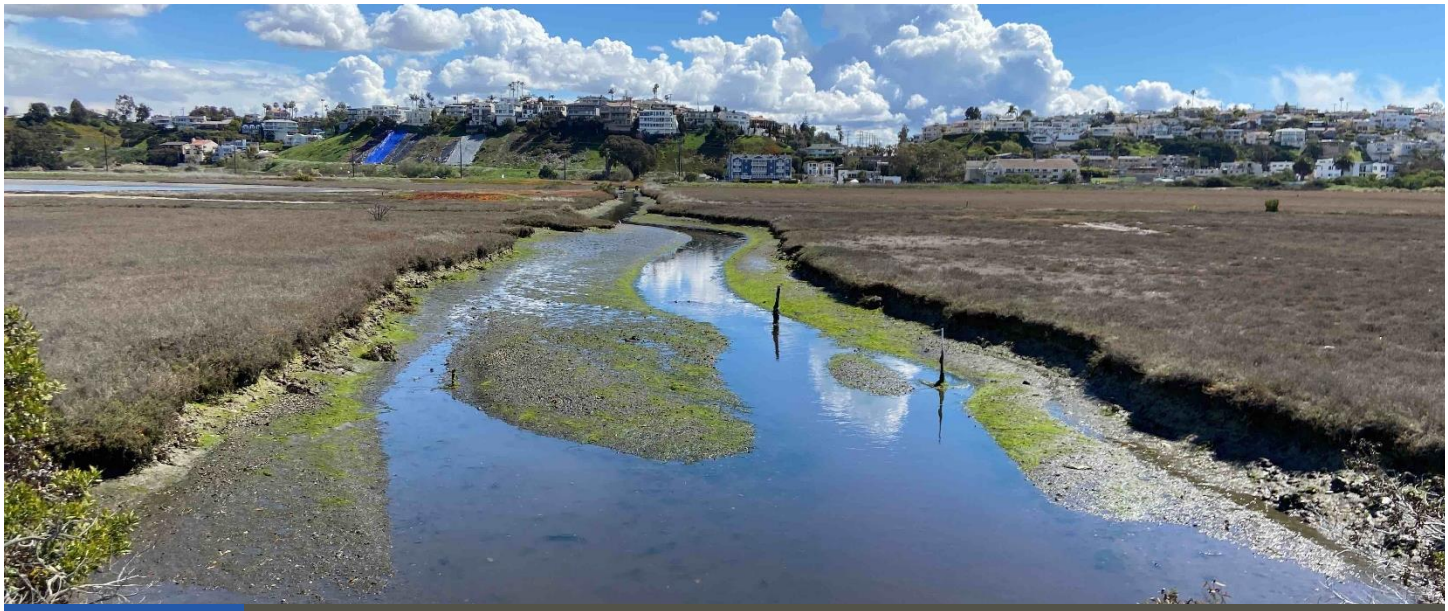

Standard Operating Procedures (6.1): Benthic Invertebrates

March 2021

Prepared for the United States Environmental Protection Agency

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Standard Operating Procedures: Benthic Invertebrates

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Protocol Suitability Evaluation

A habitat suitability table containing appropriate estuarine wetland habitat types (of those evaluated) to implement benthic invertebrate protocols is displayed in Table 1. For emergent salt marsh habitats, benthic invertebrate protocols are usually applicable in areas receiving at least partial tidal inundation, though the protocol could be used in other areas. A comparative assessment of cost, effort, and data quality are shown in Table 2. A matrix of additional detailed categorical evaluations of benthic invertebrate protocols can be found in Appendix 6.1A.

Table 1. Appropriate habitat types for benthic invertebrate survey protocols.

	Habitat Types					
Survey Protocol	**Tidal Channel**	**Mud/sand flat**	**Emergent salt marsh**	**Non-tidal salt marsh**	**Salt pan**	**'Degraded' / fill**
Benthic Cores	X	X	X	X	X	X

Table 2. Categorical assessment of cost/effort and data quality for benthic invertebrate survey protocols.

	Evaluation Metric	Benthic Cores	Notes
Time / Effort	Office Preparation Time	10-30 minutes	Identification of sampling locations and equipment collection
	Equipment Construction Time (one time)	30-60 minutes	Construction of both large and small cores
	Field Time (per station)	> 60 minutes	Coring and sieving for each station can take between 45-90 minutes, depending on the sediment grain size, number of surveyors, and the salt water source for rinsing
	Laboratory Time (per station)	> 60 minutes	Dependent on invertebrate community and familiarity with groups and taxa
	Post-Survey Processing / QAQC Time	> 30 minutes	----
	Minimum Repetition (site-dependent)	Few Repetitions	----
	Relative Cost (equipment and supplies)	$ 15-50	More expensive if samples are analyzed by professional taxonomists (laboratory)
Survey / Data Quality	Accuracy (at a survey area level)	Medium	----
	Precision (at a survey area level)	Low	Highly variable based on core placement
	Qualitative-Quantitative Score	Quantitative	----
	Subjectivity-Objectivity Score	Objective	----

Resulting Data Types

The application of benthic invertebrate survey protocols will yield quantitative data displayed in abundances by taxa or density of benthic infauna, recorded as the number of individuals per meter squared for each station. Benthic invertebrate survey protocols are intended to account for the presence of both large (e.g., bivalves, mollusks) and small (e.g., polychaetes, arthropods) infauna. These data may be used in conjunction with existing pollution tolerance indices to identify the abundance of pollution tolerant species as an indicator of habitat health (more common for freshwater species).

Objective

Benthic invertebrate taxa are useful ecological indicators because they provide a reflection of the state of the environment, especially at the transition from water to land and can indicate local biodiversity (Hilty and Merenlender 2000, Johnston et al. 2011, 2012). Long-term changes are often assessed by looking at the invertebrate community at a higher taxonomic level or by evaluating the community as a whole (Hodkinson and Jackson 2005, Johnston et al. 2011, 2012). The presence or absence of certain infauna (i.e., burrows into and lives in bottom sediments) or epifauna (i.e., lives on the surface of bottom sediments) within tidal channels can serve as indicators of water quality, anthropogenic stressors to the estuary, and the potential to support other trophic levels (WRP 2006); these benthic communities provide essential ecosystem services and support (Schreiber 1981).

The primary purpose of this sampling method is to assess the benthic invertebrate community by collecting data on the density and distribution of infauna within wetland tidal channels. Taxa will be assessed by sorting to the lowest taxonomic level possible including recognizable taxonomic units (RTU). This is discussed below, and for more details, see Monitoring Manual Version 2.0 (Johnston et al. 2021). While some protocols allow sorting to a higher taxonomic classification (e.g., order) to facilitate the use of student and volunteer (non-professional taxonomic identification) assistance, it is possible to determine morpho-species even without confirmed taxonomic assignments. Depending upon available funds, if lower level taxonomic classification is required, samples may also be sent to a qualified benthic invertebrate laboratory.

Figure 1. General items required for benthic invertebrate sampling.

Equipment

General equipment and supplies needed for benthic invertebrate surveys (Figure 1) include:

Collection:

- Sediment corers (a.k.a. Clam gun for large cores); see 'Field Methods' for sizing details
- Labeled and extra sealable bags (1 gallon)
- Pencils, permanent markers, and station data sheets (Appendix 6.1B)
- GPS with extra batteries
- Aerial Photo with stations and core locations or GPS points
- Waders (or surf/dive booties with a thick sole)
- 5-gallon buckets (3-4 recommended)
- Handheld multi-parameter water quality meter
- Cooler (to keep samples cold if an extended time period is expected between collection and preservation)

Sieving and Sorting:

- Sample Data Tracking Sheet
- Number 35 (0.5 mm for small cores) or Number 50 (0.3 mm for large cores) sieve. *Note: Choice of sieve size will affect abundance and type of invertebrates collected and ability to compare among studies and samples. For more details, see Monitoring Manual Version 2.0.*
- Glass jars, lids, and labels
- Pencils, thick permanent marker
- Waterproof paper (optional)
- 16 oz. Nalgene squirt bottles filled with DI water
- Dissecting forceps, spatula
- Benthic Sieve Bucket (optional; same mesh size as sieve)
- 70% Ethyl alcohol or 8% buffered formalin with Rose Bengal dye (Be careful, read Safety Data Sheets, SDS: https://www.osha.gov/hazcom). *Note: Rose Bengal dye can interfere with identification of some species. Ethyl alcohol can bleach animals and make identification more challenging. Formalin will prevent use for genetics.*
- Dissecting microscope with illuminator

Field Preparation

Equipment described above should be collected prior to the field shift. Batteries for all electronic devices should be checked and replaced as needed, and relevant data sheets should be printed and attached to the clipboards. *Helpful hint: Label and organize bags by station and channel location before going into the field*.

Field Methods – Station Selection and Frequency

It is important to note that specific techniques of benthic invertebrate protocols are typically unique for each monitoring program and are often targeted to particular organisms. This SOP suggests standardized protocols. Zedler (2001) recommends collecting benthic invertebrates quarterly. If that sampling frequency is not possible, then seasons should be chosen to align with monitoring program objectives, ongoing datasets, or regional studies. Some southern California monitoring programs collect data in fall to coincide with fish sampling and to avoid avian nesting season, though spring should also be considered if impacts can be minimized. Some monitoring programs (Johnston et al. 2011, 2012) suggest semi-annual sampling, once at the beginning of the wet season (September / October) and once after the wet season in approximately May (or early summer, if only collecting once). Sampling should

not occur within 72 hours of a rain event, as the freshwater input will affect abundances of certain taxa (Zedler 2001). *For more details, see Monitoring Manual Version 2.0*.

Samples from intertidal zones (e.g., mudflats) should be collected during low tides when the sediment is exposed. Samples from channels should be collected during mid to low tides when the sediment partially exposed as the channel zones may not ever be completely exposed. Again, this may vary with sampling program. Most programs prefer some water in the channel to help rinse the sediment from the sieves.

Sampling stations should be chosen (fixed) to be representative of the tidal channel and mudflat habitats of the wetland. Note: if feasible, test samples from the inundated marsh plain may also be collected. Each station consists of a cross-section transect of the tidal channel. Large and small core samples should be taken from the left, right, and thalweg of the channel [facing the outflow (Figure 2)]. The thalweg is defined as the lowest portion of the channel, and does not necessarily fall directly in the middle of the channel.

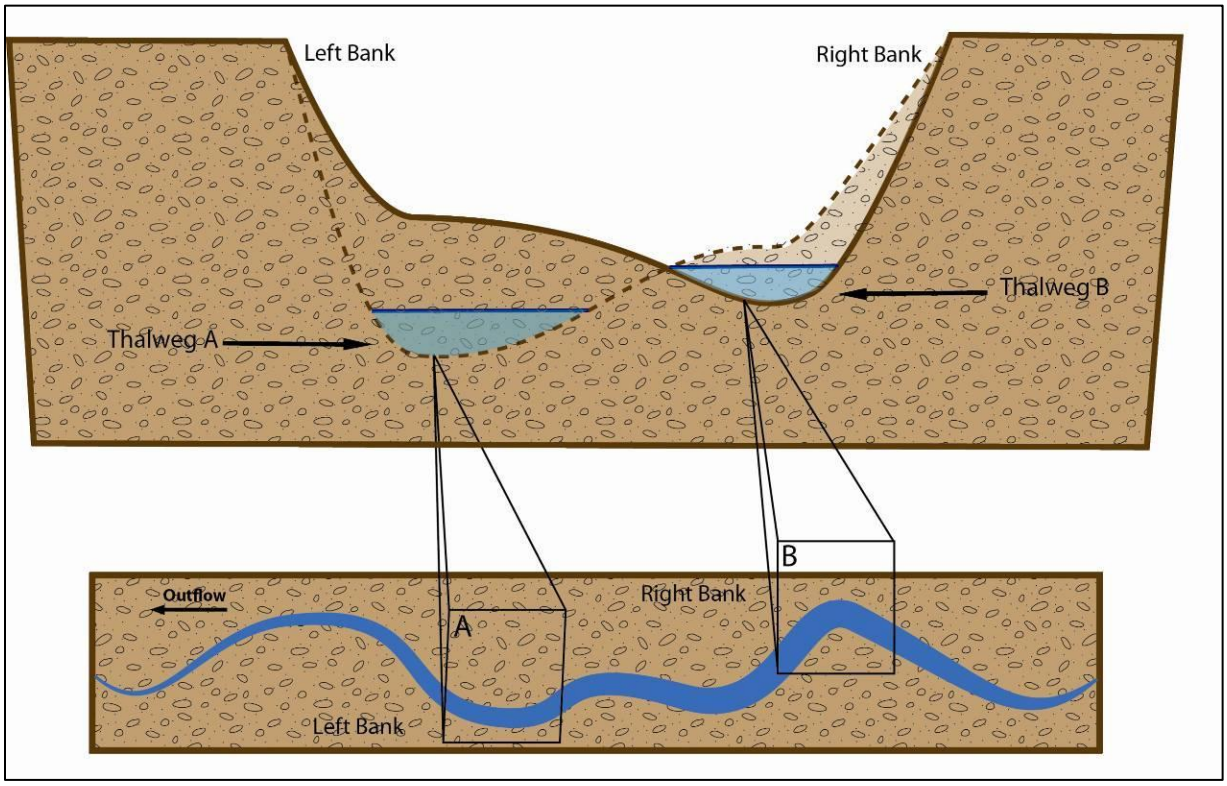

Figure 2. Depiction of cross-section transect of a tidal channel. The figure is not directly representative of a particular benthic survey station. Note: the thalweg is the deepest portion of the channel and not the midpoint.

Field Methods – Station Protocols

Readings for water temperature, salinity, dissolved oxygen (% and mg/L), and pH should be taken with a handheld multi-probe sonde at each station before entering the water (for details, refer to SOP 1.1 – continuous sonde monitoring). *Helpful hint: reaching from the shore using a pole or other device may help avoid trampling. Water should not be disturbed prior to water quality readings.*

Figure 3. Example of large core sediment extraction.

Core size and depth should be chosen to be consistent with existing datasets and/or published literature on macrobenthos from the site and nearby marshes (Levin et al. 1998, Talley and Levin 1999, Levin and Talley 2002, Levin and Currin 2005). Most (78–89%) of the macrofauna in southern California *Spartina foliosa* marshes are found in the top 2 cm of sediment (Levin et al. 1998). Deeper dwelling infauna (e.g., bivalves and shrimp) will need to be collected using a handheld, 10 cm diameter corer pushed into the sediment to a depth of approximately 30 cm (Figure 3). One core should be taken at the left, right, and thalweg of the channel (facing the outflow) (Figure 4). Core size/area will need to be recorded so that values for abundance can be adjusted for core area.

Smaller invertebrate infauna (e.g., polychaetes and amphipods) are often found in the top 5cm of sediment (Zedler 2001). Small infauna should be collected using a 6 cm diameter corer pushed into the sediment to a depth of at least 2 cm but ideally for certain organisms and depending on available processing time, 5 or 6 cm (Figure 5). Three small cores should be collected and composited from the left, right, and thalweg of the channel (Figure 4). Each set of composited cores covers an area of 0.00848 m^2.

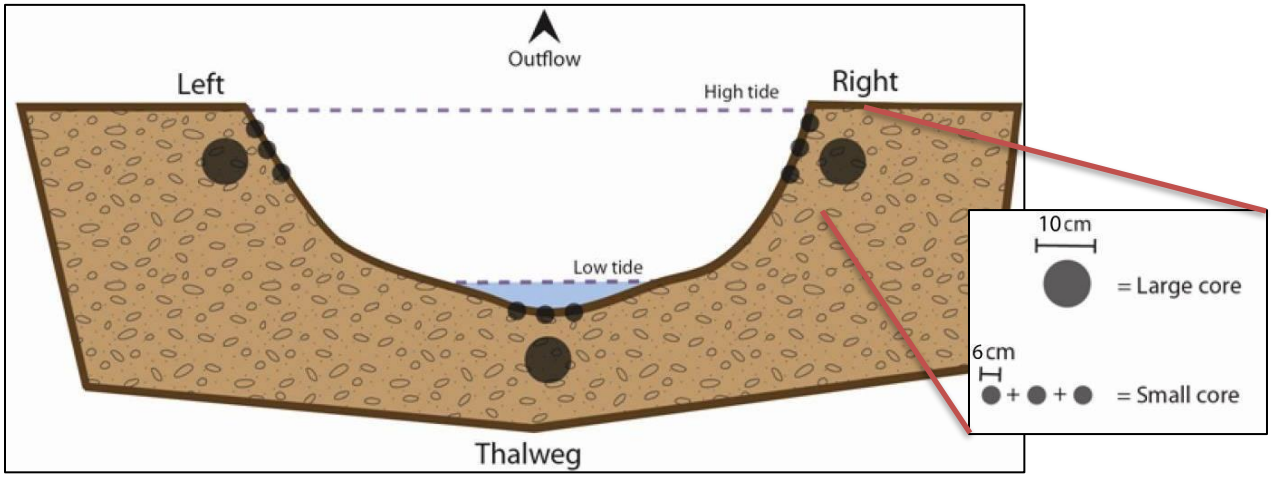

Figure 4. Diagram of benthic infauna core sizes and locations. Note: figure not drawn to scale.

Samples can either be wet-sieved in the field (see below) or carried back to the laboratory for preservation and then sieved. In this case, this choice of method will impact the type and abundance of invertebrates captured in the samples. Qualitatively, wet-sieving results in loss of the more mobile organisms like polychaetes and oligochaetes.

***Laboratory Sieving* –** The samples, placed in appropriate jars, should be kept cool in the field until being transported back to the laboratory. Once back in the laboratory (within 12 hours), the cores should be preserved with 8% buffered formalin with or without Rose Bengal (see note above). If not immediately processed, reserved samples should be stored in formalin approximately 2-5 days, and then transferred to ethanol before being processed. Longer-term storage in ethanol is appropriate up to several months (with checks to make sure the ethanol has not evaporated). At time of processing in an appropriate laboratory hood, formalin should be poured off through appropriate sieve (see note above) into waste storage jar. Once formalin is poured off, DI water can be used to rinse sediment through the sieve until the DI water runs clear out of the bottom of sieve. Material retained on the sieve is then sorted as discussed below.

Field Methods – Wet-sieving

The samples should undergo a wet-sieving process in a bucket filled with salt water, to separate infauna from sediment. Small cores should be processed using a 0.5 mm mesh sieve, and large cores should be processed using a 2.5 mm mesh sieve. As noted above, sieve size will have a significant impact on organisms retained (Johnston et al. 2021).

Figure 5. Small core pushed into the sediment.

It is important to perform all rinsing using salt water to maintain the correct osmotic pressure for the invertebrates (or brackish water from the surveyed channel). Once wet-sieved, the remaining material on the screen of the sieve (organisms, large sediment, and debris) should be carefully transferred using forceps into labeled, screw top glass jars. One option is to identify the larger organisms in the field, and if this can be done with confidence, these organisms can be released instead of brought back to the laboratory. The sieves should then be rinsed and scrubbed, to avoid cross contamination. *Helpful hint: large rocks may be discarded after being thoroughly inspected for benthic invertebrates and noted on the data sheets*. If a salt water source is present in the laboratory, it is recommended that samples undergo an initial wet sieving process to remove the bulk of the sediment and debris followed by the final sieving and labeling process being performed in the controlled laboratory environment.

Laboratory Methods – Wet Sieved samples

Following the final wet-sieving process, all samples should be transferred to labeled glass jars. The jars should be filled with sample material to 50-70% capacity, leaving at least 30% uncovered space for further processing. The jar should then be filled with salt water leaving 10% available open space. If more than one jar is needed to hold the entire sample, label as follows: 1 of 2, 2 of 2, etc. Each label should include the station ID, sample location within the channel (i.e., left, right, thalweg), date, and the split number (as applicable). *Helpful hint:* a label written in pencil on waterproof paper and placed inside the jar provides a failsafe against losing or damaging the outer label.

In the laboratory, jars should be initially preserved with a 10% formalin saltwater solution. Between two and five days after fixation, the formalin should be removed, properly disposed of, and the samples and jars should be rinsed with tap water. Samples should then be transferred back to the formalin-free jars and filled with a 70% ethyl alcohol (ethanol) solution to a level that completely immerses the sample. Samples should remain stored in the ethanol solution until sorting and analysis.

Sorting Methods – All Samples

To facilitate sorting, samples should be placed on white plastic plates and divided into small sorting trays using an illuminator, dissecting scope, spatula, and forceps. Benthic invertebrates should be sorted into the following categories: bivalves (subdivided into ridged and smooth clams, razor clams, and mussels), *C. californica*, other gastropods, worms, and amphipods (Figure 6; WRA 2004). All shelled organisms should be recorded as dead or alive, determined by the presence of muscle tissue in the bivalves. Each gastropod should be checked for an intact operculum. All unknown invertebrates (heads only to prevent overcounting and to facilitate identification) should be placed in vials and labeled for later taxonomic identification. Several examples of each taxon should be photographed, labeled, and preserved in a 70% ethanol solution as voucher specimens. The presence of wood and algae should be noted, as well as general grain size of the remaining rocky substrate (e.g., sand or pebbles). If present, algae and sea grass should be collected and placed in small aluminum pie tins. Tins should then be placed in a dehydrator for 24 hours, weighed, and the value recorded to determine dry algal weight per sample.

Taxonomic Identification – *For more details, see Monitoring Manual Version 2.0 (Johnston et al. 2021).* Trying to identify small marine animals can be challenging, time-consuming as well as require additional training and expertise in taxonomy. In addition, the dirt on sieved organisms often sticks onto the specimens thereby masking key features such as hairs, chaetae, bristles, spicules, plates, and many other features. Second, animals that are fixed in formalin or alcohol often lose color, a key feature for some organisms. In this manual, it is recommended to identify organisms to the lowest possible taxonomic group or parataxonomic sorting of samples to recognizable taxonomic units (RTUs). RTUs are also called morphospecies, morphotypes, and are generally considered to be a sufficiently reliable and conservative approach in ecological biodiversity studies or conservation biology (e.g., Krell 2004). It should be noted that evaluations of RTUs or morphospecies show many overestimations of species numbers. Use of multivariate or benthic indices may overcome some of the challenges.

Figure 6. Large core benthic invertebrate sample sorted in the lab showing bivalves (A) (D), *C. californica* (C), and other gastropods (B).

Data Entry and QAQC Procedures

Data should be entered in the field using the appropriate data sheet (Appendix 6.1B). All required fields should be completed in full, and the data recorder should assign their name at the top of the document(s). Data should be transferred to the appropriate electronic database within three days, and the hard copies filed in labeled binders. Electronic copies of all data should be housed on an in-house dedicated server and backed up to a cloud-based or off-site server nightly. Hard copies should be saved for five years. Electronic copies should be saved indefinitely.

Quality Assurance and Quality Control (QAQC) procedures should be conducted on all data. QAQC procedures should be conducted by the QA Officer and include a thorough review of all entries, double checking of all formulas or macros, and a confirmation that all data sheets, Chain-of-Custody forms, and field notes are filed appropriately with electronic back-up copies available. QAQC should verify that the entered data match the hard copies of the field data sheets. Additionally, every 30th sample should be sorted and recounted and all voucher specimens should be double checked for QAQC purposes. Any discrepancies should be corrected, and the initial data entry or sorting technician notified.

Data Analyses

After data have been entered, corrections made, and QAQC procedure completed, data can be used in multiple analyses. The resulting data can be analyzed to determine the density of benthic infauna, recorded as the number of individuals per meter squared for each station. Data can be combined for

each portion of the creek sampled (i.e., left, right, and thalweg), and analyzed separately for both large and small cores. Using the recommended protocols above, each station will sample a total area of 0.023562 m^2 for the large cores and 0.02544 m^2 for the small cores.

Presence and relative abundance of general taxonomic groups may be calculated for each location. Examples of additional analyses include abundance graphs by group or taxa or maps of distributions of each taxonomic group. For more detailed recommendations for data analyses, especially combining data between or among monitoring programs, see Johnston et al. 2021.

Health and Safety Precautions

When handling formalin, a respirator mask, latex gloves, and protective eyewear should be worn. Any formalin that comes into contact with skin should be rinsed immediately for 15 – 20 minutes to avoid irritation or other adverse effects. In the event of prolonged exposure or burning, seek immediate medical attention. Additionally, individual laboratory health and safety precautions should be always followed (e.g., closed-toed shoes, recognition of where the closest emergency equipment is located, etc). Safety Data Sheets for all chemicals should always be followed (https://www.osha.gov/hazcom).

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APPENDIX 6.1A

	Evaluation Metric	Benthic Cores	Notes
	Correlation to L2 CRAM	Not Applicable	Benthic invertebrate community is tied to hydrology and water circulation patterns
Personnel Requirements	Specialty Equipment or Clothing Required	Many Specialty Items	Large and small cores, sieves, and formalin
	Ease of Transport (amount or weight of supplies)	Many or Heavy Items / Difficult	See above, plus buckets
	Ease of Implementation	Difficult	For time-intensive coring efforts and sediment sieving
	Expertise / Skill Level	Some Technical Knowledge	No technical knowledge required if samples are sent to a lab for processing
	Number of Personnel	> 2	---
	Training Requirements	None	---
	Seasonality of Survey Time	Early Summer and Fall (beginning of wet season)	Both seasons are required to capture the breadth of benthic invertebrate species diversity; or late spring/early summer if only one sampling event is conducted; must not collect samples within 72 hours of a rain event
	Suggested Frequency	Semi-annual	---
Survey / Data Quality	Type of Output	Numerical	---
	Active or Passive Monitoring Style	Active	---
	Specialty Computer Software Required	No	---
	Availability of Online / External Resources	Many	Invertebrate guides are recommended for in-house processing
Potential Limitations	Wetland Type Applicability	Estuarine and Bar-built	Must have tidal influence or ponded water
	Images or Multi-Media Required	Images Required	Particularly for the voucher database
	Degree of Impact / Disturbance	High Disturbance	Walking and coring in tidal channels will severely disturb sediments
	Vegetation Height Limitation	Not Applicable	---
	Appropriate for Tidal / Wet Habitats	Yes	---
	Tide Height	Low to Mid-Tide	Depending on site, implementation during flood and ebb tides may be advisable to facilitate easier sample processing
	Regional or Broad Implementation *	Almost Always Used	---
	Potential for Hazards / Risk	Medium Risk	Caution must be exercised when using formalin and handling sharp inverts
	Restrictions	Special Status Species	---

** based on monitoring literature review*

APPENDIX 6.1B

BENTHIC INVERT SAMPLING DATASHEET

Sampling Program Information			
DATE:		LOCATION:	
TIME (start):	(end):	WEATHER:	
STAFF:		PAGE:	_____ of _____
GPS LAT:	GPS LONG:		

YSI PROBE MEASUREMENTS

Time _____ : _____ am / pm (circle one)
Temp _____ ºC
Turbidity _____ TDS g/L
Salinity _____ ppt
DO _____ % _____ mg/L
pH _____ pH
Notes:

SEDIMENT INFORMATION

Soil type: _____ Algae: **YES** **NO**
Soil moisture: _____ Species: _____
Soil color: _____ Thickness: _____mm Notes:

SAMPLE COLLECTION - SMALL PVC

Number of samples collected: _____ (3 cores per sample)

jars (Sample 1): _____ Bank: **LEFT** **RIGHT** **MID** Notes:
jars (Sample 2): _____ Bank: **LEFT** **RIGHT** **MID** Notes:
jars (Sample 3): _____ Bank: **LEFT** **RIGHT** **MID** Notes:

Number of jars (total): _____ Notes:
_____

SAMPLE COLLECTION - LARGE CORE

Number of samples collected: _____ (1 core per sample)

jars (Sample 1): _____ Bank: **LEFT** **RIGHT** **MID** Notes:
jars (Sample 2): _____ Bank: **LEFT** **RIGHT** **MID** Notes:
jars (Sample 3): _____ Bank: **LEFT** **RIGHT** **MID** Notes:

Number of jars (total): _____