CHAPTER 3.
INVERTEBRATE D-FRAME SAMPLING PROCEDURES FOR GREAT LAKES COASTAL WETLANDS

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I. Purpose

To describe D-Frame collection methods for the collection of invertebrates from Great Lakes Coastal Wetlands for the purpose of developing biological criteria. This method is adapted from Burton et al. (1999) for protected and open wetland types and from Chirhart (1998).

II. Scope/ Limitations

This procedure applies to all sites that Great Lakes Coastal Wetland invertebrates are collected to develop biological criteria and assess water quality in the Great Lakes.

III. General Sampling Procedures

The methods described are to be applied to all phase II invertebrate collections in the Great Lakes Coastal Wetland project. Data generated from this methodology will be used to determine final collection methodology (D-frame and/or activity trap) throughout the remainder of the Coastal Wetlands project.

IV. Requirements

A. Qualification of crew leaders: The crew leader must be a professional aquatic biologist with a minimum of a Bachelor of Science degree in biology with an aquatic entomology, invertebrate zoology, fisheries, or closely related field. Additionally, they must have at least six months experience working under a macroinvertebrate biologist in the areas of invertebrate sampling methodology and taxonomy.

B. Qualification of field technicians/ interns: A field technician/ intern must have at least one year of college education and had coursework in environmental and/ or biological science.

C. General qualifications: All personnel conducting this procedure must have excellent map reading skills and a demonstrated proficiency in the use of a GPS receiver and an orienteering compass. Sites may be located far from the nearest road access point. It maybe necessary to hike in over long distances with field equipment. High physical condition is mandatory.

V. Responsibilities

A. Field crew leader: Ensures that data generated using this procedure meet the standards and objectives of the study. Carries out the procedures in the action steps.

B. Technical personnel: Carries out he procedures outlined in the action steps, including maintenance and stocking of equipment, data collection and recording.

VI. Quality Assurance and Quality Control

Compliance with this procedure will be maintained through annual internal reviews by state and federal partners. Technical personnel will conduct periodic self-checks by comparing their results with other trained personnel. Calibration and maintenance of equipment will be conducted according to the guidelines specified in the manufacture's manuals.

In addition to adhering to the specific requirements of the sampling protocol and any supplementary site specific procedures, the QA/QC requirements are as follows:

A. Control of deviations: Deviations from the procedure shall be sufficiently documented to allow repetition on the activity as actually preformed.

B. QC samples: Ten percent of all sites sampled on any given year are resampled as a means of determining sampling error.

C. Verification: The state and federal partners will conduct periodic reviews of field personnel to ensure that technical personnel are following the procedures outlined by this SOP.

VII. Training

A. All personnel will receive training annually from a trainer designated by the program manager. Major revisions in this procedure will require that all personnel be trained in the revised procedure by an authorized trainer.

B. Training activities will include instruction in the field as well as a field test to ensure that personnel can implement this procedure.

VIII. Action steps

A. Equipment list: Ensure that all of the following items are present before implementing this procedure:

1. Two D-frame dipnets with 600 micron mesh
2. Two sieve buckets with 500 micron mesh
3. Wetland habitat partitioning form
4. Wetland verification form, previously completed with attached copies of 1:24,000 USGS topographical map
5. State specific Atlas and Gazetteer (Delorme)
6. Pencils
7. Permanent/ alcohol proof markers
8. Labeling tape
9. Coastal Wetland sample identification labels
10. 100% reagent alcohol, enough to preserve one days worth of samples, ca. 4 L/ site.
11. Waterproof notebook
12. Chest waders
13. Rain gear
14. Jars or bottles in which sample is to be preserved; preferably non-breakable synthetic, minimum 1 L capacity
15. Box or crate to store samples
16. Boat
17. Backpack
18. Entomological forceps

B. Data collection method: The multihabitat method consists of collecting a composite sample from up to five habitat types. The goal of this methodology is to collect a representative sample of the invertebrate community within a sampling reach in a timely and cost effective manner. The categories listed below a relatively non-specific, being chosen to represent broad categories instead of microhabitats. These categories will obviously encompass a variety of microhabitats in the field. It is to the discretion of the sampler on which microhabitats are to be sampled. As a general rule, the most dominant microhabitats within a category should be selected. The habitats to be sampled include:

1. *Aquatic macrophytes (submerged and emergent)*

Any vegetation found at or below the water surface should be considered for this category. Emergent vegetation is included due the stems extended bellow the surface offering suitable habitat for invertebrate colonization. Emergent plants should not be sampled above the waterline.

2. *Soft bottom (sand/clay/mud/unconsolidated detritus)*
Any area composed of sand, silt, clay (<1mm), or loosely consolidated organic matter should be considered for this category.

3. *Snags (woody debris)*
Snags include any large piece of woody debris found within the wetland. Logs, trees, branches, rootwads, beaver dams and lodges should be included in this category.

4. *Undercut banks (undercut banks/overhanging vegetation)*
This category is meant to encompass cover in-bank or near-bank habitats. These are shaded areas away from the main channel that are buffered from high flow rates.

5. *Hard bottom (cobble/boulders)*
This habitat includes any bottom substrate composed of cobble or other large rocks.

Invertebrate sampling should follow fish and habitat sampling in mid to late summer. If a habitat assessment is to be done while invertebrate sampling, it should follow invertebrate sample collection.

A sample effort is defined as taking two kick nets in a common habitat. Each effort should cover approximately 0.18 m^2 of substrate. Total area sampled is ca. 3.6 m^2. Sampler judgement will have to be used in categories that are three dimensional in structure.

Sampling consist of dividing 20 sampling efforts among the 5 habitat types. The number of samples of each habitat type is determined by the relative proportions of the habitat type. Ten percent receives two sampling efforts, 50 percent receives 10, etc. Not all habitat types may be present within the sampling zone, but in order to be included in the final assessment, a habitat must make up at least five percent of the total productive habitat.

Unlike the invertebrate sampling procedures for REMAP: Northern Lakes and Forest streams, soft-bottom substrates will be sampled in accordance to its dominance. This habitat will likely be most represented in the open waters of the sublittoral and profundal zones, the most stable zones for water levels and exposure. Failure to sample this habitat type could negatively bias against collections of important taxa such as chironomids.

Before sampling can begin, the crew leader and field technician must estimate the relative proportions of the habitat types. This should be a cooperative effort and is done by walking or floating the length of the sampling zone and assigning proportions based on visual inspection. A habitat partitioning form should be filled out during this process. Effort should be made to incorporate depth zones, frequently delineated by shifts in the vegetative community, into the sampling effort. King and Brazner, and Burton et al. used a vegetation density or vegetation dominance type classification for invertebrate sampling. During the summer, three general zone were established; dense emergent (depth = 10-25 cm), sparse emergent (depth = 25-50 cm), and open water (depth > 50 cm)(King and Brazner, 1999). These depth classifications could be marked off on the D-frame handle with colored tape or paint as a general guide.

It is difficult to estimate proportions of certain habitats due to their linear and three dimensional natures. Undercut banks and overhanging vegetation appear linear, and snags are three dimensional, as are vegetation mats, and emergent vegetation. For these reasons, best professional judgement must be used to determine what level of effort is adequate to equal one "sample effort" for any given habitat. Following are some suggestions on how to proportion and sample each of the five habitat types.

Hard bottom: It is relatively easy to estimate proportions of this habitat. It should be thought of as a two-dimensional area. If ample flow exists the D-frame net should be placed firmly, and squarely on the substrate downstream of the area to be sampled. If the water is to deep or no to little flow exists, disturb the substrate with your foot and sweep the net through the area repetitively in a "figure-eight" motion.

Vegetation: Aquatic vegetation is either completely submerged, mostly submerged and partially floating on the water's surface, or partially submerged and mostly extended above the water's surface. Weed beds should be viewed from the waters surface as two-dimensional areas, and proportioned as a function of surface area. Plants such as *Potamageton spp.*, coontail, and milfoil tend to clump and float at the water's surface. These should be sampled with an upward sweep of the net. If the net fills with weeds, the weeds should be hand washed vigorously or jostled in the net for a few moments and then discarded. Emergent plants such as reed canary grass and plants in the rush family, should be sampled with horizontal and vertical sweeps of the net until it is thought that the area being swept has been adequately sampled. Plants such as floating bur-reed and water celery tend to float in long strands with the current. They can be floating on the surface or be completely submerged. These plants should be sampled with horizontal and vertical sweeps in a downstream or upstream movement.

Undercut banks: This habitat is difficult to assign a proportion because it exists as a linear entity. Undercut banks and overhanging vegetation follow the line of the stream bank. Undercut banks can very in their degree of undercut. For this reason, banks must be prodded to determine the degree of under cutting. Overhanging vegetation should be treated in a similar fashion. Sampling should consist of upward thrusts of the net, beating the undercut portion of the bank or the overhanging vegetation to dislodge any attached organisms.

Snags: Snags and rootwads are the most difficult of the habitats to accurately proportion. They can be large or small, long or wide, simple or twisted masses that have no consistent shape. Judgement must be used to determine an appropriate sampling effort. Approximately the amount of sampleable surface area is a sensible method with larger tree trunks or branches. Masses of smaller branches and twigs must be given a best guess. Given their variable nature, there is not one method best for sampling snags. Using something like a brush works well for large pieces of wood, whereas kicking and beating with the net works best for masses of smaller branches.

Soft bottom: This habitat will always be two-dimensional and should be proportioned as a function of surface area. The procedure for sampling is variable. A swift current demands that the sample be taken by placing the net downstream of the sample area and disturbing the area in front of the net. A slow current, or no current calls for a different technique. The substrate should be disturbed with the foot, followed by immediately sweeping the net through the disturbed material until the sampler thinks that an adequate sample has been taken. This, like all of the techniques is quite subjective and demands experienced field personnel and professional judgement.

1. After relative proportions have been established, the sampling team should proceed in a downstream to upstream manner, the sampling the various habitats according to the agreed upon proportions.

2. Once sampling is complete, the sample material should be preserved as quickly as possible. Transfer the sample material from the sieve bucket to the sample containers. Fill the sample containers to the top with 100% reagent alcohol. Be sure to thoroughly clean the bucket as well as the sampling nets of all invertebrates. Forceps may be necessary to pick and dislodge some of the smaller organisms.

3. With labeling tape, label the outside of the container with field number, date, site name, and number of containers (i.e., 1 of 3). Place a properly filled out sample label in each sample container.

IX. Required Records

Habitat Partitioning Form

A. The habitat partitioning form will be filled out during the habitat partitioning. This information will be placed in the biological database.

X. References

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Merritt, R.W. and K.W. Cummins (eds.). 1996. An introduction to the aquatic insects of North America, third edition. Kendall/Hunt Publishing Company, Dubuque, IA, USA.

Thorp, J.H. and A.P. Covich. 1991. Ecology and classification of North American freshwater invertebrates. Academic Press, Inc., San Diego, CA, USA.

Invertebrate Field Sheet- Habitat Partitioning Form

(Front Page)

Site name:___________________________________ Date:_____/_____/_____

Site ID number:______________________________

X-site found? _____ (Yes) _____ (No), if no is checked record new x-site coordinates

Lat.:______________________ Long.:______________________

Sampling team:____

No. of sample containers:____________ **Net mesh size**:__________**micron**

Habitat proportion

Habitat	**% of wetland**	**# of samples**
Aquatic macrophytes		
Soft bottom		
Snags		
Undercut banks		
Hard bottom		
Other:____________		

Reach used in sampling:_________**m** **Transect length:**__________**m**

Notes

Invertebrate Field Sheet- Habitat Partitioning Form
(Back Page)

Sketch of wetland and sampling zone(s): Include basic depth/ vegetation zones using contour lines and habitat types with listed codes.

Depth:	**Habitat:**	
Dense emergent (DE) 10-25 cm	Aquatic macrophytes (veg)	Undercut banks (bank)
Sparse Emergent (SE) 25-50 cm	Soft Bottom (soft)	Hard bottom (hard)
Open Water (OW) > 50 cm	Snags (snags)	