# CALIFORNIA STREAM BIOASSESSMENT PROCEDURE

**(Protocol Brief for Biological and Physical/Habitat Assessment in Wadeable Streams)**

The California Stream Bioassessment Procedure (CSBP) is a standardized protocol for assessing biological and physical/habitat conditions of wadeable streams in California. The CSBP is a regional adaptation of the national Rapid Bioassessment Protocols outlined by the U.S. Environmental Protection Agency in "Rapid Bioassessment Protocols for use in Streams and Rivers" (**EPA/841-B-99-002**). The CSBP is a cost-effective tool that utilizes measures of the stream's benthic macroinvertebrate (BMI) community and its physical/habitat characteristics to determine the stream's biological and physical integrity. The purpose of this Protocol Brief is to introduce the techniques of bioassessment to aquatic resource professionals and help standardize data for statewide bioassessment efforts. The Protocol Brief is only a summary and does not contain all the necessary information that may be required to understand the concepts of bioassessment and to implement a successful monitoring program. Additional information and updates on bioassessment can be obtained by visiting the **DFG Aquatic Bioassessment Laboratory website at www.dfg.ca.gov/cabw/cabwhome.html**.

## History of the CSBP

The CSBP was originally developed in 1993 to measure biological response from point-source discharges of chemical contaminants, inorganic sediment and elements of organic enrichment. The method was based on sampling the single richest habitat in a stream reach; this was the most common technique at the time (Rosenberg and Resh 1993, Loeb and Spacie 1994, Lenat and Barbour 1994) and consistent with the U.S. EPA's Rapid Bioassessment Protocols (RBP) (Plafkin et al. 1989). In 1995, the CSBP was adapted for use in ambient and non-point source pollution monitoring programs and this version was reviewed by a Technical Advisory Committee assembled by DFG and the U.S. EPA. The 1996 edition of the CSBP was widely distributed in California and accepted as the state's standardized RBP protocol (Davis et al. 1996 U.S. EPA 2002). A 1999 revision added quality assurance and control (QA/QC) techniques to ensure high quality field collections, laboratory analysis and taxonomic consistency.

As of 2003, the CSBP is the most often used RBP protocol in California (Barbour and Hill, 2003). This unique protocol allows the user to produce biological and physical/habitat data that can be used to measure differences between sites, compare to a regional Index of Biological Integrity (IBI) (Ode *et al.* 2003) and help diagnose response to individual stressors. In addition to the high gradient riffle based procedure, the 2003 edition of the CSBP describes techniques for use in unique channels and a technique for low gradient channels that blends elements of the CSBP with those of a multi-habitat technique recommended by the U.S. EPA (Barbour et al. 1999).

The CSBP 2003 has four notable changes to the existing protocol; 1) the stream reach for the assessment is no longer defined by a set of five pool-riffle sequences, but rather by a discreet length of 100 m (300 ft); 2) the area of benthos sampled has been reduced from 1.6 $m^2$ (18 $ft^2$) to 0.8 $m^2$ (9 $ft^2$); 3) although 3 independent samples will be collected at each reach, there is now an option to composite the 3 samples in the laboratory and reduce the total number of BMIs identified at each reach from 900 to 500; and 4) there is a new QA/QC procedure to collect a set of duplicate samples

at 10% of the reaches for projects with more than 20 sites. These changes were based on experiences gained from several years of field testing, changes in the national RBP (Barbour et al. 1999), recommendations from Barbour and Hill (2003) and methods comparison studies conducted by DFG. **Data collected with these modifications can easily be made compatible with previous CSBP data and these changes make the CSBP more consistent with other BMI protocols used in the western US**.

## OVERVIEW OF THE CSBP

The CSBP can be used to measure biological and physical/habitat condition in all freshwater lotic environments (streams and rivers) shallow enough to allow safe wading (≤1.5 m). The CSBP samples benthic macroinvertebrates with a 0.5mm mesh net from the richest habitat along 3 randomly selected transects within a 100 m (300 ft) reach of stream or river. The 3 transects are placed within shallow-fast water habitat (usually riffle) for high gradient channels and throughout the entire reach for low gradient channels. At each transect, three 0.09 $m^2$ (1 $ft^2$) areas of stream benthos are sampled and composited into a single sample. In low gradient channels, the 3 collections along the transect are selected to represent the relative proportions of the different richest habitat categories present (submerged vegetation, hard substrate of natural rock or concrete, soft substrate of sand or mud, stream bank vegetation and woody debris). Physical/habitat is measured using a qualitative U.S. EPA procedure throughout the entire reach and additional quantitative measures within the vicinity of the BMI samples. Taxonomic identification of the BMI samples is performed on a fixed count of 300 organisms from the 3 samples (total of 900 for the entire reach) or 500 from the composite of the 3 samples. There are two standard levels of taxonomic identification: one standardized for the state by the California Bioassessment Laboratory Network (CAMLnet; www.dfg.ca.gov/cabw/camlnetste.pdf) and a more precise level based on the U.S. EPA's Environmental Monitoring and Assessment Program (EMAP).

### CALIFORNIA DEPARTMENT OF FISH AND GAME SCIENTIFIC COLLECTING PERMIT

Anyone who collects fish, amphibians, or invertebrates from the waters of the state must have a DFG Scientific Collecting Permit in their possession. The permit can be obtained from the DFG License and Revenue Branch in Sacramento (916-227-2225). Those conducting bioassessment in California should specify on the permit application that they will take freshwater invertebrates (authorization 5), incidental fish (authorization 6) and amphibians (authorization 8). It is also advisable to contact the local Game Warden and District Fisheries Biologist at the closest Regional Office prior to collecting.

## FIELD PROCEDURES FOR COLLECTING BMI SAMPLES

The CSBP can be used to sample BMIs from all streams and rivers where the access and depth (≤1.5 m) do not require the use of a boat. The step-by-step procedures described in this document have been divided into three sections: high gradient channels, low gradient channels and considerations for unusual channel conditions. **Contact DFG or visit the DFG Aquatic Bioassessment Laboratory website for more information on Rapid Bioassessment procedures for boatable streams and rivers and lentic or still water environments.**

**CSBP for High Gradient Channels**

High gradient channels usually have greater than a 1% slope and will always contain pool-riffle sequences with a ratio high enough to contain at least 3 riffles per 100 m (300 ft) reach. Riffle substrate could be rock, sand or mud, but must be at least 1 m (3 ft) wide with flow velocities greater than 0.3 m/sec (1 ft/sec).

**Biological and Physical/Habitat Equipment List**

Measuring tape (300 ft or 100 m)
D-shaped kick net (0.5 mm mesh)
Standard size 35 sieve (0.5 mm)
Wide-mouth 500 ml plastic jars
White enameled pan and forceps
95% ethanol
California Bioassessment Worksheet (CBW)
Physical/Habitat Quality Form
Chain of Custody Form (COC)
Random Number Table
pH, temp, DO and conductivity meter
Stadia rod and hand level or clinometer
Densiometer

Step 1. Measure a 100 m (300 ft) reach of channel and count the number of riffles greater than 1 m (3 ft) wide and 1 m (3 ft) long. Randomly choose 3 of the riffles within the stream reach.

Step 2. Starting with the downstream riffle, place the measuring tape along the bank of the entire riffle while being careful not to walk in the stream. Select one transect from all possible 1/3 m (1 ft) marks using a random number table. For riffles longer than 10 m (30 ft), randomly place the transect within the top third of the riffle.

Step 3. Inspect the transect before collecting BMIs by imagining a line going from one bank to the other, perpendicular to the flow. Choose 3 locations along that line where you will place your net to collect BMIs. If the substrate is fairly similar and there is no structure along the transect, the 3 locations will be on the side margins and the center of the stream. If the substrate is structurally complex along the transect, then place the 3 collections to reflect it.

Step 4. Collect BMIs at the 3 locations along the transect by placing the D-shaped net on the substrate and disturbing an area as wide as the net and 1 ft upstream. Excavate the 0.09 $m^2$ ($1ft^2$) area to an approximate depth of 10-15 cm (4-6 in) by kicking or by using a tool to loosen the substrate. Pick-up and scrub large rocks by hand under water in front of the net. If the substrate is sand or mud, a hand rake can be used to prevent substrate from filling the net. Maintain a consistent sampling effort (approximately 1-3 minutes) at each area. Combine the 3 collections within the net to make one "composite" sample.

Step 5. Place the contents of the net in a standard size 35 sieve (0.5 mm mesh) or white enameled tray. Remove the larger twigs, leaves and rocks by hand after carefully inspecting for clinging organisms. If the pan is used, place the material through the sieve to remove excess water before placing the material in the jar. Place the sampled material in a jar and completely fill with 95% ethanol. Never fill a jar more than 2/3 full with course sampled material or 1/2 full with sand or mud. **Gently** agitate jars that contain primarily mud or sand to help mix the alcohol, taking care to not damage any organisms present.

Step 6. Place a label containing descriptive information about the sites (see box) in each jar. An additional label can be taped to the outside of the jar to help with the sample log-in process at the laboratory. A Chain of Custody (COC) should accompany the samples during transportation to the laboratory.

**Bioassessment Sample Label**
Project Name:
Site Name/Code:
County:
Riffle/Reach Number:
Transect Number:
Date/Time:
Sampled by:

Step 7. Proceeding upstream, Repeat Steps 2 through 5 for the next two riffles within the stream reach.

Step 8. **QA/QC Repeat Sampling Procedure. For projects with 20 or more sites, duplicate samples must be collected at 10% of the reaches.** For reaches containing more than six riffles, randomly choose 3 riffles for the primary set of samples and randomly choose 3 more riffles for the duplicate set of samples. For reaches that contain 6 or less riffles, measure the entire length of all riffle habitat and randomly select 3 transects from the total length for the primary samples and randomly select 3 for the duplicate samples. For both methods, start at the downstream riffle or transect, proceeding upstream collecting the 6 samples designating them as primary or duplicate.

**CSBP for Low Gradient Channels**

Low gradient channels usually have less than a 1% grade and will never have more than two riffles. These channels can be as deep as 1.5 m, but with low enough water velocity to allow safe wading. **Channels greater than 1.5 m deep, with swift water velocities and/or which can not be accessed on at least one bank will require a boat**.

Step 1. Measure a 100 m (300 ft) section of channel trying to avoid large human-made structures such as bridges or dams. The stream reach can be less than 100 m (300 ft) if access or obstacles are a problem, especially if the channel is morphologically homogeneous.

Step 2. Without entering the water, survey the entire reach for approximate percentages of 5 generalized habitat categories: a. submerged vegetation, b. hard substrate of natural rock or concrete, c. soft substrate of sand or mud, d. stream bank vegetation and e. woody debris. Record the proportions and make note if it was difficult to determine depth and habitat type (e.g. water was highly turbid).

Step 3. Determine how many 2 m (6 ft) intervals can be established along the entire length of the reach. Randomly select 3 of the intervals and using a range finder or measuring tape, locate the three points on the bank of the reach.

Step 4. Starting with the downstream point, establish a transect across the channel perpendicular to the flow. Sample BMIs at 3 locations along that transect, choosing areas representing the generalized habitats identified in Step 2. Collect BMIs by placing the D-shaped kick-net on the substrate or vegetation and disturb a 0.09 $m^2$ (1 $ft^2$) portion of habitat upstream of the kick-net. Maintain a consistent sampling effort (approximately 1-3 minutes) at each site. Combine the 3 collections within the kick-net to make one "composite" sample. Note the 3 generalized habitats that were sampled along the transect on the field form.

Step 5. Place the contents of the kick-net in a standard size 35 sieve (0.5 mm mesh) or white enameled tray. Remove the larger twigs, leaves and rocks by hand after carefully inspecting for clinging organisms. If the pan is used, place the material through the sieve to remove excess water before placing the material in the jar. Place the sampled material and label (see box) in a jar and completely fill with 95% ethanol. Never fill a jar more than 2/3 full with course sampled material or 1/2 full with sand or mud. **Gently** agitate jars that contain primarily mud or sand to help mix the alcohol, taking care to not damage any organisms present.

Step 6. Place a label containing descriptive information about the sites (see page 4 box) in each jar. An additional label can be taped to the outside of the jar to help with the sample log-in process at the laboratory. A Chain of Custody (COC) should accompany the samples during transportation to the laboratory.

Step 7. Proceeding upstream, Repeat Steps 4 and 5 for the next two transects within the reach. Try to choose generalized habitats for the 9 collections (3 areas along 3 transects) in proportion to what was determined in Step 2.

Step 8. **QA/QC Repeat Sampling Procedure. For projects with 20 or more sites, duplicate samples must be collected at 10% of the reaches.** After determining how many 2 m (6 ft) intervals can be established along the entire length of the reach, randomly select 3 of the intervals for collecting the primary samples and randomly select 3 more intervals for the duplicate samples. Starting with the downstream transect, proceed upstream collecting the 6 samples and designating them as primary or duplicate.

**PROTOCOL CONSIDERATIONS FOR UNUSUAL CHANNEL CONDITIONS**

**CSBP for Intermittent or Ephemeral Channels:** Intermittent or ephemeral channels will have flowing water during the rainy season and be dry during mid to late summer. These channels can be sampled using the CSBP for high or low gradient streams, but must be sampled in a spring (March through May) index period or at the end of the wet period.

**CSBP for No Flow Conditions in High and Low Gradient Channels:** Although this is very problematic for sampling BMIs, sometimes sampling areas in high gradient streams have pocket water with little or no flow. In this case, put the net at the downstream portion of the sampling area, disturb the substrate and push the water into the net with vigorous hand motions. Strained water from the surface of a nearby pool with a bucket can be used to move organisms into the net by pouring the water into the pocket area in front of the net. In low gradient channels, low flow or no flow conditions can be quite common. In this case, put the net downstream of the sampling area, get in front of the net and agitate the substrate with a twisting foot motion for 30 seconds. At 5-10 second intervals throughout the agitation, step aside and swiftly move the net in a "figure eight" motion through the cloud of suspended substrate.

**CSBP for Bifurcated or Braided Channels:** Low gradient channels can have two or more channels flowing through a typically wide riparian corridor. There is no need to extend the transect through islands or sand bars separating these bifurcated or braided high gradient channels. Use the

standard procedure for sampling the dominant channel or randomly selected one channel if there are more than 2 similar channels >1 m (>3 ft) wide.

**CSBP for Channels <1 M (3 ft) Wide (the "Spot-Sampling" modification):** High gradient channels <1 m (<3 ft) wide can not be sampled using the 1/3 m (1 ft) wide D-frame net at three places along the transect. In this case, divide the channel into an upper, middle and lower section, relative to the flow. Each section should be approximately 30 m long, but could be divided by natural breaks in the morphology of the channel. Survey each section, without stepping into the channel for all 0.09 $m^2$ (1 $ft^2$) areas where the substrate and flow resemble a riffle. Randomly select 3 of these "sampleable areas" in the lower section and composite them into one sample. Proceed upstream and repeat for each section.

**CSBP for Large Boulder Channels:** High gradient channels that are dominated by boulder substrates too large to move, but with enough gravel substrate in patches between the boulder can be sampled similarly to the previous modification. After dividing the channel into three sections, count the patches of substrate small enough to sample and randomly select three patches. Composite the three samples and proceed upstream to sample the next two sections.

**CSBP for Channels Immediately Below Water Impoundments:** High gradient channels immediately below a water impoundment structure that prevents gravels and fines from moving downstream will often not contain shallow-fast water habitats with gravel or cobble substrates. These channels can be sampled either using the modification for large boulder channels or by using the low gradient procedure where 3 transects are chosen randomly from the entire reach.

**CSBP for Cement Channels:** Cement channels in urban areas will typically have uniform shape and depth with no natural habitat. These channels should be sampled using the low gradient protocol of 3 randomly selected transects along 100 m (300 ft) of channel. The 3 collections can be simply taken from the left margin, center and right margin of the channel. Try to avoid human made habitats such as shopping carts and other transient debris.

**CSBP for Channels with Gradient Controls:** Some low gradient urban streams will have low level dams to control the gradient. The channel will be transformed into small impoundments separated by extremely high gradient sections of large boulders to dissipate the energy. Do not sample the high gradient sections. Sample the impounded areas using the low gradient protocol or if the impoundments are too deep to wade, sample along the littoral zone of one bank. Divide the bank into upper, middle and lower sections, randomly pick three points at 1 m (3 ft) intervals and at each point, take a 0.09 $m^2$ (1$ft^2$) sweep through the vegetation trying to disturb the sediment if present. Composite the 3 collections and repeat for each section.

**CSBP for Channels with Three or Fewer Riffles:** High gradient channels that are wider than 1 m (3 ft), but have 3 or fewer riffles within the 100 m (300 ft) reach will not allow for an independent sample from several riffles. In these cases, measure the entire length of all riffle habitat and select the 3 transects randomly from the total length.

**CSBP for Channels with Continuous Riffle Habitat:** Stream reaches (usually very high gradient) that have continuous riffle habitat should be sampled using the low gradient procedure where 3 transects are chosen randomly from the entire reach.

**CSBP for Channels with Transitional Gradient:** Large watersheds can have wide channels where the gradient transitions from high to low. Riffle pool sequences can be present, but further apart than in higher gradient channels. In these cases, expand the reach length to 40 times the average width to allow for an adequate number of riffles to sample. If riffle habitat is limited to one or two riffles in a greater than 100 m (300 ft) transitional gradient reach, then consider the riffle to be hard substrate and use the low gradient procedures.

**FIELD PROCEDURES FOR MEASURING CHEMICAL AND PHYSICAL/HABITAT QUALITY**

The EPA's physical/habitat scoring criteria is a nationally standardized method (Barbour et al. 1999). It is used to measure the physical integrity of a stream and can provide a stand alone evaluation or used in conjunction with a bioassessment sampling event. DFG recommends that this procedure be conducted on every 100 m (300 ft) reach as part of a bioassessment program. A detailed description of the scoring criteria is available through the DFG Aquatic Bioassessment Laboratory website. **This procedure is an effective measure of a stream's physical/habitat quality, but can produce inconsistent measures if QA/QC measures are not regularly implemented. This procedure requires field training prior to its use and field audits throughout the program.**

The following list of quantitative measures of chemical and physical/habitat characteristics are considered minimal and should be measured when rapid bioassessments are not part of an existing chemical or fisheries habitat program where a more extensive list of parameters are measured. The information produced from measuring chemical and physical/habitat characteristics can be used to classify stream reaches and to help explain data anomalies.

**Reach-Wide Parameters:**

- GPS coordinates at the top and bottom of the reach
- Water temperature, specific conductance, pH, alkalinity and dissolved oxygen at the center of the reach using approved standardized procedures and instruments
- Reach length, average width and gradient
- Visually estimated substrate composition using the following categories: fines (<0.25 cm) (<0.1in.), gravel (0.25-0.8 cm) (0.1-2 in.), cobble (0.8-25 cm) (2-10 in.), boulder (>25 cm) (>10 in.) and bedrock (solid)

**Sample Site Specific Parameters:**

- Average length, width and depth for each of the 3 randomly chosen riffles (for unmodified high gradient protocol only)

- Water velocity immediately upstream of the three composite samples along each of the 3 transects

- Percent cover upstream of the three composite samples along each of the 3 transects. Measure this parameter using a densiometer 1/3 m (1 ft) above the water surface and averaged for each transect

- Substrate consolidation at the three sample excavations along the 3 transects. Estimates are obtained while collecting the BMI sample by noting whether the substrate is loosely, moderately or tightly cemented

- Pebble count and percent embeddedness immediately upstream of the 3 transects where BMI samples were collected. Measure this parameter by establishing a transect approximately 1/3 m (1 ft) upstream of the sample transect, randomly choosing 10 points along the transect, reaching down to the point at the end of a wooden dowel or tip of the boot and measure the width of the particle. For every third particle (3 on each transect), estimate percent embeddedness by noting how much of the particle was surrounded by fine substrate.

## LABORATORY PROCEDURES FOR ANALYZING BMI SAMPLES

DFG recommends that taxonomic identification of BMI samples collected using the CSBP is performed by a professional or permanent university laboratory with extensive experience with California taxa. **These bioassessment laboratories should participate in the California Bioassessment Laboratories Network (CAMLnet) to ensure that they are aware of the standardized level of taxonomy and QA/QC procedures recommended for bioassessments conducted in California.** To ensure a high quality product, all contracts to a bioassessment laboratory should require:

1. A Laboratory Standard Operation Procedure (SOP) document and Quality Assurance Protection Plan (QAPP)

2. A list of all taxonomists that will work on the samples including their education, years of experience and any specialized training they have received.

3. Internal QA/QC documentation for sub-sampling and taxonomic validation (can be specified to provide this information upon request);

4. Be able and willing to perform taxonomy consistent with the CAMLnet Taxonomic Effort Standards (www.dfg.ca.gov/cabw/camlnetste.pdf ).

Project managers are encouraged to subject all laboratory data to an external review by an independent laboratory at the rate of 10% to 20% (depending on experience and nature of the project) of the project samples. The DFG Aquatic Bioassessment Laboratory performs this QC procedure and can be contacted about information on the procedure requirements and costs.

**Taxonomic Level of BMI Identification**

There are two levels of taxonomic identification for samples collected using the CSBP. It is the ultimate responsibly of the contractor or project manager to guarantee that the level of taxonomy reported is consistent with the CSBP standards.

**CSBP Level 1** is used for most state-wide rapid bioassessment projects and it is imperative when comparing data to the Southern California IBI. In general, Level 1 taxonomic effort is to genera where possible for most taxonomic groups, order for oligochaetes and family for chironomids.

**CSBP Level 2** is based on the taxonomic effort levels established by the U.S. EPA for the Western Pilot EMAP. In general, Level 2 taxonomic effort identifies insects to species level where possible and the Dipteran Family: Chironomidae to genus.

**Compositing Samples or Data**

There will always be 3 samples collected at each sampling reach when using the CSBP. Depending on the objectives of the project, the samples can be processed as individual samples and subsampled for 300 organisms/sample (900 organisms total per site) or **composited at the laboratory** and subsampled for 500 organisms.

**Subsampling**

The CSBP requires fixed count subsampling with a +/- 10% accuracy. The total count of BMIs must come from at least 3 randomly selected grids within a subsampling tray. The last grid must be fully counted to get an estimate of relative abundance. The debris from processed grids should be put in a clean "remnant" jar and the remaining contents of the tray should be placed back into the original sample jar. If a "large and rare" survey is preformed on the sample, it should be conducted after the subsampling procedure and counted separately.

**Data Production, Storage and Analysis**

DFG has developed a Microsoft Access® database based loosely on the U.S. EPA's Environmental Data Analysis System (EDAS). The structure of the CalEDAS database is available through the DFG Aquatic Bioassessment Laboratory website, but it does not currently come with end-user support. Whether using the DFG database or other software, the laboratory analysis should produce a BMI taxa list that is consistent with CAMLnet (see above) for all samples and a list of common or project specific biological metrics. Many common biological metrics are listed in the U.S. EPA's RBP document (Barbour et al 1999) and several other sources of bioassessment literature. When BMI samples are processed independently, there are two options for calculating metrics depending on the needs of the project:

1. Calculate metrics for all three samples independently and calculate metric averages at each site

2. The three samples can be composited in the analysis stage, and a 500 count subsample of the 900 organisms can be used to generate one set of **cumulative** metrics for each site.

## QA/QC CONSIDERATIONS FOR USING THE CSBP

All private and public entities conducting bioassessment using the CSBP should have a Standard Operating Procedures document (SOP) and a Quality Assurance Protection Plan (QAPP). Large programs and laboratories can have a quality assurance officer and some smaller operations may only have a field or laboratory supervisor. In either case, those individuals responsible for assuring the quality of samples collected in the field and processed in the laboratory should be trained on all aspects of the CSBP. Two 3-day courses on bioassessment concepts and the use of the CSBP are available through the American Fisheries Society (CalNeva AFS) and the Society of Environmental Toxicology and Chemistry (NorCal and SoCal SETAC). Information on these courses can be found at www.slsii.org

The details of a QAPP should be tailored for particular bioassessment operations. Depending on the nature of the project, appropriate boiler plate for QAPPs may be available through Regional Water Quality Control Boards or the State Water Resources Control Board. These agencies should be contacted before developing a QAPP and initiating a bioassessment program.

## REFERENCES USED IN THIS DOCUMENT

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