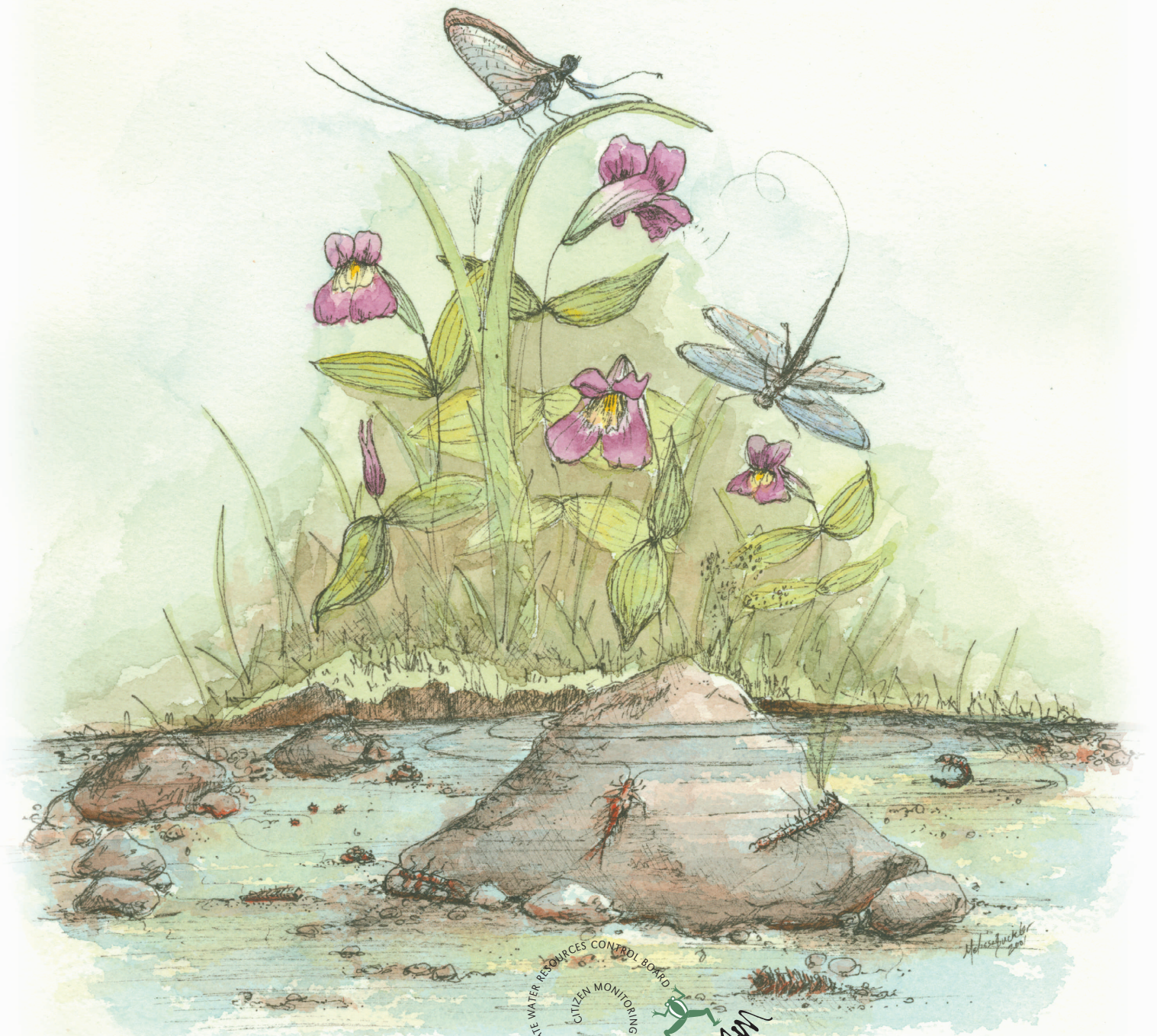


# THE CALIFORNIA STREAMSIDE BIOSURVEY

AN INTRODUCTION TO USING AQUATIC  
INVERTEBRATES AS WATER QUALITY INDICATORS

SEPTEMBER, 2001



STATE WATER RESOURCES CONTROL BOARD  
CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY

# The California Streamside Biosurvey

## *An Introduction to Using Aquatic Invertebrates as Water Quality Indicators*

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September, 2001

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# **The California Streamside Biosurvey: Using Aquatic Invertebrates as Water Quality Indicators**

## ***What is a Biosurvey?***

Streams are often described in terms of size and flow, dissolved chemicals, temperature, riparian plants along the banks, and the shape of the channel. But these are measures of the environment or setting of the stream and tell us little about the health of the stream as a place for aquatic life.

Aquatic insects and other invertebrates are the most common form of animal life in streams. They live among algae, aquatic plants, and many microscopic organisms (like bacteria). Macroinvertebrates (those invertebrates visible to the unaided eye) play many roles in the aquatic food web--they help break down organic debris, recycle nutrients, and provide food for fish, amphibians and riparian birds. Some of these organisms can live and even thrive under polluted conditions but many others require clean and cold water to survive. The variety and types of organisms present are indicators of the health of the stream. A biosurvey is literally a "life-survey", a simple test of stream health that involves collecting and classifying stream life. The biosurvey described here, and more extensive sampling known as bioassessment, are tools for measuring stream water quality and habitat health based on the types of invertebrate organisms that live on the stream bottom.

## ***Why is there a Need for Biological Monitoring of Streams?***

Since streams have sometimes been used as conduits to carry waste away from cities or factories, water quality standards are needed to prevent streams from becoming harmed by toxic chemicals or disease organisms such as bacteria. These standards typically are based on minimum drinking water quality for human consumption. However, monitoring of water quality problems requires not only an evaluation of human health risks, but of the biological health of the stream as well. Biological health is not always an obvious feature of the environment so practical field techniques are needed to assess the well-being of streams. Bioassessment can fill this need by providing direct measures of the types of life inhabiting streams.

The approach outlined here is a simple technique using aquatic insects and other invertebrates as indicators of whether a stream is sustaining a viable habitat and living up to its potential. This approach should only be used in streams, brooks and other waterways where there is *flowing* water. It is not appropriate in still waters such as ponds or lakes. The "bugs" collected in the survey are identified using a picture key, placed into indicator groups, and given scores based on their relative abundance. The overall stream water quality is then rated according to a standard scale. The scoring system and rating scale were developed using actual data sets from across California, representing a range of some of the least and most impacted streams known.

The health or *biological integrity* of a stream, lake or other body of water is its ability to function naturally and support life in the way that it would without any human impact. Biological integrity is usually reflected in diverse and sustainable *communities* of organisms, which can include macroinvertebrates, fish and algae. The macroinvertebrate portion of a community is

influenced by a wide range of physical, chemical and biological factors, most of which can be affected by human activities.

### ***What Are Aquatic Invertebrates and Why Use Them to Measure Water Quality?***

Aquatic invertebrates are familiar to fly-fishing enthusiasts or anyone who turns over rocks in streams. Insects are the main types present and examples include mayflies, stoneflies, caddisflies and black flies. Non-insect invertebrates include snails, leeches, worms, and scuds. Aquatic insects and other invertebrates are central to the proper function of streams. These creatures keep streams clean by consuming decomposing organic matter (detritus, wood and leaf debris) and algae, and provide food to other wildlife such as fish and riparian birds. Aquatic invertebrates also have varying degrees of ability to withstand pollution and so may be used as indicators of water quality and habitat condition. For example, sediments from erosion may bury the rocks used as homes by insects and other invertebrates, forcing many to escape or die, signaling a loss of biological health.

The California Streamside Biosurvey technique described here uses collections of the bottom-dwelling (*benthic*) organisms to detect changes in health based on the number of different types present (*diversity*), and how tolerant they are of pollution. Collecting stream invertebrates in one location and comparing them to those in another less disturbed location with a similar physical setting (a *reference site*), allows estimation of damages, or recovery from past water quality problems.

The California Streamside Biosurvey may be used together with traditional stream channel and riparian monitoring to provide a tool that measures the response of stream life to habitat changes caused by different forms of land use or pollution. When pollution does not originate from a single point ("*non-point*"), it can be difficult to measure using chemical methods because this type of pollution usually does not occur at all times and could be missed in a single water sample. Problems may also exist upstream of a location and not be reflected in the channel or riparian conditions at that site. The advantage of using stream invertebrates is that they live in the stream and experience everything that flows over and around them and so incorporate and embody pollution effects that occur over time and in local and upstream areas of the watershed.

### ***The Purpose of the Streamside Biosurvey***

The simplified approach presented in this workbook is intended to be an elementary introduction to stream bioassessment. It may be most useful to volunteer groups conducting monitoring demonstration projects or as an educational curriculum guide for school groups. When only limited field time is available for monitoring and no laboratory work is possible, or for those not wishing to preserve and kill collected organisms, this method provides an alternative. For those wanting to conduct further more intensive studies, the protocols outlined in "Measuring the Health of California Streams and Rivers" (Harrington and Born, 2000) should be used. The California Streamside Biosurvey serves as an entry level guide to these more detailed methods.

The California Streamside Biosurvey provides an appropriate introduction to the biological monitoring of streams for the following reasons:



- No taxonomy is involved (minimal or no experience is needed),
- “Catch and Release” sampling: samples are sorted in the field, counted, and returned to the stream (no need to preserve samples in alcohol or sort in the lab/classroom, though this remains an option),
- A water quality rating can be obtained without a reference site(s) (a reference is optional),
- Test of the technique with data sets from throughout California covering a range of “least” to “most” degraded streams show the ratings to have high fidelity with ratings generated using more detailed bioassessment analysis of the same data (see appendix section describing the beta testing comparison of the data sets).

Different protocols (standardized methods for sampling macroinvertebrates) provide different levels of information, at different levels of effort and cost. Table 1 summarizes the main elements of two protocols that are distributed for use by volunteer monitors in California: the California Streamside Biosurvey (an adaptation of EPA streamside biosurvey procedure) and the Citizen Monitor’s version of the California Stream Bioassessment Procedure (CSBP). Actual sampling is similar for both protocols: a D-shaped net is held against the streambed and the rocks and other substrate are disturbed by hands and feet, so the clinging invertebrates are swept into the net by the current.

The major difference between these protocols is the way that the organisms are identified and counted. The CSBP approach uses family-level taxonomy on counts of 300 organisms for assessment of biological integrity. It requires a significant investment of time and training to sort and identify preserved samples in a laboratory setting. The Streamside Biosurvey approach is simpler, requires fewer organisms, produces data in the field without the need for preservation, but may be less accurate as a biological assessment. The typical California Streamside Biosurvey requires 2-3 hours to complete, including sample collection, sorting, classification, and completing the habitat features checklist. When sample preservation is an acceptable option to a monitoring group, further taxonomic analysis can be conducted on the preserved sample by taxonomists. This can provide the group with verification of their results and yield more detailed information on the condition of the aquatic insect community.

**Table 1. Overview of Differences in Introductory Bioassessment Protocols in California**

<b>Protocol Procedures</b>	<b>California Streamside Biosurvey</b>	<b>CSBP-Citizen Monitoring</b>
Sampling locations	Collect 3 composited samples from transects across each of 3 riffle segments in defined study reach	
Macroinvertebrate collection	Kick sampling using 500 micron D-frame net and hand-rubbing of substrates in square-foot area for constant effort time period per sample	
Sample handling	Spread live sub-sample in pan of water (or preserve – optional)	Preserve sample in alcohol in labeled sample containers
Sorting and sub-sampling	Pick 100 live animals at streamside and sort by group into ice-cube tray	Pick 100 to 300 organisms from grids on tray in laboratory setting
Identification and counting	Sort by picture-key groupings of organisms into abundance classes	Identify with microscope to family-level, relative abundance of each
Assessment of impairment	Derive a single score from weighted counts and compare to rating scale	Calculate multiple metrics, form biotic score, compare reference / IBI

### ***When And How To Conduct Bioassessment On Streams***

Bioassessment is useful for surveying along streams to localize problem spots where aquatic habitat has been degraded and to track the progress of habitat recovery where land management practices have been changed. In order to have a standard for stream health it is desirable to compare the stream of interest to nearby streams which are similar, but where impacts have not occurred or have been minimal (*reference streams*). If this is not possible then all stream sites surveyed can be ranked from best to worst and divided into quartiles (quartered-groups), rated as excellent (top 25%), good, fair, and poor (lowest 25%). Improvement in these sites over time can then be determined from regular sampling surveys (always taken at the same time of year or in the same season). Progress toward attaining the best conditions can then be measured according to a relative scale.

Taking a walk beside a stream of concern is a good way to first identify areas where problems may occur. Degraded habitats may be found in places where bare and caving stream banks exist, in wide and shallow channels, below livestock and road crossings, where waters are often turbid and muddy, or below pollution outfalls. Bioassessments should be used together with measurements of channel features, streamside vegetation, and water chemistry. This information will contribute to your ability to assess the causes of observed differences in the types of stream organisms found. For example, you could use this information in evaluating whether eroding stream banks and sediment need to be controlled to improve stream habitat conditions. The California Streamside Biosurvey includes a Habitat Features Checklist to help you collect this information.

### ***Designing a study to use the California Streamside Biosurvey***

The usefulness of a macroinvertebrate survey depends on how well the study plan matches the purposes of the study and the resources available. There are different ways to apply bioassessment methods, depending on the main purpose of each project (Table 2). Each study question can be approached in several different ways, depending on who will use the data (e.g. volunteer groups, watershed scientists, and management agencies). In general, as questions become more specific and data become more critical for decision-making, macroinvertebrate surveys require more rigorous protocols and collection of supporting data. Project planning should involve the users of the data in determining the standards for data quality.

**Table 2. Design Considerations in Planning Bioassessment Studies**

PURPOSE	QUESTION	PROTOCOL	STUDY COMPONENTS REQUIRED TO ANSWER QUESTION		
			COMPARISON?	CLASSIFY STREAMS?	USE METRICS?
Education	What kinds of animals live in stream?	Informal streamwalk	Not required	Not required	Not required
Evaluate point-source pollutants	Does outfall discharge affect life in the creek?	CA Biosurvey Or CSBP	Upstream-downstream contrast	Not usually required since test and control sites are close together	Single biosurvey score or multiple metrics
Evaluate non-point source pollutants	Does dispersed land use affect life in the creek?	CA Biosurvey Or CSBP	Regional reference data or rating scale	Chosen based on size, flows, elevation, or gradient for example	Single biosurvey score or multiple metrics
Identify trends	Is the creek habitat changing over time?	CA Biosurvey Or CSBP	Monitor a set location over time (seasonally or yearly)	Not required (habitat assessment should also be conducted)	Single biosurvey score or multiple metrics
Assess level of impairment (or screening)	Is the creek impaired or unimpaired?	CA Biosurvey Or CSBP, Other methods	Regional reference data or rating scale, biocriteria	Chosen based on size, flows, elevation, or gradient for example	Single biosurvey score or multiple metrics, other methods
Problem identification	Which factors are causing the observed impact on stream life?	Use biological and physical habitat data to diagnose the problem(s)	Contrasts with sites not exposed to suspected specific source of problem	Control for stream type: Chosen based on size, flows, elevation, or gradient for example	Metrics and other data used to identify type of impact
Guidance for restoration	What actions could improve stream habitat?	Management to remove or limit identified problem	Other case histories used to inform decisions	Restoration appropriate to stream type?	Metrics and other data used to define needs for better habitat
Effectiveness monitoring for restoration	Are specific changes in management helping the creek?	CA Biosurvey Or CSBP, Other methods	Regional reference data or rating scale, or trend monitoring before-after	Not required if trend monitoring only but should be compared to conditions on similar reference sites	Single biosurvey score or multiple metrics, other methods

### Components of Study Design

The design of a bioassessment project includes several components that work together: protocols, comparisons to reference conditions, stream classification, and metrics or indices describing the composition of the macroinvertebrate community.

- *Protocols* are standardized methods for collecting data. Informal surveys and the EPA Streamwalk are used for education or for reconnaissance when planning a study. The California Streamside Biosurvey is a semi-quantitative but introductory protocol.
- *Comparison* of data from a particular site with some kind of standard. The unimpaired or least impaired condition of these streams is called the *reference condition*. Reference conditions apply to a particular region and position of the sample site within the watershed (e.g. headwaters vs. main stem). When evaluating a suspected point source of pollution such as a pipe discharging to the stream, comparison is usually with a site-specific control located just upstream of the pipe. Trends may also be compared as change over time within a site. The California Streamside Biosurvey approach requires no reference data --a water quality rating scale is used based on a range of data from known impaired to unimpaired streams. However, specialized habitats such as springs or seeps may not fit well into this scoring system.
- *Stream Classification* is a way of grouping sites or sections of streams so that data comparisons are only made among sites that are physically similar. A system of classification categories that has been found useful in other situations includes stream size and channel form (e.g. rocky bottom canyon streams vs. soft-bottom lowland streams). For more precise assessments, *validation* of the classification system involves collecting and reviewing different kinds of data to be sure that the categories are appropriate.
- *Metrics* or indices are numerical descriptions of the composition of the macroinvertebrate community. The California Streamside Biosurvey uses a single metric that takes into account the abundance, diversity and general tolerance to pollution of the macroinvertebrates found in the sample. Other methods use a series of individual metrics that represent each of these attributes as well as others, such as the proportions of stoneflies or other specific groups of organisms. The reliability of individual metrics varies at different scales and in different conditions, so multiple metrics allow more refined interpretation of results. It may also be helpful if previous studies in the same watershed or region have tested the metrics against other indicators of watershed health and validate their response to different types of problems.

By examining bioassessment datasets gathered by systematic sampling throughout a region, biologists can establish numerical *biocriteria*--benchmark values for individual metrics that represent the reference condition for each class of stream. The Streamside Biosurvey scoring system, which assigns each stream a rating such as Excellent or Fair, attempts to define approximate biocriteria that will be applicable throughout California, and in different types of streams. These ratings should be considered as suggestions, which must be independently confirmed by further investigation of your stream or by comparison with professional-level biocriteria established in your region. For general screening and preliminary testing the Biosurvey is an appropriate tool. Study of particular problems or sites may, however, require more advanced procedures that give detailed information on the physical, chemical, and biological characteristics of test sites.



## Choosing a Final Study Design

After determining the general goals of the project, additional choices must be made to establish the final sampling design:

- *Site selection* should consider two main questions: are the sites representative of the kinds of conditions you are interested in, and will you be able to make the kinds of comparisons that you want? Sites may be chosen because they are the focus of community or management interest, or because they are needed for comparison (reference or control sites).
- *Sampling dates* affect the results, because insect life cycles are generally seasonal. Initial characterization of a site, stream or region can examine the community composition at different seasons; more rigorous comparisons require that sampling be done at the same season, usually either in spring or in late summer-fall. Because weather fluctuations and other random variables cause macroinvertebrate populations to vary from year to year, several years of sampling data may be needed to determine how much natural variation is typical at a site.
- *Microhabitats* are distinct stream environments chosen for sampling. Riffles, pools, runs, leaf packs or woody debris each support a different cross-section of the macroinvertebrate community. Most protocols sample either riffles only or a combination of several microhabitats.
- *Habitat assessment* provides an overview of physical conditions that are likely to affect stream organisms. Bioassessment surveys usually incorporate a procedure for general habitat assessment, which helps characterize sites and interpret data. These procedures rely on some subjective evaluations, and experience with streams in the region is required to apply them consistently. (See *Habitat Features Checklist*, page 16)
- *Effort level per sample* depends mostly on sample processing and data analysis procedures. Laboratory analysis is more time consuming than streamside identification, but has advantages:
  - a) Improved taxonomic resolution (going from *Order* to *Family* to *Genus* to *Species*) may distinguish biological differences among sites.
  - b) Preservation of the sample allows all data to be checked and verified or even re-analyzed at a later date.
- If collected samples are preserved, they should be organized and archived for record-keeping. If non-experts do taxonomic identification, trained taxonomists should verify the specimens. Refer to Harrington and Born (2000) for detailed instructions and considerations in using alcohol to preserve samples. Project plans should also consider the effort needed for data analysis and presentation, which depend on project goals and the level of guidance available from regional or state biocriteria.

## The Procedure for the California Streamside Biosurvey

The California Streamside Biosurvey is conducted by collecting bottom-dwelling invertebrates from riffle habitats in wade-able streams. Riffles are characterized by turbulent flows of water over rocky, shallow sections of streams. Pools and runs are the other main types of physical stream habitats but do not contain the abundance and diversity of invertebrates found in riffles. Riffles must be shallow enough to wade in so that samples can be taken by kicking and flushing

organisms from rocks into a net held just downstream of the sample area. Large wood or rock debris is removed and washed into the net, and then the entire sample can either be preserved (in alcohol) or sorted live in the field.

The sample collected will contain invertebrates in proportion to their abundance within the riffle sample areas. Using the picture keys and worksheets provided, identification and counting of invertebrates can be done in the field. The picture key groups represent some of the major orders and common families of aquatic invertebrates. Where possible, preserved specimens may be kept for later confirmation and quality control checks. For each monitoring project, a reference collection including each type of organism present should be preserved and saved with proper identification and labeling. This provides a resource for use with any future collections for comparison and verification of identifications. Selection of survey sites and preparation of a reference collection should be done with the help of aquatic biologists or a project supervisor.

### **Equipment and Supplies**

The equipment and supplies needed include waders, sample net with a 0.5 mm mesh (D-frame net, 12" wide), buckets and fine-mesh aquarium net (for holding and sub-sampling), shallow white plastic pans (for sorting invertebrates), tweezers /forceps or eye-droppers (to grasp and pick out the bugs), ice cube trays (to hold the sorted invertebrate groups), containers for storing the samples or the sorted bugs (with good seal), alcohol (optional-either rubbing alcohol or ethyl alcohol if preserving the invertebrate sample), magnifying lens (easier to see and identify small organisms), and small squirt bottle to wash off rocks, leaves and wood collected.

### **Step-by- Step Procedure**

The procedure described here is based in part on draft guidelines for Volunteer Stream Monitoring developed by the U.S. Environmental Protection Agency for mid-Atlantic states. Modifications have been made to account for regional differences in the common types and tolerance of fauna present (found in California and other parts of the west), and some additional indicators are suggested.

#### **I. Sample Collection and Counting**

1. Select sample location (mid-stream riffles in a rocky stream, or mixed submerged substrates such as aquatic plants, wood, bank margins, or gravel/sand/silt deposits in a muddy stream).
2. Thoroughly kick and rub substrates in the sample area (one square foot), collecting dislodged organisms in the D-frame net held securely against the streambed (this should be a sample from near the middle of the stream)\*.
3. Collect another two samples at new locations (repeat steps 1-2 for each) into the same net (locations should be towards the stream margins, on either side of the mid-stream sample).
4. Place the full collection from the net into a bucket half-filled with water (pick off any animals that cling to the net), and rinse the net in the bucket.

5. Remove a portion of the contents of the bucket using an aquarium net to scoop up part of the sample, including sand, gravel, and leaf/wood debris within which the animals are mixed. To make this sampling easier you may want to use a squirt bottle to wash off larger rocks, leaves, twigs, and wood into the bucket, then discard these rinsed objects.
6. Empty the aquarium net into a shallow pan of water and shake the pan to evenly distribute the sample; then use the net to again scoop up another small portion (sub-sample) of the collection. Scrape the net across the bottom of the pan so that animals in mineral cases or shells are also picked up.

\* When sampling mixed substrates in a muddy stream it is best to kick or rub the surface of plants, logs or the stream bottom as with rocky streams but combine these for each sample and record on the data sheet which types of substrates make up each sample.

7. Place the sub-sample into another white pan of water and use forceps or an eyedropper to pick out organisms into separate look-alike groups in the cups of an ice cube tray. Start on one side of the tray, picking each organism you see, and move across the tray until 100 total organisms have been picked out (*pick each one seen, large or small*). Take additional sub-samples if needed to reach 100 but count all organisms present in the last sub-sample (even if they exceed 100). Keep the ice cube trays filled with fresh water and out of direct sun. It's a good idea to have spare trays to avoid overcrowding and make counting easier.
8. Identify each group according to **Invertebrate Picture Key** (pages 13-15) and count and enter numbers on the **Biosurvey Data Worksheet** (page 17).
9. **Optional.** Preserve the collection of organisms in a container of alcohol, and label with pencil or alcohol-proof pen (include a clean paper tag with stream, site location, date, sample # 1, 2 or 3). Consult with stream ecology specialists to obtain further or verified identifications of the invertebrates collected.
10. Repeat the complete sampling two more times (at the next riffles upstream) so that a total of three samples are taken to allow a measure of variability (with each sample containing collections from both sides and the middle of the stream).

## II. Habitat Assessment

Observations of other environmental features will help in interpreting why there may be differences in biological indicators among different stream sites. Such habitat features as the size of rocks in the stream bed and whether they are partly buried in silt (embedded), the condition of the stream bank, amount of riparian cover, width and depth of the stream, and growth of algae, all can provide insight into the causes of degraded water quality shown by (use the **Habitat Features Checklist**, page 16).

To fill out the Habitat Features Checklist you should look over about 100 meters of stream length to evaluate the percent cover of different substrate sizes that cover the stream bottom over that length of stream; the cover of algae, aquatic plants or decomposing leaves/wood present; the bankside cover of riparian plants; and the width and depth range found. What length is riffle vs. pools? Are the banks stable or eroding? Can you measure the flow using a float and stopwatch over a measured length of stream? What is the temperature, color, and chemistry of the water? You'll need a meter stick, tape measure, thermometer, and some optional chemistry test kits.

### ***Rating Water Quality and Stream Health with Your Data***

1. For each Indicator Group identified on your Biosurvey Data Worksheet, assign a "S-score" from the table below, based on Abundance and the appropriate category. For example, if your worksheet says that mayflies, a sensitive category bug, are common, the S-score is 5. Note that a very high proportion (dominance) of one group will score less than more moderate numbers, even for sensitive organisms.

**TABLE of INDEX SCORES (S values)**

(Provisional Scoring System for Indicators and Ranking of Water Quality)

<b>Abundance</b>	<b>Category I Sensitive</b>	<b>Category II Intermediate</b>	<b>Category III Tolerant</b>
<b>Rare</b> (<5%)	<b>3</b>	<b>2</b>	<b>1</b>
<b>Common</b> (5-25%)	<b>5</b>	<b>3</b>	<b>1</b>
<b>Dominant</b> (>25%)	<b>4</b>	<b>2</b>	<b>0</b>

2. Referring to your Data Worksheet, calculate the Biological Index of Water Quality as the sum of index scores from all indicator categories. You can then assign a quality level from the Water Quality Rating Scale, below. The Biological Index can also be used to contrast with previous or future samples taken at the same site to see how water quality has changed (see Example Calculations).

**Water Quality Rating Scale** (Provisional, use as an approximate guide)

<b>Biological Index</b>	<b>Rating</b>	<b>Description</b>
<15	<b>Poor</b>	Indicates degraded water quality & habitat problems needing repair.
15 - 20	<b>Fair</b>	Conditions improved but still somewhat impaired.
21 - 25	<b>Good</b>	Only slightly reduced stream health indicated.
>25	<b>Excellent</b>	Indicates the best water and habitat quality for biological conditions.

**Example Calculations # A S**  
**Indicator Group:**

Mayflies	32	D	4
Stoneflies	10	C	5
Caddisflies	3	R	3
Flatworms	4	R	2
Crane Flies	3	R	2
Caddis (net-spinners)	13	C	3
Riffle Beetles	30	D	2
Midges	10	C	1
<b>Total</b>	<b>100</b>		<b>22</b>
<b>Biological Index = 22</b>		<b>Water Quality Rating = Good</b>	

These ratings assume that a reference stream would have an excellent rating. Water quality assessments should be based on actual reference comparisons whenever possible. If a reference site does not have an excellent rating, then impaired conditions at other sites should be evaluated relative to the standard established by this reference. This may be the best attainable condition if some level of impact already exists even on reference streams.

**Other Biological Indicators (optional):**

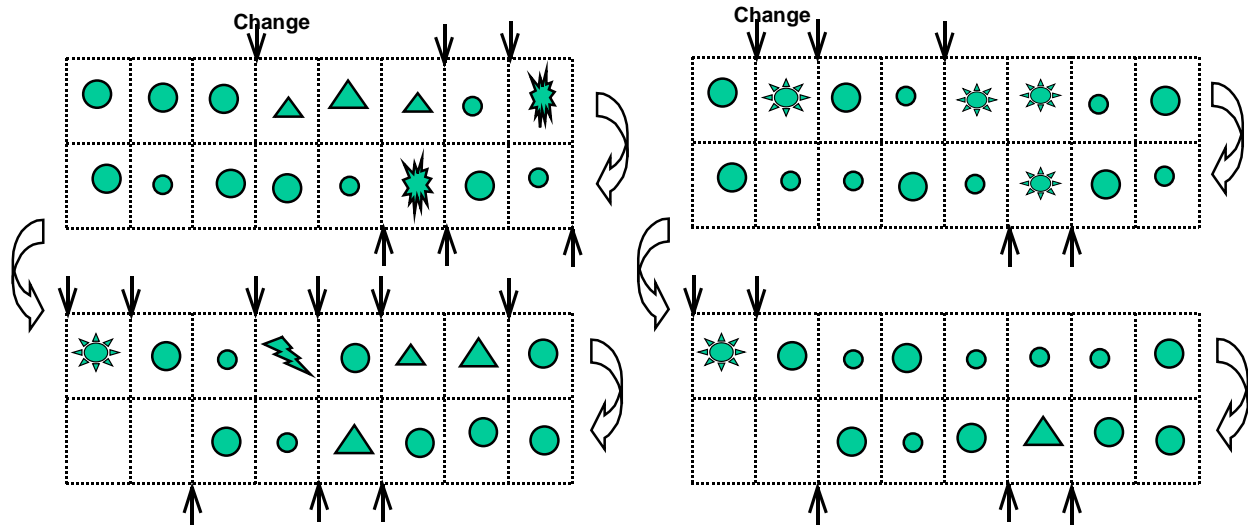
1. Measure the size range (smallest to largest length), of organisms in each sorted group and record on the data sheet (pollution often can eliminate larger invertebrates or produce small body sizes in a species that would otherwise be larger in unpolluted conditions).
2. Ratio of numbers in category I+II to category III (high ratios indicate better water quality).
3. Percent of the total made up by the most dominant group (if more than 50% of the total number belong to a single group, this may indicate a disturbed or unbalanced community).
4. Sequential Comparisons Index groups organisms by differences in their appearance, without requiring any knowledge of what taxonomic classes those differences represent.

*A Simple Measure of Diversity: the Sequential Comparisons Index*

Collect a sample and subsample it as described in steps 1-6 above. Randomly pick out 50 organisms, but instead of organizing them into groups, randomly place them in individual cells of several ice cube trays. Beginning at one end of one tray and working systematically through the rows, examine each organism and decide if it looks similar to the previous one or if it is different. Use a piece of paper to tally the number of times there is a change between successive organisms, and add one for the end. This is the number of “runs” of similar organisms. Then divide this number by the total number of organisms (50) to calculate the Sequential Comparisons Index.

In the illustration below, each of two subsamples contains 30 shapes representing different types of organisms, and the first run starts at the upper left cell. In the subsample on the left, the SCI is  $15/30 = 0.50$ . The SCI for the subsample on the right is  $10/30 = 0.33$ . If these were from the same sample, they would be averaged to get a more reliable SCI for the site. Sites with better water and habitat quality will generally have higher SCI scores.



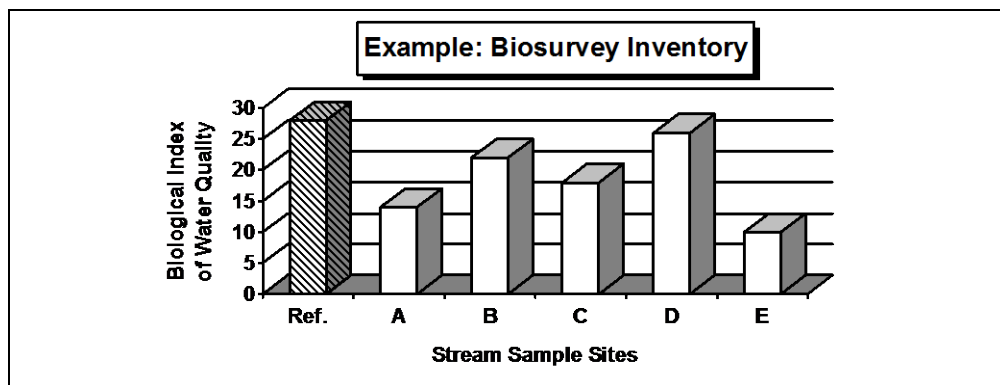


Some tips: size differences do not count, as they may represent different age groups within one species. Insects that have recently molted (shed their external body covering) may be paler but will usually have the same pattern of markings. It may be easier to start with immobile organisms that have been preserved in alcohol; when your recognition skills have improved, try sorting live animals.

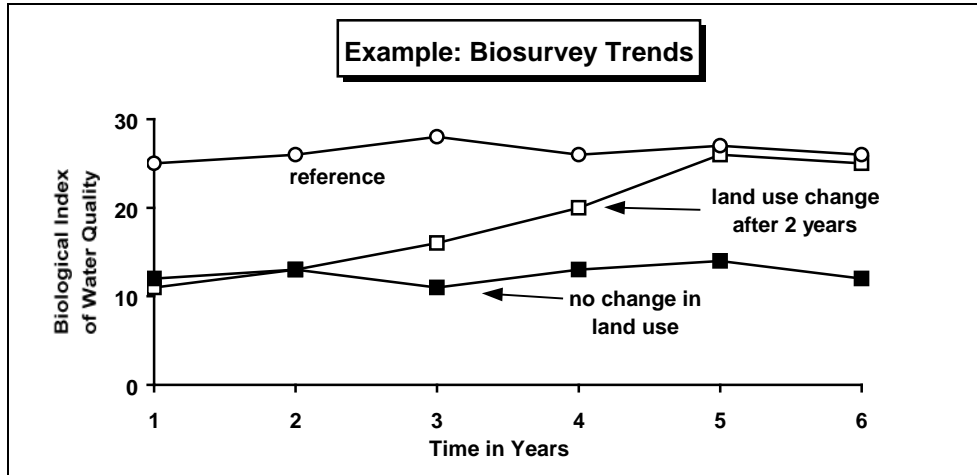
### Possible Comparisons of Biological Indicator Values

Here are some of the ways to compare biomonitoring data in order to spot problem areas, inventory water quality at various locations, and track habitat recovery (see graphs below):

1. Compare test site to the water quality rating table (a general scale for rating stream quality).
2. Compare test to reference site(s) selected to represent relatively undisturbed conditions. (or to the best conditions found in the area on a similar stream, Graph 1).
3. Trends at the test sample site over time--before and after water quality treatment relative to reference stream sample sites. (Graph 2).



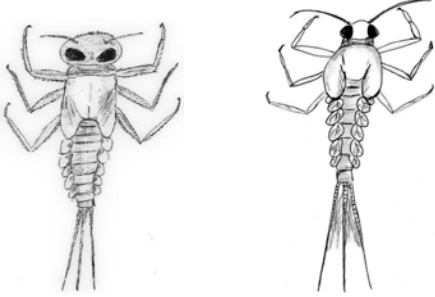
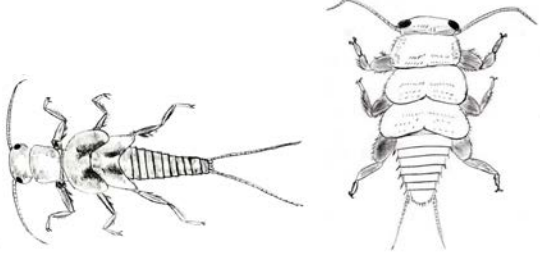
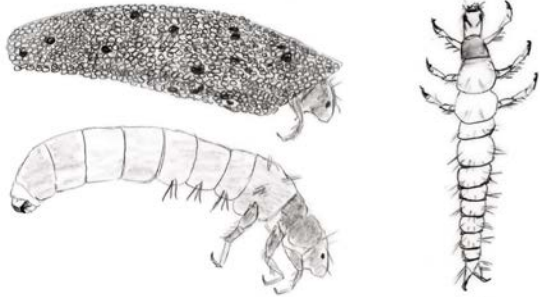
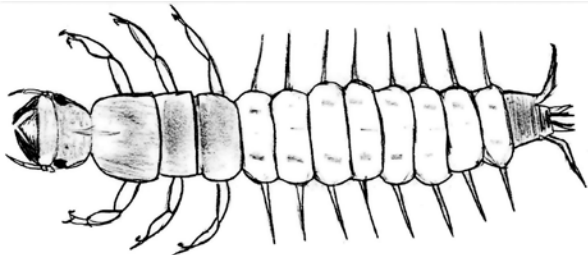
**Graph 1.** Using Streamside Biosurvey to inventory stream quality at various sites and locate problem spots that may need to be protected from damage in some way. Ref. is the reference site or sites used to establish a baseline condition for water quality. This inventory shows that conditions at sites A & E are poor, C & B fair to good, and excellent at D.



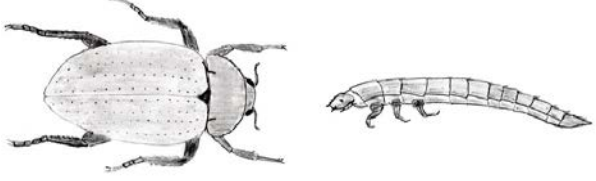
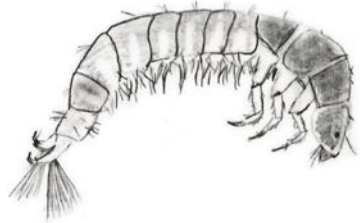
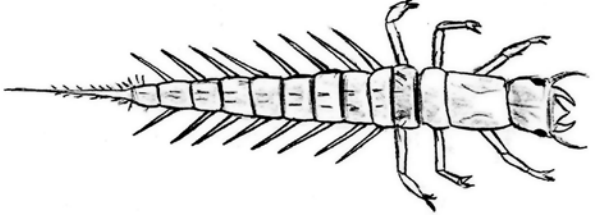

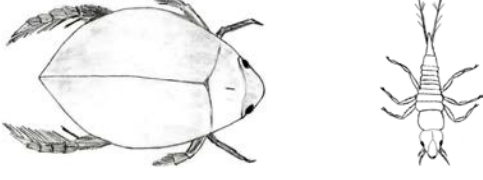
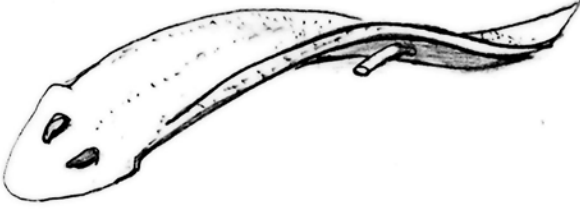
**Graph 2.** Shows how Streamside Biosurvey can be used to track trends in stream recovery by comparing two impacted sites, one of which is placed under new land use practices after a second year of monitoring. The graph shows how conditions might improve relative to the unchanged site and a reference site over time.

## INVERTEBRATE PICTURE KEY

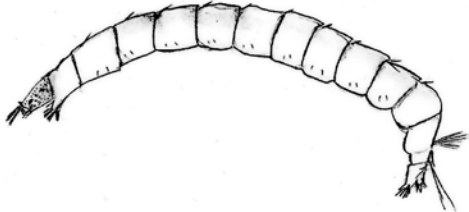

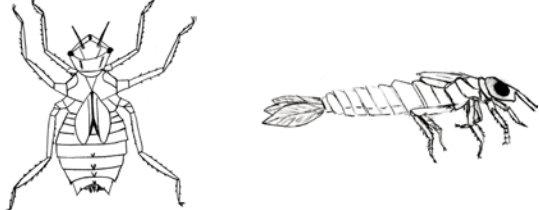
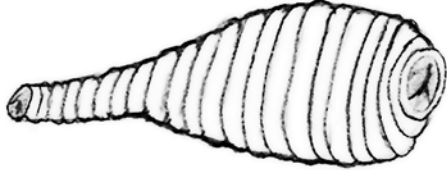
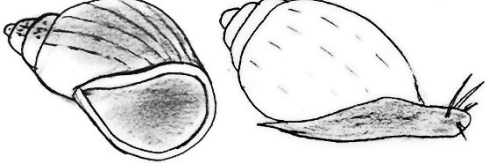
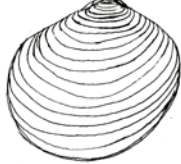


### I. SENSITIVE CATEGORY

<p><b>Mayflies</b>          Insect order Ephemeroptera          Usually have 3 tails, gills on abdomen, and feed on algae or organic particles (detritus).          Size: Usual- <u>5-10</u> mm, Range- <u>2-15</u> mm</p>	
<p><b>Stoneflies</b>          Insect order Plecoptera          Two tails, gills on thorax but not abdomen.          Large predators and small detritivores.          Size: Usual- <u>10-20</u> mm, Range- <u>5-30</u> mm</p>	
<p><b>Caddisflies</b>          Insect order Trichoptera          Usually construct a case but may be without, and commonly feed on detritus or algae.          Size: Usual- <u>5-10</u> mm, Range- <u>2-25</u> mm</p>	
<p><b>Hellgrammites (fishflies or dobsonflies)</b>          Insect order Megaloptera, Family Corydalidae          The larvae are large and active predators, with dark heads and long gills along the abdomen.          Size: Usual- <u>20-30</u> mm, Range- <u>10-70</u> mm</p>	

## II. INTERMEDIATE CATEGORY

<p><b>Riffle Beetles</b>            Insect order Coleoptera, Family Elmidae            Adults &amp; larvae of these small stout beetles are dark brown, and feed on detritus &amp; algae.            Size: Usual- <u>3-5</u> mm, Range- <u>2-10</u> mm</p>	 <p style="text-align: center;">ADULT                      LARVA</p>
<p><b>Net-Spinning Caddisflies</b>            Order Trichoptera, Family Hydropsychidae            These caddisflies attach their case to rocks and spin a net to capture fine particle food.            Size: Usual- <u>5-10</u> mm, Range- <u>3-15</u> mm</p>	
<p><b>Alderflies</b>            Order Megaloptera, Family Sialidae            Medium size predators with gill filaments on the sides and tip of abdomen.            Size: Usual- <u>5-15</u> mm, Range- <u>up to 25</u> mm</p>	
<p><b>Crane Flies (and other Diptera)</b>            Insect order Diptera, Family Tipulidae            Larvae maggot-like, segmented, gills at hind end finger-like, often feed on detritus.            Size: Usual- <u>5-10</u> mm, Range- <u>up to 50</u> mm</p>	
<p><b>Other Water Beetles</b> (Order Coleoptera)            Families Dytiscidae &amp; Hydrophilidae            Adults are active swimmers, both adults and larvae are predators or scavengers.            Size: Usual <u>3-10</u> mm, Range- <u>2-30</u> mm</p>	 <p style="text-align: center;">ADULT                      LARVA</p>
<p><b>Flatworms</b> (Class Turbellaria)            Order Tricladida (commonly Planarians)            No segments, often darker above and lighter below, predators and scavengers.            Size: Usual- <u>5-10</u> mm, Range- <u>3-20</u> mm</p>	
<p><b>Other / Unknown Invertebrates</b>            Includes mostly rare taxa of insects and non-insects but should be counted.</p>	<p style="text-align: center;">NO PICTURE</p>

### III. TOLERANT CATEGORY

<p><b>Midges</b>          Insect order Diptera, Family Chironomidae          Small fly larvae, often feed on fine particles.          Size: Usual- <u>2-4</u> mm, Range- <u>1-10</u> mm</p>	
<p><b>Black Flies</b>          Insect order Diptera, Family Simuliidae          Hourglass body-shape, particle filter-feeder.          Size: Usual- <u>4-8</u> mm, Range- <u>2-12</u> mm</p>	
<p><b>Dragon- and Damselflies</b>          Insect order Odonata          Stout-bodied predators with extendable jaws.          Size: Usual- <u>10-15</u> Range- <u>5-40</u> mm</p>	
<p><b>Leeches</b>          Class Hirudinea          Body flattened, segments &amp; sucker, predators.          Size: Usual- <u>10-20</u> mm, Range- <u>5-40</u> mm</p>	
<p><b>Snails</b>          Class Gastropoda          Turban/ spiral-shaped shell, feed on algae.          Size: Usual- <u>5-10</u> mm, Range- <u>2-20</u> mm</p>	
<p><b>Clams ("fingernail")</b>          Class Bivalvia, Family Sphaeriidae          Shells speckled black / white, feed on detritus.          Size: Usual- <u>2-5</u> mm, Range- <u>2-15</u> mm</p>	
<p><b>Scuds ("sideswimmers")</b>          Crustacean order Amphipoda          White-yellow, flat, many legs, varied food.          Size: Usual- <u>4-8</u> mm, Range- <u>2-12</u> mm</p>	
<p><b>Segmented Worms</b>          Class Oligochaeta          Round body many segments, feed on detritus.          Size: Usual- <u>3-10</u> mm, Range- <u>up to 50</u> mm</p>	

## HABITAT FEATURES CHECKLIST

(make copies of this blank data sheet for each study site)

Date:	Stream Name:	Location:
Observers:	Group Name:	County:
		Supervisor:

Survey 100 meters of stream. Measure the length in meters (use a tape measure) of each habitat type present:

Riffles/Rapids	Pools	Runs/Glides
----------------	-------	-------------

1. Particle sizes on stream bottom: estimate percent area for each						4. Stream size: in cm			
Percent:	Fines <0.25 mm	Sand 0.25-2 mm	Gravel 2-65 mm	Cobble 65-250 mm	Boulder >250 mm	Order: (use map)			
<5%						Width of water:			
5-25%						min	max		
25-50%						Width of channel:			
>50%						min	max		
2. Bottom cover on stream: estimate percent area for each						Mid-stream depth range:			
Percent:	Algae (slick film or strands)	Twigs and leaf debris (<10 cm)	Woody debris (>10 cm)	Aquatic plants (rooted)	Other:	5. Embeddedness %: burial of gravel and cobble by fine and sand sediment			
<5%						<5%			
5-25%						5-25%			
25-50%						25-50%			
>50%						>50%			
3. Streambank cover, slopes and erosion: estimate percent area on both banks for each									
Percent:	Grasses	Brush	Trees	Rocks	Soil	undercut	steep	shallow	Eroding
<5%									
5-25%									
25-50%									
>50%									

Note: undercut slope angle = <90° to water; steep slope = >90°-150°; shallow or gentle slope = 150°-180° flat to water

**Flow Conditions:**     slow/smooth     moderate/rippling     rapid/turbulent

or Current velocity estimate (using meter or timed float):    **Average cm / second:** \_\_\_\_\_

**Discharge estimate:** \_\_\_\_\_ [avg. velocity (cm/sec) X cross-section area (avg. depth x width, cm<sup>2</sup>)]

Measures of water quality (if test kits available):

Temperature	Dissolved Oxygen	Alkalinity	Turbidity	Other?

Appearance of water:     clear     turbid     foam     oils     greenish

Description of other surrounding environmental features such as landscape and land:

**The Clean Water Team Guidance Compendium for Watershed Monitoring and Assessment**  
**State Water Resources Control Board**    CA streamside biosurvey    4/17/2013



# CALIFORNIA STREAMSIDE BIOSURVEY DATA WORKSHEET

(make multiple copies of this blank data sheet, one for each sample)

Date	Stream	Location	Sample No.
------	--------	----------	------------

Stream Type Sampled:

- Rock-bottom Riffle  
 Mud-bottom Channel (circle substrates sampled: wood / bank / plants / bottom sediment)

INVERTEBRATE INDICATOR GROUP COUNTS:

# = Counts (actual number counted in each group, out of a minimum 100 total)  
A = Abundance category (R = rare <5%; C = common 5-25%; D = dominant >25%)  
S = Index Score (from Table of Index Scores)

#	% A	S	Sensitive Category I
	—		Mayflies
	—		Stoneflies
	—		Caddisflies (w/o net-spinners)
	—		Hellgrammites (Dobson - Fishflies)

Sum  
Score =       

#	% A	S	Intermediate Category II
	—		Riffle Beetles
	—		Netspinning Caddisflies
	—		Alderflies
	—		Crane Flies & Other Diptera
	—		Other Water Beetles
	—		Flatworms
	—		Other/Unknown Invertebrates

Sum  
Score =       

#	% A	S	Tolerant Category III
	—		Midges
	—		Black Flies
	—		Dragon- or damselflies
	—		Leeches
	—		Snails
	—		Clams
	—		Scuds
	—		Segmented Worms

Sum  
Score =       

**Biological Index of Water Quality** =        [Sum Scores of categories I + II + III ]

Water Quality Rating [        ] (see Rating Table) Comparison to Reference Biol. Index [        ]

Total Number # Groups Counted (all categories) =       

SCI (sequential comparison index for n=50) =

## **Annotated Bibliography**

- D.M. Rosenberg and V.H. Resh (Eds). 1992. *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman and Hall. [Technical reviews of status, approaches, and problems for biomonitoring.]
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- USEPA. 1999. *Revisions to Rapid Bioassessment Protocols for Use in Streams and Rivers: periphyton, benthic macroinvertebrates, and fish*. (2nd edition). EPA 841-B99-002. Also available online at <http://www.epa.gov/owow/monitoring/rbp/>  
[The first edition presents detailed methods for several levels and types of bioassessment, with interpretation and case histories. The second edition adds new protocol options and includes examples and background on conducting habitat assessment]
- R.W. Merritt and K.W. Cummins (Eds.). 1995. *An Introduction to the Aquatic Insects of North America*. Third edition, Kendall/Hall Publishing Company. [The most complete general taxonomic reference including keys, illustrations and natural history information, feeding ecology, and biomonitoring. Oriented towards professional level assessments]
- Oregon Department of Fish & Wildlife. 1992. *The Stream Scene: Watersheds, Wildlife and People*. [Watershed education project of the Oregon Department of Fish and Wildlife, P.O. Box 59, Portland, OR 97207]
- Volunteer Stream Monitoring: A Methods Manual*. 1997. U.S. Environmental Protection Agency, Office of Water, EPA 841 B 97-003. [Contains background and procedures for various levels of biomonitoring as well as habitat and water quality assessment, oriented towards the needs of volunteer monitoring programs]
- D.M. Lehmkuhl. 1979. *How to Know the Aquatic Insects*. Wm. C. Brown Publishers, Dubuque, Iowa. [An excellent introductory book to the biology of aquatic insects with easy to follow family keys and sections on anatomy and insects as pollution indicators].
- W.P. McCafferty. 1981. *Aquatic Entomology*. Jones and Bartlett Publishers, Boston (original publisher: Science Books International). [Written for "fishermen and ecologists", this contains useful illustrations and picture keys for aquatic insects, as well as descriptions of common non-insect groups.]
- J. Harrington and M. Born. 2000. *Measuring the Health of California's Streams and Rivers*. Sustainable Land Stewardship international Institute. Sacramento, California.

V.H. Resh, M.J. Myers, and M.J. Hannaford. 1996. Macroinvertebrates as indicators of environmental quality. Pp. 647-667. In: Methods in Stream Ecology, ed. F.R. Hauer and G.A. Lamberti, Academic Press. [Introduction to stream habitat and invertebrate monitoring]

**Resource Contacts for Bioassessment in California**

California Aquatic Bioassessment Workgroup. California Department of Fish and Game.

Internet: <http://www.dfg.ca.gov/cabw/cabwhome.html>

Clean Water Team. Watershed Pollution Prevention Section, Division of Water Quality, State Water Resources Control Board, 1001 I Street, Sacramento, CA 95814

Internet: <http://www.swrcb.ca.gov/nps/volunteer.html>

**Acknowledgements:**

We would like to recognize the following individuals for their contributions: Dominic Roques for editorial assistance and initial planning of this document; Jim Harrington, Marilyn Myers, and Chris Rosamond for reviews; Heather Perry for scanning and sizing the picture key images; Peter Ode and Frazier Syme for providing data for the beta test; and the many individuals active in the California Aquatic Bioassessment Workgroup who have provided enthusiastic encouragement of this project over the last several years. Lastly we thank all the readers of this document and encourage you to help us improve future editions with any advice, observations and suggestions you may have after using the Biosurvey.

## **APPENDIX: California Streamside Biosurvey: Outline and Beta-Test Simplified Approach for Biological Monitoring of Streams**

### Outline

This simplified approach for evaluating water quality or biological integrity of streams involves use of a picture key of stream invertebrates grouped into sensitive, intermediate and tolerant organisms. A weighted score is assigned to each depending on relative abundance and the sum of these scores compared to a water quality rating scale divided into excellent-good-fair and poor ranges.

This method is intended for use by volunteer monitoring groups who are being introduced to bioassessment and may only be involved in stream surveys for a limited time or as a demonstration. It is also designed as a teaching curriculum for school groups involved in environmental education and field science. It is suited to these applications because:

- no taxonomy is involved (minimal or no experience is needed) – invertebrates are sorted into groups by reference to picture-keys
- “catch-and-release” samples are sorted in the field, counted, and returned to the stream (there is no need to preserve samples in alcohol and sort in the lab/classroom)
- a water quality rating can be obtained without a reference sample
- test of the technique with data sets from throughout California show the ratings to have a high fidelity with ratings generated from a more detailed bioassessment analysis of the same data sets

### Beta-Test

The biosurvey is a modification of an approach used in mid-Atlantic states and outlined in the EPA volunteer monitoring guidelines. The approach presented in that document was inappropriate for use in California (regional differences in stream biology and need for adjustments in scoring/rating system) so the streamside biosurvey was developed and tested using actual data sets from throughout California. This test consisted of comparing the fidelity of water quality ratings between a higher-resolution multimetric analysis and the lower-resolution simplified biosurvey approach presented in this document.

- Data from 25 streams were obtained from different sources, selected to represent a wide range of conditions from sites of known pollution impact to near-pristine habitat.
- The data sets were used to calculate bioassessment metrics based on genus-and family-level taxonomic resolution. Five metrics included taxa richness, EPT richness, % EPT, dominance (one taxon), and biotic index (HBI).
- These metrics were rated on a percentage scale over the range of the data and summed to form a multimetric score for each site. The sum values were divided into quartiles for the potential range of data (0-5), and rated as poor, fair, good, and excellent.
- From these data, based on sample sizes of 300, a simple data analysis for a 100-organism count was performed (thus removing from counts any taxa that comprised less than 1% of the original sample size).
- Taxa were assigned to picture key groups, given weighted scores according to the following indicator groups and relative abundance, and total scores ranked:

**Categories For Picture-Key Tolerance Groups**

GROUP	Common Name	Latin Name
Group I - Sensitive	Mayflies	Ephemeroptera
	Stoneflies	Plecoptera
	Caddisflies (w/o net-spinners)	Trichoptera (except Hydropsychidae)
	Fishflies/Dobsonflies	Corydalidae
Group II - Intermediate	Riffle Beetles	Elmidae
	Other Water Beetles	Dytiscidae and Hydrophilidae
	Crane Flies / other Diptera	Tipulidae + others
	Net-spinning Caddisflies	Hydropsychidae
	Alderflies	Sialidae
	Flatworms	Tricladida
Group III - Tolerant	Other/Unknown Inverts	Undetermined
	Midges	Chironomidae
	Black Flies	Simuliidae
	Dragon- or Damselflies	Odonata
	Leeches	Hirudinea
	Snails	Gastropoda
	Clams	Sphaeriidae
	Segmented Worms	Oligochaeta
Scuds	Amphipoda	

**WEIGHTED INDEX SCORES:**

Index Scores	Group I	Group II	Group III
Rare (1-5%)	3	2	1
Common (5-25%)	5	3	1
Dominant (>25%)	4	2	0

Biological Integrity Water Quality Rating Scores

Excellent	>25
Good	21-25
Fair	15-20
Poor	<15

Results of Beta-Test shown below. Methods are in agreement at 17 of 25 sites. When not, the simple approach appears to be more conservative in rating water quality or biological integrity - under-rating usually by only one rank and mostly when the multimetric would rate at excellent or when at the boundary between ranks. The Biosurvey is not likely to over-rate water quality.

