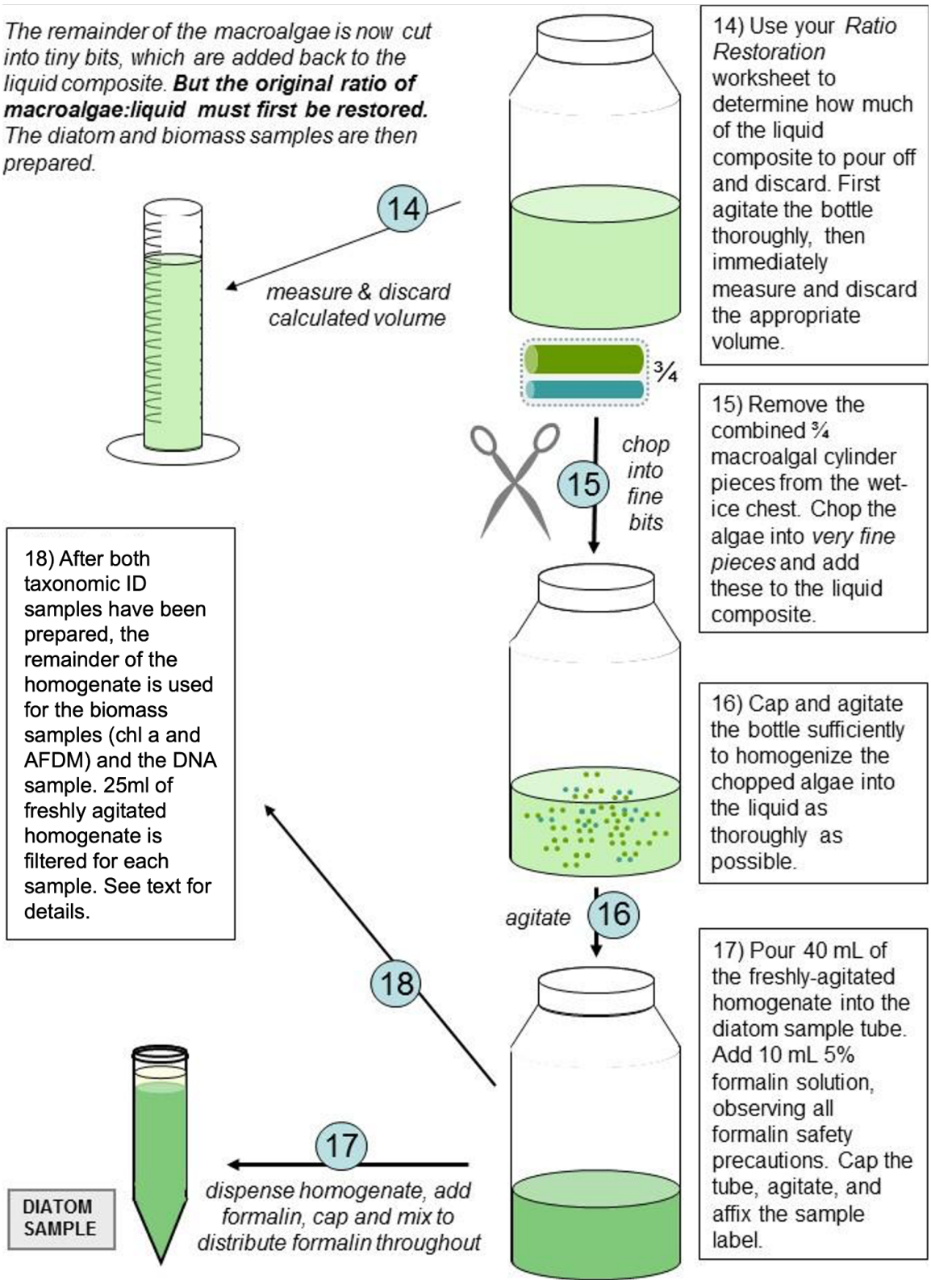

Figure 12. Processing soft-bodied algal and diatom samples when macroalgal clumps are in the sample (Steps 14-18). *Note that diatom samples are now 45 ml and fixed with Lugol's (not formalin) as described in the main SOP.*

A7. Procedure for Collecting and Storing Qualitative Soft Algae Samples

Whenever quantitative soft algae samples (Section 5) are collected for taxonomic analysis, a "qualitative" soft algae sample may also be collected in certain situations. The qualitative sample consists of a composite of all types of soft-bodied algae observed within the reach. The qualitative sample can aid identification of taxa captured in the RWB sampling, since it allows larger, more intact specimens to be collected than those that may end up in the more heavily processed quantitative sample. In addition, if the qualitative sample is kept cool and in the dark and is delivered to the laboratory in a timely manner (i.e., within two weeks of collection), there is a possibility of culturing live specimens, which is sometimes essential for standard taxonomic effort-level identifications and can provide material for subsequent gene sequencing.

Collection of the qualitative soft-bodied algae sample can be conducted at any time during the field visit, as long as its collection does not in any way interfere with the water chemistry, biotic, and PHab sampling/data collection (i.e., by kicking up sediment, displacing BMIs, and/or disturbing the stream bottom). It helps to have the collection bag on hand at all times so that it can be used for spontaneous grabs of specimens that are spotted during the course of the other fieldwork (e.g., conducting PHab data collection). However, the entire sampling reach should be visually scoured at least one time during the course of the day's fieldwork in an effort to see, and collect samples from, all patches of distinct soft-bodied-algae specimens therein.

Step 1. Using a thick, waterproof marker, label a Whirl-Pak™ bag with the Station Code, Date, and Sample ID.

Step 2. Hand-pick specimens of all visibly different types of macroalgal filaments and mats, as well as microalgae (in the forms of scrapings using a razor blade or knife), and depositional samples (suctioned from along the surface of sediments using a clean turkey baster). See Section A-9 for photos that will help collectors develop an eye for the variety of types of algae that may be encountered in streams. A few helpful tips:

Some algae (e.g., species of *Chara*, *Paralemanea*, and *Vaucheria*) look like submerged macrophytes or mosses. Algae come in many colors, and may be green, dark-brown, golden, red, black, or bluish-green. Some cyanobacteria, such *Nostoc* spp., look like gelatinous globules or "deflated" sacs, ranging in size from smaller than a pea to larger than a lime. Collect from as many distinct locations as possible throughout the reach so as to capture as much of the apparent diversity as possible. Include any holdfast structures that had attached the macroalgae to the substrate, as these structures can be useful for taxonomic identification. Since these samples are merely qualitative, it is not necessary to collect them in a manner that is representative of their relative abundances within the reach. When in doubt as to whether a candidate specimen qualifies as "algae", add it to the sample; final determinations will be

made by the taxonomist. In the absence of macroalgae, rock scrapings, substrate particles, and CPOM can still be collected (as described above). Macroalgae growing within 10 m of the reach can also be added to the qualitative sample.

Step 3. Fill the bag with a total volume of up to 100 mL of qualitative algae sample + stream water. Purge most of the extra air from the bag, and seal with the wire tabs by twisting them together (not just folding them over, as this can result in leakage). Tuck the ends of the wire tabs inward so that they cannot poke holes in the bag. Collect as many bags as needed, based on the variety of algae visible in the stream reach. If multiple bags are collected, number them accordingly (e.g., "bag 2 of 4") so that the laboratory will know how many bags to process for that site.

Step 4. Double-bag the qualitative samples, and slip a filled-out (with pencil) label ( ) printed on waterproof paper into the outer bag. Store in cool, dark conditions (i.e., in the wet ice cooler, not on dry ice). Do not let the bags touch ice (or 'blue-ice" packs) directly, which could cause the samples to freeze, thus destroying them. Do not add any fixative to these samples.

Step 5. Refrigerate the qualitative samples immediately upon return to the laboratory. Because they are not preserved, these samples should be examined by a taxonomist as soon as possible (and within two weeks, at most), as they can decompose rapidly. Coordinate beforehand with the receiving laboratory, as necessary, in order to ensure that samples are processed in a timely fashion.

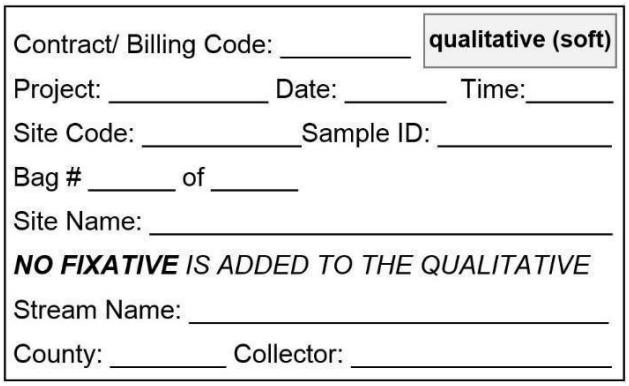

Figure 13. Label for soft-bodied algae qualitative sample.

Figure 14. Photographs of representative macroalgae observed in California streams.

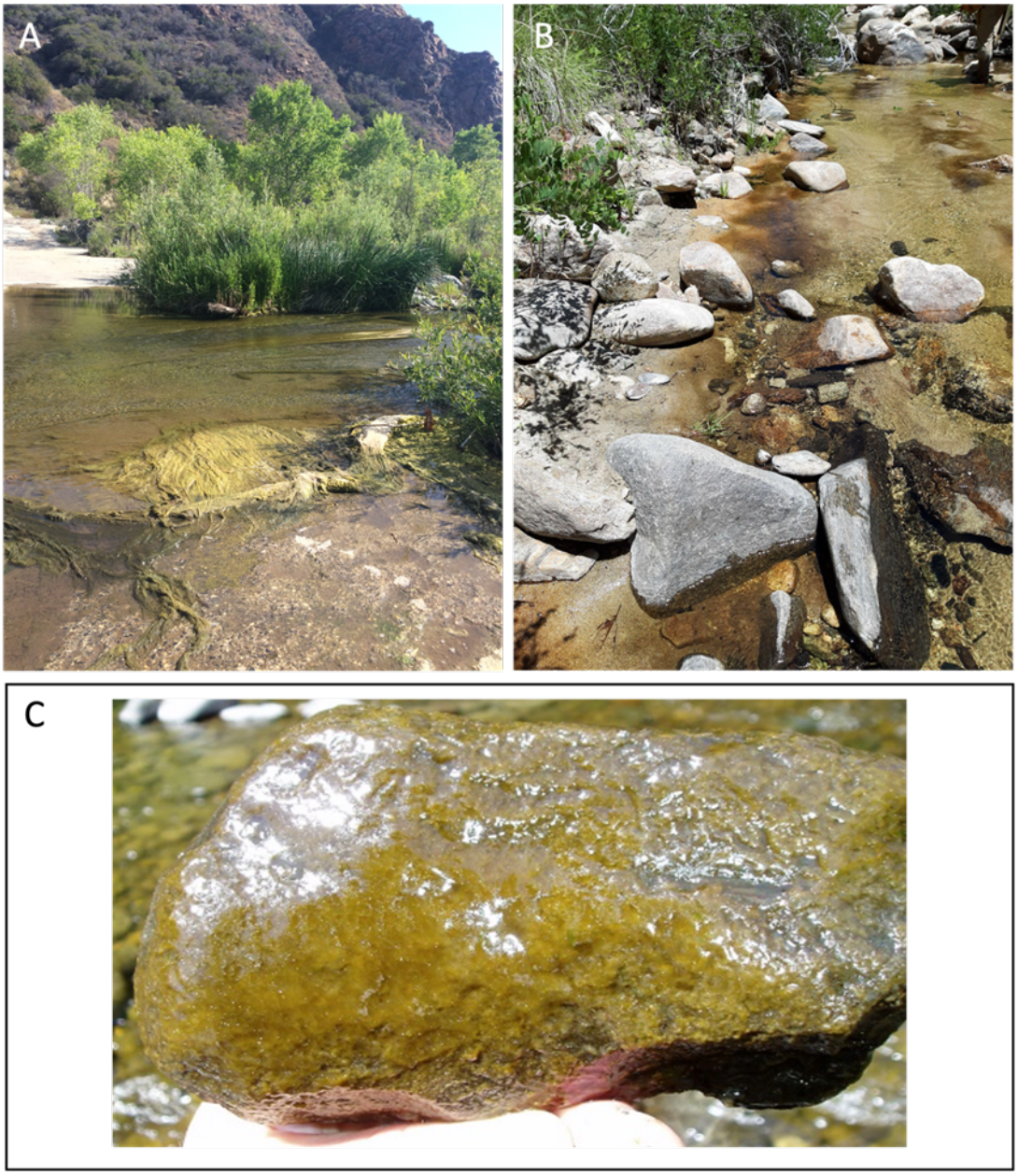

Figure 15. Distinguishing macro from microalgae: A) Soft-bodied algae form large mats, filaments, or globose structures that remain cohesive when picked up. They may exhibit a range of colors, including green, brown, yellow, red, and near-black. B) Diatoms may sometimes form large films or filaments that are visible without magnification. Diatoms tend to be golden-brown in color and are usually less cohesive than non-diatom macroalgae. Unlike other algae, diatoms secrete mucus that gives them a slippery texture. C) Microalgae: diatoms and microscopic soft-bodied algae that form a seemingly “amorphous”, slimy coating on substrate, like icing on a cake (photo credit USGS).

A8. Collection of Algal Samples for molecular Analysis

Materials:

- 47mm Whatman/Swinnex filter holders* -OR- Filter funnel*
- 47mm 0.45um cellulose nitrate filters (Millipore HAWP04700)
- 2ml screw cap tube pre-loaded with preservation solution (bead solution, Qiagen # 12955-4-BS) labeled with sample site code, date, and replicate number
- 60ml Syringe with luer lock* (for syringe filtering only)
- 25mm Swinnex filter holder with luer lock* (for syringe filtering only)
- 500ml or 1L bottle*
- 100ml deionized water (DI H_2O)
- Latex gloves
- Tweezers/forceps*
- Whirlpaks labeled with sample site code, date, and replicate number

* Items should be sterilized before use and between sampling sites to prevent cross-contamination. To sterilize, soak in acid wash (1% solution of hydrochloric or nitric acid), rinse in DI H_2O, and autoclave OR soak in 10% bleach and rinse with DI H_2O.

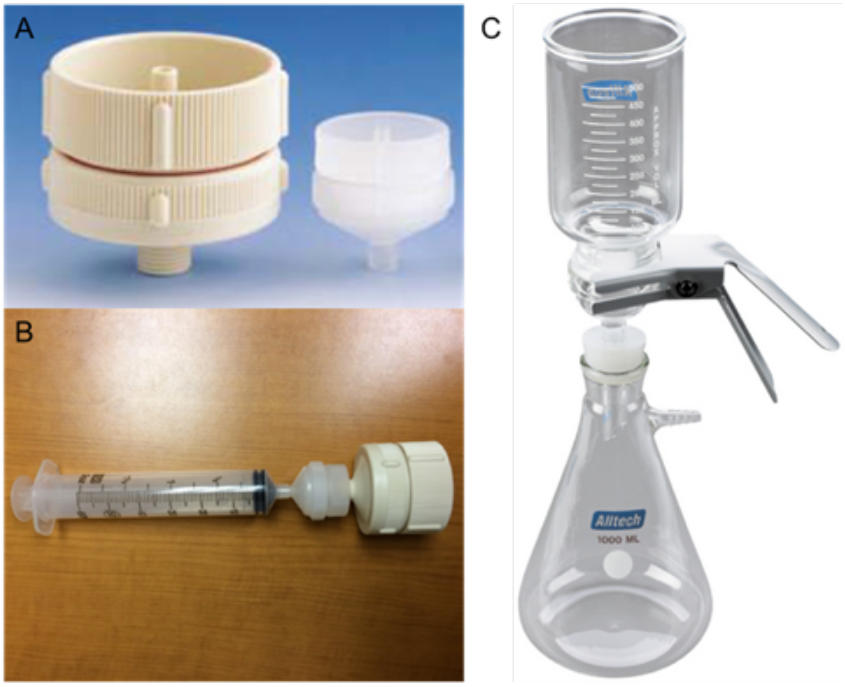

Figure 16. A: 47mm Swinnex, 25mm Swinnex. B: Assembled syringe, 25mm Swinnex and 47mm Swinnex. The 25mm Swinnex is used as a connector between the syringe and 47mm Swinnex. C. Filter funnel assembled.

Sample collection and processing:

1. **Sample collection:** The sample for DNA sequencing should be sourced from the bioassessment composite bucket (after the total composite volume has been recorded).
    a. To prepare the sample, mix the bioassessment composite bucket to re-suspend any settled particles, and allow sand and other large particles to briefly settle. Pour the entire supernatant into a 1L bottle, retaining particulate matter in the bucket.
    b. Homogenize sample in a 1L bottle by vigorously shaking the bottle. If necessary, remove large clumps of biomass to individually cut-up and place back in the 1L bottle.
2. **Filtering**:
    a. Using forceps to avoid contamination, place filter on to Swinnex or filter funnel (Figure 16). Ensure that the filter is secured evenly and held in place by rubber gaskets. If the filter is slipping, you can use sterile DI H2O to help secure the filter in place.
    b. Vigorously shake 1L bottle with sample to homogenize. Filter 25 ml of homogenized sample. If the filter becomes clogged, make note of the total volume of sample filtered. Record this volume on the worksheet. *If your filter is clogging: Take replicate filters of small volume to ensure there is adequate biomass being preserved (remember to record volumes filtered).*
3. **After filtering**:
    a. To remove the filter from the filter-holder, gently fold the filter using forceps. Ideally, fold the filter paper three times to create a triangle shape with filtrate protected on the inside of the triangle. Place the filter in a pre-loaded screw cap tube and cap. Label tube. Filter must be submerged in the preservation solution; shake vigorously if needed. Place the tube in a labeled Whirlpak bag.
    b. Keep tubes with filters on ice until being transferred to a -20°C or -80°C freezer. Samples at -20°C are stable for six months.
    c. If there is adequate material, taking three replicate filters from each composite sample is preferred. Simply repeat Steps #1-3 for the two additional filters. When possible, freeze (-20°C) any remaining composite algal sample in the event of low biomass DNA extractions.
4. **Collecting a field blank:** Collect a field blank at each sampling site. Filter 50ml of DI H_2O onto a clean filter and place the filter in a tube with preservation solution.

Table 3. Example label for molecular algae samples

Station code	Date	Replicate	Vol (ml)
Notes	Collection time	Filtration time	Preservation solution (Y/N)

A9. Collection of Water Environmental DNA (eDNA) Samples for Molecular Analysis

Environmental DNA (eDNA) monitoring analyzes DNA present in environmental samples (e.g., water, soil, air) to detect species and/or assess biodiversity. eDNA methods are commonly used to evaluate the presence of organisms of concern (e.g., special status species or invasive species) as well as to assess overall biodiversity of specific taxonomic groups (USGS 2013). eDNA sampling associated with bioassessments typically involves the field collection and/or filtration of a water sample. eDNA samples should be consistent with traditional water chemistry sampling, with care taken to collect samples prior to field crew entry into the stream or placement of any field equipment into the stream.

Notes on sterile technique: Avoid cross-contamination between samples. Contamination can result from a variety of factors, such as touching sterile gloves to dirty equipment, reusing forceps without sterilizing, and field gear coming in contact with sampling equipment. Refer to the California Molecular Methods Workgroup guidance for details (https://mywaterquality.ca.gov/monitoring_council/mmw.html).

Materials:

- Filtration apparatus, such as:
  - Vacuum manifold and filter funnel*
  - Flask, hand pump, and filter funnel*
- Filter, such as:
  - Stericup 0.45µ PES or MCE filter (Thermo Scientific 1450045)
  - Millipore MCE 0.45µ filter (HAWP04700)
- Preservation:
  - Preservation solution (bead solution, Qiagen #12855-50-BS) pre-loaded in 2ml labelled screwcap tube
  - No preservation solution needed if filter is frozen at -80°C immediately after collection.
- 500 ml or 1L bottle* or 1L WhirlPaks
- MilliQ water
- Nitrile gloves
- Tweezers/forceps*

** Items should be sterilized before use and between sampling sites to prevent cross-contamination. To sterilize, soak in acid wash (1% solution of hydrochloric or nitric acid) or bleach (final concentration 1-5%), rinse 3x in DI H2O, and autoclave (if possible).*

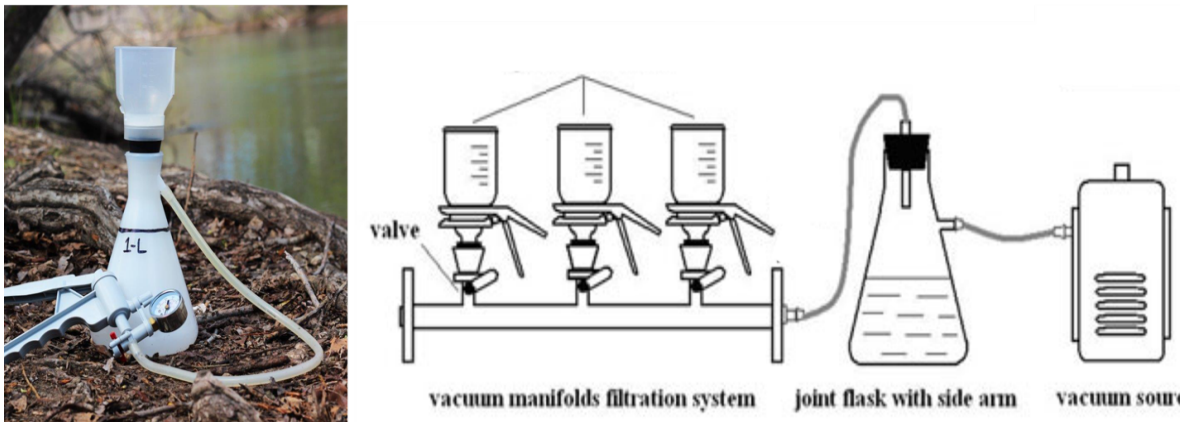

Figure 17. eDNA filtering options. Left: Hand-pump filtration units (cr. USGS). Right: vacuum manifold filtration set-up

1. **Sample collection:**
    a. Rinse sample bottle 3x with ~10ml of sample water; discard rinse water onshore or away from sampling site. Fill the bottle with 1L of sample water. If bottle numbers are limited, large WhirlPaks can be used for water collection instead.
    b. If you cannot filter immediately, place the sample on ice and in the dark for up to 6 hours.
2. **Filtering:**
    a. Place clean filter funnel on vacuum pump. Vigorously shake bottle with sample to homogenize. Pour sample into filter cup and cover. Turn on vacuum pump and filter.
    b. If the filter becomes clogged, record total volume of sample filtered on worksheet.
3. **After filtering:**
    a. When filtration is complete, let the pump run 5-10 more seconds to dry filter.
    b. To remove filter from the manifold, remove the filter funnel and gently fold filter using clean forceps, optimally folding three times to create triangle-shaped filter with filtrate protected on the inside of the triangle. Place filter in pre-labeled, pre-loaded 2ml screw cap tubes and cap. Filter must be submerged in preservation solution, shake vigorously if necessary.
    c. Keep tubes with filters on ice until able to be transported to -20°C or -80°C freezer. Samples at -20°C are stable for six months.
    d. Repeat all steps for as many replicates as requested (default = 3 replicates).
4. **Blanks:** Filter ~100ml of MilliQ water through a clean filter and preserve filter. Process one blank for each sampling day or site, whichever is greater.

Table 4. Example eDNA sample labels

Station code	Date	Replicate	Vol (L)
Notes	Collection time	Filtration time	Preservation solution (Y/N)

A10. Monitoring Stream Flow with Loggers

In certain situations, it may be useful to monitor stream flow at bioassessment sampling sites. For example, while natural stream intermittency has been documented to not impact bioassessment scoring in reference southern California streams using the California Stream Condition Index (Mazor et al. 2015, Rehn 2016), natural stream intermittency may result in naturally depressed CSCI scores in other parts of California (Mazor et al. in review). Anthropogenic activities can also result in modified flow regimes that can impact stream bioassessment. These include converting perennial streams to intermittent by dewatering for short or long durations and increasing or decreasing the magnitude and duration of peak flows (e.g., downstream of dams).

Methods for deploying and retaining water level logging equipment were developed using Onset branded equipment. Other manufacturers' equipment may require altering the size or shape of items described. Some devices are designed to record only the presence or absence of water, while others measure water depth using pressure sensors. For the latter, two loggers are necessary: one placed in the channel to record water depth (commonly referred to as the "wet" logger) and another placed on land to compensate for barometric pressure changes (the "dry" logger).

Materials

- Case, made from a section of plastic pipe.
- 3-foot concrete stake
- Stainless steel chain
- Clevis pins
- Cotter pins
- Shackle
- Pliers (Channel Lock style)
- Small sledgehammer
- Camera
- GPS
- Meter stick
- Compass
- HoboShuttle and all attachments
- HoboWare® software (update frequently)

Tracking Logger Data

Thorough record keeping is essential for tracking logger data. Serial numbers of loggers should be used as file names when launching devices. Pre-assigning loggers to locations with specific file names (e.g., "DeerCreekWet") can lead to confusion during data analysis if field crews accidentally switch loggers. Field crews should use a standardized data recording method for each visit to a data logger (Figure 21). Paper and electronic field forms are both acceptable.

Procedures for installing new water level loggers

1. Launch loggers using HoboWare® software prior to visiting site. Detailed instructions for launching loggers are included in HoboWare®. Details for using the shuttle are in the HOBO® Waterproof Shuttle (U-DTW-1) manual.
2. At site, bring all equipment listed except Shuttle.
3. Record basic site information (i.e., lat/long, StationCode, Site Name, Date) and the serial numbers of loggers being installed.
4. Select a location near the thalweg of the channel to install the logger. Avoid pools if possible since they may remain wet after all other flow has ceased. The location should be stable and able to withstand high flows. Locations immediately downstream of boulders have proven effective.
5. Install the concrete stake into the substrate of the stream using the small sledgehammer. Make sure the stake is embedded at least 2 feet in the substrate or until it feels very secure. Attempt to remove the stake by hand to test how secure it is. If the stake can be removed, try another spot. If there is no area in the stream that can retain the stake against one handed removal, consider not installing loggers as they may be difficult to retrieve.
6. Place the logger in the case and insert clevis pins through the holes top and bottom, being sure that the one clevis pins pass through a link of chain (Figure 18).

7. Slide the shackle over the top or around the stake and place the set pin through the shackle making sure the pin also goes through a link in the chain. Place a pin through holes in the stake above the shackle to insure it does not slide up and off. Bend one arm of each cotter pin to secure (Figure 19).
8. Measure and record depth from the bottom of the logger to the surface of the water. This will be used for indexing water level data.
9. Place large rocks to conceal and protect the loggers from people and high flows.
10. Record GPS coordinates (NAD 83) directly above the wet logger. Hold the GPS still and allow it to acquire several satellite signals. This can take a while in canyons. Coordinates should be as accurate as possible.
11. Take a series of photos of the logger's location. It is easiest to start at close range, then move back, panning out. When taking photos from farther away it is helpful to have someone else in frame pointing to the location or leave a larger bright object very near the logger for reference. Another option is to place flagging directly above the logger. Think about the likely direction someone will be approaching the site when taking photos.
12. In addition to taking photographs of the logger's location unique objects can be visual cues to the logger's location. Record their location using GPS, description and photograph. Measure the distance and compass bearing from the unique object to the water level logger. If future crews are having trouble finding loggers, this information will literally point them in the right direction.

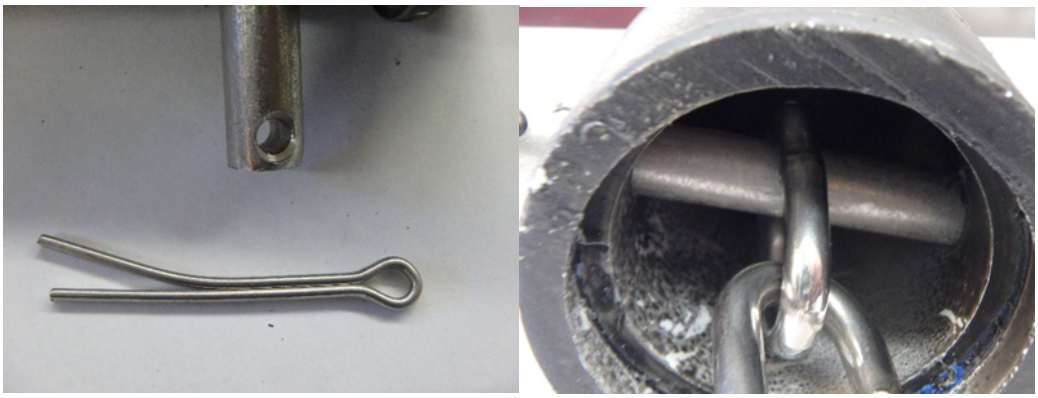

Figure 18. Clevis pin attachment to logger case, showing cotter pin (left) and chain link attachment to clevis pin (right).

Figure 19. Detail of shackle attachment to stake

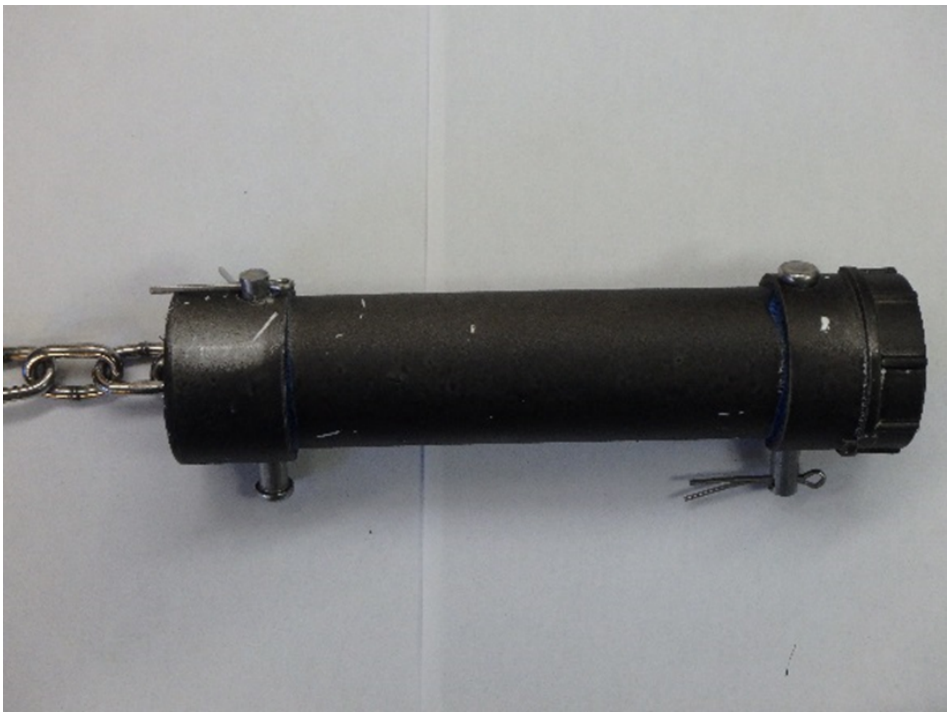

Figure 20. Competed logger case

Procedures for installing DRY logger:

1. Complete steps 1, 2 and 3 from above.
2. Choose a location to install the dry logger on the bank that is higher in elevation than bankfull or flood heights to ensure it will not get swept away during winter high flow events. Ideal locations are the base of large trees and/or under piles of rocks above the bank. The air logger should be hidden from sight.
3. Record GPS coordinates directly above the dry logger as you did for the wet logger.
4. Take photographs of the dry logger as you did for the wet logger.
5. If unique objects are used for locating the wet logger record their bearing and distance to the dry logger as well.

Procedures for reading dry and wet loggers

Bring all equipment, including shuttle and adapters, previous pictures, and completed logger worksheets.

Wet logger

1. Find the wet logger using the pictures and completed logger worksheets. Be sure to measure depth before retrieving the logger and after replacing.
2. Leave the stake in place. Remove the logger from the case.
3. Attach the appropriate coupler to the shuttle.
4. Read the logger with the shuttle by depressing the control arm to the translucent case. If there are any issues with the shuttle, refer to the HOBO® Waterproof Shuttle (U-DTW-1) Manual for troubleshooting details.
5. Record date, time, serial number and depth of logger. Take additional photographs of logger location. Over time, creek appearances change with the addition or loss of sediment and riparian growth.
6. Return the logger to the case and examine all materials for any corrosion or wear. If there is damage, replace with extra parts brought to the site.
7. Return the wet logger to the original location.
8. Measure placement depth

IF THE WET LOGGER IS MISSING: Replace the logger and case if the stake is still stable and in place. Ensure that all deployment measures are followed for wet and dry logger deployment and record new depths if necessary. If the stake is missing or removed do not replace any equipment. Take detailed notes and photos of the area. Note whether the stake was missing or still present/intact.

Dry logger

1. Locate the dry logger using the pictures and recorded notes from deployment visit.
2. Read the logger with the shuttle. If there are any issues with the shuttle, refer to the HOBO® Waterproof Shuttle (U-DTW-1) Manual for troubleshooting details.
3. Record date, time, serial number and depth of logger. Take additional photographs of logger location. Over time, creek appearances change with the addition or loss of sediment and riparian growth.
4. Return the air logger to the original location.

StationCode		Notes:
StationName		
Date		

Location

Wet Logger			Dry logger	
SN			SN	
Depth before moved				
Depth after placed				
Lat			Lat	
Long			Long	
Pic #			Pic #	

Object 1

Description			
Lat			
Long			
Pic #			
Distance to Wet logger		Distance to Dry logger	
Bearing to Wet logger		Bearing to Dry logger	

Object 2

Description			
Lat			
Long			
Pic #			
Distance to Wet logger		Distance to Dry logger	
Bearing to Wet logger		Bearing to Dry logger	

Figure 21. Data sheets for recording logger location and deployment information

A11. Collection of Bedded Sediment for Chemistry and Toxicity

Bioassessment sampling may also involve the collection of bedded sediment for additional chemistry and toxicity analysis, particularly for those pollutants that may impact the benthos and readily bind to sediments (e.g., pyrethroid pesticides). Sampling sediments for chemistry and toxicity should be done in accordance with the methods prescribed by the SWAMP Stream Pollution Trends Monitoring Program (see SPOT QAPP, SWAMP 2021). Generally, multiple sediment sampling locations along the bioassessment reach should be collected and composited, with sampling targeting areas with the finest newly deposited sediment. Sampling efforts must take care not to disturb potential BMI, algae, or physical habitat sampling locations. Sampling ideally should occur alongside bioassessment sampling efforts but may occur prior to or after depending on site-specific conditions. For sites with limited fine-grained sediments, coarse-grained sediments can still be sampled for analysis (see Phillips 2021).

A12. Collecting Permits Required for Sampling of Benthic Macroinvertebrates

Introduction

As specified in California Code of Regulations Title 14, Section 650 (Fish and Game Code § 1002), it is unlawful for any person or entity to take and/or possess live or dead wildlife, or wildlife parts, except as authorized by a permit issued by the California Department of Fish and Wildlife (CDFW). Individuals or entities wanting to collect wildlife for scientific, educational, or propagation purposes must apply for and obtain an approved Scientific Collecting Permit (SCP).

Any individual or organization collecting benthic macroinvertebrates using SWAMP protocol must obtain a SCP. Permits are valid for 3 years once approved and are non-transferrable. Projects solely conducting bioassessment are required to obtain a General Use Permit-Inland Fisheries. If the project also includes the take of other species, such as fish, amphibians, or listed species (e.g., vernal pool fairy shrimp, California freshwater shrimp), a separate Specific Use Permit may also be required. This guidance document only covers information on General Use permits. **The application to apply for is the CDFW General Use- SCP Application (Inland Fisheries), DFW 1379GF**.

Permit timeline

Applications may take over 100 days for review (Figure 22). Following receipt of an application, CDFW will determine whether an application is complete within 40 calendar days from the date the application fee clears payment. If the application is determined to be incomplete, CDFW will notify the applicant in writing of any deficiencies in the application or information provided. The applicant shall provide any outstanding information or related documents to the department within 30 calendar days of the notification, or the application may be denied. When the application is determined to be complete, the department will approve or deny the permit within 60 calendar days of that determination.

Contacting the Aquatic Bioassessment Lab

Prior to submitting the SCP, applicants are encouraged to contact CDFW's Aquatic Bioassessment Lab (ABL) at bioassessment@wildlife.ca.gov . In your email inquiry, please provide a copy of the documents prepared for your SCP, such as a project description, summary of staff experience with bioassessment and/or aquatic fieldwork, and a list of proposed sampling locations. ABL staff may provide prompt, valuable feedback to applicants prior to application submission that could prevent future delay or denial of the SCP.

Communication with the ABL is an opportunity for applicants to ask professionally trained staff questions and usually shortens the overall SCP application process. It also allows ABL to track where data are being collected throughout California and coordinate sampling activities. Based upon the information provided, ABL may request a follow-up meeting. ABL may also suggest

that an ABL staff member assist in project implementation to ensure the Bioassessment Standard Operating Procedures are employed correctly. Failure to communicate with the ABL may result in delay, revocation of the SCP permit and/or flagging of their data in CEDEN.

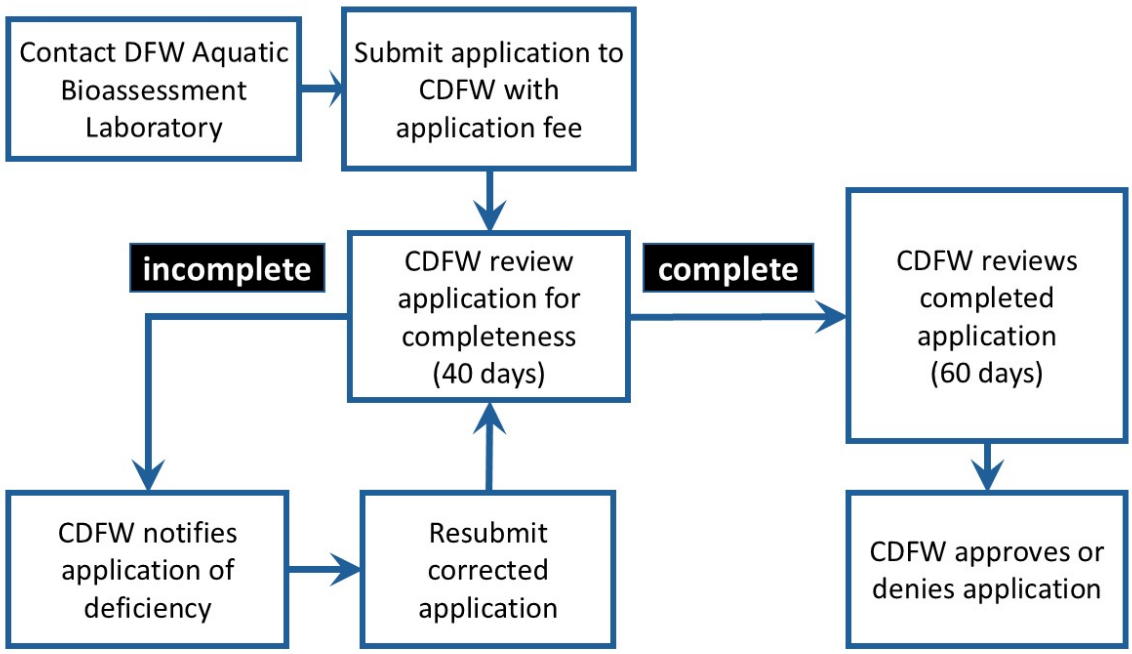

Figure 22. SCP application process and timeline.

Applying for a SCP

Information on how to apply for SCPs and permit fees are available at the website: https://wildlife.ca.gov/Licensing/Scientific-Collecting . This document provides brief pointers on how to apply for a General Use-Inland Fisheries permits only. All permits must be submitted online through the Scientific Collecting Permit Portal (https://scpapp.wildlife.ca.gov/).

Any individual listed on the permit application (Principal investigator and any Authorized individuals) must create an individual profile through the SCP portal. They will be required to provide the following:

- A Statement of Qualifications (SOQ) that quantifies and describes experience with requested wildlife and/or similar wildlife
- A resume or curriculum vitae that describes the educational background and wildlife-related experience, including a list of any relevant publications
- Contact information for two (2) references (for example, other Permit holders or experts) who can verify the applicant's experience with requested wildlife.

The information provided in profiles is reviewed during permit review. Once the permit and the listed individuals are approved by CDFW, they are authorized to supervise any non-listed individuals who may assist with field collections. Only one listed individual needs to be present while Benthic Macroinvertebrate (BMI) sampling takes place.

Applicants will be required to provide the following information when submitting the SCP application:

- The latitudes and longitudes of the target sampling locations in decimal degree format. Since applicants are required to submit applications before site evaluations are completed, it is recommended that applicants list more sampling locations than anticipated to make up for potential site losses and/or project changes. It is more difficult to go through the amendment process to add different sites than those previously proposed.
- What types of work or activities are expected to be conducted (e.g., research, surveys and monitoring for permit compliance, etc.). Please ensure that you will be using the most current Bioassessment SOP if you are submitting data to CEDEN.
- Information about any study(ies) or project(s) collaborated on with other researchers
- If applicable, what species are being targeted, or what species could be encountered with authorized methods, if known. You do not need to list each benthic macroinvertebrate species you expect to encounter or the number of individuals. Instead, you may list the Standard Level of Taxonomic Effort that will be employed (e.g., "SAFIT Level 2")
- What measures will be taken to minimize the incidental harm or death to non-target wildlife.

After permit approval, investigators with an approved SCP are expected to follow all conditions outlined in the permit, including:

- Adhering to CDFW's Aquatic Invasive Species Decontamination Protocol
- Submitting a Notification of Fieldwork (DFW1379b) at least 36 hours prior to conducting Bioassessment activities
- Submitting a quarterly Mandatory Wildlife Report
- Submitting a Mandatory Wildlife Report within 30 days of permit expiration or upon submission of renewal application.

BMI samples may be transported to other entities or individuals specified in the SCP or with CDFW permission. Samples should be accompanied by a Chain of Custody Form that includes the name, address, and permit number of the individual holding the SCP.

Permit Updates and Amendments

Applications to amend an existing permit are subject to the same review procedures and timeline as new General Use SCP applications. Updating affiliation information or removing Authorized Individuals from a permit do not require an amendment. However, activities such as changing the PI, adding more authorized individuals, and adding sampling locations to a permit would require an amendment.

A13. References

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