GULFWATCH PROJECT

STANDARD PROCEDURES
FOR
FIELD SAMPLING, MEASUREMENT
AND SAMPLE PREPARATION

GULFWATCH PILOT PROJECT PERIOD 1991-1992

Gulf of Maine Council on the Marine Environment
Monitoring Committee
-1992-
(Second Edition)

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Site Selection

Within each of the states of Maine, New Hampshire, Massachusetts, and the provinces of New Brunswick and Nova Scotia two sites will be selected to represent two posited extremes of marine environmental health. One of each pair should consist of a reference site free of known contamination. The second, a test site, should be located in an area known to receive an active discharge of contaminants.

Since mussel growth and contaminant burden are affected by many different variables, each site selected must meet certain criteria to minimize variability.

Station Standards

1. Sites should be subtidal. However, in locations with an extreme tidal flux, i.e. N.B. and N.S., mussels can be collected from exposed areas just above the low low tide level.

2. Sites should be adjacent to the mainland (no offshore islands). Water quality varies from offshore to near shore due to upwelling and currents. We are attempting to compare landside anthropogenic factors with natural factors.

3. Natural indigenous mussels in the 50-60 mm shell length range must be present so that caged mussels can be deployed adjacent to them.

4. Deployment site suitability must be confirmed by a visit or prior knowledge of the area. What appears to be suitable from a chart may violate site criteria or even lack mussels. Unless you are familiar with the site, it is best to check it out beforehand.

Station Preferences

1. Circulation should be roughly understood in relation to factors influencing growth and/or contaminant accumulation. Reference sites should be located at least 5 miles from known sources of contamination such as sewage outfalls, sludge discharge, combined sewer overflows, population centers, etc. Conversely, the test sites should be located within one quarter mile of an active contamination source.

2. Wave exposure affects mussel condition. Sites should not be located in areas constantly subjected to heavy swells and wind/wave action.

3. Freshwater inflow should be minimal, with salinity above 26 ppt.

4. Turbidity due to sediment resuspension or landside discharges should be minimal. However, it is recognized that the Bay of Fundy region is subject to considerable tidal and current effects that can cause significant levels of turbidity.

5. Vandalism potential should be balanced with ease of access for collection and retrieval.

Schedule

A 60 day deployment period between the beginning of August and end of October will be used in all jurisdictions in order ensure a comparable and adequate time period for the equilibration of tissue contaminants. Some jurisdictions may also be testing at shorter intervals in addition to the standard 60 day period. All participants should therefore prepare for field work prior to August in anticipation of an early August deployment.

Collection of Transplant Stock

Stock for caged mussels are to be collected from an uncontaminated site within each jurisdiction and transplanted to the test and reference site cages. As previously mentioned all mussels should be collected subtidally or at low low tide. Their length should be in the 50-60 mm length range which can be facilitated by constructing a wooden length gauge for this size range (fig. 1). If feasible, wash and clean the mussels at the time of collection but be careful that the byssus thread is left intact. Place the mussels on a bed of seaweed and transport the mussels to the lab in clean coolers with freeze packs. It is recommended not to use ice since melt water will kill the animals.

A total of 40 mussels per cage will be deployed and three cages moored at each site. An additional 20 mussels from the stock source should be collected for speciation analysis and another 90 mussels collected for metal and organic preset analyses. The

grand total collected from the stock site is 350 mussels.

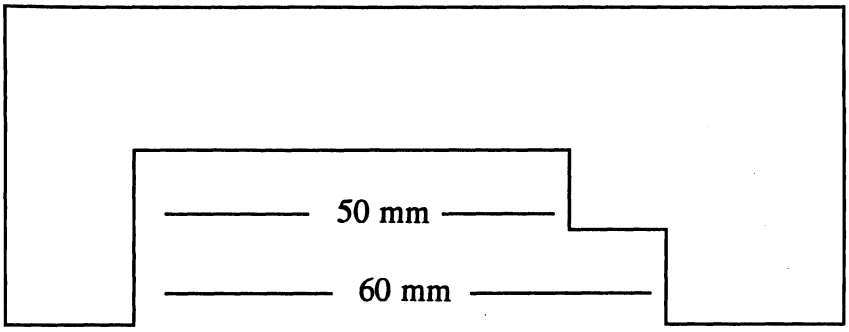


Figure 1
Mussel Length Gage

Within each cage, 10 mussels will be used for growth and condition index. Those 10 will be supplemented by 5 additional mussels and processed for metals. An additional 15 will be processed for organics. The remaining 10 should account for mortality. Figure 2 illustrates the distribution of mussels for condition index and contaminant analysis. In those jurisdictions where mussels are sampled at both 30 days and 60 days, additional mussels must be collected accordingly.

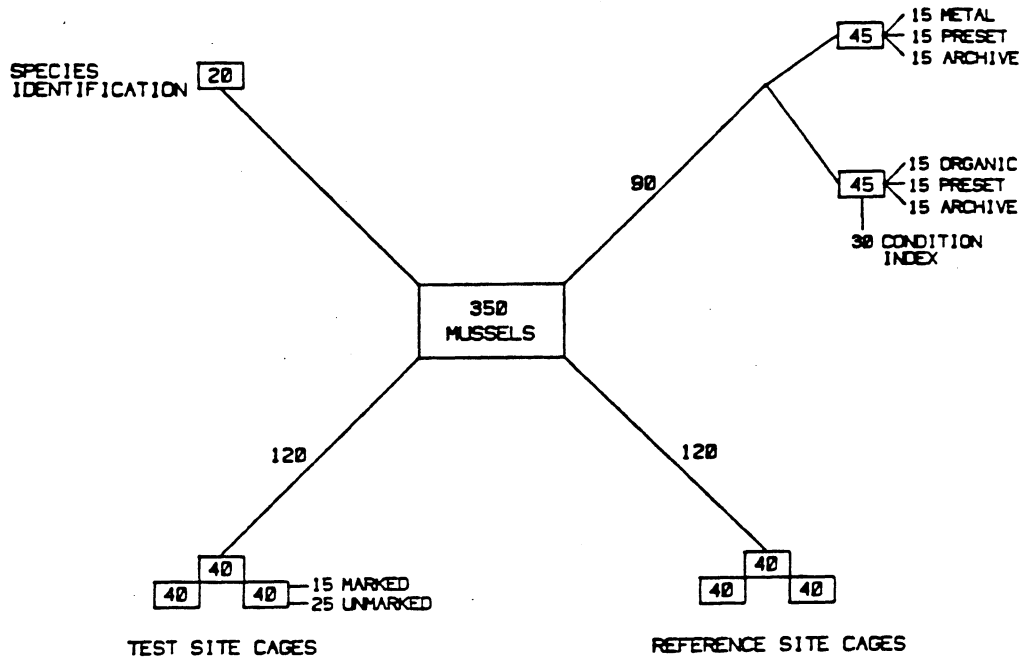
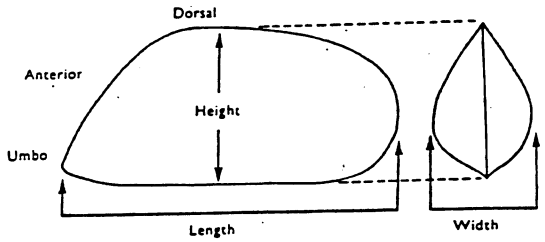


Figure 2.
Distribution of Stock Mussels

Marking, Measuring and Handling

In the lab or field, 15 (including 5 for mortality) of the 40 mussels per cage are marked on the left posterior side of the shell (Fig. 3) using a high speed engraving tool ("Dremel" type) with a diamond bit. Care must be taken to mark the shell deep enough to be able to read the numbers on retrieval but not so deep as to penetrate the shell and injure or kill the animal. It is recommended to mark the mussels with a sequential number system, i.e. cage #1 (1-15) ... cage #6 (76-90).



CONDITION INDEX

$$CI = \frac{WT}{LE * WI * HE}$$

WT = Soft tissue wet weight
LE = Length (mm)
WI = Width (mm)
HE = Height (mm)

Figure 3.
Mussel Dimensions and Condition Index (after Seed, 1968)

For each numbered mussel, measure and record the length, width and height to the nearest whole millimeter using a vernier caliper. These measurements will be used later to determination growth and condition index. When the marking and sorting of the replicate samples has been completed, place the mussels in separate containers or directly into their respective cages. It is advisable to mark the cages (1-6) with either waterproof ink, a tag, or the engraving bit. Place the mussels in a cooler with freeze packs but do not hold for more that 2 days out of water. If the mussels cannot be deployed within 2 days then place the mussels in a filtered sea water aquarium with an uncontaminated water source until deployment.

Preset analyses

Of the 350 mussels collected from the transplant site for a typical 60 day cage deployment, 90 mussels are to be archived for triplicate metals and triplicate organics analyses in composites of 15 individuals. See the two sections below on "Measurement, Draining and Preparation of Composites" and "Labeling." These samples will be archived frozen and shipped with the final samples at the end of the 60 day deployment. We recommend that as these samples are being processed for archiving, that condition index be obtained from at least 30 of the transplant mussels.

Deployment

Assign and place 15 marked and 25 unmarked mussels into each of the 6 cages (23mm x 23mm x 23mm polypropylene molded baskets). Secure the lids with nylon "pull ties". Cages are fastened together with the pull ties and the anchor line run through the cages. In areas of strong current, we recommend polypropylene encased steel cable as an anchor line. Depending on currents, either 1 or 2 concrete blocks can be used as the anchor. Consider using steel cable reinforced line to ensure that chafing does not sever the anchor line to the cages.

Any instrumentation such as the recording thermometers should be attached at this time outside of the cage to the anchor or buoy line. The cages are suspended above the anchor by means of a subsurface 8" trawl float. The anchor line should be arranged so that the cages will be suspended 1 meter off the bottom and at a depth that will ensure that the cages will always be underwater. Preferably the cages should be deployed at low, low tide. A marker buoy may be attached directly to the cages where vandalism or tampering is not likely. However, where vandalism or tampering is likely, the following procedures are suggested (Fig. 4):

1. Run a grappling line out from the anchor. A non-floating line such as nylon or kevlar can be used which is fastened to the cage anchor and anchored by a concrete block some distance away (60 meters).

2. With a compass, determine and record the bearings of the cage site relative to three land marks and record the direction the grappling line runs.

3. Separate surface marker buoys may be deployed close to the cage. These buoys should not be directly attached to the cages to prevent the cages from being hauled up.

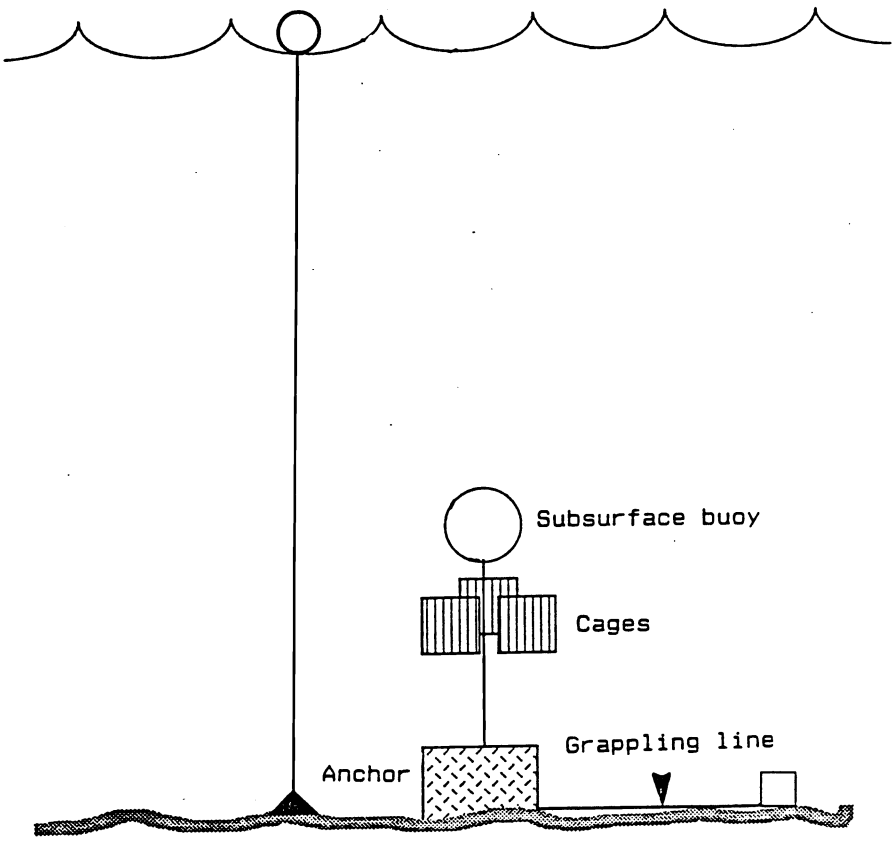


Figure 4.
Mussel Cage Deployment Configuration

Maintenance

Mussel cages which are covered by fouling organisms have a reduced flow of sea water through them. Because fouling can occur in a short time, we recommend check visits every 2 to 4 weeks depending on the location. At the check, cages should be cleaned of all growth interfering with the free exchange of sea water. Lines and fastenings should also be inspected for chafing and security and repaired as necessary.

Collection and Retrieval of Caged Mussels

After the mussels have been deployed for 60 days, it is time for final retrieval. Collection should be at least three days after any storms so as to minimize the impact of sediment resuspension or river sediments to be introduced to the water column.

Each mussel in each of the triplicate cages should be cleaned of external debris and placed in their respective labeled containers (Fig. 5).

Collection of Indigenous Mussels

Indigenous mussels will also be collected in triplicate samples at the time of cage retrieval. The sampling technique is to sample 50-60 mm mussels from three discrete areas of the subtidal zone, within a 50 meter section of the shore. Each set should contain at least 30 live mussels (15 for metals, C.I., and growth, and 15 for organics, Fig. 5) and it is recommended to gather a few extra in case dead or substandard mussels are collected accidentally. In the Bay of Fundy or other areas which have extreme tides, mussels can be sampled from just above the low low tidal level.

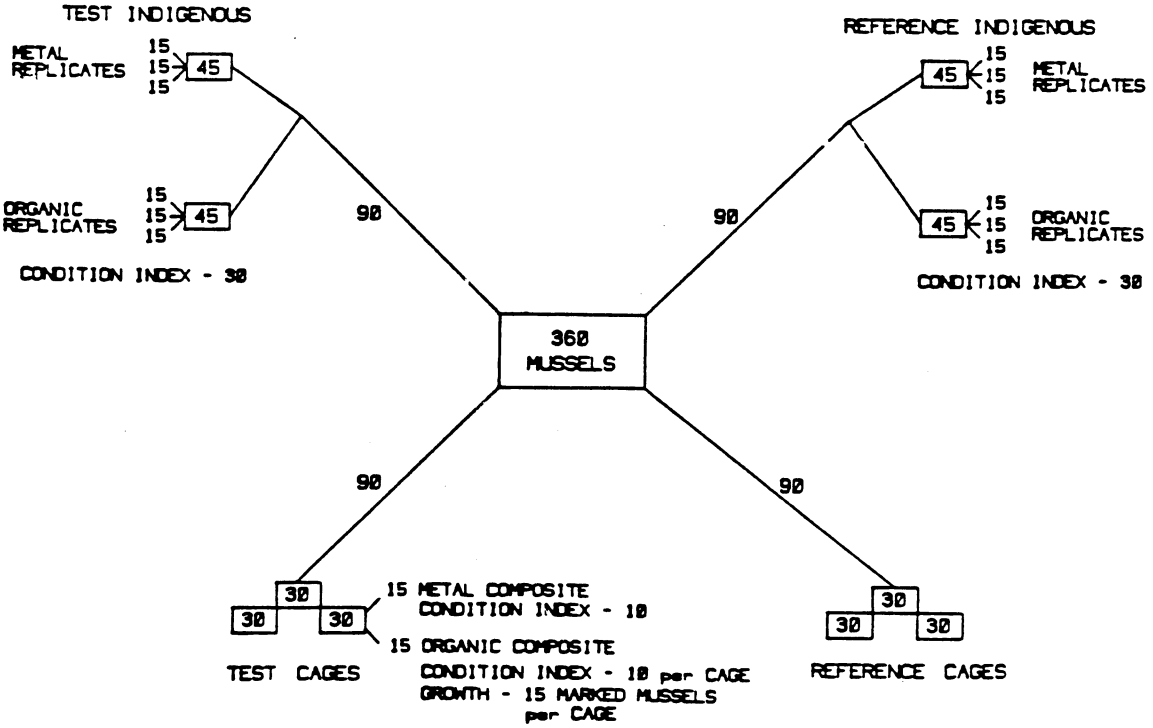


Figure 5.
Distribution of Caged and Indigenous Mussel Samples

Handling and Transport

All mussels should be stored live without water in clean labeled containers. Clean solvent rinsed quart mason jars with an aluminum foil liners on the lid work well and may be used for both metals and organics as long as the mussels are not in contact with the tops. The quart size will generally hold 15 to 20 animals. The containers placed upright in coolers with freeze packs for transport back to the lab. If using glass, be sure that glass to glass contact is avoided to prevent breakage. We recommend placing a sheet of cardboard or several thicknesses of newspaper between all glass.

Measurement, Draining and Preparation of Composites

For each population of indigenous and caged mussels at the test and reference sites, measurements for condition index are taken on a subsample of 30 mussels. This is easiest to do as they are prepared for metal analyses by taking measurements on 10 individuals from each of the triplicate 15 mussel metal composites. With the valves fully closed, lengths, widths, and heights are measured with a vernier caliper to the nearest 1 mm.

The mussels are then opened with an acid washed lucite wedge for metal analysis and a solvent rinsed stainless steel blade for organic analysis and the valves pried open to release any remaining free sea water. Further details are provided in Appendix A. The animals are gently shaken and drained of free sea water for about thirty seconds. Be sure not to cut into the soft tissues.

Although we strongly recommend final preparation of the mussels for chemical analyses while the animals are alive and fresh, should time not allow it, mussels may be drained as described above and placed back into their respective labeled jars and frozen in a standard freezer until measuring and shucking is possible. When measuring frozen mussels, pay particular attention that they have thawed sufficiently to allow the valves to fully close when measuring width.

For the metal composite, shuck 15 mussels (10 of which can contribute to the growth and condition index 30 mussel sample) using an acid washed non-metallic knife into clean 500 ml glass mason jars. The metal analysis jars are prepared by washing first with soap and water followed by a warm 10% nitric acid rinse and rinsed three times

each mussel added to the jar. Cap the jars with a plastic liner, e.g. saran wrap, and store at -15oC until shipping is arranged.

For the organic composite use a stainless steel knife in the shucking procedure (15 mussels per composite). The mason jars are prepared by washing with soap, rinsing with deionized water, rinsed with acetone followed by hexane and then heated to dryness. The caps are lined with hexane washed aluminum foil. As with the metal samples store the mason jars at -15oC until shipping is arranged.

Labels and Field Form Format

All samples will be labeled in the field and again on tissue composite jars for laboratory analyses according to the minimal 12 digit format below. Please adhere to this to avoid confusion. Samples will <u>not</u> be analyzed without this label.

Station - 4 letters,	1st and 2nd = state or province
	3rd and 4th = waterbody
Replicate #	1,2, or 3
Sample Type	C= cage, N= Indigenous
Date of collection	YY/MM/DD (note that <u>year</u> is first)

Optional information such as collector and NOAA Status and Trends basin may <u>follow</u>.

An example of a proper label for a Maine indigenous replicate #2 sample from the Kennebec River on October 12, 1993 collected by Susan Murray would be MEKN2N931012-SM.

The Data Information and Management Committee is developing a spreadsheet format for entering the station description and sample data. In the event that this format is not available then use the forms that were developed for the 1991 project.

Additional Monitoring

<u>Temperature</u> - Small temperature recording units (Hobo Temp) will be available for placement at each site. These units will be capable of recording temperature every 24 minutes over a 30-day deployment period or 48 minutes for 60 days. The temperature information can then be down-loaded to a PC or Mac for analysis and graphing. The

temperature unit fits inside a 35 mm film canister. Fill the canister with silicone oil to equalize pressure and protect the circuits from water. The canister and temperature unit can then be placed inside a waterproof container made from plumbing parts available at hardware stores (Fig. 6). Seal the end-cap with a PVC glue and use teflon tape or plumbers putty on the threads of the cap section. A bead of silicon caulk should then be placed around the cap joint. The outer container can also be filled with silicone to ensure pressure equalization. Fasten the temperature unit to the line extending from the anchor to the subsurface buoy. Stainless steel automotive hose clamps work well as an attachment point for a small line.

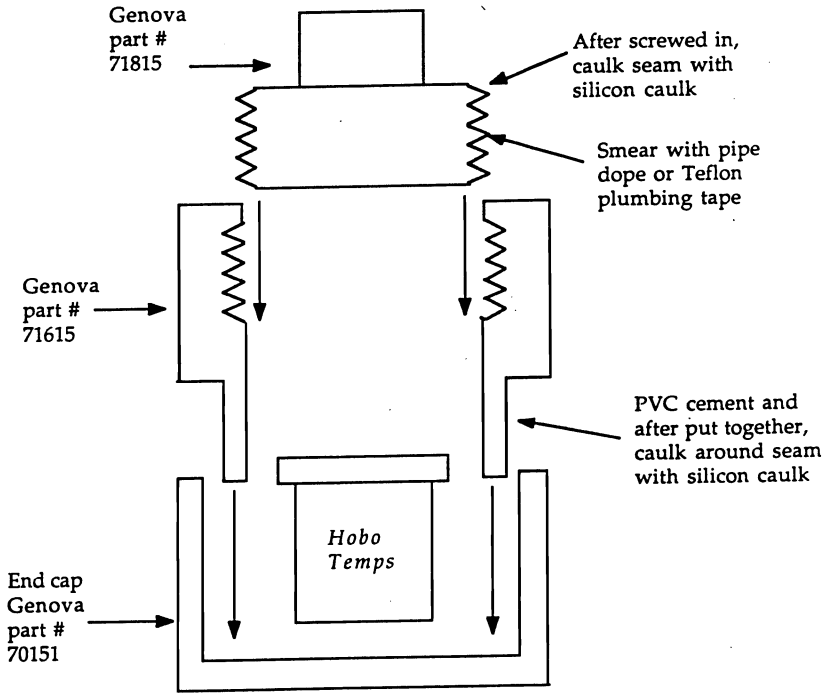


Figure 6.
Waterproof Case for HoboTemps

Salinity - Use a salinometer to measure the vertical salinity profile at each station. Depth intervals will depend on the proximity of freshwater discharge relative to the site.

Turbidity - Use a Secchi disc to measure the water turbidity. The standard Secchi disc is 8" (20.5cm) in diameter, with four quadrants of white and black. In order to standardize viewing of the disc underwater and minimize surface reflections, it is

advisable to make a viewing scope. This can be made from a two foot length of 4" PVC pipe that has a plexiglas insert on one end. The disc is lowered until it is no longer visible and this depth recorded. The disc is then slowly raised until it just becomes visible. The Secchi disk transparency is the average of the two readings.

Chlorophyll, Nutrients, Phytoplankton - These items will be left to each jurisdiction for measurement if possible.

Shipping

All samples will be held at -15oC by each jurisdiction until arrangements for shipping have been made with the receiving laboratories. An itemized packing slip must accompany each shipment to avoid confusion by the receiver and help correct descrepancies should any arise.

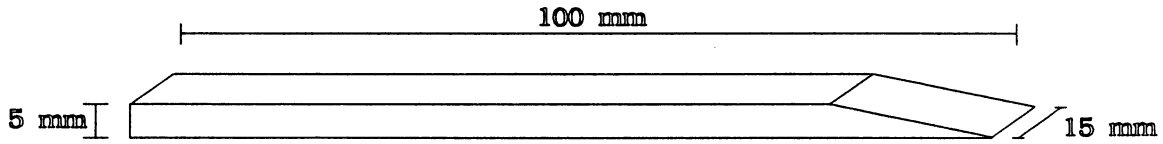
Special care should be taken to prevent breakage. Glass containers should not be shipped loose in "peanuts." Jars settle during transit to the bottom where they strike one another and break. We have had excellent results if each glass jar is wrapped in a single sheet of newspaper or separated by a single "tube" of cardboard. Glass against glass breaks easily. The jars are then placed in a cardboard box cut to size. All voids in the box are stuffed with paper to prevent the jars from shifting and a packing slip enclosed or taped to the top. The box may be placed in a freezer overnight to refreeze it if necessary or dry ice wrapped in paper and packed in the box (do not let dry ice touch the glass directly as the glass will shatter). Once ready for shipment, the frozen box is placed in a cooler large enough to hold the box and either icepacks or more dry ice depending on the time needed to reach the destination freezer.

APPENDIX A

MUSSEL DRAINING PROCEDURES

Because marine mussels can adduct their valves (shells) tightly for long periods of time, varying amounts of sea water can be retained in their mantle cavities. At a minimum, this sea water must be excluded from all measurements of wet tissue weights and mussel total wet weights (as used for condition index calculations). Therefore, valves should be forced open and the sea water drained prior to these measurements. Ideally, it would be best to drain the sea water from all mussels prior to dissection and analysis for both metals and organics.

A simple tool can be fashioned from a sheet of 5mm thick methyl methacrylate (=Plexiglas, Perspex, Lucite) for use on mussels that will later be analyzed for metals. Teflon is a little too soft for this application. Cut out a rectangle 1.5cm wide by 10cm long. File down the narrow dimension to form a long, flat wedge:



The wedge should be soap and water washed, rinsed three times in glass distilled deionized water, acid soaked for at least 12 h in 4 N HNO_3 and rinsed six times in glass distilled deionized water. It should be rinsed briefly with 6 N HCL and distilled deionized water between each use (i.e. between using on each mussel).

Turn a mussel over on a flat clean surface, dorsal side down (hinge side down) and position the tip of the wedge against the line where the opposed valves meet. Give the top of the wedge a smart rap with the palm of your hand or with a plastic/Teflon block, forcing the wedge into the mantle cavity no less than 5mm and no more than 10mm. Hold the mussel dorsal side up (hinge side up) for 20 seconds, shaking the mussel 20 - 30 times. If done correctly the procedure will allow the vast majority of the mantle cavity sea water to drain, but will not damage the mantle tissues along the edge of the valves (when you later dissect the mussel, the mantle will still be attached to the shell at the site where the sedge was inserted). Since tissues are not damaged, blood loss will be minimal or absent.

If you wish to drain the mantle cavity sea water from mussels which will be analyzed for organics, a stainless steel wedge can be fashioned. The wedge should be soap and water washed, rinsed three times with glass distilled deionized water, rinsed three times with methanol, three times with toluene and finally three times with hexane prior to use. Between each use, the wedge can be rinsed briefly with glass distilled water, methanol, toluene and hexane.