Stream Physical Habitat Survey - Standard Operating Procedures
Bioassessment Surveys

Summary outline of methods: 15 transects spaced at 10 meter intervals along the 150 meter delineated reach length (starting at 0). Bank and channel features are measured (wetted perimeter width, bank cover category, bank angles, and vegetation cover (using densiometer) across each transect and at 5 equal-spaced points within each transect the depth, current velocity (60% depth), and substrate type (size class) are measured. **Location** of each site (mid-reach) is determined with a **GPS** unit, and **elevation** determined from map location (and/or barometer). Slope is measured using a hand-held leveling scope sighted on a stadia rod over a series of intervals over the 150 meter reach length. Sinuosity is determined from the ratio of reach length to minimum linear distance from the bottom to top points of the reach. Percent riparian canopy cover by type (within 1 meter on the bank) is visually estimated for the reach. Temperature, pH, conductivity and turbidity are measured using calibrated field meters. Dissolved oxygen is determined in the field using a standard test kit. Alkalinity, nitrogen, phosphate, and hardness are measured in the lab from field samples. General types of algae present are noted for each reach (algae samples from rock surfaces are also collected and preserved). **Photo** documentation of each reach is also made at 4 points: mid-stream looking upstream at 0, 50, 100, and at 150 meters looking downsteam.

Reach and Riffle-Pool Delineation

The first step in description of physical habitat is **delineation** of the 150 meter length of the stream reach along an approximation of the thalweg of the channel. To the extent possible, this measurement should be made by following along the bank contours of the channel, laying out the meter tape (50 m on a reel). This may require crossing the channel or even walking in the stream if bank vegetation cover is too dense – but this should be kept to a minimum to avoid disturbance of benthic habitat. Fish survey counting (Yosemite) should be done just ahead of the layout of the 150 meter reach. For each 25 meter length a flag should be placed to serve as a monument for marking locations and later measurement of gradient. Over the 150 meter reach delineation, the primary data to be recorded is the position along the meter tape (to the nearest meter) where erosional and depositional habitat types begin and end – **riffles and pools**, respectively. This data provides an indication of the distribution and length of these major geomorphic units within each reach. The position of these habitat features will also be used to determine where the benthic invertebrate samples are to be taken by using a random number table (0-150) to assign a riffle or pool location to be sampled. Any habitat not assigned to the riffle-pool categories may be regarded as transitional glide or run habitat type. Depending on the criteria for reach selection, the starting point of a reach may be established to maintain the reach within a certain zone defined by the problem of interest, the gradient, vegetation cover, or accessibility. Selection may also be random, using preliminary map information on the target area.

Bank and Channel Features

As outlined on the data forms and code sheet, bank features on each transect are identified according to **bank cover** categories (substrate type, vegetation present and eroded, stable or incised). The intersect of interest is between the water level and an approximation of the bank full height of the channel. **Bank angle** is also rated categorically as shallow (less than 30 degrees), moderate (30-90) or undercut (>90). Riparian **vegetation cover** over and next to the channel is determined using a concave mirror **densiometer**, taped to view the canopy in the facing direction of the measure. There are 17 grid points and vegetation reflected at those grid points is recorded at the left and right banks, and mid-stream facing up- and downstream.

Transect Measures

After measuring stream **width** (wetted perimeter), the transect is visually divided into 5 equally spaced points (visualize the mid-point as 3, and equally divide the left and right sides into points 1 and 2 and points 4 and 5). At each point, the **depth** and substrate type at the point of contact are recorded (recorder on bank) using a meter stick. **Substrate types** are grouped by size class for the mineral type, and also according to algal, vegetation or detrital components present at the point (see codes). At 60% depth the **current velocity** is also measured at each point (also record current meter type used and units). **Discharge** is calculated later for each of the 5 cells measured (current x cross-section area). Any cobble encountered is also rated according to the volume of rock **embedded** by fine / sand substrates (a visual estimate, calibrated among observers).

Overall Reach Features

The **gradient** of the channel is measured using a hand-held leveling scope (5X magnification) to sight off a 5 meter leveling rod. The observer serves as the tripod and so should find a position where both upstream and downstream position of the rod can be clearly observed without moving except to turn the upper body. Most readings will be taken over 25 meter intervals but where possible should be taken over 50 meter intervals to save time. The sum difference in up-down readings over 150 will give the percent slope or gradient. The **sinuosity** of the channel is measured as the ratio of the 150 meter thalweg stream length to the direct line distance from the top to bottom flags defining the reach. This is done by sighting to the leveling rod held at one end of the reach and walking a direct line of sight to the rod, measuring distance with a reel tape over the distance (a person to hold the tape end facilitates the several walks needed to measure the full distance). **Riparian vegetation** cover is visually estimated as morphological categories of cover (grass, bush, tree) and type (see data sheets). This provides another measure of shading, riparian development and potential inputs. **Algae** type present is also qualitatively scored. Notes should also be kept on any aquatic vegetation present.

Water Quality Measures

Using an Oakton conductivity/ pH/ temperature meter, these three measures are taken prior to any disturbance through the physical habitat surveys. In addition, LaMotte test kits are used to determine alkalinity and dissolved oxygen. Duplicate water samples are collected in acid/DI rinsed bottles, acidified for separate N, P analyses and turbidity analyses (60 ml HDPE bottles).

Benthic Macroinvertebrate Sampling Procedure

Select 5 riffles from a random number table along the 150 meter reach. Use the D-net to collect kick samples at ¼, ½ and ¾ of the stream width (always start at the location furthest downstream and work up). Kick an area approximately 30 square centimeters directly above the net (a square area with sides equal to net width). Continue this kick for about 10-15 seconds, then rub the rocks by hand for an additional 10-15 seconds (total 20-30 seconds at each of 3 positions = 1-1.5 minutes). If shallow enough just use hands for the full time. After each sample position remove large rocks or wood debris after washing them in the current into the net. For streams less than 1-2 meters wide, take the 3 kick samples from both sides and middle above or singly one above another at the random number location (instead of taking all 3 across the stream when widths are greater than 1-2 meters). Keep in mind that we are trying to sample across different microhabitat types in the stream including varied depth, current, substrate types – the three composited samples should represent the variety of habitat present. One or two composits may be taken if samples are dense with debris. The label should then indicate the number of kicks used (1 or 2), assume 3 if not noted on label. If riffle is not available across the line of each transect, select representative locations to collect the needed composite sample.

If sampling in pools, take only a single collection within the tail zone of the pool (i.e. downstream third of pool zone) by sweeping or brushing the sample area into the mouth of the net -this flushing by hand will facilitate collection of the invertebrates. The net may also be used to scoop through sample area after the sweep. More than a single area sampled will usually produce too much sample volume to process and preserve.

Quickly dip the net into the stream to consolidate the material to the bottom of the D-net. Pick out any remaining large debris being sure to remove any attached insects. Invert the net into a bucket with 1/4 to 1/3 full of water. Shake out the net to collect all the debris and insects (do not dip in bucket water since insects will adhere). Dip net into the stream again to consolidate remaining contents and flick inverted net into the bucket.

Elutriate (pour off lighter material) with a swirling motion into the other bucket five times. Use only a small volume of water in each elutriation so the receiving bucket does not overflow. Only rocks and sand should be left in the original bucket. Empty these rocks into a shallow white pan (or closely examine the bottom of the bucket). Search for cased caddisflies/snails and add to sample if found (they are heavier and may not pour-off).

Strain collected material through a fine mesh aquarium net supported on one bucket (this may also serve as elutriation since some sand usually remains). Empty contents of aquarium net into a sample container. Use BioQuip forceps to scrape any remaining debris into vial. Fill container with ethanol to preserve the bugs. Fill to a level that just covers the amount of debris. Add a small volume of rose bengal stain.

Label sample jar as shown below, and move on to next sample. (identify as riffle or pool if both are sampled e.g. riffle #1)

Stream / Date / Site / Replicate #

Example: Convict Creek
lower SNARL
6 VII 95 #1

Equipment

Waders and D-net (250 micron)
Buckets (2)and aquarium nets
BioQuip forceps and white exam tray
100% ethanol and Rose Bengal stain

PROTOCOLS FOR CPOM AND FPOM SAMPLING (Coarse and Fine Particulate Organic Matter)

Place D-Net firmly onto stream substrate at ¼ distance from left shore. Dislodge any organic matter trapped among the substrates by kicking and shuffling among rocks and debris for 20-30 seconds. Disturb an area that creates a square, using the D-Net as a guide (30x30 cm). Repeat for ½and ¾distance from left shore, retaining all material collected in net except large rocks which should be washed in net and discarded. A glove (rubber or neoprene) may make it easier to vigorously sample. This is one of three replicates taken at the bottom, middle, and top of the 5 random riffle transects also selected for macroinvertebrate sampling.

Invert contents of D-Net into 1/4 full bucket, being sure to get all detritus off the net. Invertebrates that are clinging to the net can be ignored. Wash and remove large stones from the bucket, being sure to remove detritus from the rocks. Elutriate into 2nd bucket by swirling bucket and pouring off lighter material while leaving behind gravel and sand. Repeat 2-3 times. Discard the cleaned rocks and sand left behind in the rinsing bucket.

Place 1 mm mesh screen over empty bucket, and secure with cord lock. It helps to create a small depression in the mesh. Pour contents of 2nd bucket onto mesh screen. Use this as another elutriation- when close to the end, swirl bucket and stop pouring when only sand is left.

Pick invertebrates from 1mm mesh screen for 3 minutes. Use constant effort for all samples. The biggest and heaviest bugs should be removed first. Squirt the mesh with a rinse bottle so that all particles <1mm wash through (=FPOM fraction of 0.25 to 1.0 mm). Carefully remove the cord-locked mesh from the top of the bucket and fold corners of the 1 mm mesh screen to create a bag. Shake bag gently to remove excess water. Wrap mesh in towel to remove any remaining water.

Weigh the 1mm mesh bag using the appropriate Pesola scale. If the scale has a hook instead of a clip, secure the mesh bag using a rubber band, then hook the band. Record weight on FPOM vial label, then rinse bag. Shake excess water from mesh and dry in towel, then obtain a tare weight of the mesh bag. If a rubber band was used, include this in the tare. Record tare weight T.
[Ex. label for a 40.3 gram gross weight: CPOM = 40.3 - 26.5T]

From bucket containing FPOM, remove small mayflies or other insects swimming or floating (plastic pipette works well). Do this for no more than a few minutes and be careful not to remove FPOM. Strain FPOM through 100 µm handnet (using empty bucket as support). This can act as an elutriation step as well. Use BioQuip forceps to remove FPOM from net and place into scintillation vial. Use wash bottle to gather FPOM in bottom of net to facilitate removal. Add water to vial to ½full and add 1-2 mL of formaldehyde to preserve. Store in field ice chest. This FPOM fraction is for the size range 250 - 1000 µm since particles less than 250 will have passed through the sampling D-net.

Vial Label: stream, site, date (day- month (roman numeral)- year), and replicate # (1-3)

Example:

FPOM #1
Convict Creek
lower SNARL
6 VII 96
CPOM = 40.3 - 26.5T

Materials Needed:

D-Net
1 mm mesh square (1.5 x 1.5 feet)
2 buckets, 100 µm handnet
BioQuip forceps, label tape, sharpies
500 mL washbottle
Pesola scales 50, 100, 500 g
Hand towel (to dry mesh)
Cord (w/ cord loc to tighten around bucket)
Scintillation vials, rubber bands
Formalin (buffered over $MgCO_3$)
Plastic pipettes for small insect removal

ALGAE SAMPLING PROTOCOL

Select cobble size substrate (5-25 cm range) from center of transect line (upper, middle and lower random transects for 3 replicates total). Lift cobble from bed into submerged collecting tray. Pay attention to rock orientation to assist with measurements. Pour off all but a small volume of water from the collecting tray.

Remove algae from substrate using wire or nylon brush until all algae is visibly removed (brush down, into water in the sample container for 3-5 minutes). Rinse substrate, brush, and fingers into collection container (w/ about 50-100 mL).

Measure exposed surfaces of substrate using plastic ruler to record length, width, and height along the mid-lines of the rock. Only the height of the exposed upper surface should be recorded (or half the height if the exposed surface is not obvious). Measure the circumference (=the longest perimeter) using the tape measure. Record measurements on sample collection bag for the filter (see below). [For example as 14L, 8W, 4H, 34LP]. Only now should you discard the sampled rock.

Measure total volume of sample by pouring through fine net into graduated cylinder. This will remove sand/rock/bugs but if filamentous or mat algae are present, they should be gently elutriated, leaving behind sand/rock. Rinse collection container and pour sample back into it. Record total volume on sample bag.

Homogenize sample using repeated suction-expulsion with a 60 mL syringe. Use scissors to cut large pieces of algae if necessary and use syringe with cut-off tip to prevent clogging of needle-connector. Filamentous algae may be set aside to dry in the sun for about 5 minutes and can then be crushed/crumbled with fingers into the processing water. Use syringe to measure 20 mL of sample into 25 mL scintillation vial. Add 2 mL formaldehyde and 0.5 ml Lugols soln. Store in ice chest. Label bottle with date, stream, site, and sample # (1,2,3). This is for later algal ID and biomass (AFDM), or direct cell counts and sizes as an index of algal food resource density.

Re-homogenize sample and fill syringe with 50 mL of sample (or use 10 mL syringe for dense algal samples). Place 25 mm glass fiber filter on metal screen filter holder (use BioQuip forceps), place O-ring and gently secure syringe adapter. Holding onto filter adapter pointed down, expel sample until resistance to filtration occurs (do not force further volume through). Use an empty syring to push air through filter assembly to expel any remaining water. Record mL of sample expelled as well as the total volume on sample bag.*
Remove filter, fold in half, place on aluminum foil square, fold foil and place in sample bag. Label with date, site, and sample #. Place bag in field ice chest. This filtered sample is for later chlorophyll analysis or AFDM burning. If for AFDM analysis in the lab, each filter should be pre-tared and a drop of buffered formalin placed on filter to preserve (drop on chl filter also). Discard remaining sample. Rinse materials thoroughly, proceed to next sample.

*Completed sample bag should be labeled with date, stream, site, sample #, substrate dimensions, and volume measurements (the 20 mL vial only with date, stream, site and replicate #):
Example Label for filter storage bag:

Convict Creek	Algae #1
lower SNARL	14L, 8W, 4H, 34LP
6 VII 96	8 filt. / 175 vol.

Materials Needed for Algae Sampling:

Sample collection box (tupperware type)	Metric ruler
Wire brush	Metric measure tape
250 mL rinse bottle	Formalin (over $MgCO_3$) and dropper pipette
250 mL graduated cylinder	Labeling tape and sharpie pen
60 mL syringes (cut/uncut tips)	Scintillation vials
25 mm GF/A filters	Plastic storage bags
Filter holder assembly	Aluminum foil squares
Fine mesh net (100 µm)	Small scissors
Small ice chest (w/blue ice)	BioQuip forceps

Standard Operating Procedures:
Laboratory Bioassessment Sample Processing and Identifications

Fill out log sheet records of samples processed (including initials of sorter/IDer and checker). Each individual should process and ID all replicates from a single sample reach site.

Minimum count of 250 organisms. Complete counts of any subsamples taken. [average counts will be in the 300-500 range]

Sample Splitting – Split sample to get into target range of organism count (250) but make no more than 3 splits ($1/8^{th}$ the sample) on the first 2 samples, other samples may use more splits. Use Folsom plankton splitter (rotating drum) and record number of splits on post-it labels in pencil for sorted samples (post-it placed on sorting tray lids), and on invertebrate ID record sheets when doing identifications and counts. This is essential to making density calculations from samples. Use a random number chart to select the sample split to be sorted, and save unprocessed splits until sorting is complete. One of the unprocessed fractions from the 5 samples taken at a site is saved as a sample voucher (this may be stored in the field sample container). Adjust sample splits after initial sample to be in the range of 300-500 counted. Be sure splitter is centered/balanced and inspect splits to verify that splits were equally divided.

Sample Sorting:
Each subsample may be sorted initially by eye or with the aid of an Optivisor or swing-arm lens. Search may then continue or even begin with microscope scan of samples (10-15X). Small portions of subsamples will be placed into gridded trays for picking out invertebrates. Only nematodes should not be picked - all others including mites and ostracods should be removed. Look-alike taxa or broad taxonomic groupings should be placed into separate cells of sorting trays for subsequent identification. One cell of each tray should be reserved for midges and mites (placed together for later IDs by Herbst). The cells of this tray should be about half-full with 80% ethanol/ 5% glycerol mix. Very small or early instars of abundant insects may not be removed completely but as many as practical should be sorted. Following sorting of the subsample, the unprocessed portion of each full sample should be scanned for large and rare taxa not encountered in the sorted subsample. A representative specimen of each large/rare (if present) should be put into the sample sorting tray. For one of five sample replicates sorted (20%), a verification check by another lab worker is required (w/inititals) to ensure that a sample has been completely picked. This should be done by pooling of the contents of a sample as it is picked, then having a co-worker examine this with an optivisor or under a 10-15X microscope. Records of incomplete sorts (number missed) should be kept in the log records. This cross-check should be done on the first of each of the five replicates taken for a stream site (this will establish a search image for the remainder of the set of samples). One of the 5 unprocessed sample fractions is to be saved as a sample voucher (record the fraction remaining).

If the sample is to be stored in the refrigerator, place a zip-lock plastic bag over the tray and tray lid (w/field bottle label and # of splits on post-it note) placed over the bag. All 5 samples from a site should be stacked so they can be IDed together without having to search for each. All placed inside a storage tub.

Sample Identification:
Sorted specimens will be IDed to the lowest taxonomic level possible given the availability of keys. Each identification will have a taxonomic certainty rating attached to it for use in evaluating problems with taxonomy (see taxa record sheets). Also record life stage(s) and any notes on ID traits or specimen condition. Uncertain identifications should be checked with other technicians and the reference collection. **Each** IDed replicate is stored in a 20 mL vial, then reviewed for QC verification of identifications with Herbst (technician checking off each ID sheet). The conservative guidance for IDs is not to push rare organisms beyond a verifiable identity (or exclude/pool). Uncertain taxa and 10% of all samples will be sent to taxonomic authorities and external labs for final verification and used as part of an archived reference collection. Representative identified specimens are archived in a reference collection. After data compilation and verifications, all replicates from a single site will eventually be pooled and permanently archived (80% EtOH-5% glycerol). All midges/mites from each replicate should be placed in a separate small glass vials, labeled with stream/site/date/ #splits, and stored in a rack for further ID. Ostracods and oligochaetes are not IDed further; copepods are recorded when present but not counted.

Water Chemistry Protocol for Bioassessment Surveys

For each survey reach, record measurements of pH, temperature (°C), conductivity [µS (Siemens)] using an Oakton meter, and conduct titrations for dissolved oxygen and alkalinity using LaMotte kits.

Oakton meter (pH, temperature, conductivity)

Locate yourself at a convenient location and place the Oakton meter probe in the stream for 15 minutes to allow for equilibration to stable readings. Place the meter in a shaded area of the bank. Flag the site in case you leave the meter unattended and be careful that it does not get stepped on. During this time, run titrations for dissolved oxygen and alkalinity. After this time, record pH, temperature, and conductivity.

Alkalinity (LaMotte kit)

1. Rinse titration tube with stream water three times before taking 5ml sample.
2. Add one Phenolphthalein tablet. Cap and shake until tablet disintegrates. If solution does not turn red, P alkalinity is 0 (discard the sample, collect another, and proceed to steps 6 and 7b -most cases of low alkalinity). If solution turns red, proceed to step 3.
3. Fill the direct reading titrator syringe with Alkalinity Titration Reagent B (insert syringe in reagent bottle, turn upside down, draw-in sample and eject back into bottle to remove bubbles, then draw reagent into syringe to zero fill-line). Insert titrator syringe into center hole of cap in titation bottle.
4. While gently swirling tube, slowly press plunger to titrate until red color disappears. Read test result to the nearest mark where plunger tip lines up with titrator scale or it may be necessary to estimate between subdivisions as each line equals 4. Record as mg/l P Alkalinity as $CaCO_3$.
5. Being careful not to move plunger, remove titrator and titration tube cap from titration tube.
6. Add one BCG-MR tablet. Cap and shake until tablet disintegrates. Solution will turn green-blue.

7a. Reinsert titrator syringe in cap and continue titration until color changes from green-blue to pink. Read test result to the nearest mark where plunger tip lines up with titrator scale or it may be necessary to estimate between subdivisions as each line equals 4. Record as mg/l T Alkalinity as $CaCO_3$. Be sure to include in test result value the total amount of titration reagent dispensed (i.e., see step 5).

7b. Fill the direct reading titrator syringe with Alkalinity Titration Reagent B (insert syringe in reagent bottle, turn upside down, draw-in sample and eject back into bottle to remove bubbles, then draw reagent into syringe to zero fill-line). Insert titrator syringe into center hole of cap in titation bottle. Add titrant drop by drop (swirling with each drop) until green-blue color changes completely to pink. Read the titrant added from syringe at the tip of the syringe plunger.

Dissolved oxygen (LaMotte kit)

1. Rinse sample bottle three times with stream water before taking sample.
2. Submerge bottle, then remove cap. Tap sides of bottle to remove air bubbles and cap while submerged.
3. Add 8 drops of manganous sulfate solution. Add 8 drops of alkaline potassium iodide azide. Cap and mix. Allow precipitate to settle.
4. Use spoon to add 1g of sulfamic acid powder (tap in carefully). Cap and mix until reagent and precipitate dissolve. Sample is now fixed.
5. Rinse titration tube w/ small volume fixed sample, then fill titration tube to 20 ml line and cap.
6. Fill titrator with sodium thiosulfate (0.025N) adding one drop at a time to sample and swirl between each addition until color is very faint yellow.
7. Remove titrator and cap. Add 8 drops of starch indicator solution. Replace cap and titrator. Titrate sample until blue color disappears. Read titrator scale and record result as mg/l dissolved oxygen.

Turbidity

Collect an unfiltered sample of stream water from the center of the flow (below water surface but off the bottom) in a 60 ml HDPE bottle and record stream/site/date and turbidity on the label. Determine in lab using turbidimeter.

Water Sampling for Nutrient Analyses

- collect water samples for nutrient analysis from a location above the delineated reach (avoids sampling from the disturbed reach area)
- collect duplicate samples, each in a 175 ml HDPE bottle (acid cleaned and stored with DI)
- samples are filtered and acidified so that they may be stored (up to 30 d) prior to analysis

At the collection location:

1. Draw stream water into a 60 ml syringe and eject several times (rinses)
2. Collect sample in syringe and filter through a GF/F filter (in filter holder) into a 250 ml beaker (or other stream-rinsed container) – 3 syringe volumes required
3. Insert stream-rinsed Oakton pH probe into beaker and add a drop of concentrated sulfuric acid – stir and note pH – adjust in this way to a pH around 2.0 (no more than 2-3 drops required for adjustment of poorly buffered stream water in many Yosemite watersheds)
4. Pour the filtered-acidified water into the sample container
5. Label as stream/site/date nutirient #1, and repeat for duplicate nutrient sample (#2)