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-D-97-002). The CSBP is a cost-effective tool which utilizes measures of the stream's benthic macroinvertebrate (BMI) community and its physical/habitat characteristics to determine the stream's biological and physical integrity. BMIs can have a diverse community structure with individual species residing within the stream for a period of months to several years. They are also sensitive, in varying degrees, to temperature, dissolved oxygen, sedimentation, scouring, nutrient enrichment and chemical and organic pollution. Biological and physical assessment measures integrate the effects of water quality over time, are sensitive to multiple aspects of water and habitat quality and can provide the public with a familiar expression of ecological health.

The use of this procedure will ensure that the data generated can be used by state regulatory agencies and will be compatible with a statewide bioassessment effort. The Protocol Brief is only a summary and does not contain all the information that may be required to implement a bioassessment program.

CALIFORNIA DEPARTMENT OF FISH AND GAME SCIENTIFIC COLLECTING PERMIT

Anyone who collects fish, amphibians, or invertebrates from the waters of the state must have in their possession a DFG Scientific Collecting Permit. The permit can be obtained from the DFG License and Revenue Branch in Sacramento (916 227-2225). Those people conducting bioassessment in California should specify on the permit application, that they will take freshwater invertebrates (authorization 5) and 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. Starting in summer 1999, everyone indicating that they will be conducting bioassessment in California will receive the most recent version of the CSBP Protocol Brief and an Access® database program to store, process and return a copy of the collected data.

FIELD PROCEDURES FOR COLLECTING BMI SAMPLES AND ASSESSING PHYSICAL/ HABITAT QUALITY

The CSBP can be used to detect aquatic impacts from point and non-point sources of pollution and for assessing ambient biological condition. The sampling unit is an individual riffle or riffles within a reach of stream depending on the type of sampling design used. Riffles are used for collecting biological samples because they are the richest habitat for BMIs in wadeable streams. **The BMI sampling procedures described in this Protocol Brief are intended for sampling wadeable, running water streams with available riffle habitats.** There are approved modifications of this procedure for narrow (< 1m) streams, wadeable streams with sand or mud bottoms and channelized streams. There are also procedures for lentic or still water environments.

Point Source Sampling Design

There will be discernable perturbations, impacting structures or discharges into the stream with point sources of pollution. The sampling units will be individual riffles within the affected section of stream and an upstream unaffected section. At least one riffle in the unaffected section should be sampled and one or more riffles in the affected section depending on the amount of detail that is required on downstream recovery. The riffles used for sampling BMIs should have relatively similar gradient, substrate and physical/habitat characteristics and quality. **One sample will be collected from 3 randomly chosen transects in each riffle.**

Use the following step-by-step procedures for collecting BMIs using the point source sampling design:

Step 1. Place the measuring tape along the bank of the entire riffle while being careful not to walk in the stream. Each meter or 3 foot mark represents a possible transect location. Select 3 transects from all possible meter marks along the measuring tape using a random number table. Walk to the downstream transect before proceeding to Step 2.

Step 2. 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 there is substrate and structure complexity along the transect, then as much as possible, select the 3 collections to reflect it.

Step 3. After mentally locating the 3 areas, collect BMIs by placing the D-shaped kick-net on the substrate and disturbing a 1x2 foot portion of substrate upstream of the kick-net to approximately 4-6 inches in depth. Pick-up and scrub large rocks by hand under water in front of the 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.

Step 4. 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 the 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 sampled material and gently agitate jars that contain primarily mud or sand.

Step 5. Proceeding upstream, repeat Steps 2 through 4 for the next two randomly chosen transects within the riffle.

Non-point Source Sampling Design

There will be no obvious perturbations or discharges into the stream with non-point sources of pollution. This sampling design is appropriate for assessing an entire stream or large section of stream. The sampling units will be riffles within a reach of stream. The stream reach must contain at least 5 riffles within the same stream order and relative gradient. **One sample will be collected from the upstream third of 3 randomly chosen riffles.**

Use the following step-by-step procedures for collecting BMIs using the non-point source sampling design:

Step 1. Randomly choose 3 of the 5 riffles within the stream reach using the random number table.

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 1 transect from all possible meter marks along the top third of the riffle using a random number table.

Step 3. (See Point Source Sampling Design Step 2)

Step 4. (See Point Source Sampling Design Step 3)

Step 5. (See Point Source Sampling Design Step 4)

Step 6. Proceeding upstream, Repeat Steps 2 through 5 for the next two riffles within the stream reach.

Sampling Design for Assessing Ambient Biological Conditions

Assessment of ambient biological condition utilizes both the point and non-point source sampling designs to cover an entire watershed or larger regional area. Ambient bioassessment programs are used to evaluate the biological and physical integrity of targeted inland surface waters. Stream reaches should be established in the upper, middle and lower portions of each watershed and above and below areas of particular interest. Quite often bioassessment is incorporated into an existing chemical or toxicological sampling design. In most cases, the water quality information is being collected at a particular point on the stream. Although there will be the tendency to use the point source design, try to convert to a non-point reach design for biological sampling.

Measuring Physical/Habitat Quality

The physical/habitat scoring criteria is an EPA nationally standardized method. It is used to measure the physical integrity of a stream and can be a stand alone evaluation or used in conjunction with a bioassessment sampling event. DFG recommends that this procedure be conducted on every reach of stream sampled as part of a bioassessment program. Fill out the Physical/Habitat Quality Form for the entire reach where the BMI samples were collected as part of a non-point source sampling design. Some of the parameters do not apply to a single riffle, so this procedure is usually not performed as part of the point source sampling design. **This procedure is an effective measure of a stream's physical/habitat quality, but requires field training prior to using it and implementation of quality assurance measures throughout the field season.**

Measuring Chemical and Physical/Habitat Characteristics

Measurements of the chemical and physical/habitat characteristics are used to describe the riffle environment and help the water resource specialist interpret the BMI data. The information can be used to classify stream reaches and to explain anomalies that might occur in the data. **They are not necessarily a good substitute for a quantitative fisheries habitat survey.**

Use the following step-by-step procedures to measure chemical and physical/habitat characteristics:

Step 1. Water temperature, specific conductance, pH and dissolved oxygen should be measured at the sampling site using approved standardized procedures and instruments.

Step 2. Record the riffle length determine for the procedure to choose the transect locations. Estimate the average riffle width by averaging several measurements along its length. Measure the riffle depth by placing the stadia rod at several places within the riffle and averaging the measurements.

Step 3. Estimate or measure the entire length of the reach where the three riffles are chosen as part of the non-point source sampling design.

Step 4. Measure the riffle velocity using a flow meter placed in front of the three locations along the transect(s) where the BMI samples were collected. Average the readings.

Step 5. Estimate the percent of the riffle surface which is covered by shade from streamside vegetation (canopy cover) using a densiometer at several places along the riffle and averaging the readings.

Step 6. Determine substrate complexity and embeddedness by applying Parameters 1 and 2, respectively from the Physical/Habitat Quality Form to the riffle where the BMI sample was collected. Use the entire riffle to assess these parameters and make note if the area along the transect(s) are considerably different from the rest of the riffle.

Step 7. Visually estimate the percent of riffle in each of the following substrate categories: fines (<0.1"), gravel (0.1-2"), cobble (2-10"), boulder (>10") and bedrock (solid). Use the entire riffle to assess this parameter and make note if the area along the transect(s) are considerable different from the rest of the riffle.

Step 8. Estimate substrate consolidation by kicking the substrate with the heel of your wader boots to note whether it is loosely, moderately or tightly cemented. The estimate should also take into consideration the hands-on experience obtained from collecting the BMI sample.

Step 9. Measure the gradient or slope of the riffle using a stadia rod and hand level or a clinometer.

Using the California Bioassessment Worksheet

A California Bioassessment Worksheet (CBW) should be filled out for each individual riffle when following the Point Source Sampling Design and for the entire reach when using the Non-point Sampling Design. Use the following step-by-step procedures for filling out the CBW:

Step 1. Enter the watershed and stream name, date and time of sample collection, name of the company or agency collecting the samples, sample identification number(s), and a short site description on the CBW.

Step 2. Enter the names of each crew member in the Crew Member Box.

Step 3. Determine the longitude and latitude coordinates and elevation from a GPS unit or watershed topographic map. Determine which California ecoregion or sub-ecoregion the site is located in by using the U.S. Forest Service map obtained by visiting the California Aquatic Bioassessment Web Site. Record this information and any other comments on the sampling site in the Site Location Box.

Step 4. Record the water temperature, specific conductance, pH and dissolved oxygen measurements in the Chemical Characteristics Box.

Step 5. Record the physical/habitat characteristics in the Riffle/Reach Characteristics Box. For the Point Source Sampling Design, record the riffle length, the 3 transect locations along the riffle and the physical/habitat characteristics information (starting with Ave. Riffle Width) on the lines below the "riffle 1" column. For the Non- point Source Sampling Design, record the reach length, the total score from the Physical/Habitat Quality Form and all physical/habitat characteristics information on the lines below the "riffle 1" through "riffle 3" columns.

Step 6. Record the name and address of the Bioassessment Laboratory that received the samples along with the laboratory sample numbers if they are different than the field sample identification numbers.

Using the Chain of Custody (COC) Form

The Chain of Custody (COC) form is a necessary part of collecting BMI samples. It is an official document for tracking the samples from the field to the laboratory and then to their final storage area. The COC will also provide important information if samples are lost or misplaced. Use the following step-by-step procedures for using the COC:

Step 1. At the end of the field day, record the following information on the COC for each group of BMI samples: program name;

watershed name; field ID numbers; sampling dates; and name, address, telephone number and signature of one of the crew members collecting the sample.

Step 2. Field samples and COCs must remain in a locked sample depository until a decision has been made to send them to a bioassessment laboratory for processing.

Step 3. When transporting to a bioassessment laboratory, each group of samples must be accompanied by a COC. Upon delivery, a Bioassessment Laboratory Number will be assigned to each sample. Record this number on the COC and each individual CBW along with the name and address of the bioassessment laboratory. When all samples listed on the COC are accounted for, then the individual delivering the samples will sign the "Released By" portion and the laboratory personnel will sign the "Received By" portion of the COC. The original COC will remain at the laboratory and a copy will be retained by the project supervisor.

PROFESSIONAL (LEVEL 3) LABORATORY PROCEDURES

The CSBP has three levels of BMI identification. Level 3 is the professional level equivalent and requires identification of BMIs to a standard level of taxonomy, usually to genus and/or species level.

Subsampling

Step 1. Retrieve the sample from the sample depository and cross-check the sample number with the bioassessment laboratory number on the COC.

Step 2. Empty the contents of the sample jar into the # 35 sieve (0.5 mm mesh) and thoroughly rinse with water.

Step 3. Once the sample is rinsed, clean and remove debris larger than ½ inch. Remove and discard green leaves, twigs and rocks. Do not remove filamentous algae and skeletonized leaves.

Step 4. After cleaning, place the material into a plastic tray marked with equally sized, numbered grids (approximately 2x2 inches). Do not allow any excess water into the tray. Spread the moist, cleaned debris on the bottom of the tray using as many grids necessary to obtain an approximate thickness of ½ inch. Make an effort to distribute the material as evenly as possible.

Step 5. Remove and count macroinvertebrates from randomly chosen grids until 300 BMIs are removed. Place the BMIs in a clean petri dish containing 70% ethanol/5% glycerin. Completely count the remaining organisms in the last grid but do not include them with the 300 used for identification. The final count should be recorded on the benchsheet for eventual abundance calculations.

Step 6. 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. Both jars should be filled with fresh 70% ethanol, labeled (bioassessment laboratory number and either "original" or "remnant") and returned to the sample depository.

Identification of BMIs

Step 7. Identify the 300 BMIs from each sample to the standardized level recommended by CAMLnet using appropriate taxonomic keys.

Step 8. Place identified BMIs in individual glass vials for each taxon. Each vial should contain a label with taxonomic name, bioassessment laboratory number, stream, county, collection date and collector's name. This voucher collection should be labeled and returned to the Sample Depository.

Step 9. Record taxonomic information on a Macroinvertebrate Laboratory Bench Sheet. The bench sheet should include the following information: watershed or project name; sampling date; sample ID number; bioassessment laboratory number; date of subsampling; name of subsampler; remnant jar number; taxonomy completion date; name of taxonomist; taxonomic list of organism and enumeration; total number of organisms; total number of taxa; list of unknowns, problem groups and comments.

Step 10. Maintain a reference collection of representative specimens of all accurately identified BMI taxa.

QUALITY ASSURANCE (QA) PROCEDURES FOR THE FIELD AND LABORATORY

QA for Collecting BMIs

The CSBP is designed to produce consistent, random samples of BMIs. It is important to prevent bias in riffle choice and transect placement. The following procedures will help field crews collect unbiased and consistent BMI samples:

. In using the CSBP, most sampling reaches should contain riffles that are at least 10 meters long, one meter wide and have a homogenous gravel/cobble substrate with swift water velocity. **There are approved modifications of the CSBP when these**

conditions do not exist. Sampling personnel should be familiar with methods to sample narrow streams, wadeable streams with muddy bottoms and channelized streams.

2. A DFG biologists or project supervisor should train field crews in the use of the BMI sampling procedures described in the CSBP. Field personnel should review the CSBPs before each field season.

3. During the training, crew members should practice collecting BMI samples as described in the CSBP. The 2 ft^2 area upstream of the sampling device should be delineated using the measuring tape or a metal grid and the collection effort should be timed. Practice repeatedly until each crew member has demonstrated sampling consistency. Throughout the sampling season, assure that effort and sampling area remain consistent by timing sampling effort and measuring sampled area for approximately 20% of the sampling events. The results should be discussed immediately and need not be reported.

QA for Measuring Physical/Habitat Quality

Physical/habitat parameters are assessed using a ranking system ranging from optimal to poor condition. This rapid ranking system relies on visual evaluation and is inherently subjective. The following procedures will help to standardize individual observations to reduce differences in scores:

1. A DFG biologist or a project supervisor should train field crews in the use of the EPA physical/habitat assessment procedures. Contact DFG or visit the California Aquatic Bioassessment Web Site for a detailed description of the procedures. Field personnel should review these procedures before each field season.

2. At the beginning of each field season, all crew members should conduct a physical/habitat assessment of two practice stream reaches. Assess the first stream reach as a team and discuss in detail each of the 10 physical/habitat parameters described in the EPA procedure. Assess the second stream reach individually and when members are finished, discuss the 10 parameters and resolve discrepancies.

3.Crews or individuals assessing physical/habitat quality should frequently mix personnel or alternate assessment responsibilities. At the end of each field day, crew members should discuss habitat assessment results and resolve discrepancies.

4.The Project Supervisor should randomly pre-select 10 - 20% of the stream reaches where each crew member will be asked to assess the physical/habitat parameters separately. The discrepancies in individual crew member scores should be discussed and resolved with the Project Supervisor.

QA for the Laboratory

Laboratory analysis of macroinvertebrate samples can be a significant cost for bioassessment programs. The CSBP specifies identification of BMIs to a standard level of taxonomy, usually to genus and/or species level. The CSBP also requires subsampling procedures using a fixed count of 300 organisms. Employing these procedures with confidence requires an effective quality assurance program. Complete quality assurance compliance will require a minimal 10% cost overhead. However, it will allow for testing whether subsampling, organism enumeration and taxonomic identification are consistent and accurate. Use the following procedures in the bioassessment laboratory to ensuring that quality data is produced:

The California Macroinvertebrate Laboratory Network (CAMLnet) - All individuals, private consulting firms and agency personnel using the CSBP laboratory procedures should contact the WPCL for information on CAMLnet. This group consists of personnel from bioassessment laboratories throughout California. The group provides a forum where laboratory procedures are discussed and the BMI taxonomic levels are determined. It also provides taxonomic workshops and assistance with interlaboratory taxonomic verification.

tandard Operation Procedures (SOP) - Each bioassessment laboratory should produce an SOP manual following the procedures utlined in the CSBP, but with detailed instructions specific to each laboratory. The SOP manual should be maintained for all boratory operations and updated regularly. The assigned personnel and the duties of a Laboratory Supervisor and QA Taxonomist ıould be specified in the SOP manual. Customized benchsheets should be developed for each phase of subsampling and entification.

ımple Handling and Custody - When samples arrive, laboratory staff should inspect the samples for a sufficient volume of ethanol d labels for pertinent information including water-body name, sample date and time, location, transect number and sampler name. e steps discussed in the "Using the Chain of Custody (COC)" section in this protocol should be followed. The sample description ormation should be recorded in the Laboratory Sample Inventory Log and each sample given a unique identification number. A itten and electronic record should be maintained to trace the samples from entry into the laboratory through final analysis. Samples

should be stored in the a Sample Repository until processing and returned after processing.

Subsampling - Subsampling involves removing 300 organisms from each sample, or all organisms if the entire sample contains fewer than 300. The procedure to estimate abundance usually requires removing more than 300 organisms from each sample; however, only 300 are retained for identification. The Subsampling Technician systematically transfers organisms from the sample to a collection vial then transfers the processed sample debris (remnant) into a Remnant jar. At least 10% of the Remnant samples should be examined by the QA Taxonomist for organisms that may have been overlooked during subsampling. For subsamples containing 300 or more organisms, the Remnant sample should contain fewer than 10% of the total organisms subsampled. The Remnant for samples containing fewer than 300 organisms should contain fewer than 30 organisms.

Taxonomic Identification and Enumeration - The CSBP requires that all organisms are identified to a standardized taxonomic level using established taxonomic keys and references. The QA Taxonomist should check at least 10% of the samples for taxonomic accuracy and enumeration of individuals within each taxon. The same sample numbers that were selected randomly for the subsampling quality control should be used for this procedure. Misidentifications and/or taxonomic discrepancies as well as enumeration errors should be noted on the laboratory benchsheets. The Laboratory Supervisor determines if the errors warrant corrective action.

Organism Recovery - During the sorting and identification process organisms may be lost, miscounted or discarded. Taxonomists will record the number of organisms discarded and a justification for discarding on the laboratory benchsheets. Organisms may be discarded for several reasons including: 1) subsampler mistakes (e.g. inclusion of terrestrial or semi-aquatic organisms or exuviae), 2) small size (< 0.5 mm), 3) poor condition or 4) fragments of organisms. The number of organisms recovered at the end of sample processing will also be recorded and a percent recovery determined for all samples. Concern is warranted when organism recoveries fall below 90%. Samples with recoveries below 90% should be checked for counting errors and laboratory benchsheets should be checked to determine the number of discarded organisms. If the number of discarded organisms is high, then the technician that performed the subsampling should be informed and re-trained if necessary.

Corrective Action - Any quality control parameter that is considered out of range should be followed by a standard corrective action process that includes two levels. Level I corrective action includes an investigation for the source of error or discrepancy derived from the quality control parameter. Level II corrective action includes checking all samples for the error derived from the quality control parameter but is initiated only after the results of the Level I process justify it. The decision to initiate Level II corrective action and reanalyze samples or conduct quality control on additional samples should be made by the Laboratory Supervisor.

Interlaboratory Taxonomic Validation - An external laboratory or taxonomic specialist should be consulted on a regular basis to verify taxonomic accuracy. External validation can be performed on selected taxa to help the laboratory taxonomists with problem groups of BMIs and to verify representative specimens of all taxa assembled in a reference collection.

Bioassessment Validation - The CSBP recommends at least 10% bioassessment validation where whole samples of 300 identified BMIs are randomly selected from all samples either for a particular project or for all samples processed within a set time period such as each 6 months or a year. The labels should be removed from the vials and replaced with a coded label that does not show the taxonomic name of the BMIs. The validation laboratory or specialist should be instructed to identify and enumerate all specimens in each vial and produce a taxonomic list. There will inevitably be some disagreements between the bioassessment and the external laboratory on taxonomic identification. These taxa should be reexamined by both parties and a resolution reached before a final QA report is written. **DFG is working on this QA technique to determine the acceptable level of misidentification and appropriate corrective actions.**

DATA DEVELOPMENT AND ANALYSIS

The CSBP analysis procedures are based on the EPA's multi-metric approach to bioassessment data analysis. The EPA is developing procedures for multi-variate analysis of bioassessment data, but that method is not presented here. However, the sampling protocols presented in this document were designed to facilitate the use of multi-variate analysis and more information will be presented when standardizes techniques for California become available.

A taxonomic list of the BMIs identified for each sample should be generated for each project along with a table of sample values and means for the biological metrics listed on the last page of this document. Variability of the sample values should be expressed as the coefficient of variability (CV). Significance testing can be use for point source sampling programs and ranking procedures can be used to compare sites sampled using the non-point sampling design (contact DFG for information on ranking formulas). Ultimately, there will be a regional Index of Biological Integrity (IBI) to compare sample site mean values.

Starting in summer 1999, an Access® database program to store, process and return a copy of the collected data will be available. Contact DFG or visit the California Aquatic Bioassessment Web Site to learn more about the availability of regional IBIs and the database program.

FIELD EQUIPMENT AND SUPPLIES

Measuring tape (300 ft or 100 meter)
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
Random Number Table
Ph, temp, DO and conductivity meter
Stadia rod and hand level or clinometer
Densiometer
GPS unit or watershed topographic map

Bioassessment Sample Label
Riffle/Reach Number:
Transect Number:
Stream Name:
Date/Time:
Sampled by:

LABORATORY EQUIPMENT AND SUPPLIES

Dissecting microscope
Standard size 35 sieve (0.5 mm)
Gridded white enameled pan
Wide-mouth glass jars
Plastic petri dish
Vials
Taxonomic keys
70% ethanol/5% glycerin solution
Forceps
List of Standardized Taxonomic Levels
Water-proof paper and pencils
Laboratory benchsheets
Random Number Table
Chain of Custody Form