

Supplemental Guidance for the SWAMP Bioassessment Field Protocol

March 2016

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1. Field Guidance

1.1 Labor division for efficiency in the field

To maximize efficiency in the field, a workable division of labor is as follows (where Team "1" and "2" could consist of one or more people): Team 1 collects water chemistry samples and data, and measures discharge along a "flow transect" (as long as a good one can be located near, but downstream of, Transect A), while Team 2 lays out the transects for the sampling reach (remember: those setting up the transects must stay out of the stream). Then Team 1 begins collecting biota (one person focuses on BMIs and the other focuses on algae). Once Team 1 is done collecting at Transect A, Team 2 begins collecting PHab data at that Transect, then follows Team 1 as they proceed from transect to transect. Team 2 collects slope and sinuosity data while Team 1 processes the biotic samples.

1.2 Memo: Amendment to SWAMP Interim Guidance on Quality Assurance for SWAMP Bioassessments

Surface Water Ambient Monitoring Program Quality Assurance Program Memorandum *(Approved by the Interim SWAMP Coordinator)*

To: SWAMP Round Table

From: Beverly H. van Buuren, SWAMP Quality Assurance Officer and
Peter R. Ode, SWAMP Bioassessment Lead Scientist

Date: September 17, 2008

Re: Ammendment to SWAMP Interim Guidance on Quality Assurance for
SWAMP Bioassessments

On May 21, 2007, the SWAMP Quality Assurance (QA) Officer and SWAMP Bioassessment Coordinator issued interim guidance for all SWAMP-funded bioassessment projects defining QA requirements for several key elements of SWAMP's Bioassessment Program. This memorandum, effective September 17, 2008, amends the May 2007 document with updated guidance on macroinvertebrate sample collection.

If you have any questions regarding this guidance, please contact the SWAMP QA Officer, Beverly H. van Buuren at (206) 297-1378, or via email at bvanbuuren@mlml.calstate.edu or the SWAMP Bioassessment Lead Scientist, Peter Ode, at (916) 358-0316, or via email at pode@ospr.dfg.ca.gov.

Macroinvertebrate Sample Collection

The previous SWAMP policy (Ode 2007, <http://www.swrcb.ca.gov/swamp/biocalstreams.html>) was to collect two field methods under most circumstances: a targeted habitat method (“targeted riffle composite”, or TRC) and a systematic representation of multiple habitats in a reach (“reachwide benthos”, or RWB). Recent published and unpublished analyses (Gerth and Herlihy 2007, Rehn et al. 2007, Mazor et al. 2008) provide evidence that RWB and TRC methods can produce generally comparable results across a broad range of settings within California. Based on these analyses, SWAMP is now adopting a single field method in MOST streams statewide. However, because the comparability data are equivocal in some settings, SWAMP policy includes two specific exceptions.

SWAMP-funded bioassessments shall adhere to the following guidelines:

1. In most regions of the state (see exceptions listed in 2 and 3 below), SWAMP funded programs shall collect bioassessment samples using the RWB method (sometimes referred to as multi-habitat or MH).
2. SWAMP programs shall continue to collect both TRC and RWB samples in environmental settings where method comparability results are equivocal. While SWAMP will define specific criteria for these settings in a future guidance memo, the current interim policy is to collect both methods at high elevation pool-dominated streams (>2000 m elevation, >80% pool reaches, boulder cascades).
3. SWAMP programs shall employ a modified version of the RWB in large, low-gradient streams dominated by sandy bottoms (e.g., low gradient coastal streams, large Central Valley streams). The modification is to collect subsamples at 0%, 50%, and 100% of stream width instead of 25%, 50%, and 75% of stream width) to ensure collection of marginal habitats.
4. SWAMP programs may choose (at their discretion) to continue to collect a second method at any site where additional sampling data is likely to produce complementary information.

The SWAMP Bioassessment Program will establish a Technical Advisory Committee (TAC) to provide additional guidance on the following topics:

1. *Refined criteria for defining when the exceptions described in 2 and 3 above should be applied*
2. *Analytical considerations for combining RWB and TRC datasets or applying data collected with one method to an indicator (e.g., index of biotic integrity (IBI) or observed/expected (O/E) model) derived from another method*

References

- Gerth, W.J., and A.T. Herlihy. 2006. The effect of sampling different habitat types in regional macroinvertebrate bioassessment surveys. *Journal of the North American Benthological Society* 25:501-512.
- Ode, P.R. 2007. Standard operating procedures for collecting macroinvertebrate samples and associated physical and chemical data for ambient bioassessments in California. California State Water Resources Control Board Surface Water Ambient Monitoring Program (SWAMP) Bioassessment SOP 001.
- Rehn, A.C., P.R. Ode and C.P. Hawkins. 2007. Comparisons of targeted-riffle and reach-wide benthic macroinvertebrate samples: implications for data sharing in stream-condition assessments. *Journal of the North American Benthological Society* 26: 332-348.
- Mazor, R.D, K. Schiff, A.C. Rehn, P.R. Ode and K. Ritter. 2008. Bioassessment tools in novel habitats: An evaluation of indices and sampling methods in low-gradient streams in California. *In preparation*.

1.3 Note for delineating BMI sample area

If desired, a 1ft² wire frame can be placed on the stream bottom in front of the D-frame net to delineate the area to be sampled for BMIs, but it is often sufficient to visually estimate the prescribed sampling area (based on the net dimensions) in order to keep sampling effort consistent between transects and between sites.

1.4 Applying the Basic SOP and recognizing situations in which modifications to procedures may be made

The SOP is geared toward ambient assessment (i.e., monitoring a large number of probabilistically selected sites in order to arrive at regional condition estimate with a known level of confidence). Other applications, such as site-specific compliance monitoring, may benefit from augmentation to aspects of this protocol. For example, if the monitoring goal is to determine, with high precision, the amount of algal biomass in a particular stream reach in order to understand nutrient impacts, a higher spatial density of algae sampling (such as 33, instead of 11, subsamples to create the composite sample) may be desirable.

Although individual modules can be added or eliminated from the SOP to reflect project objectives requiring different levels of intensity for characterizing the chemical and physical habitat data, a core set of PHab measures is recommended to accompany all bioassessment samples. The following “**Basic**” list of PHab measurements reflects the minimum amount of physical and chemical data that should be taken along with any ambient bioassessment sample.

- Layout of reach, marking transects, recording GPS coordinates
- Temperature, pH, specific conductance, salinity, DO, alkalinity

- Notable field conditions
- Wetted Width
- Stream Shading
- Bank Stability
- Percent Algal Cover
- Flow Habitat Delineation
- Slope (%): conducted at *reach* scale (i.e., a *single* slope measurement for the *entire* reach, as opposed to one for each transect as described in the SOP)
- Photo documentation

However, note that the "Full" PHab characterization (i.e., the full suite of Modules described in the SOP) represents the data that should be collected with most professional-level bioassessment sampling efforts (and Full PHab is required for SWAMP regional monitoring programs). A single set of field sheets is used in conjunction with this SOP, regardless of whether the Basic or Full level of effort for the PHab measures and water chemistry is carried out. For the Basic level, fill in "NR" (for "Not Recorded") for the PHab measures that are not taken.

When there are some dry transects in the sampling reach, bioassessment practices for the two assemblages (BMIs and algae) are different. In the case of algae, the crew should collect the subsample at the closest wet place (i.e., the goal is to achieve 11 subsamples for the composite, even if sampling locations must be shifted to some degree). For BMIs, crews should skip the dry transect and make a note of how many subsamples were ultimately collected (i.e., some number < 11).

Note that it is acceptable to sample biota in natural pools, because a reach-wide benthos (RWB) method is being used to collect samples. If it is necessary to shorten the sampling reach, note that if it is < 100 m long, the "representativeness" of the sample may be compromised.

1.5 Information resources for avoiding introduction of invasive species and pathogens into streams

The following is an adaptation of an excerpt taken from an EMAP-based Quality Assurance Project Plan developed by the California Department of Fish and Game Aquatic Bioassessment Laboratory (2008).

Organisms of concern in the U.S. include, but may not be limited to, Eurasian watermilfoil (*Myriophyllum spicatum*), New Zealand mud snail (*Potamopyrgus antipodarum*), zebra mussel (*Dreissena polymorpha*), *Myxobolus cerebralis* (the sporozoan parasite that causes salmonid whirling disease), and *Batrachochytrium dendrobatidis* (a chytrid fungus that threatens amphibian populations).

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 be 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 (e.g., Schisler et al., 2008).

There are several online sources of information regarding invasive species, including information on cleaning and disinfecting gear, such as the Whirling Disease Foundation (www.whirling-disease.org), the USDA Forest Service (*Preventing Accidental Introductions of Freshwater Invasive Species*, available from http://www.fs.fed.us/invasivespecies/documents/Aquatic_is_prevention.pdf), and the California Department of Fish and Wildlife (Hosea and Finlayson 2005 and <https://www.dfg.ca.gov/invasives/>). 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 *Protect Your Waters* website (<http://www.protectyourwaters.net/hitchhikers>) that is co-sponsored by the U.S. Fish and Wildlife Service. The California State Water Resources Control Board Aquatic Invasive Species website (http://www.swrcb.ca.gov/water_issues/programs/swamp/ais/) should also be consulted regularly for updates.

YouTube videos are also available for viewing. These include the Invasive Species Playlist:

<https://www.youtube.com/playlist?list=PLMSa5d-iIl6Nyoo0daTJLeXOan3dj7OCl>

and Cleaning and Treating Water Quality Monitoring Field Gear for Freshwater Aquatic Invasive Species:

<https://www.youtube.com/watch?v=YMI4hgeMMAw&index=2&list=PLMSa5diIl6Nyoo0daTJLeXOan3dj7OCl>

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

<http://seagrant.oregonstate.edu/sgpubs/onlinepubs/g10001.pdf>

The following procedures for decontamination of equipment to prevent the spread of chytrid fungus is 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 USFS Region 4, which has an excellent summary table of viable methods for multiple types of AIS (USFS 2013;

http://www.fs.fed.us/r4/resources/aquatic/guidelines/2011techguidelines_fire_AIS.pdf).

A 5% 1 Quat 128 solution requires 30 seconds of soak time to kill chytrid fungus.

However, New Zealand mud snail (NZMS), although unlikely to be found in wetlands, could nonetheless be present, and requires 10 minutes of soak time at the same concentration. Because lakes and reservoirs may have NZMS and be sampled with this protocol, a 10 min soak time for all gear in 5% Quat 128 solution or similar Quat-related product should prevent movement of all potential invasive species, including aquatic diseases. If a non-chemical solution is preferred, waders can be fully cleaned of mud and debris, then exposed to sun for three hours and allowed to rest completely dry for 48 hrs. Please refer to the USFS guidance for specifics. *Note: freezing gear alone will not kill chytrid fungus, so this is not an acceptable method of decontamination for wetland sampling.*

An alternative method for decontaminating gear for chytrid fungus is via the use of a bleach solution, along with freezing to kill any NZMS that may be present. In consideration of the difficult logistics of field decontamination, a potential approach would be to have 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 (USFS 2013; http://www.fs.fed.us/r4/resources/aquatic/guidelines/2011techguidelines_fire_AIS.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.

References

- Hosea, R.C. and B. Finlayson. 2005. Controlling the spread of New Zealand mudsnails of wading gear. California Department of Fish and Game, Office of Spill Prevention and Response, Administrative Report 2005-02, Sacramento.
- Schisler, G.J., N.K.M. Vieira, and P.G. Walker. 2008. Application of Household Disinfectants to Control New Zealand Mudsnails. *North American Journal of Fisheries Management* 28:1171-1176.

¹ Test strips are available to test the concentration.

1.6 Targeted riffle composite procedure

The Targeted Riffle Composite (TRC) method is designed for sampling BMIs in wadeable streams that contain fast-water (riffle/run) habitats and is not appropriate for water bodies without fastwater habitats. The Regional Board protocol should be used in these situations. 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 Regional Board protocol should be applied in these situations.*

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. 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.*

Sampling Locations – Selecting Habitat Units

A TRC sample is a composite of eight individual kick samples of 1 ft² (0.09 m²) 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 1). Unless you are sampling in small streams, try to avoid very small riffle units (i.e., <5 ft²). 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.

Flow Habitat Type	Description
Cascades	Short, high gradient drop in stream bed elevation often accompanied by boulders and considerable turbulence
Falls	High gradient drop in elevation of the stream bed associated with an abrupt change in the bedrock
Rapids	Sections of stream with swiftly flowing water and considerable surface turbulence. Rapids tend to have larger substrate sizes than riffles
Riffles	Shallow sections where the water flows over coarse stream bed particles that create mild to moderate surface turbulence
Step-Runs	A series of runs that are separated by short riffles or flow obstructions that cause discontinuous breaks in slope
Runs	Long, relatively straight, low-gradient sections without flow obstructions. The stream bed is typically even and the water flows faster than it does in a pool
Glides	A section of stream with little or no turbulence, but faster velocity than pools
Pools	A reach of stream that is characterized by deep, low-velocity water and a smooth surface

Table 1. Common habitat types in stream channels, arranged in decreasing order of energy

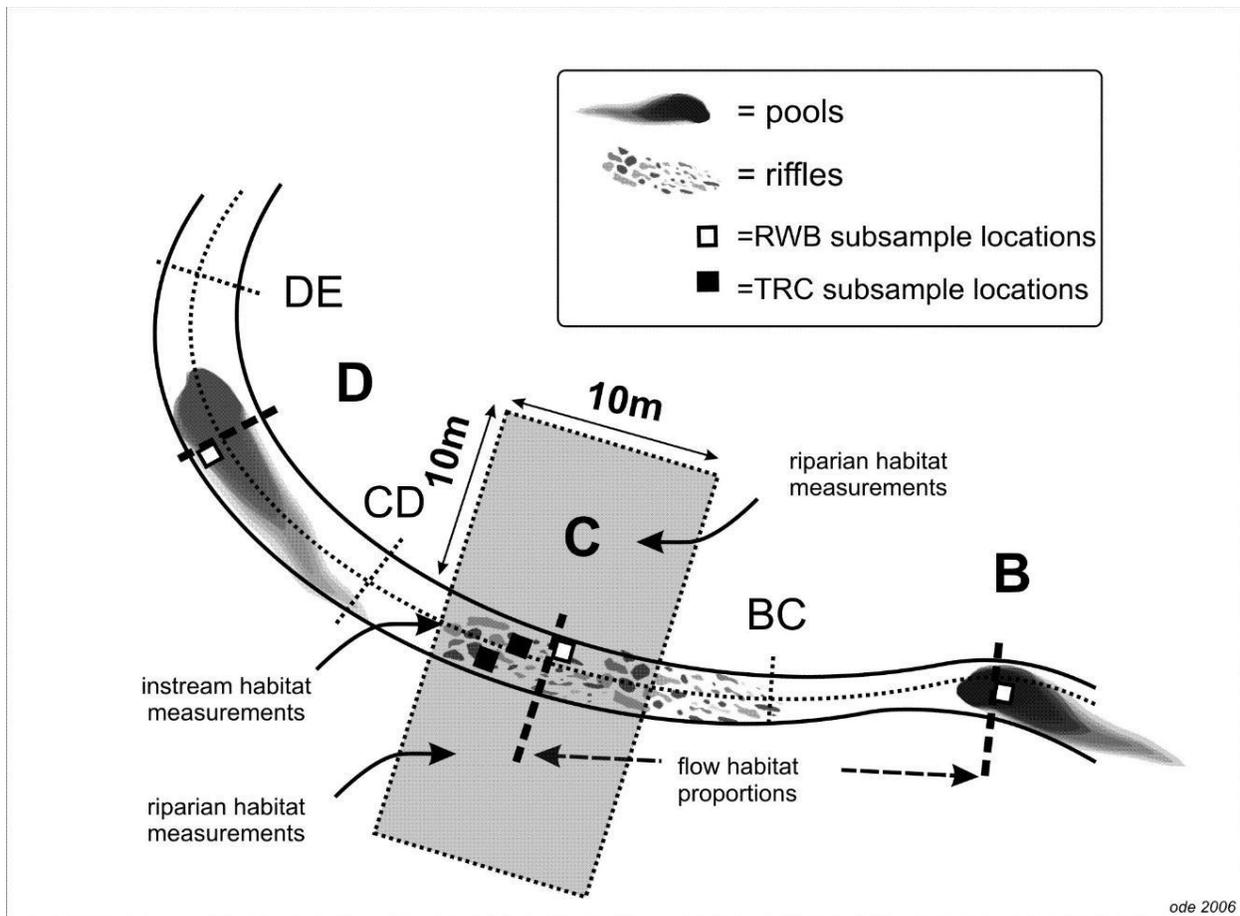


Figure 1. Section of the standard reach expanded from Figure 2 (in the SOP) showing the appropriate positions for collecting benthic macroinvertebrate samples, instream and riparian habitat measurements and flow habitat proportion measurements.

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 ft² (0.09 m²) 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 1ft² (0.09 m²). 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 insects. As you are disturbing the plot, let the water current carry all loosened material into the net.

Note 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.

Note 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 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.

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 1ft² (0.09 m²) of benthos.

TRC-Step 8. PROCEED to Section IIc. Filling and Labeling BMI Sample Jars.

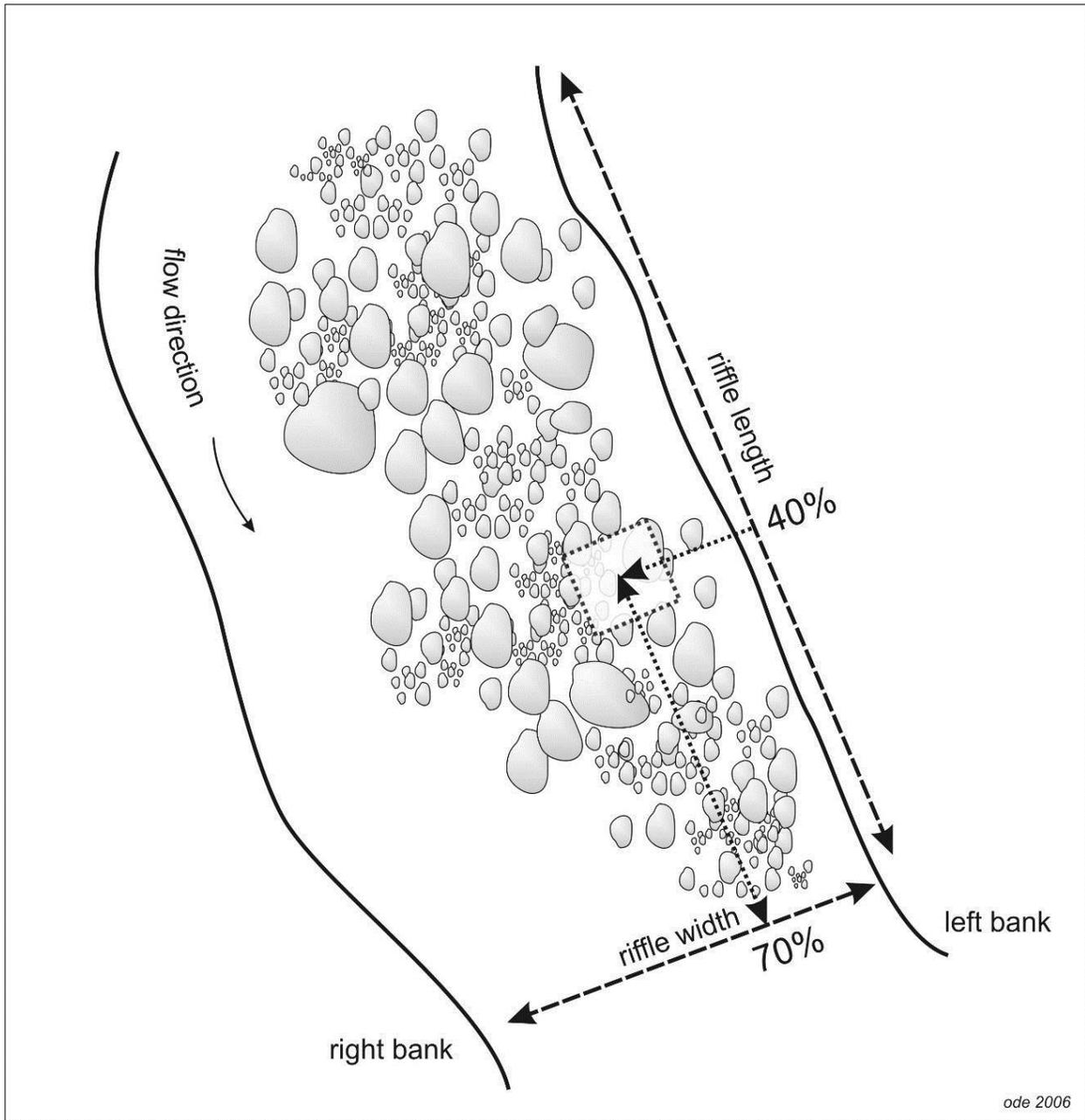


Figure 2. Example showing the method for selecting a subsampling position within a selected riffle under the TRC method. In this example, the random numbers 4 and 7 were selected.

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 for methods for BMI sampling use 500- μ m 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 1.

1.7 Determining bankfull position in streams

BANKFULL: WHAT IT IS AND HOW TO LOCATE IT

WHY BANKFULL?

Several procedures in this manual require you to locate what is known as the “bankfull channel edge,” or more simply as “bankfull.” This is an important concept in understanding the workings of a stream.

HOW DOES A “BANKFULL” GET CREATED?

Most lower portions of streams in our area are alluvial, meaning that they create their own channels by moving sediment from the surrounding hillslopes and from the stream channel itself. Major episodes of such movement occur during floods and are called “channelforming 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 1.5 years (which we call 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. So usually, what you’ll see if you look at 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 site.

HOW SHOULD I LOCATE BANKFULL?

The following method was found by the TFW program to maximize data precision and minimize bias toward over- or under-estimation of bankfull elevation:

1. Start on the bank with the best bankfull indicators.
 - a. Move up the bank from the channel, observing the indicators listed above. When you reach a point at which you're no longer 100% sure that you're below bankfull, mark that level with a flag or stick.
 - b. Then walk up to what is clearly dry land, and walk around, observing indicators and moving back toward the bankfull edge. When you're no longer 100% confident that you're above bankfull, mark that point.
 - c. Reassess the indicators and your confidence levels, and consult with your fellow samplers, and make adjustments as needed.
 - d. The bankfull channel edge is at the elevation point midway between these two points.
2. Now follow the same procedure on the other bank. If it is not possible to accurately identify the bankfull level on that bank (which often happens on the outside bank of a meander bend), locate it using a level line from the bankfull point on the first bank.
(Also referenced for this section: Harrelson et al., 1994.)¹

Identifying the Boundaries of the Bankfull Channel

To measure bankfull width and depth, you must first determine the edge of the bankfull channel. Unfortunately, the boundaries of the bankfull channel are not always easy to identify. Geomorphologists have used many methods to delineate the bankfull channel. None are without shortcomings, and the most accurate methods are not feasible for stream surveys on remote and engaged stream reaches because they require long-term discharge records or the use of surveying techniques (Williams, 1978).

The TFW Ambient Monitoring Program uses a combination of indicators developed by Dunne and Leopold (1978) to delineate the bankfull channels. The indicators include floodplain level, the shape of the bank, and changes in vegetation.

Floodplain indicators- In channels with natural (un-diked) riparian areas and a low, flat flood-plain, the boundary of the bankfull channel corresponds with the top of the low bank between the active channel and the floodplain. The floodplain must be frequently flooded, i.e., during floods with a recurrence interval of approximately 1.5 - 2 years.

In many streams in forested parts of the state, frequently inundated floodplains are often absent, particularly when the channel is confined between steep hillslopes or is incised into an elevated terrace deposit that is not frequently flooded. This indicator is also not appropriate for streams that have been artificially diked or channelized.

¹ Field crews may also want to view this instructional video: <http://www.youtube.com/watch?v=UuS7H2NxJIM>. Often, the bankfull position is easier to interpret from one bank than from the other; in these cases, it is easiest to infer the opposite bank's bankfull position by projecting a level line across the channel. Additionally, height can be verified by measuring the height from both edges of the wetted channel to the bankfull height (i.e., these heights should be equal).

Vegetative indicators- The bankfull channel boundary is often marked by a distinct demarcation line in the vegetation between lower areas that are either bare or have aquatic vegetation, and higher areas vegetated with perennial vegetation such as shrubs, grasses or trees. In boulder or bedrock confined channels, it may be marked by the line between bare rock and moss. Unfortunately, the vegetation line changes over time, retreating due to disturbance during large peak flow events, and advancing during periods between floods.

Identifying the bankfull channel boundary using vegetative indicators requires caution. The vegetation line can be deceptively low when moisture-tolerant species are present. Reed canary-grass, willow and sedges are examples of plants that may actively invade and colonize areas within the bankfull channel. When using vegetative indicators, use only perennial vegetation greater than 1 meter in height of species that do not invade the active channel.

Other situations- Sometimes it may be possible to identify the height of the bankfull channel on one side of the channel but not the other. For example, this occurs when there is a low floodplain with vegetative indicators on one side of the stream and a steep, eroding bank on the other. In these cases, extend a level line horizontally across the channel from the side with good indicators to determine bankfull height on the side lacking indicators.

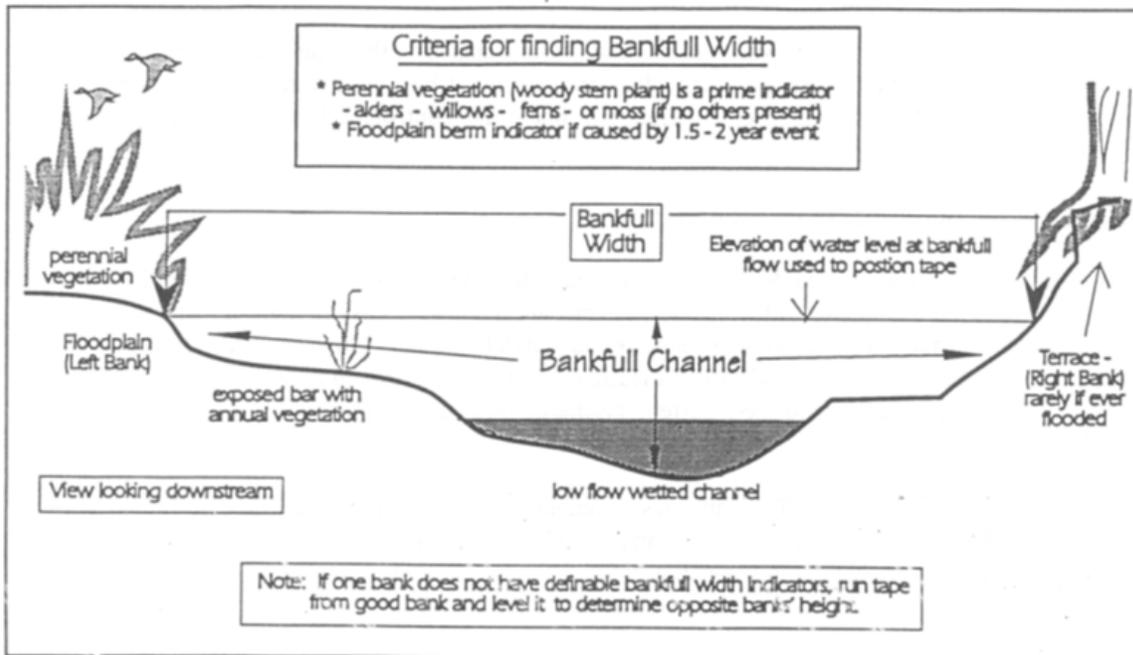


Figure 4. Determining the bankfull width of channels.

One of the most difficult situations is encountered in stream reaches where large gravel bars have been deposited by floods. It can be very difficult to determine if the tops of newly deposited bars protrude above the level of the bankfull channel. Vegetative indicators are 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 other cases, physical obstructions such as debris jams, undercut banks, or complete lack of indicators may make determination or measurement of bankfull dimensions impossible at the reference point. In these cases, take the measurement at the nearest place where it is feasible.

Taking Bankfull Width and Depth Measurements

To measure bankfull width, securely attach the end of the fiberglass tape measure at one boundary of the bankfull channel. Extend the tape across the channel to the other boundary of the bankfull channel. This distance is the bankfull width. If a side-channel is present, add the bankfull width of the side-channel to that of the main channel.

While the tape is stretched between these two points, determine the average bankfull depth. Bankfull depth measurements are taken at regular intervals across the stream channel. The number of measurements, and distance between them, depends on the width of the channel (Figure 4). Take

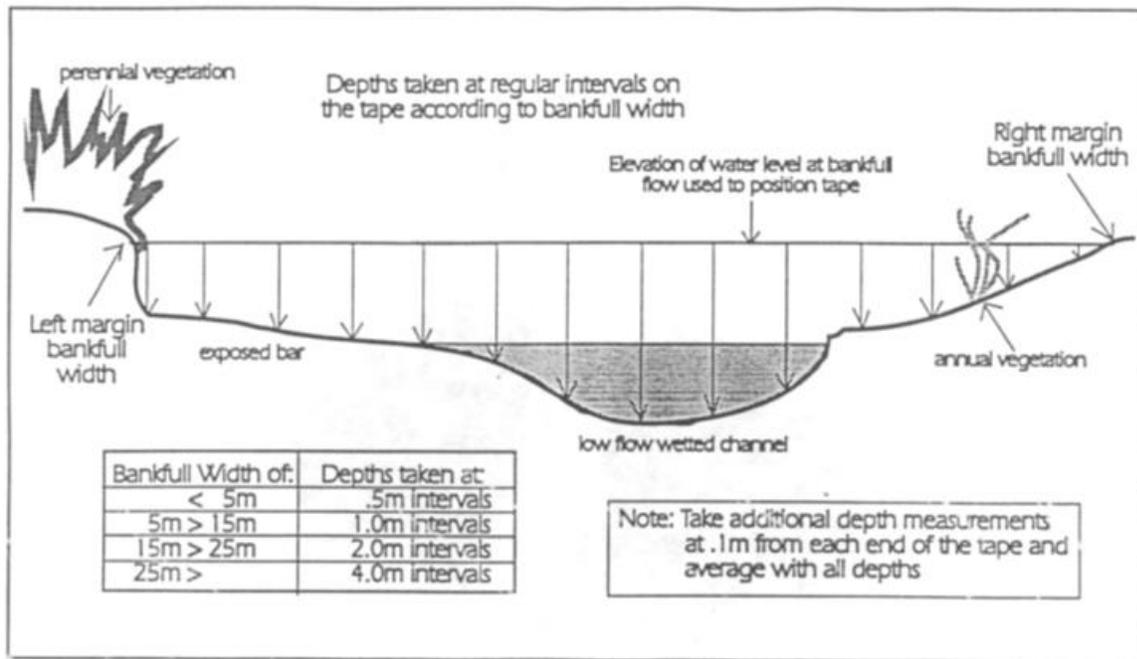


Figure 5. Measuring bankfull depth.

measurements at 0.5 meter intervals in channels less than 5 meters in width, at 1 meter intervals in channels between 5 and 15 meters in width, at 2 meter intervals for channels between 15 and 25 meters, and every 4 meters in channels greater than 25 meters in width. In addition, take an initial measurement 0.1 meter out from the starting point, and 0.1 meter before the endpoint.

Bankfull depth is the distance from the channel bed to the estimated water surface elevation at bankfull flow, represented by a tape stretched horizontally between the bankfull boundaries. The depth of water at the time of the survey, or its absence, does not affect this measurement. The sum of all depth measurements are then divided by the number of measurements taken to compute average bankfull depth.

Canopy Closure Measurement

Canopy closure measurements are taken at every reference point. To measure canopy closure, stand in the middle of the wetted portion of the channel and take four readings with the densiometer. Begin with a reading facing directly downstream. Turn clockwise 90 degrees and take a reading facing the right bank. Turn another 90 degrees clockwise and take a reading facing upstream, and finally turn clockwise another 90 degrees and take a reading facing the left bank.

To take a densiometer reading, hold the densiometer 12-18" in front of you at elbow height. Use the circular bubble-level to ensure that it is level. Look down on the surface of the densiometer, which has 24 squares etched into its reflective face. The reflection of your head should be just outside the grid. Imagine that each square is sub-divided into four additional squares, so that there are 96 smaller quarter-squares. Envision a dot in the center of each quarter-square. Count the total number.

1.8 Recommendations for Preparing for Fieldwork

- Have in mind at least three sites to visit per day (target two, but plan for at least one additional site as a backup if one of the first two sites is not useable.) Sites should be safe to sample and legally accessible. The time required to access the sampling sites should be a consideration in planning which sites to visit, in order to ensure that sample holding times can be met
- Use the equipment checklist provided below to make sure all necessary supplies are brought along.
- Check with contract laboratory on 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 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 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).

LIST OF SUPPLIES

The first column indicates what 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; “C” = chlorophyll *a* sampling; “A” = AFDM sampling

Table 2. List of Supplies

Needed for:	Item	Quantity / Site	Specifications, Comments
G	Sampling SOP (this document)	1/person	
G	Equipment decontamination supplies		
G	Hip or chest waders, or wading boots/shoes (not felt-soled)	at least 1 pair/person	
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	Add a 150-m scale line to aerials adjacent to stream
G	Thomas Guide, 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	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 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	
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)	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

Needed for:	Item	Quantity / Site	Specifications, Comments
P	10-sided die or random number table (if no digital watch available)	1	For selecting locations to place net for TRC sampling
P	Stadia rod	1	
P	Marked ski pole (or waterproof meter stick)	1	Mark pole with cm graduations to measure water depth during pebble count
P	Clinometer	1	
P	Autolevel and tripod	1	Required 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	Flagging tape	1 strip	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 large, heavy washers each tied with a strip of flagging tape	21 total	Two colors; label with main transect (11 ct.) and inter-transect (10 ct.) names
P	Small/slender rod with 1, 5, and 20 mm marks	1	For measuring microalgal thickness
P	Rangefinder & spare batteries	1	
P	Fresh orange peel OR plastic film canister partially full of water OR ice cube	1	Use as neutrally buoyant object
P	Small metric ruler or gravelometer for substrate measurements	1	
P, S	Algae viewing bucket (optional)	1	
B	D-frame kick net (fitted with 500- μ m mesh bag)	1	
B	Standard #35 sieve (500- μ m mesh)	1	
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	

Needed for:	Item	Quantity / Site	Specifications, Comments
B	Large spill tray	1	Used when transferring the BMIs from the D-frame net to the sample jar in order to avoid any 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		
D, S, C, A	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 <i>a</i> analysis
D, S, C, A	Scrubbing brush or scouring pad to clean dish tub or bucket, etc.	1	
D, S, C, A	Composite sample receiving bottle (wide-mouth HDPE jar with cap, 1 L)	1	Fisher 05-719-239
D, S, C, A	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	Bottle brush to clean graduated cylinders, etc.	1 sm, 1 lg	
D, S, C, A	PVC delimitter, 12.6 cm ² area	1	
D, S, C, A	Masonry trowel (flat, <i>pointed</i> , with a surface area > 12.6 cm ²)	1	
D, S, C, A	Rubber delimitter, 12.6 cm ² area	1	
D, S, C, A	Toothbrush, firm-bristled	1	
D, S, C, A	Syringe scrubber, 60 mL syringe, 5.3 cm ² area	1	
D, S, C, A	White (non-pigmented) scrubbing-pad circles	11 per replicate	
D, S, C, A	Tally meter (optional)	1	Ben Meadows 9JB-102385
D, S, C, A	Scissors	1	
D, S, C, A	Wash bottles	2	Label bottles with “stream water”, and “DI water”
D, S, C, A	Exacto™ or Swiss-army-style knife	1	
D, S, C, A	Sample labels (printed on waterproof paper)	4 per replicate	
D, S, C, A	Clear plastic tape, 5 cm wide	Length of ~20 cm per replicate	
D, S, C, A	Ice chest with wet ice	1 (2 preferred if multiple sites to be sampled)	
D, S, C, A	Fisherman’s vest (optional)	1	
D, S, C, A	Tarp, plastic, clean	1	To cover the ground at the algae processing station

Needed for:	Item	Quantity / Site	Specifications, Comments
D, C, A	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	5% formalin solution	10 mL per replicate	
D	Formalin-resistant gloves	1 pair	
D	Safety goggles or face shield	1	
D	Small syringe or bulb pipette	1	
D	Vermiculite packing material	as needed	
S	Turkey baster	1	
S (see note)	20% glutaraldehyde solution (<i>to be dispensed by trained individual using a laboratory fume hood, and wearing appropriate safety gear</i>)	5 mL per replicate	Note: glutaraldehyde could be added by taxonomy lab, with prior notification
S	Calculator	1	
S	Small metric ruler (waterproof)	1	
S	Small Ziploc bag	1	
S, C, A	Whirl-Pak bag, 100 mL	3 per replicate	Cole Parmer 06498-00
S, C	Umbrella	1	To shade processing station when shade is not available at site
C, A	Filter forceps	1	Fisher 0975350
C, A	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	Snapping Petri dish, 47 mm	2 per replicate	Fisher 08-757-105
C	Glass fiber filter, 47 mm, 0.7 μ m pore size	1 per replicate	Fisher 09804142H
C	Aluminum foil	~100 cm ² per replicate	
A	Glass fiber filter, 47 mm, 0.7 μ m pore size; pre-combusted	1 per replicate	Check with analytical laboratory ahead of time; they should be able to supply these

2. Algae Sampling

2.1 Notes for processing soft-bodied algal and diatom samples when macroalgal clumps are in the sample

Note 1: Whenever aliquoting the homogenate, make sure that it is well mixed, and that there are no “rafts” of algal fragments coalescing at the surface or behind the lip of the bottle such that they compromise sample representativeness when aliquoting the diatom and/or biomass samples. In order to prevent this, it is recommended that the well-mixed homogenate be poured quickly from the bottle into a wide-mouthed measuring cup with a broad pouring spout. From this vessel, the sample can then be poured out more deliberately for careful measurement and maintenance of homogeneity.

Note 2: Formalin can be added to the diatom sample tube using a small syringe or bulb pipette. Alternatively, if preferred, the tubes for diatom samples can be pre-loaded (in the laboratory) with 10 mL of the 5% formalin such that 40 mL of sample can be added to the fixative (thus avoiding having to dispense the fixative in the field).

2.2 Instructions for Constructing Algae Sampling Devices

Rubber Delimiter:

The rubber delimiter for use on “erosional”/hard substrates like cobbles and wood is made from a sliced-open mountain bike inner tube that has a 4-cm diameter hole cut in the middle. The hole should be reinforced with a rubber gasket affixed to the inner tube with rubber cement.



ABS Delimiter:

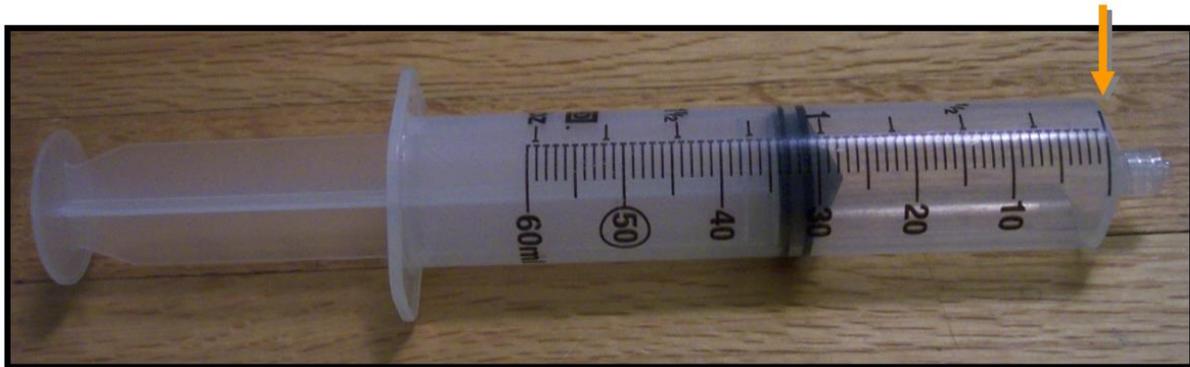
The ABS delimiter for “depositional”/soft substrates like sand, small gravel, and silt is made from a 1½” sewer cleanout, which can be found at a home-improvement or plumbing supply store. The hole in the bottom of the cleanout is 4 cm in diameter. The bottom edge of the cleanout is filed to make it 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. This indicates the depth to which to insert the delimiter when sampling.



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.

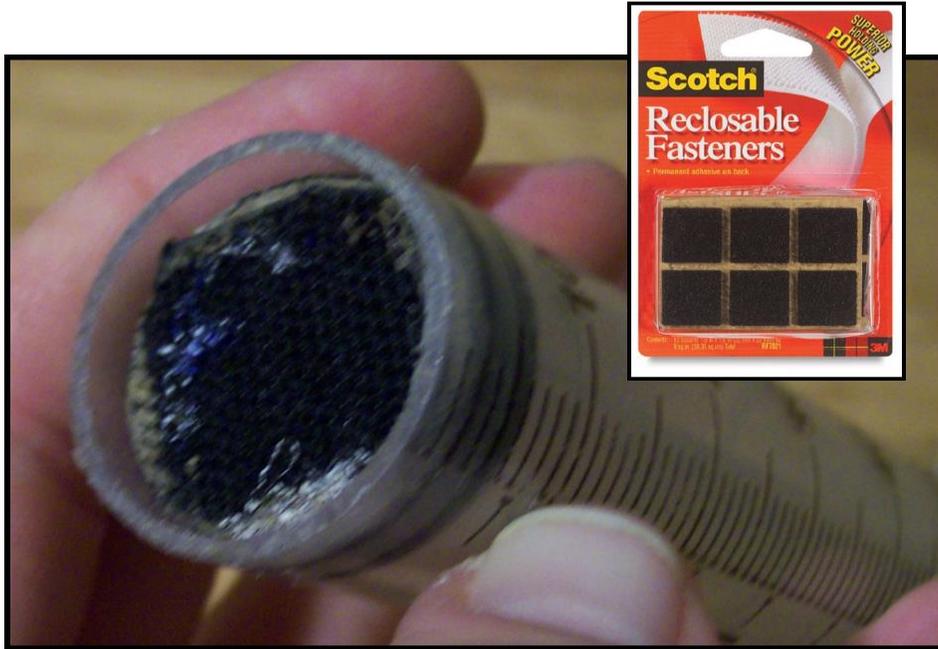
You will need a 60-mL plastic syringe for each sampler you want to make. Remove the plunger and saw the conical end off the plastic syringe, then sand the bottom so it is flat all the way around and fits tightly against a flat surface.



Firmly affix the rubber end to the plastic plunger by removing the rubber tip, applying glue to the “naked” end of the plunger, and *replacing the rubber cap*. Allow glue to cure. Then cut the conical part off the plunger tip so that only a flat surface of rubber remains.



Cut a circle of Velcro-style hook material to fit the size of the plunger. Use a waterproof adhesive to affix the “Velcro” circle to the end of the plunger.



Obtain some white scrubbing pad material (make sure it is not pigmented so it will not end up interfering with eventual chlorophyll *a* analysis of the samples collected.) Cut a supply of circles to fit the size of the plunger.



Before each sampling event, attach a fresh circular scrubbing pad to the end of the plunger. This is a head-on view of the plunger, with the scrubbing pad circle attached.



This is what the syringe sampler looks like when it is ready to be used.



Viewing bucket:

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



For additional guidance, the following section is a highly detailed set of instructions for manufacture of the algal sampling devices. This write-up has been provided courtesy of David Williams of the San Francisco Bay Regional Water Quality Control Board

2.3 Tips and Tricks for Fabrication of SWAMP Algae Sampling Tools

February 2012

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This document supplements Appendix D of SWAMP's "Standard Operating Procedures for Collecting Stream Algae Samples and Associated Physical Habitat and Chemical Data for Ambient Bioassessments in California" (Fetscher *et al* 2009). Three key field sampling devices required for algae sampling are not available for purchase and must be assembled from readily available materials. The writers of the original SOP did a good job of describing how to construct the field sampling devices for experienced technicians; however, not everyone is handy with tools or savvy about glues. This document is intended to provide detailed instructions for fabrication of high-quality field equipment. It is critical for field personnel performing the SWAMP Algae Collection SOP to be using uniform, correctly fabricated sampling devices to ensure collection of robust and comparable data. Attention to detail while preparing these devices will mitigate sampling error.

The majority 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 or sourcing from colleagues. 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.

I. 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 enforce the hole. Fabrication of this device involves steps similar to patching a bicycle inner tube. Recommend two per field crew.

Required supplies (makes 2):

- 1 x 26inch diameter (mountain bike) inner tube
- 2 x 66mm outer diameter rubber washer ¹
- Rubber cement or Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) ²
- 4cm diameter circle template (Burt's Beeswax Lip Balm Tin (.30 oz) works well)
- Razor blade or X-acto™ knife (must be very sharp)

- Coarse - Medium (40-80) grit sand paper
- Acetone or rubbing alcohol (90%)
- Coin or bicycle tire iron
- Heavy object (not made out of rubber)

¹ DO NOT use a metal washer – the washer must be rubber so that it can conform to irregular shaped surfaces. The washer will be found at a home improvement store (e.g., Home Depot) 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.

² Use a vulcanizing compound from a bike repair kit or Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201). The product can be found in a three-pack with two other types of Liquid Nails™ adhesive or purchased separately. **Do NOT use Liquid Nails™ construction adhesive.** Make sure the adhesive you use is designed to glue rubber to rubber; super glue won't work.

1. Cut the inner tube:
 - a. Remove the valve stem (where the air goes in) by cutting across the tube on both sides of the valve.
 - i. Discard the valve stem.
 - b. Cut the remaining tube in half to make two tubes each about 0.75m long.

Repeat the remaining steps below for each half
 - c. Slice along one seam of the tube (the long way) to create a flat strap.
 - d. Cut the hole in the center of the strap.
 - i. Position the 4cm template (lip balm container) in the very center of the strap.
 - ii. Follow around the edge of the container with the X-acto™ knife to slice the hole. (Note: as long as the knife is very sharp this produces a much cleaner cut than tracing the hole with a pen first.)
2. Affix the rubber washer to the hole:
 - a. Sand both surfaces to be glued together until visibly scuffed.
 - i. Sand the inner tube surrounding the hole to an area slightly larger than the washer will cover.
 - ii. Sand one side of the washer and the inner/outer edges.
 - b. Clean all sanded surfaces with the acetone or alcohol. Allow to dry a couple minutes.
 - c. Apply rubber cement to both clean flat sanded surfaces.
 - i. Allow to dry just until the rubber cement feels tacky. DO NOT allow to dry completely.
 - d. 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.)
 - i. 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.
 - e. Put the heavy object on top of the washer and leave to dry overnight
 - f. Next morning, run a bead of rubber cement around both the inner and outer edge of the washer and allow to dry completely for at least 24 hours.

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

II. ABS Delimiter:

For use in small “depositional” / soft substrates like sand, small gravel, and silt, the delimiter is made from an **ABS** sewer cleanout fitting filed or ground sharp to ease insertion into the substrate. A bright line marked around the outside surface of the delimiter, 1cm up from the bottom edge, helps facilitate consistent sampling. Creation of this device requires access to power tools. You can file the edge sharp (bevel), but this will take a long time. Always be sure to wear proper personal protective equipment.

Recommend two per field crew. This tool does not float!

Required supplies (makes 2):

- (2) 1½ inch ABS sewer cleanout fitting⁴
- (2) 1½ inch ABS cleanout fitting cap⁵
- Paint pen in bright color⁶
- 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 is a small black 1½ inch ABS end piece available at most hardware stores.

It has a threaded fitting (threaded on inside) on one side and tubing (to slide in or over a length of tubing) on the other side. **The inner diameter is 4cm.**

⁵ The cleanout cap screws into the threaded end of the sewer cleanout and has a square top. It may be sold separately from the cleanout so we have listed it as a separate line item. It makes a handle for the delimiter.

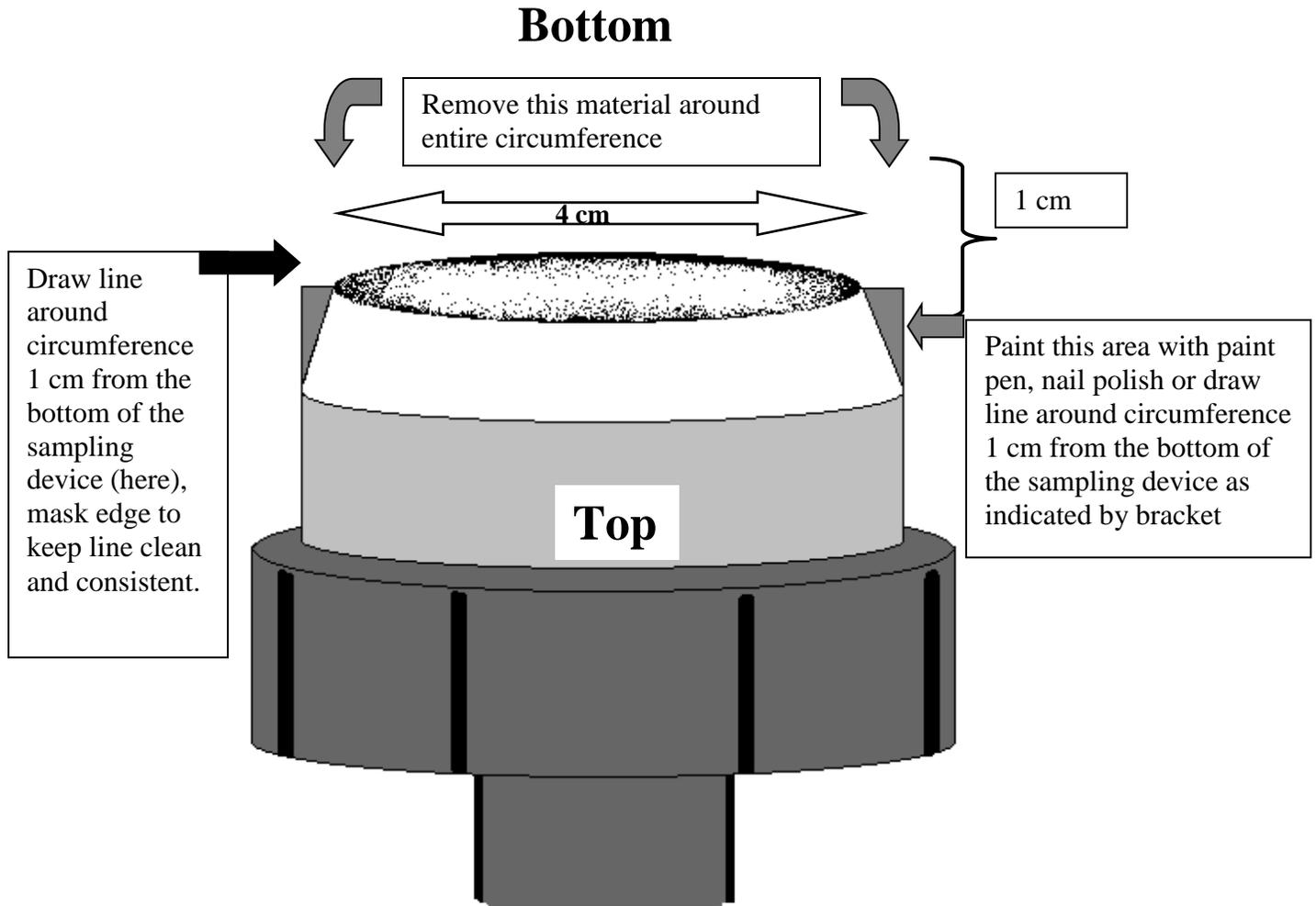
⁶ Also sold at most hardware stores in silver or gold.

1. See Figure #1 (next page)
2. Sharpen (bevel) the edge.
 - a. Using the fine point permanent marker, mark a line around the outer edge of the cleanout’s smooth end’s outer circumference (tubing connecting 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 the electric sander to sharpen (bevel) the bottom outer diameter of the cleanout, below the 1cm line.
 - i. 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 (please see figure 1).
 - ii. Clean the delimiter with acetone or rubbing alcohol (90%).
 - iii. Allow to dry for a few minutes.

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 1 cm from the edge in the plane parallel to the fitting.
 - b. Use the paint pen to draw a bright line around delimiter’s outer circumference, 1cm up from the bottom edge.
 - c. Allow to dry completely.
 - d. Repeat if necessary.

The masked edge indicates the depth to which to insert the delimiter into the substrate when sampling. Appendix D of the SWAMP Algae Collection SOP calls for a line to be drawn here; however, free painting a consistent clean line can be difficult. Masking this line with tape, before marking / painting helps to make the line clean and consistent. Use the masked edge as the 1cm depth indicator.

The area sampled must be correct to calculate biomass accurately and consistently.



III. Syringe Scrubber:

For use on hard substrates that can't be picked up out of the stream, like submerged bedrock, concrete channel bottoms, or logs. The syringe scrubber is made from a sawed-off syringe barrel and its plunger fitted with the hook side of a hook and loop fastener (Velcro®). Small circular scrubber pads cut from large squares of disposable white scrubbing pads are affixed to the end of the plunger and used to scrub the algae from the substrate. This is the only device with materials that are not readily available for in-store purchase – allow time for shipping. Recommend two per field crew.

Required supplies:

- (2) 60-mL plastic syringe (Cole Parmer EW-07940-30) ⁷
- (Velcro®) Package of Scotch™ 3M Reclosable Fasteners, multipurpose ¾ inch squares ⁸
- Package of white scrubber pads ⁹
- Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) ¹⁰
- Acetone or rubbing alcohol (90%)
- Super fine point permanent marker

- Medium (80-100) grit sand paper
- Sharp knife
- Cutting board
- Ruler with mm increments
- Calculator

⁷ The syringes are available from a laboratory supply company, such as Cole-Parmer. The outlet is the twist-in style. Some programs may have these syringes that have been used for water sampling and it is advisable to recycle and reuse. You can also ask around the bioassessment community to see if anyone has extra syringes lying around.

!!!!!!BE SURE TO VERIFY THAT THE INNER DIAMETER OF THE SYRINGE BARREL IS 26mm.

⁸ Other brands/types of hook and loop fasteners (Velcro®) won't stick to the scrubber pads as well as the 3M offerings.

⁹ The scrubber pads must be white (non-pigmented) because colored pads will interfere with the chlorophyll α analysis. Scotch Brite™ pads work well: they are 15cm wide by 20cm tall by ~1cm thick.

¹⁰ DO NOT use Gorilla Glue™ as it tends to foam up and throw off the alignment of the hook fastener pad.

1. Cut the outlet end of the syringe.
 - a. Fully depress the plunger. Use the bottom edge of the plunger as a template/ guide to draw or score a circle around the outside of the end of the syringe. (Note: You will be cutting below the 10ml line.)
 - b. Remove the plunger.
 - c. Roll the syringe on the cutting board with the blade of the knife, scoring around the template/ guide line.
 - i. 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. Similar to the way a tubing cutter works for those of you with any plumbing experience.
 - ii. Sand the cut end of the syringe with sand paper 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.
 - d. Measure and note the inner diameter of the syringe

The SWAMP data sheets have an area sampled for the syringe scrubber of 5.3cm² in the algae samples collection devices section on page 24. The inner diameter of the barrel of your syringe **MUST HAVE** an inner diameter of 26mm for the area listed in the SWAMP data sheets, 5.3cm², to be correct. Notice that the diameter unit here is mm and the area unit is cm. So, if you want to do the calculation you can use 2.6cm for the diameter, but using a measuring device capable of measuring in millimeters is preferably used to verify the inner diameter of the syringe barrel. ***The area sampled must be correct to calculate biomass accurately and consistently.***

Remember: Circle Area = $\pi \cdot r^2 = \frac{1}{4} \cdot \pi \cdot d^2$ (d= diameter, r= radius (1/2 diameter), $\pi= 3.14159$) There are also many on-line calculators (e.g., <http://www.1728.com/diam.htm>).

2. Firmly affix the rubber tip to the plastic plunger. If your syringe has a rubber end on the plunger, you must firmly affix it to the plunger. When you are sampling with this device

you must rotate the device 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 legitimate 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).
 - c. Clean the plunger and inside of the rubber tip with acetone or rubbing alcohol (90%). Allow to dry for a couple minutes.
 - d. Use Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) to glue the rubber tip to the plunger.
 - e. Allow an hour or more for the adhesive to dry before step 3.
3. Affix the Scotch™ pads to the plunger.
- a. Use the hook side of the material only, not the fuzzy side. Cut the tips off all four corners of the square pieces, sized to just cover 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 Liquid Nails™ HOME PROJECTS REPAIR ADHESIVE (LN-201) over the nose of the plunger. Firmly press trimmed Scotch™ pad into place. Allow to dry.
4. Cut white scrub pad circles for use in the field.
- a. 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.
 - b. Make at least two pads for each planned site. **Do not reuse pads from site to site; this would result in sample contamination.**

The area sampled must be correct to calculate biomass accurately and consistently.

2.4 Standard Operating Procedures for Glutaraldehyde for the Preservation of Soft Algae

STANDARD OPERATING PROCEDURES (SOP) FOR 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 Wildlife 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.

2.5 Preparation of formalin solution

Preparing a 1-L solution of 5-percent (buffered²) Formalin (modified from Moulton et al. 2002):

- Add 50 mL of formaldehyde (37-40%) to 950 mL of water in a chemically resistant, non-breakable bottle.
- Tightly seal the bottle and mix by carefully inverting the bottle several times.
- Label the outside of the bottle with "5-percent formalin," the date of preparation, and related hazardous chemical stickers. *If* the solution has been buffered with phosphate (it is not required to be buffered), indicate this on the label as well.

² Do not use borax to buffer formalin, as indicated in earlier versions. Use either unbuffered formalin or phosphate-buffered formalin. Be sure to indicate clearly on the diatom sample label and chain-of-custody form whether or not the formalin used in each sample has been phosphate-buffered.

2.6 SOP for the use of formalin for fixing diatoms (adapted from Peck, et al. 2006)

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 immediately 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 5% 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 5% formalin in a laboratory fume hood prior to going into the field.

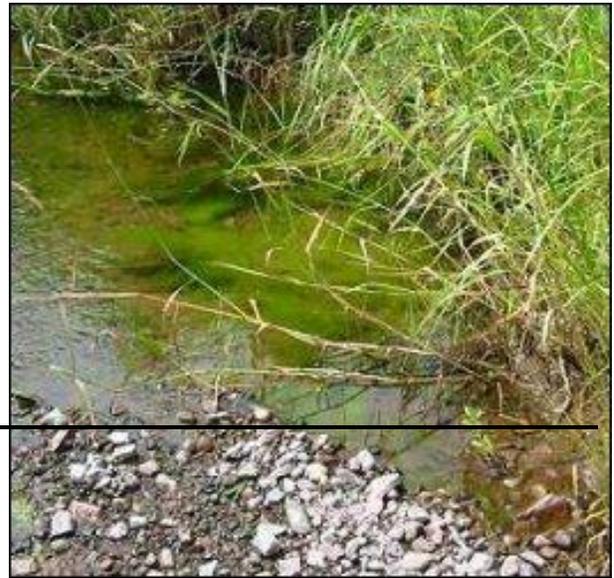
The preparation of the 5% formalin stock solution should always be done by trained personnel under a laboratory fume hood while wearing protective gloves, clothing, and goggles.

2.7 Examples of macroalgae



2.8 Distinguishing macro from microalgae

Macroalgae: soft-bodied algae that form macroscopically discernible filaments, mats, or globose structures that remain cohesive when picked up



Microalgae: diatoms and microscopic soft-bodied algae that form a seemingly “amorphous”, slimy coating on substrate, like icing on a cake.

3. Information about scientific collecting permits

2013 Requirements for Conducting Routine Monitoring Using SWAMP Bioassessment Procedures

1. Go to the following link on the CDFW website to download the application and instructions:
http://www.dfg.ca.gov/wildlife/nongame/research_permit/scp/scp_aplic_procs.html
2. Fill out Section 1 if you are working by yourself and Section 2 if you are an entity. The SCP calls it the Principle Scientific Investigator or PI. The PI is responsible for the crew and must be a crew leader and not a manager or someone who will never be in the field.
3. The important part of Section 3 is indicating that you are only sampling for freshwater invertebrates. If you check any other box then there will be others besides Jim Harrington (of CDFW), who will need to approve your application.
4. Section 5 is where you describe what you will be doing and where you will be doing it. Write in this section that you will be using SWAMP protocols and submitting your data to CEDEN. Then list the sites including GPS coordinates along with the approximate dates you will be sampling.
5. When your application is submitted to Jim Harrington, who will have the SCP staff put the following requirements on your permit.
 - The individual listed in Section 1 or the Principle Scientific Investigator (PI) and field crew listed in Section 2 must have SWAMP bioassessment and algae training and/or related work experience prior to the 2013 field season. They must also meet all SWAMP training requirements throughout the life of the permit.
 - The individual listed in Section 1 or the entity listed in Section 2 must have a SWAMP Bioassessment QAPP or similar documentation describing the procedures used by them to insure the data collected was of the quality acceptable by the SWAMP program.
 - The QAPP must describe the level of taxonomic effort and subsampling size as described in SAFIT document <http://www.safit.org/ste.php> and decontamination techniques for sampling equipment and procedures for reporting Aquatic Invasive Species (AIS).
 - Within one year of sample collection or upon the request of CDFW, all data produced under this permit must be in a format that can be uploaded to CEDEN.



CDFW’s Aquatic Bioassessment Laboratory (ABL) is working with the Fisheries Branch to ensure that the data you collect can be used by CDFW and others to help us protect and preserve aquatic resources in California. We appreciate that you agree to use the standardized bioassessment procedure developed by the ABL for the State Water Resources Control Board’s Surface Water Ambient Monitoring Program (SWAMP).

The following list of bioassessment specific conditions is a requirement of your Scientific Collectors Permit (SCP):

The individual listed in Section 1 or the Principle Scientific Investigator (PI) and field crew listed in Section 2 must have SWAMP bioassessment and algae training and/or related work experience prior to the first sampling of the year. They must also meet all SWAMP training requirements throughout the life of the permit.

The individual listed in Section 1 or the entity listed in Section 2 must have a SWAMP Bioassessment QAPP or equivalent documentation describing the procedures used by them to ensure the data collected was of the quality acceptable by the SWAMP program.

The QAPP must describe the level of taxonomic effort and subsampling size as described in the SAFIT Standard Taxonomic Effort document* and decontamination techniques for sampling equipment and procedures for reporting Aquatic Invasive Species (AIS).

GIS coordinates for the sites sampled during each field season must be sent to James.Harrington@wildlife.ca.gov no later than 14 days before they are sampled.

At the end of the field season, the final list of sites sampled must be confirmed in an e-mail to James.Harrington@wildlife.ca.gov . Within one year of sample collection or upon the request of CDFW, all data produced for the current year under this permit must be in a format that can be uploaded to the California Environmental Database Exchange Network.

* The Southwest Association of Freshwater Invertebrate Taxonomy documents are available at <http://www.safit.org/ste.html> .

SWAMP standard procedure documents and other related bioassessment technical reports related to conducting bioassessment are located at http://www.waterboards.ca.gov/water_issues/programs/swamp/tools.shtml#methods

Any questions on bioassessment procedures or with these specific conditions can be addressed to James Harrington by e-mail James.Harrington@wildlife.ca.gov or by calling (916) 358-2862.