Workplan:
Stream Bioassessment in the Santa Ana Region

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1. Introduction:

This retrospective workplan references two documents that have been approved, signed and executed by the SWRCB Contracts Section and the California State University Long Beach Foundation. Further information regarding this project can be obtained by referencing SWRCB standard agreement 05-321-250-0 and the California State University, Long Beach Stream Ecology and Assessment Laboratory Quality Assurance Project Plan (QAPP) for Aquatic Invertebrate Bioassessment Monitoring for the Santa Ana Regional Water Quality Control Board (Region 8) approved 5/19/06.

The Santa Ana Region is one of the smallest of the nine regions in the state. Although small, the region's four million residents make it one of the most densely populated regions. The climate of the region is classified as Mediterranean: generally dry in the summer with mild, wet winters. The average annual rainfall in the region is about fifteen inches, most of it occurring between November and March. The Santa Ana regional stream network consists of first order coastal streams that flow directly into the ocean as well as a network of first, second, third, and fourth order streams that are tributary to the two major rivers in the area, the Santa Ana and San Jacinto Rivers. The Santa Ana River cuts through the San Bernardino Mountains and the Santa Ana Mountains and flows down to the ocean and is effluent dominated during dry weather. The San Jacinto River has no flow during the dry season, flowing during large storm events. The terminus of the San Jacinto River is Canyon Lake during small storm events and Lake Elsinore during large storm events. These streams are designated to support several beneficial uses, including that for aquatic life, but at this point in time there is limited information on the water quality of this stream network.

Understanding the quality of the rivers, streams and other water sources is important for the development of management plans to protect the nations vital water resources. One approach that has been advocated for determining water quality is "Aquatic Life Use

Assessment" (ALUA). ALUA is one of the Environmental Protection Indicators for California (EPIC) adopted by the California Environmental Protection Agency (Cal/EPA) for determining water quality. This bioassessment tool utilizes the biological integrity of biological assemblages occupying various trophic niches and can include algae, macroinvertebrates and vertebrates, such as fishes, as a direct method for assessing the biological health of stream ecosystems. As defined by the 2006 EPA Wadeable Streams Assessment (WSA) document, *"biological integrity represents the capability of supporting and maintaining a balanced, integrated, adaptive community of organisms having a species composition, diversity and functional organization comparable to that of the natural habitat of the region".* Bioassessment is a proxy for determining stream water quality and habitat quality based on the types and numbers of organisms living there.

2. Problem Statement:

Water quality information for the streams in the Santa Ana Region is currently based mostly on discharger data from NPDES permits, and volunteer monitoring efforts of selected streams. This information focuses on problem areas within the region or areas where permits have been issued. Consequently, there are a large number of streams in the region that lack water quality information. Due to lack of available funding to implement a fully comprehensive "multiple biological assemblage model" to assess the biotic integrity, a decision was made by the Santa Ana Regional Water Quality Control Board (SARWQCB) to initially focus on using a macroinvertebrate bioassessment tool to assess the biotic integrity of the wadeable streams in Region 8.

3. Project Objectives:

The objectives of the bioassessment program described in this workplan are to address the federal EPA-mandated requirement (EPA requirement 305(b)) for an assessment of the integrity of surface waters in Region 8 (Santa Ana Region) of California. Specifically, this project aims to meet this objective by collecting and subsequently analyzing macroinvertebrate data using two indices: an Index of Biological Integrity (IBI) and a River Invertebrate Prediction and Classification System (RIVPACS) model. Each of these methods yields a single score of the biological integrity of a site. The IBI model provides a score based on the combination of a variety of independent biological metrics. These scores can then be ranked, and compared to sites that are independently designated as high-quality "reference" sites. The RIVPACS model predicts the expected occurrence of species at an unimpaired site based on physical parameters; expected occurrence can be compared to observed occurrence at the site, yielding a measure of deviation from reference conditions.

The data collected using these analyses will be used to identify streams that may require improvement of water quality. It will also be used to refine and compare several

methods of analysis and interpretation of bioassessment data. Although not comprehensive by nature, the design of the proposed workplan will also provide a basis for managers to estimate the percentage of stream kilometers in the region that are meeting the aquatic life beneficial use. The region's Basin Plan related to beneficial use is as follows:

"Inland surface water communities and populations including vertebrate, invertebrate and plant species shall not be degraded as a result of the discharge of waste. Degradation is damage to an aquatic community or population with the result that a balanced community no longer exists. A balanced community is one that is diverse, has the ability to sustain itself through cyclic seasonal changes, includes necessary food chain species, and is not dominated by pollution tolerant species, unless that domination is caused by physical habitat limitations. A balanced community also may include historically introduced non-native species but does not include species present because best available technology has not been implemented or because site-specific objectives have been adopted or because of thermal discharges".

4. General Description of Methods to Achieve the Project's Objectives:

This workplan advocates the use of an invertebrate bioassessment paradigm to investigate the biological health of the rivers in the Santa Ana region. This procedure will use the IBI and RIVPACS models to measure key metrics of benthic community structure (e.g. richness, composition and diversity) and function to establish the integrity of the intrinsic invertebrate community. Although this methodology does not include vertebrate and plant species, and is therefore not a fully comprehensive "multiple biological assemblage" approach for determining the percentage of the linear km meeting aquatic life beneficial life use, it does offer a scientifically defensible and cost-effective monitoring tool that has been widely adopted throughout the State of California to identify causes and sources of water impairment and to evaluate and gauge changes in water quality in response to management actions.

Macroinvertebrates are an integral part of the trophic web and have been adopted by numerous agencies as an index of biological condition that is directly related to the overall biological integrity of the stream. As described in the recent 2006 collaborative report by the EPA Wadeable Streams Assessment (WSA) (EPA 841-B-06-002) macroinvertebrates assemblages respond in a reasonably predictable and defined manner to water quality impairment and human disturbance. They are sensitive to physical, chemical and biological parameters and can therefore integrate the temporal effects of a wide range of influential stressors. Moreover, because some invertebrates are more sensitive than others to specific stressors, the composition of the macroinvertebrate assemblages can provide important diagnostic information that can potentially narrow down the identify of the stressor.

5. Experimental Design:

The following workplan conforms to the following standardized procedures to ensure consistency and comparability with other assessments occurring simultaneously within the State.

i) the methods comply with those prescribed by the California Monitoring and Assessment Program for Perennial Streams (CMAP). CMAP was initiated in 2004 and was formulated from the Environmental Monitoring and Monitoring and Assessment Program –Western Pilot (EMAP-West) for inland surface waters implemented in California from 1999-2003. The workplan will use the Generalized Random Tessellation Stratified (GRTS) sampling design of CMAP. This probability-based survey design uses the data obtained from a subset of sampling sites to statistically make estimates about the population with a known level of uncertainty.

ii) the workplan uses the California Stream Bioassessment Procedure (CSBP, Harrington, 2003) standardized protocols for assessing biological and physical conditions of California. The CSBP is a regional adaptation of the U.S. EPA protocol EPA/841-B-99-002 (Rapid Bioassessment Protocols for use in Streams and Rivers).

iii) the workplan involves a bioassessment laboratory and personnel that have participated in the California Bioassessment Laboratories Network (CAMLnet) to ensure compliance with the recommended QA/QC procedures for CAMLnet taxonomic effort standards (www.dfg.ca.gov.cabw/camlnetste.pdf). CAMLnet was recently expanded to include all of the southwestern states and has been renamed Southwestern Aquatic Freshwater Invertebrate Taxonomists (SAFIT). A revised Standard Taxonomic Effort (STE) draft was circulated to the SAFIT membership by Joseph Slusark on September 27, 2006. This workplan will adopt the final STE rules once approved by SAFIT.

iv) the workplan adheres to a documented Standard Operation Procedures (SOPs) and an approved Quality Assurance Project Plan (QAPP). Please see appendix 1 and 2 for the SOPs and the California State University, Long Beach Stream Ecology and Assessment Laboratory QAPP for Aquatic Invertebrate Bioassessment Monitoring for the SARWQCB (Region 8) approved 5/19/06.

6. Sample Site Selection.

The SARWQCB worked with statistician Tony Olsen from EPA at Corvallis to design a cost effective, randomized sampling design based upon EMAP criteria that could be used to representatively sub-sample the various streams in the region (see Appendix 3 for parameters used to define the sampling model). Dr. Olsen provided a list of coordinates for

750 potential locations to select for sampling. Under the original sampling design, each year fifty sites would be randomly selected from a 250-site subset of these locations annually for a period of 5 years to provide a total of 250 sites that would be considered statistically representative of the 1302 linear stream kilometers covering the Santa Ana regional stream network. This sampling density provided a level of statistical precision of ±12% with at a spatial coverage resolution of approximately 1.6 linear kilometers. The original sampling study also did not include any stratification elements and was designed for perennial streams coded as "R", "S", "T", "N", and "W" and non-perennial streams that were 3rd and higher Strahler order coded as "R", "S", "T", "N", and "W". Given the nature of the terrain and the xeric conditions in southern California not all sites were considered viable for the study. Consequently prior to collecting any environmental measurements or infauna samples, the sites from within the list were prescreened by first undertaking reconnaissance of each of the sampling location to determine accessibility and suitability for benthic macroinvertebrate sampling. Elements that were deemed essential for an accessible site to be considered suitable for sampling were based upon criteria that led to the development of the California Index of Biotic Integrity (IBI) (Ode *et al*., 2005).

Subsequently, two approved modifications were made to the design in the sampling study outlined above.

First, due to the constraints in the available funds for the project, the numbers of number of sampling sites were reduced from 50 to 30 for the 2005-2006 sampling year. Statistical analyses show that this reduction in sampling effort increased the level of imprecision regarding the representation of the sub samples by 4% (Tony Olsen, personal communication). While not desirable, this difference was not considered to unduly compromise the objectives of the study. Furthermore it was concluded that additional sampling or an extension to the duration of the study could ultimately be undertaken to restore the original level of precision in the sampling design.

Second, the initial experimental design involved dividing Region 8 into two hydrological units (Santa Ana unit and the San Jacinto unit). Because the portion of the San Gabriel hydrological units included in Region 8 is so small, those sites were combined with those in the Santa Ana hydrological units. The two hydrologic units (Santa Ana and San Jacinto, with the former including the San Gabriel) were subsequently divided into three elevation strata – 0-350 meters, 350-700 m, and 700+. Randomly generated GPS coordinates were used to determine the location of sites (evenly distributed throughout defined categories). The purpose of dividing the region into three elevation categories was to ensure that sampling occurred throughout the entire region each year. It was determined that not dividing the region into these biologically relevant strata might have resulted in analytical bias due to intensive sampling in a small subset of the region one year and no sampling in this subset the following year.

7. Physical Habitat Information.

Benthic macro invertebrate community assemblages are known to be affected by a number of physical parameters such as riparian vegetative cover, riparian disturbance, aspect, flow and streambed substrate to mention a few (U.S. EPA protocol EPA/841-B-99-002). Consequently each sampling site requires a comprehensive description of physical habitat. This workplan incorporates the standardized basic CSBP habitat scoring criteria as references in Appendix 2. Data from the survey are recorded in the basic physical data log sheet shown in Appendix 3. Tom Suk of the Surface Water Ambient Monitoring Program (SWAMP) distributed a FINAL DRAFT of protocols for ambient bioassessment of freshwater wadeable streams in California on September 15, 2006. The workplan complies with the "BASIC level" of physical habitat measurements.

8. Water Chemistry

Benthic macroinvertebrate community assemblages are potentially influenced by variety of abiotic chemical stressors that can be measured relatively easily to provide information on eutrophication and the degree of chemical degradation when compared to reference streams (WSA EPA 841-B-06-002). For all sampling sites water chemistry and physical measurements will be taken using a YSI (or equivalent) environmental monitoring unit for pH, dissolved oxygen (mg/l), conductivity (mS/cm), water temperature (°C), turbidity (NTU), and alkalinity. In addition to these on site measurements, a 500ml water sample will also be collected at each site for laboratory analysis to test for other parameters that will be used to describe the general chemical status of the streams. Samples transported to the laboratory for chemical analysis will be processed within three days of collection. These measurements will be performed by CRG Marine Laboratories, Inc. and will include the quantification of ammonia nitrogen, dissolved orthophosphate, nitrate-nitrogen, nitrite-nitrogen, and total suspended solids (Table 1). .

This information will be used to calculate the percent number of streams that are of concern and require further study. Correlations between physical parameters, physical habitat, nutrient results, and bioassessment results will be done to determine possible relationships between IBI scores and the water column indicators.

Table 1. Water chemistry analyses conducted by CRG Marine Laboratories, Inc.

CONSTITUENT	UNITS
Ammonia-N	mg/L

Conductivity	mS
Nitrate-N	mg/L
Nitrite-N	mg/L
Orthophosphate as P	mg/L
Total Suspended Solids	mg/L
Turbidity	NTU

9. Benthic macroinvertebrate collection and identification:

The bioassessment procedures used by this study and by all organizations to sample BMI communities follows the current CSBP established by the Department of Fish and Game. This workplan calls for "Multihabitat" sampling at each site as well as "Targeted Riffle" sampling if the site conforms to the necessary specifications for this alternative sampling procedure. Specific information on each procedure in given in Appendix 1. For the multihabitat sampling procedure, a 150-meter reach, parallel to the stream, is established. The 150-meter reach is then subdivided into 11 transect cross-sections spaced at 15 meters. BMIs are sampled at each of the 11 transects following an alternating pattern (right margin; 75% width, center 50% width; left margin; 25% width) with the starting position decided by flipping a coin for either left and right banks. Samples are collected by firmly placing a D-frame kick-net (0.3 meter wide with 0.5 mm mesh) on the streambed perpendicular to the channel flow, with the opening facing upstream. A one-foot-square area directly in front of the net is sampled by manually disrupting the substrate and scrubbing any cobble or boulders within the sample area for 15-45 seconds (depending on the complexity of the substrate) to a depth of 4 cm. The 11 samples are combined into one composite sample for the 150-meter reach (Detailed methodology can be found in Appendix 2 (Field Standard Operating Procedures [SOP]). In the laboratory, the composite sample is sub-sampled and sorted to obtain 500 BMIs that will be keyed to the standard taxonomic level established by California Aquatic Bioassessment Laboratory Network (CAMLnet); detailed methodology can be found in Appendix 2 (Lab SOP).

10. References

Harrington, J. M. 2003. California stream bioassessment procedures. California Department of Fish and Game, Water Pollution Control Laboratory, Rancho Cordova, California.

Ode, P. R., Rehn, A. C., and May, J. T. 2005. A quantitative tool for assessing the integrity of southern coastal California streams. Environmental Management 35: 493-504.

Appendix 1: CSULB-SEAL Field Sampling SOP.

EQUIPMENT AND SUPPLIES REQUIRED

- Field Sampling SOP (this document)
- water chemistry box
  - pH/dissolved oxygen meter (1 9V)
  - autocalibration solution
  - Meter Calibration Log
  - water chemistry sample jars
  - pencils
- physical habitat kit
  - flow meter (1 lithium CR2032)
  - extensible stadia rod
  - transect tape
  - rangefinder (1 lithium CR2)
  - densiometer
  - inclinometer
  - graduated "dowel" for stream depth
  - graduated tent stake for sediment depth
- tool box
  - pencils/sharpies
  - wax pencils
  - spare batteries
  - screwdrivers (Phillips and flathead)
  - duct tape
  - flagging tape
  - sunblock/bug repellent
- DC to AC inverter
- walkie-talkies with charger
- form/label envelope

  Field Datasheets (3 blank)
  Primary Sample Labels (5)
- clipboards (3)
- machetes (2)
- rope (at least 2 lengths)
- pepper spray
- plastic sample jars (10-15, 1/2 gal)
- plastic buckets (2)
- 95% ethanol (20 liters)
- WAAS-enabled GPS (2 AA)
- regional Thomas guide
- GIS maps of region with sites plotted
- digital camera with charger
- D net, 500 µm mesh
- numbered transect stakes, mallet
- 30 meter transect tape
- white plastic trays (2)
- large cooler with ice
- extra sample containers, Ziploc bags
- waders
- standard size #35 Tyler sieve (500 µm)
- cell phone
- rite in the rain field book
- first aid kit (see separate list)

STANDARD OPERATING PROCEDURES

1. Turn on and calibrate pH/dissolved oxygen meter

Use instructions associated with the meter (HORIBA Water Quality Checker U-10) to auto calibrate it for pH, DO, conductivity, and turbidity. The meter should be calibrated at each site. Log calibration information in the log sheet associated with the meter.

2. Locate the site by GPS

Note: GPS coordinates often fall on dry land. If the coordinates fall near a stream and were apparently intended to sample that stream, find the actual stream site nearest to the given coordinates and use that stream site as the actual (alternative) sampling site. Name this new site XXX-A, where "XXX" is the original site number (e.g. 123-A if the original site number was 123). If the given GPS coordinates fall more than 500 m from a stream or are otherwise not clearly intended to fall on that stream, treat the site as invalid and move to the next site. Mark the site with the "0" stake. Save the site position as a waypoint in the GPS. Fill out the top box of the Field Datasheet.

3. Lay out the transect

One person should stand at the zero transect marker while the other person walks upstream ~30 meters and uses the rangefinder (Bushnell YardagePro Scout, model 20-001) to sight back to the first person. The person with the rangefinder should repeat the measurement, adjusting position as needed, until the 30-meter point has been located. Mark the 30-meter point with the "30" stake. Repeat this process, moving upstream in 30-meter increments, until the four remaining transects (60, 90, 120, 150) have been staked out. Use the GPS to save the location of the 150-meter transect as a waypoint. Note that if the stream is sinuous or the banks are heavily vegetated, a pair of people may need to use transect tape instead of the rangefinder to locate transects.

4. Water chemistry

Water sampler should wear latex gloves to prevent sunblock, insect repellent, etc. from contaminating samples. Approaching the 0-transect from downstream, fill a plastic 500 ml sampling container with surface water for immediate measurements. Also fill a 1 liter plastic bottle with surface water to be returned to the laboratory for further water chemistry analyses. Label this bottle using a sharpie, and place it immediately in a cooler with ice. Use the calibrated meter to measure temperature, pH, conductivity, salinity, turbidity, and dissolved oxygen of the water in the plastic sample jar. Record data in the "In situ measurements" box of the Field Datasheet. Rinse the meter probe with tap water and return it to its box.

5. Biological sampling

Use targeted riffle or multi-habitat methods depending on the stream. *Make sure to note (in the "In Situ Measurements" box on the Field Sampling Sheet) which method you used!* Start at the downstream end of the reach and move upstream.

Targeted Riffle – Composite 1 ft^2 of substrate from each of eight fast water habitats distributed throughout the reach. To determine the sampling position within the riffle, take two random numbers between 1 and 10 with the first number representing the percent of the length (from the bottom), and the second number representing the percent of the width (from the right bank).

Multi-Habitat - Composite 1 ft^2 of substrate from each of 11 transects (six primary transects and five intermediate transects [e.g., 15 m, 45 m, etc.), alternating from right margin, center, and left margin. The position of the first sample is decided by flipping a coin for either left and right banks.

Sample by placing the D-net against the substratum, with the opening facing upstream. Use gloved hands or feet to stir up the sediment in a 1 ft^2 sampling quadrat upstream from D-net opening for about 30 seconds. If the sampling quadrat contains rocks, pick these up and rub them with gloved hands to remove adhering macroinvertebrates. After each sampling, sweep the net through the water to concentrate material in the back end of the net. When all the quadrats (either 8 or 11, depending on the type of stream) have been sampled, carefully remove all of the material from the net into a plastic tray. Pour the contents of this tray, with as little water as possible, into plastic sample jars. Each jar should be no more than 50% full of material; if there is too much material, use additional jars. Cover the samples with 95% ethanol. Make sure to include a completed Primary Sample Label inside each jar; use a wax pencil to label the outside of each jar with the site code and stream name.

In some cases, samples will contain a large volume of sand. Rather than preserving all of this sand as part of the primary sample, separate the organic material from it by elutriation using the following method (adapted from the Sierra Nevada Aquatic Research Laboratory protocols, June 2005, and the 2005 SWAMP Bioassessment protocols). When all the quadrats have been sampled, remove all of the material from the net into a plastic bucket of water. Stir vigorously, then elutriate (pour off the lighter material) with a swirling motion into another bucket. Repeat this process five times, using only a small amount of water in each elutriation so the receiving bucket doesn't overflow. Only rocks and sand should be left in the original bucket. Empty these into a shallow white pan, doing this in several iterations if there is a large volume of sand or rocks. Search for remaining organisms (dense organisms like cased caddisflies, snails, and clams will often not be removed during elutriation), and place them in the receiving bucket. When all the organisms have been removed from the sand/rocks, throw the latter out. Pour the elutriate through a 500 µm Tyler sieve

to concentrate organisms, then empty the contents of the sieve into a sample container and In some cases, samples will contain a large volume of sand. Rather than preserving all of this sand as part of the primary sample, separate the organic material from it by elutriation using the following method (adapted from the Sierra Nevada Aquatic Research Laboratory protocols, June 2005, and the 2005 SWAMP Bioassessment protocols). When all the quadrats have been sampled, remove all of the material from the net into a plastic bucket of water. Stir vigorously, then elutriate (pour off the lighter material) with a swirling motion into another bucket. Repeat this process five times, using only a small amount of water in each elutriation so the receiving bucket doesn't overflow. Only rocks and sand should be left in the original bucket. Empty these into a shallow white pan, doing this in several iterations if there is a large volume of sand or rocks. Search for remaining organisms (dense organisms like cased caddisflies, snails, and clams will often not be removed during elutriation), and place them in the receiving bucket. When all the organisms have been removed from the sand/rocks, throw the latter out. Pour the elutriate through a 500 µm Tyler sieve to concentrate organisms, then empty the contents of the sieve into a sample container and proceed to label and preserve the sample as described above.

6. Physical habitat sampling

Three people are required here – one data recorder, one flow meter operator, and one general phab measurer.

As soon as the biological sampling has occurred at lowest part of the reach, lay out a stadia rod across the stream at transect 0 and measure stream width. Measure width of the main channel – that is, if there are side channels separated from the main channel by emergent substratum, do not include them in the width measurement (but note this in the site comments). With the rod in place, begin measuring transect physical habitat parameters (see below) and recording them in the "Substrate Cross-Sectional Information" boxes. Parameters include: water depth, sediment depth, substrate size class, water velocity, and densitometer readings. All of these measurements should be done at: the left bank, 25% stream width, 50%, 75%, and right bank (facing upstream). Exceptions are flow measurements, which are generally impossible at the banks because of shallow water, and densitometry, which should be done from the center of the stream facing the left bank, upstream, downstream, and right bank.

Water depth – hold graduated dowel on streambed at the correct point on the stadia rod and read off depth.

Sediment depth – at the point of contact of the dowel with the streambed, insert the graduated stake into the sediment, and read off sediment depth. If the stake falls on a cobble or other hard substrate, the sediment depth is zero.

Substrate size class – identify the size class (using the table on the datasheet) of the substrate sediment/stones at the point of contact of the dowel with the streambed.

Embeddedness – do this only if the dowel falls on cobble substrate (stones ~2.5-10 inches diameter, 6-25 cm). If so, remove the cobble from the substrate and visually estimate the percentage of its volume that was embedded in sediment.

Flow velocity – hold the flowmeter (Flowatch) at the correct point on the stadia rod. Use the graduated markings on its rod to hold it so that the propeller is 60% of stream depth below the water surface. Make sure the LCD is zeroed. Record the number of propeller turns for one minute (time using a stopwatch) and record this value on the Field Sampling Sheet. Later this value can be

converted to an actual velocity using the calibration curve.

When all of these parameters are recorded, pick up the stadia rod and walk upstream to the next transect to repeat the process. While walking up to the next transect, the data recorder should fill out the "Human Influence" and "Indication of Trophic Complexity" sections of the Field Datasheet.

7. Photography

While physical habitat measurements are being taken, one person should walk the transect and take four digital photographs (transect 0, facing upstream; 60, upstream; 120, upstream; and 150, downstream). Record image numbers in the "In Situ Measurements" box on p. 1 of the Field Sampling Sheet. Take more photographs if interesting land use, biological, or hydrological features are noted; make sure to record their image numbers and short descriptions of them in one of the "Site Comments" sections of the Field Datasheet.

8. Walk the transect to retrieve stakes and to make sure that no gear has been left behind

Back at the vehicle, check to make sure that the samples are appropriately labeled, preserved and safely stowed, that meter probes are in appropriate storage solutions, and that all gear is stowed in the vehicle.

9. Post-sampling discussion and note-taking

Briefly convene the entire team for a quick discussion of the site. Specifically, ask if anyone noted anything unusual about the site; if people noted organisms outside of the formal reach; and solicit comments on how to improve protocols.

Field Datasheet

Project Name: CSULB RIVERS	Date:	
Stream Name:	Time:	
Site Code:	Crew Members	
Initial GPS Latitude: °N		
Initial GPS Longitude: °W		
Elevation:		
Final GPS Latitude: °N		
Final GPS Longitude: °W	Distance Between Initial and Final GPS Coordinates:	

In Situ Measurements					
pH:		Conductivity (mS/cm):		Turbidity (NTU):	
Dissolved O_2 (mg/l):		Water temperature		Alkalinity:	
Sampling method (circle	TARGETED RIFFLE or MULTIHABITAT				
Photograph	Trans 0 up:	Trans 30 up:	Trans 60 up:	Trans 90 up:	Trans 150 down:
Additional Photographs (Optional):					

General Habitat Characterization (Reach Wide)				
Habitat Parameter	Optimal	Suboptimal	Marginal	**Poor**
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; most favorable is a mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE ___	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

Site Code: _____ Date: _____ Page 2

Transect 0

Substrate Cross-Sectional Information					
Estimated Stream Width		Depth XXX cm	Size Class Code	Sediment Depth (cm)	Velocity Counts/min
Left	Bank				N/A
L Ctr	25%				
Ctr	50%				
R Ctr	75%				
Right	bank				N/A

Densiometer (0-17 Max)	
Left Bank	
Ctr Up	
Ctr Dwn	
Rt. Bank	

Comments: _____

Human Influence	0 - Not Present CH - Within Channel B - On Bank C - Within 10m of Channel P - >10m of Channel								
	Left Bank					Right Bank			
Wall/Tyhs/Rip-rap/ Revetment/Dam	0	B	C	P	CH	0	B	C	P
Buildings	0	B	C	P	CH	0	B	C	P
Pavement/Cleared Lot	0	B	C	P	CH	0	B	C	P
Road/Railroad	0	B	C	P	CH	0	B	C	P
Pipes (Inlet/Outlet)	0	B	C	P	CH	0	B	C	P
Landfill/ Trash	0	B	C	P	CH	0	B	C	P
Park/Lawn	0	B	C	P	CH	0	B	C	P
Row Crops	0	B	C	P	CH	0	B	C	P
Pasture/Range/ Hayfield	0	B	C	P	CH	0	B	C	P
Logging Operations	0	B	C	P	CH	0	B	C	P
Mining Activity	0	B	C	P	CH	0	B	C	P

Transect 30

Substrate Cross-Sectional Information					
Estimated Stream Width		Depth XXX cm	Size Class Code	Sediment Depth (cm)	Velocity Counts/min
Left	Bank				N/A
L Ctr	25%				
Ctr	50%				
R Ctr	75%				
Right	bank				N/A

Densiometer (0-17 Max)	
Left Bank	
Ctr Up	
Ctr Dwn	
Rt. Bank	

Comments: _____

Substrate Size Class Codes
RS = Bedrock (Smooth) - Larger Than a Car
RR = Bedrock (Rough) - Larger Than a Car
RC = Concrete/ Asphalt
LB = Larger Boulder (1000 to 4000 mm) - Meterstick to Car
SB = Small Boulder (250 to 1000 mm) - Basketball to Meterstick
CB = Cobble (64 to 250 mm) - Tennis Ball to Basketball
GC = Coarse Gravel (16 to 64 mm) - Marble to Tennis Ball
GF = Fine Gravel (2 to 16 mm) - Ladybug to Marble
SA = Sand (0.06 to 2 mm) - Gritty up to Lady Bug
FN = Silt/ Clay/ Muck - Not Gritty
HP = Hardpan - Firm Consolidated Fine Substrate
WD = Wood - Any Size
OT = Other (Write comment below)

Indication of Trophic Complexity	Y	N
Amphibians		
Fishes		
Water Striders		
Adult Insects (Dragons/Damsels/Butterflies/Moths/etc.)		

Site Code: _____ Date: _____ Page 3

Transect 60

Substrate Cross-Sectional Information					
Estimated Stream Width		Depth XXX cm	Size Class Code	Sediment Depth (cm)	Velocity Counts/min
Left	Bank				N/A
L Ctr	25%				
Ctr	50%				
R Ctr	75%				
Right	bank				N/A

Transect 90

Substrate Cross-Sectional Information					
Estimated Stream Width		Depth XXX cm	Size Class Code	Sediment Depth (cm)	Velocity Counts/min
Left	Bank				N/A
L Ctr	25%				
Ctr	50%				
R Ctr	75%				
Right	bank				N/A

Densiometer (0-17 Max)	
Left Bank	
Ctr Up	
Ctr Dwn	
Rt. Bank	

Comments: _____

Human Influence	0 - Not Present CH - Within Channel B - On Bank C - Within 10m of Channel P - >10m of Channel								
	Left Bank					Right Bank			
Wall/Dyke/Rip-rap/ Revetment/Dam	0	B	C	P	CH	0	B	C	P
Buildings	0	B	C	P	CH	0	B	C	P
Pavement/Paved Lot	0	B	C	P	CH	0	B	C	P
Road/Railroad	0	B	C	P	CH	0	B	C	P
Pipes (Inlet/Outlet)	0	B	C	P	CH	0	B	C	P
Landfill/ Trash	0	B	C	P	CH	0	B	C	P
Park/Lawn	0	B	C	P	CH	0	B	C	P
Row Crops	0	B	C	P	CH	0	B	C	P
Pasture/Range/ Hayfield	0	B	C	P	CH	0	B	C	P
Logging Operations	0	B	C	P	CH	0	B	C	P
Mining Activity	0	B	C	P	CH	0	B	C	P

Densiometer (0-17 Max)	
Left Bank	
Ctr Up	
Ctr Dwn	
Rt. Bank	

Comments: _____

Substrate Size Class Codes
RS = Bedrock (Smooth) - Larger Than a Car
RR = Bedrock (Rough) - Larger Than a Car
RC = Concrete/ Asphalt
LB = Larger Boulder (1000 to 4000 mm) - Meterstick to Car
SB = Small Boulder (250 to 1000 mm) - Basketball to Meterstick
CB = Cobble (64 to 250 mm) - Tennis Ball to Basketball
GC = Coarse Gravel (16 to 64 mm) - Marble to Tennis Ball
GF = Fine Gravel (2 to 16 mm) - Ladybug to Marble
SA = Sand (0.06 to 2 mm) - Gritty up to Lady Bug
FN = Silt/ Clay/ Muck - Not Gritty
HP = Hardpan - Firm Consolidated Fine Substrate
WD = Wood - Any Size
OT = Other (Write comment below)

Indication of Trophic Complexity	Y	N
Amphibians		
Fishes		
Water Striders		
Adult Insects (Dragons/Damsels/Butterflies/Moths/etc.)		

Appendix 1.1 – Field Datasheet

Site Code: _____ Date: _____ Page 4

Transect 120

Substrate Cross-Sectional Information					
Estimated Stream Width		Depth XXX cm	Size Class Code	Sediment Depth (cm)	Velocity Counts/min
Left	Bank				N/A
L Ctr	25%				
Ctr	50%				
R Ctr	75%				
Right	bank				N/A

Transect 150

Substrate Cross-Sectional Information					
Estimated Stream Width		Depth XXX cm	Size Class Code	Sediment Depth (cm)	Velocity Counts/min
Left	Bank				N/A
L Ctr	25%				
Ctr	50%				
R Ctr	75%				
Right	bank				N/A

Densiometer (0-17 Max)	
Left Bank	
Ctr Up	
Ctr Dwn	
Rt. Bank	

Human Influence	0 - Not Present CH - Within Channel B - On Bank C - Within 10m of Channel P - >10m of Channel								
	Left Bank					Right Bank			
Wall/Dyke/Rip-rap/ Revetment/Dam	0	B	C	P	CH	0	B	C	P
Buildings	0	B	C	P	CH	0	B	C	P
Pavement/Channel Lot	0	B	C	P	CH	0	B	C	P
Road/Railroad	0	B	C	P	CH	0	B	C	P
Pipes (Inlet/Outlet)	0	B	C	P	CH	0	B	C	P
Landfill/Trash	0	B	C	P	CH	0	B	C	P
Park/Lawn	0	B	C	P	CH	0	B	C	P
Row Crops	0	B	C	P	CH	0	B	C	P
Pasture/Range/ Hayfield	0	B	C	P	CH	0	B	C	P
Logging Operations	0	B	C	P	CH	0	B	C	P
Mining Activity	0	B	C	P	CH	0	B	C	P

Densiometer (0-17 Max)	
Left Bank	
Ctr Up	
Ctr Dwn	
Rt. Bank	

Comments: _____

Comments: _____

Substrate Size Class Codes
RS = Bedrock (Smooth) - Larger Than a Car
RR = Bedrock (Rough) - Larger Than a Car
RC = Concrete/ Asphalt
LB = Larger Boulder (1000 to 4000 mm) - Meterstick to Car
SB = Small Boulder (250 to 1000 mm) - Basketball to Meterstick
CB = Cobble (64 to 250 mm) - Tennis Ball to Basketball
GC = Coarse Gravel (16 to 64 mm) - Marble to Tennis Ball
GF = Fine Gravel (2 to 16 mm) - Ladybug to Marble
SA = Sand (0.06 to 2 mm) - Gritty up to Lady Bug
FN = Silt/ Clay/ Muck - Not Gritty
HP = Hardpan - Firm Consolidated Fine Substrate
WD = Wood - Any Size
OT = Other (Write comment below)

Indication of Trophic Complexity	Y	N
Amphibians		
Fishes		
Water Striders		
Adult Insects (Dragons/Damsels/Butterflies/Moths/etc.)		

Appendix 1.1 – Field Datasheet

Appendix 2: CSULB-SEAL Laboratory SOP.

EQUIPMENT AND SUPPLIES REQUIRED

- dissecting microscope
- #35 Tyler sieve (500 µm)
- gridded tray (plastic or metal)
- wide mouth glass jars and lids
- plastic Petri dish
- five vials in vial rack
- 70% ethanol
- fine forceps (2 prs)
- Sample Processing Lab Sheet
- Subsample Labels
- random number table
- five-channel tally counter

STANDARD OPERATING PROCEDURES

1. Log the sample into the Sample Tracking Log

This helps us keep track of where the sample is in the processing and taxonomy process, and who is in charge of it.

2. Fill out the Sample Processing Lab Sheet

For consistency and QC tracking purposes, a single person should process each sample. Keep track of the total time spent processing a sample; this is important in the QC process and in helping us determine efficiency at particular types of sites.

3. Clean the sample of large debris

Empty the contents of the primary sample jar into the Tyler sieve (in a sink), making sure to rinse all the debris from the sample jar and the Primary Sample Label into the sieve (using tap water). Return the label to the empty jar. If there is too much material in the jar to fit in a thin layer in the sieve, do this in several steps. Use water from the tap and rinse the ethanol from the sample carefully, making sure not to splash organisms from the sieve.

Once the sample is thoroughly rinsed of ethanol, clean and remove debris larger than ~10 mm in size. Remove green leaves, twigs, and rocks; do not remove filamentous algae, decaying leaves, or other type of debris that may contain invertebrates. Make sure to inspect each piece of debris carefully to reduce the chance of discarding invertebrates. The removed debris can be discarded. *This is the ONLY time in this procedure that material from the sample will be discarded.*

Place the cleaned sample with as little water as possible into a gridded tray. Make sure that there are no animals left on the sieve. If there is more sample material to clean, return to the beginning of step 3 and repeat until the entire sample is clean and in the tray. *Note that if the primary sample is split into more than one jar, the contents of all of the jars must be cleaned and pooled before proceeding to step 4 below.*

4. Subsample material using a gridded tray

Spread the cleaned, damp sample out in the gridded tray using as many grids as necessary to obtain an approximate thickness of 10-15 mm. If the sample contains too much material to follow this procedure, process in a larger tray. Record the total number of grids covered on the Sample Processing Lab Sheet.

Choose 5-10 sets of grid numbers using a random number table and record these on the Sample Processing Lab Sheet. To do this, haphazardly choose a starting digit on the random number table. Reading across, record the next 40 digits on the Sample Processing Lab Sheet. Working through this list in order, take the coordinates of 5-10 grids. Digits larger than the values of the x or y axes of the grid should simply be crossed off the list. If 40 digits are not sufficient to yield 5-10 sets of grid coordinates, go back to the random number table and take more digits.

Remove all the material from the first random grid. Use a razor blade to gently cut the debris around the edge of the grid, but try to avoid cutting any animals. Place the material into a clean glass jar. Label this jar with a Subsample Label. Add ethanol to cover the sample. Repeat with the next 4-9 grids, placing contents of each grid into a separate clean glass jar, and labeling each.

Replace all the remaining sample material in the gridded tray back into the primary sample jar. Include a new Primary Sample Label, indicating when and by whom the grid samples were taken, how many grid samples were taken, and how many remained. Do not discard the original Primary Sample Label. Cover the sample with ethanol, and return it to storage.

5. <u>Pick invertebrates from the first subsample</u>

Fill five vials (in vial rack) halfway with 70% ethanol. Tape a Subsample Label in front of each vial, on the back writing one of five categories: Diptera, Ephemeroptera, Plecoptera, Other Insects, and Non-insects. You will pick specimens from the subsample into these various containers.

Empty the contents of the jar from the first subsample into a plastic Petri dish. Go through the dish systematically, removing any benthic macroinvertebrates (BMIs) encountered and placing them in the appropriate vial. As you remove BMIs, keep track of their numbers using a tally counter labeled to match the vials.

Two complications are the presence of animal fragments, and the presence of animals that are not "approved" by CAMLnet for use in benthic studies.

A. Fragments of animals. Deal with these as follows:

i. worms (annelids, nemerteans, platyhelminths) – pick it out and count it if the animal has a head.

ii. insects – pick out and count animals that have (minimally) a head, thorax, and at least three legs (one of each).

iii. miscellaneous arthropods – pick out and count whole animals only.

iv. mollusks – pick out if they were alive when collected (no empty shells).

If in doubt about whether or not a fragment is a head end, or whether or not an organism is a fragment or whole, PICK IT OUT but don't count it on the tally counter.

B. Non-CAMLnet approved animals. Some invertebrates that you encounter should not be counted towards the total 500, according to CAMLnet standards. These include: nematodes, cladocerans, copepods, branchiurans, and non-benthic insects (CAMLnet 2003, p. 5). Ignore nematode. Pick the others from your samples and include them in the Petri dish, but do not count them on the tally counter.

At the end of sorting the subsample, record the number of BMIs removed from it in each of the five groups on the Sample Processing Lab Sheet, as well as the total number. Also record the number of BMIs in each group on the appropriate label, and place it in the appropriate vial. Now sort the sample once more (quickly) as a self-QC step. If a significant number of organisms (i.e., > 5% of the total) are found during the self-QC, repeat the self-QC again.

Return the remaining debris to the jar with the original Subsample Label and cover it with ethanol. This will later be examined in a separate QC process to examine picking efficiency.

6. Pick invertebrates from additional subsamples until at least 500 BMIs are removed

Repeat step 5 with the next subsamples until you reach a count of at least 500 BMIs removed. For each subsample use a unique set of five vials. Make sure to record the total number of BMIs picked from each subsample on the Sample Processing Lab Sheet. If you reach 500 before finishing picking your last subsample, continue picking all the remaining BMIs from this last subsample and place them in a new vial (the "remnant" vial). Cover them with ethanol and label them with a completed Subsample Label on which you've also written the word "remnant". If you have extra, unpicked subsamples, return their contents to the primary sample jar.

7. Finish filling out the Sample Processing Lab Sheet and the Sample Tracking Log

Place the Sample Processing Lab Sheet in the appropriate binder. You are now done with the sorting process. The 500 (or more) BMIs are now ready to go through the identification process, and the picked over grid samples (each of which you've retained) are ready to go through the QC process to determine the efficiency of picking.

Appendix 3. California Santa Ana Region Survey Design

Description of Sample Design

Target Population: Target population consists of all streams within the Santa Ana Regional Water Board regional area.

Sample Frame: RF3 Alpha. Use perennially streams coded as "R", "S", "T", "N", and "W" and non-perennial streams that are 3rd and higher Strahler order coded as "R", "S", "T", "N", and "W". Initial frame reviewed by Region staff modified the frame to include specific streams that were known to be perennial and of interest.

Survey Design: A Generalized Random Tessellation Stratified (GRTS) survey design for a linear stream resource was used. The GRTS design includes reverse hierarchical ordering of the selected sites.

Stratification: No strata

Multi-Density Categories: Four Strahler order categories: 1st, 2nd, 3rd, and 4th+.

Panels: Five panels. Each panel will be visited once every five years.

Sample Size: Expect to sample 50 sites each year. Provide sufficient sites so that volunteers could visit a larger number of sites.

Oversample: 500 site over sample for a total of 750 sites. This results in a site approximately every 1.6 km.

Site Use: The base design has 250 sites. These sites are identified by PanelOne through PanelFive in the variable "Panel". If it is necessary for a site to be replaced, then the lowest ordered SiteID that is part of the oversample of sites (identified by "OverSamp" in variable "Panel") must be used. Subsequent replacement sites continue to be used in the same way.

Sample Frame Summary

The total stream length in the sampling frame 1301.89 km.

Site Selection Summary

	OverSamp	PanelFive	PanelFour	PanelOne	PanelThree	PanelTwo
1st	134	13	7	10	10	18
2nd	121	14	19	17	11	7
3rd	124	14	13	12	17	12
4th+	121	9	11	11	12	13

Description of Sample Design Output:

To achieve an expected sample size of sites in the target population, an appropriate sample size was selected for the study area. A Base set of sites and an Oversample of sites are included in the output. The oversample sites should be added, as needed, in numerical SiteID order. Oversample sites are identified in the "panel" data column as Oversamp. Note that sites may be used in order beginning at the first SiteID number and continuing until desired sample size is reached.

The tab-delimited, ASCII file (OutletSites.tab) has the following variable definitions:

Variable Name	Description
SiteID	Unique site identification (character)
arcid	Internal identification number
x	Albers x-coordinate
y	Albers y-coordinate
LonDD	Longitude, decimal degrees NAD27
LatDD	Latitude, decimal degrees NAD27
mdcaty	Multi-density categories used for unequal probability selection
weight	Weight (in meters), inverse of inclusion probability, to be used in statistical analyses
stratum	Strata used in the survey design
panel	Identifies base sample by panel name and Oversample by OverSamp
auxiliary variables	Remaining columns are from the sample frame provided

Projection Information

Albers projection used

Datum: NAD 27

Spheroid: Clarke1866

Units: meters

Center longitude (decimal degrees): -96

Origin latitude (decimal degrees): 23

Standard parallel 1 (decimal degrees): 29.5

Standard parallel 2 (decimal degrees): 45.5

Evaluation Process

The survey design weights that are given in the design file assume that the survey design is implemented as designed. That is, only the sites that are in the base sample (not in the over sample) are used, and all of the base sites are used. This may not occur due to (1) sites not being a member of the target population, (2) landowners deny access to a site, (3) a site is physically inaccessible (safety reasons), or (4) site not sampled for other reasons. Typically, users prefer to replace sites that can not be sampled with other sites to achieve the sample size planned. The site replacement process is described above. When sites are replaced, the survey design weights are no longer correct and must be adjusted. The weight adjustment requires knowing what happened to each site in the base design and the over sample sites. EvalStatus is initially set to "NotEval" to indicate that the site has yet to be evaluated for sampling. When a site is evaluated for sampling, then the EvalStatus for the site must be changed. Recommended codes are:

EvalStatus Code	Name	Meaning
TS	Target Sampled	site is a member of the target population and was sampled
LD	Landowner Denial	landowner denied access to the site
PB	Physical Barrier	physical barrier prevented access to the site
NT	Non-Target	site is not a member of the target population
NN	Not Needed	site is a member of the over sample and was not evaluated for sampling
Other codes		Many times useful to have other codes. For example, rather than use NT, may use specific codes indicating why the site was non-target.

Statistical Analysis

Any statistical analysis of data must incorporate information about the monitoring survey design. In particular, when estimates of characteristics for the entire target population are computed, the statistical analysis must account for any stratification or unequal probability selection in the design. Procedures for doing this are available from the Aquatic Resource Monitoring web page given in the bibliography. A statistical analysis library of functions is available from the web page to do common population estimates in the statistical software environment R.

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Stevens, D. L., Jr., and A. R. Olsen. 2004. Spatially-balanced sampling of natural resources in the presence of frame imperfections. Journal of American Statistical Association:99:262-278.

Web Page: http://www.epa.gov/nheerl/arm

Notes on importing data into ArcGIS 8.x (ArcView 8.x):

For *.tab file (note: this isn't the ONLY way to do this - just a quick example of one way to do it):

Start MS Excel and read in the *.tab file as a tab delimited file.

Change the format of the columns with decimal place (non-integer) numbers.

Select all columns with numeric values (shift + left mouse click).

Using the "right mouse click menu" and choosing "format cells", you can select the "Number" tab, then select the "Number" category and modify the number of decimal places for the columns selected.

Making them numeric fields with 10 decimal places should be sufficient.

Save the modified *.tab file as a "DBF 3 (dBase III) (*.dbf)" file type (found in the "File", "Save As" menu).

Start ArcMap as a "new empty map" (or you can .

Select the "Add Data" icon (under "File" menu or usually in the toolbar as a black "+" with a yellow triangle behind it).

Select the new *.dbf file you created.

Right-mouse-click on the table you just added to the map and choose the "Display XY Data" selection.

If you want the file in the original projection, choose the "X" and "Y" fields as they default.

If you want the file as geographic coordinates, choose the "Longdd" for the X field and the "Latdd" for the Y field.

Edit the coordinate system to match whichever you have chosen to use (original projection provided or geographic).

Click OK to show the data - this will create a "Events" dataset.

If you Right-mouse-click on the Event dataset name, and select "Data", "Export Data" on the menu and sub-menu, you can create a shape file of the XY event data you just imported.

For *.e00 file (assumes any compressed file sent has been uncompressed using something like WinZip) :

You can bring the arc export files into ArcMap - you just need to "import" the file to a coverage before being able to use it.

Using ArcGIS:

If you start up the "ArcToolbox" and look under "Conversion Tools" and then "Import to Coverage" you'll see a "ArcView Import from Interchange File" - this is the tool to bring in an arc export (interchange) file.