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## **A PROPOSED LENTIC BENTHIC BIOASSESSMENT PROCEDURE FOR CALIFORNIA**

(Protocol Brief for Biological Sampling in Lakes, Reservoirs, and Ponds)

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*Draft*  
November 2004  
SFEI Contribution No. 315



***Acknowledgements:***

We would like to thank Brian Anderson (UC Davis MPSL), Jim Harrington (CDFG ABL), Victor DeVlaming (UC Davis ATL), and Charles Goldman for their technical assistance and oversight. This protocol was produced under State Water Resources Control Board contract 01-130-250-0

***Citation:***

Hayworth, J.D. November 2004. A Proposed Lentic Benthic Bioassessment Procedure for California (Protocol Brief for Biological Sampling in Lakes, Reservoirs, and Ponds) - Draft Technical Report. San Francisco Estuary Institute, Oakland, CA.

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## **1. Introduction**

Lentic water bodies such as small ponds, lakes, reservoirs, sloughs, and lagoons, are under-represented in comprehensive statewide California bioassessment. The development of a standardized protocol to sample highly diverse lentic systems would enable the collection of high quality, comparable data needed in order to document condition of all California water body types and address the State's 305b requirements. This brief is a proposed standardized protocol utilizing measures of the benthic macroinvertebrate community to assess biological conditions of still water environments. The protocol was developed from work conducted on the California Aquatic Pesticide Monitoring Program (APMP) (Hayworth and Siemering 2003), and based on adaptations of procedures described in the U.S. Environmental Protection Agency's Lake and Reservoir Bioassessment and Biocriteria Technical Guidance Document (EPA 841-B-98-007) (Gerritsen et al. 1998), US EPA's EMAP Field Operations Manual for Lakes (EPA/620/R-97/001) (Baker et al. 1997), and those utilized by various state agencies around the country (WIDNR 2003, FDEP 2004, US EPA 1987).

## **2. Protocol Overview**

The purpose of this protocol is to allow ambient biological monitoring of lentic water bodies, although it can also be adapted for diagnostic monitoring of stressors. Study design and objectives will determine how the protocol is used, and must be considered prior to field sampling. The protocol provides for random sampling of benthic macroinvertebrates in the most stable, representative habitat within lake/reservoir/ponds, which occurs in the sublittoral zone. This zone is defined as the area immediately below the vegetated littoral zone, where little to no submerged or emergent vegetation is present. While depths and slopes of the sublittoral zone vary by site, optimal sampling depth is located in water depths ranging from 2-4 meters to ensure sampling within the oxygenated zone of most systems (US EPA 1987).

Depending on study objectives and resources, this protocol recommends selection of reference sites specifically for comparison of study data. Reference should represent a pre-determined and clearly defined idea of the 'least impacted' condition available (Gerritsen et al. 1998). Reference should be based on similar attributes (e.g. water and sediment quality) shared between the main site and the reference site, with the exception of degree of disturbance.

## **3. Field Methods**

### *3.1 Sampling summary for benthic macroinvertebrates*

<b>Sampling Habitat</b>	Sublittoral zone in approximately 2-4 meter water depth
<b>Sampling Gear</b>	Dredge grab samplers - Petite Ponar or Ekman (tall).
<b>Sample Replication</b>	Minimum 3-10 transects per site or sampling area, depending on site area. 3 replicate grabs at one station per transect.
<b>Sieve Size</b>	#35 sieve (0.5 mm mesh)

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## *3.2 Equipment and Supplies*

- 100 meter (300ft) measuring tape
- GPS unit
- Petite Ponar (6 inch grab) or Ekman Dredge (6 inch grab) w/ attached rope marked in 0.1 meter sections
- Bucket Sieve (0.5 mm mesh)
- Standard size 35 nesting sieve (0.5 mm)
- Secchi Disk
- Extendable sampling pole for depth determinations
- White enamel or plastic sorting pan
- Forceps
- 95% Ethanol
- 2 1-L Rinse Bottles, 1 filled with 95% Ethanol, 1 filled with D.I. water
- Wide mouth plastic sample containers (500-mL and/or 1-L)
- Plastic Funnel
- Cooler
- Heavy cloth gloves
- Lentic Bioassessment Worksheet (LBW)
- Chain of Custody Form (COC)
- Water quality meters (pH, DO, conductivity, temperature)
- Permanent markers, pencils
- Water proof labels, stock/heavy bond paper for labels inside benthos jars
- Site Map

## *3.3 Procedures*

1. Prior to visiting site, obtain a map of the system. At the site, make a preliminary survey of shoreline and general water body features.
2. Fill out a LBW for the site with site ID, lentic type, date, time, site acreage, surface conditions (i.e. fetch), water color, trophic state, and GPS coordinates. Sketch and mark features of the site observed within sketch area of CBLW form.
3. Measure the shoreline perimeter using the map. Assign potential transect marks at every 2 meters for sampling areas less than 300 meters, and every 4 meters for sampling areas greater than 300 meters.
4. Select the appropriate number of transects from all possible assigned transect marks using a table of random numbers. For sites or sampling areas less than 500 acres in size, a minimum 3-6 transects should be conducted. If the site or sampling area is larger than 500 acres, a minimum 6-10 transects should be conducted. Transects should represent the average conditions within the area of interest. If a transect does not meet this criteria, another transect should be chosen from among potential transect marks.
5. Delineate transects with an invisible line running perpendicular to shore towards the center of the water body, and terminating at the end of the littoral zone. One benthic invertebrate station is located at each transect's end in the sublittoral zone and encompasses a 25 m<sup>2</sup> area. Denote transects and stations on map. Measure the length of each transect to the start of the sublittoral zone and record on LBW.

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6. For each station, deploy the sampling boat to the transect end, allowing the boat to drift in or be gently paddled to the station to decrease disturbance. Place anchor outside the 25 m<sup>2</sup> station area.
7. At each station: Collect all water quality and physical habitat measures and record on CBLW (see section 5). Record water depth at each station.
8. At each station: Collect 3 replicate benthic grabs. From a boat, lower the dredge to the bottom substrate, lower the messenger (for Ekman) or pull up on rope (for Petite Ponar), and retrieve the dredge. Each grab is collected from previously undisturbed substrate, i.e. successive grabs are pulled from various spots around the perimeter of the boat within the 25 m<sup>2</sup> station. For each grab, record the water depth, volume of material in the dredge, and bottom substrate condition. On one of the grabs, insert ORP redox probe into the top 2-3 cm and record reading.

### **Guidelines for Dredge Sample Acceptability (see Figure 1)**

- Sample is not extruded from the top of the dredge
- The sediment surface is relatively uniform, flat, and the entire surface is included in the sampler.
- No rocks or debris are caught in the dredge jaws, impeding complete jaw closure.
- The presence of aquatic vegetation and large organic debris in the dredge is minimal.

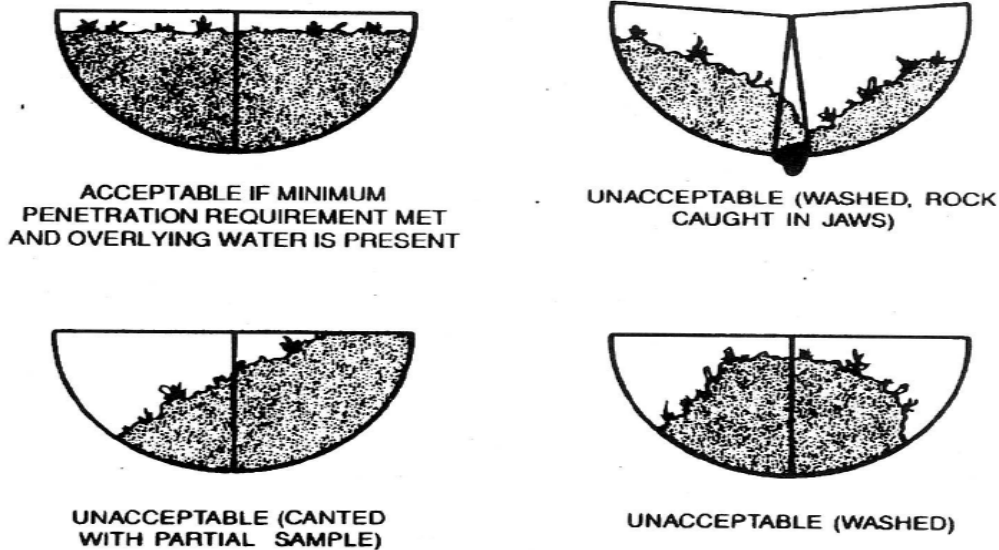
9. After collecting sample, place grab material into a 0.5mm mesh sieve bucket. Clean and remove any large debris in bucket (adding any invertebrates removed into the sample container).
10. Wash the sample through the bucket over the side of the boat until no more fine sediment washes through the mesh. Washing is accomplished by submerging the bucket half way into the water and gently lowering and raising the bucket with enough thrust to push water in from the bottom and suspend the sediment, while twisting the bucket to re-suspend the sample and allow fine sediment to fall out. Do not allow site water into the bucket from the top as this could allow non-sample organisms to contaminate the sample. If the sample contains chunks of clay, the globules must be carefully broken up by hand. Handle washing as gently as possible to avoid damage to fragile invertebrates.
11. Transfer the material in the bucket to a sample container. This is done by tipping the bucket so a bottom corner is lowest and the remaining sample is washed off the screen into the bottom corner of the bucket. Wash the concentrated material directly into the sample jar using a wash bottle and forceps or hand. Remove water in the sample jar by inverting the jar tightly over a 0.5mm mesh standard sieve and draining the water. Pick off any material left on the sieve with forceps and place back into the container. Thoroughly rinse dredges and sieves and pick free of any organisms or debris prior to use at the next sampling station.
12. Add 95% ethanol to sample containers in the following ratio: 30 % sample material and 70% ethanol. Gently invert the sample jar to mix; the sample should not be shaken.
13. Place a waterproof paper label, written in pencil, in the container indicating the sample identification code, date, water body name, and collector initials. Label

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the outside of the container with the same information and with the preservative noted. If more than one container is needed for a sample, each container label should contain all the information for the sample and should be numbered. Store samples in coolers with no ice.

14. Move the boat to the station area of each transect. Repeat procedures 6-13 for every sample.
15. On return from the field, check sample jar lids for tightness and add additional ethanol as needed prior to packing and shipping. All shipped samples must be accompanied by a 'Chain of Custody' (COC) form that indicates sample identification information and analyses requested for each sample. A copy of the COC should be maintained in the project's records.

**Figure 1: Examples of Acceptable and Unacceptable Grab Samples**  
(from U.S. EPA 1987)



## 4. Physical Habitat, Water and Sediment Quality Measures

A whole site habitat assessment is not presented in this protocol, as other efforts are underway to develop and adapt protocols for lentic systems (i.e. CRAM) (Collins et al. 2004). However, qualitative measures of physical and chemical conditions are collected in conjunction with benthic sampling.

### 4.1 Physical Habitat Measures

At each station, the approximate coverage of submerged, emergent and floating macrophyte is recorded, as well as the presence of general human disturbances (dock structures, boat density, aquatic pesticide application, trash or non-organic debris).

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## ***4.2 Water Quality Measures***

Water quality measurements are collected at every station. Water color and surface film/appearance are recorded from general observation. Dissolved oxygen, pH, temperature, and conductivity or salinity are measured from the bottom, middle and top portions of the water column using hand-held water quality meters. For each measurement, record the water depth and temperature. Conduct D.O. and temperature profiles of the water body at one station minimum at 1-meter increments throughout the water column to just above substrate surface. As sites can have highly flocculent bottom substrates, it is suggested that a weighted plate (thin, but dense material) be attached to meter probes to alleviate problems with accurate bottom measurements. Water clarity is measured with a Secchi disk.

## ***4.3 Sediment Quality Measures***

The sublittoral sediment from which the benthic samples are collected is characterized initially by visual and textural (by hand) examination of the substrate within the benthic dredge sample. For each grab, assess the general sediment composition (texture, color, odor) and record on the LBW. Redox potential (Eh) of the soil will also be measured with an ORP probe.

## **5. Laboratory Procedures**

Bioassessment laboratories chosen to process a project's benthic macroinvertebrate samples should have extensive California taxonomic experience, as well as participate in the California Bioassessment Laboratories Network (CAMLnet) (Ode 2003). CAMLnet provides standardized levels of taxonomy and QA/QC procedures to promote standardized bioassessment data in California. The California Department of Fish and Game Aquatic Bioassessment Laboratory (CDFG ABL) recommends that contracts to a bioassessment laboratory should require the following (from CDFG 1999):

1. A Laboratory Standard Operating 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](http://www.dfg.ca.gov/cabw/camlnetste.pdf)).

### ***5.1 Subsampling***

A minimum of 300 organisms should be identified and counted for each sample, with at least three randomly selected grids processed. Identified organisms should be placed in separate glass vials for each taxon. If 300 organisms are counted before a grid is completed, the remaining organisms in that grid are counted but not identified. The remaining sample (uncounted grids, and counted but unidentified organisms) is placed back in a jar labeled for the "original" sample. For subsamples containing 300 or more organisms, the remnant sample should contain fewer than 10% of the total organisms

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subsampled. Retrieved organisms and remnant samples should be preserved for long-term storage according to standard benthic laboratory procedures. It is recommended that a voucher collection for each site be maintained by the project manager.

## *5.2 Taxonomic Level for BMI Identification*

Most taxonomic groups are sorted and identified to the genus level according to CAMLnet (corresponds to CSBP Level 1). Chironomidae and oligochaetes are also taken down to the genera level where possible. Chironomidae are identified according to taxonomic levels recommended by the U.S. EPA for the Western Pilot EMAP.

## *5.3 Data Handling*

Laboratory analysis should produce a BMI taxa list that is consistent with CAMLnet (see above) for all samples, taxa counts by site, and a list of common or project specific biological metrics. Many common biological metrics are listed in the U.S. EPA's Lake and Reservoir Bioassessment and Biocriteria Technical Guidance Document (EPA 841-B-98-007), as well as in other sources of bioassessment literature. Some candidate metrics are listed below, along with their predicted response to stress.

<b>Metric</b>	<b>Predicted Response to Stress</b>
No. of taxa	Reduced
Diversity	Reduced
% Oligochaetes	Increased
% Contribution of dominant taxon	Increased
Tolerance Indices	Increased
Abundance (exclude Chironomidae and Tubificidae)	Reduced

## *5.4 General QA/QC*

Ten percent of the samples from any given project should be checked for quality assurance in taxonomic identification by an external laboratory. Individuals responsible for ensuring sample quality collected in the field and processed in the laboratory should be thoroughly trained on protocol procedures prior to use.

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[http://www.dnr.state.wi.us/org/water/wm/wqs/sediment/sampling/702\\_1.HTM](http://www.dnr.state.wi.us/org/water/wm/wqs/sediment/sampling/702_1.HTM).

### **7. Appendix**

Lentic Bioassessment Field Worksheet (LBW)

# Lentic Bioassessment Field Worksheet

San Francisco Estuary Institute, 7770 Pardee Lane, Oakland, CA

Watershed/Site Name: \_\_\_\_\_  
 Company/Agency: \_\_\_\_\_  
 System Type (circle): Lake Reservoir Pond Lagoon  
 Surface Conditions (circle): Flat Ripples Choppy Whitecaps  
 Surface Film (circle): Scum Algal mat Oily None/other  
 Air Temperature (°C): \_\_\_\_\_  
 Water Color: \_\_\_\_\_  
 Sampling Device (circle): Petite Ponar Ekman Other

Date/Time: \_\_\_\_\_  
 Crew Members: \_\_\_\_\_  
 Site Acreage: \_\_\_\_\_  
 Trophic State: \_\_\_\_\_  
 GPS Coordinates:  
 Lat \_\_\_\_\_  
 Long \_\_\_\_\_  
 Signal Quality (EPE) \_\_\_\_\_  
 Water Depth/Station \_\_\_\_\_

## Field Measurements

Depth Intervals	Depth meters	Dissolved Oxygen mg/L	Water Temp °C	pH s.u.	Water Temp °C	Conductivity/ Salinity (ppt) umhos/cm	Water Temp °C	Secchi Disk Depth meters	Eh (soil) mV	Time Collected
Surface – 1 m below										
Mid - column										
Bottom – above sediment										
Initial Calibration/ Check Value										

## Benthic Macroinvertebrate Samples Collected

Transect ID	Transect Length (m)	Station ID	Sample Grab ID	Grab Depth (m)	Grab Volume (% full)	Sediment Type (S, SC, M, CPOM, SAV)	Sediment Color (B, G, Br, R, O)	Sediment Odor (S, A, P, C, N)
1		1						
1		1						
1		1						
2		2						
2		2						
2		2						
3		3						
3		3						
3		3						
4		4						
4		4						
4		4						
5		5						
5		5						
5		5						

Sediment Type: S=sand, SC=silt/clay, M=muck, CPOM=coarse particulate organic matter, SAV=submerged vegetation  
 Sediment Color: B=black, G=gray, Br=brown, R=red, O=other  
 Sediment Odor: S=Hydrogen sulfide, A=anoxic, P=oil, C=chemical, N=none

Characterization at or within proximity to stations

Macrophyte Coverage: 0=absent, 1=sparse (<10%), 2=moderate (10 to 40%), 3=heavy (40 to 75%), 4=very heavy (>75%)					
Station ID	1	2	3	4	5
submerged					
emergent					
floating					
<b>Human Disturbances</b>					
Motor Boat Density (high, low, restricted, banned)					
Structures (docks, landing, pilings, riprap)					
Trash/Litter (high, moderate, low)					
Aquatic Pesticide Applications (Y, N)					
Drains/pipes (Y, N)					

Additional comments:

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Sketch of Water Body (or sampling area)

