GULFWATCH

MUSSEL

PILOT PROJECT

OF THE

THE GULF OF MAINE

ENVIRONMENTAL MONITORING PLAN

Monitoring Committee

of the

Gulf of Maine Council on the Marine Environment

May 1991

(Second Edition)

Introduction

In December of 1989, an agreement between Nova Scotia, New Brunswick, Maine, New Hampshire and Massachusetts established the Gulf of Maine Council on the Marine Environment. The overarching mission of this Council is to maintain and enhance the Gulf's marine ecosystem, its natural resources and environmental quality. This is to be accomplished through the Council's assistance and coordination in three areas: research, monitoring, and management; dissemination of scientific data and information; communication between state, provincial and federal levels of government; and public education.

To help meet the Council's mission statement, the Gulf of Maine Monitoring Committee was formed and charged with the development of the Gulf of Maine Environmental Monitoring Plan. The monitoring plan is based on a mission statement provided by the Council:

It is the mission of the Gulf of Maine Marine Environmental Quality Monitoring Program to provide environmental and resource managers with information to support sustainable use of the Gulf, and allow assessment and management of risk to public and environmental health from current and potential threats.

Three monitoring goals were established to meet the mission statement:

- **to provide information on the status, trends, and sources of risks to the marine environment in the Gulf of Maine.**
- **to provide information on the status, trends, and sources of marine-based human health risks in the Gulf of Maine.**
- **to provide appropriate and timely information to environmental and resource managers that will allow both efficient and effective management action and evaluation of such action.**

The final part of the monitoring plan was to identify key tasks which, once completed, would in effect implement the larger plan. Recognizing the complexity of scientific, administrative, and political issues, the Monitoring Committee endorsed a small scale Monitoring Pilot Project to occur during the first year. This project would involve all jurisdictions and determine the level of logistical cooperation, identify weaknesses, and recommend measures to strengthen the larger Gulf of Maine Environmental Monitoring Program. The Council approved the concept of a pilot and provided a budget of $36,000 U.S.

The Pilot Project

In a conference call meeting during the fall of 1990, the full Gulf of Maine Monitoring Committee decided to move forward with a Gulf-wide pilot monitoring and demonstration project using mussels as indicators of ecosystem health. Although it was the consensus of the group that integration of a suite of indicators is necessary to provide any meaningful statement on the Gulf's health, they agreed that the small scale single indicator pilot had much to offer.

The pilot was designed to test the major components of the monitoring plan:

- develop consensus among scientists, managers, and policy makers on the specific issue to be addressed by the monitoring and the information needed to resolve the issue.
- confirm the scope of existing programs and gain a consensus on cooperation among the responsible agencies including the determination of compatible methods and region-wide standards
- develop a sampling design considering ongoing and historic data and the statistical requirements of the hypothesis being tested
- develop a consensus on a priori decision points
- develop a pilot scale database management system
- develop and implement a plan for public information and citizen/volunteer participation in the project
- provide managers with information generated by monitoring and use the results to improve further monitoring activities
- use monitoring information to develop management actions and to track the effectiveness of those actions

Concept

Of the many possible environmental indicators, the Pilot Project will use a single indicator organism monitored throughout the Gulf of Maine as a vehicle to test the above eight components of the Monitoring Plan. By planning, conducting, evaluating and reporting on an actual field project, most aspects, concepts and activities of the full Monitoring Plan can be exercised in time to benefit the larger plan.

The blue mussel (Mytilus edulis) was selected over other indicators for the following reasons. Mussels are relatively abundant within and across each of the five jurisdictions of the Gulf program. Mussels are easily accessed with minimal equipment requirements. Sedentary in their habit, mussels respond to their immediate environment thereby eliminating the complications in interpretation of results introduced by mobile species. Finally, since mussels are filter feeders, it is thought that they reflect present day water column conditions which itself is a direct measure of the efficacy of current environmental management.

Mussel watch programs have not, however, been without controversy. Mussels do not integrate all contaminants of concern equally. Bioaccumulation is not always proportional to ambient water chemistry, rather it may depend more on food quantity and quality. Due to the high variability associated with growth, season, and bioaccumulation, mussels are less useful in reflecting **status** than **trends**. The pros and cons of using mussels versus other indicators were evaluated sufficiently, however, to conclude that at this point in the Monitoring Program, the advantages of using mussels outweigh those of other indicators such as sediment bulk analyses, mixed function oxidase, pathology, fish liver contaminant burdens, etc.

A second criticism of using a mussel watch approach is that the results are rarely of value to local managers. We believe that this is largely a result of differences in program objectives and perspectives. Station locations in the national and international programs, for example, are frequently not representative of local management concerns but intended to provide national and international overviews. The Gulfwatch Pilot will attempt to overcome these differences.

Objectives

The objectives of the Pilot Project are three-fold:

1.) to evaluate the feasibility of using a mussel watch approach as but one means of Gulf-wide environmental assessment,

2.) to determine the level of logistical cooperation between jurisdictions, identify weaknesses and recommend measures to strengthen cooperation, and

3.) to initiate testing of simple hypotheses and collect comparative data from different locations in the Gulf of Maine.

It is important, however, to clearly state that the ability of this pilot demonstration project to assess "environmental health" based on a single year's sample is extremely limited. Rather, its value lies within the exercise itself, which will provide the critique necessary for the full Monitoring Program to succeed.

WORK PLAN

Second Edition Note: Methods were subsequently revised prior to the 1991 field trial (see Appendix B). Final methods employed are documented in "Standard Procedures for Field Sampling, Measurement and Sample Preparation - Gulfwatch Pilot Project Period 1991-1992."

Experimental Design

Three hypotheses are to be tested in the Gulfwatch Pilot Project:

Ho 1. Growth of caged mussels from "clean" reference areas equals that of mussels from "dirty" test areas.
Ho 2. Select contaminant body burdens of mussels grown in submerged cages are equal to those collected from adjacent subtidal areas.
Ho 3. Select contaminant body burdens of mussels from "clean" reference areas equals those of mussels from "dirty" test areas.

Four treatments are prescribed Gulfwide:

Caged and "clean" reference
Caged and "dirty" test
Indigenous and "clean" reference
Indigenous and "dirty" test

Each treatment will be replicated 3 times by each jurisdiction for a total of 15 replicates Gulfwide per treatment. The present budget provides sufficient funds to analyze about 76 samples for heavy metals. Should additional funds become available, either more replicates or more stations will be added. With this number of samples, it is expected that we will have the opportunity to analyze the data using a variety of statistical techniques. At a minimum, non-parametric tests such as Student-Newman-Keuls are possible to determine differences between and among jurisdiction treatments.

Site Selection

Within each of the states of Maine, New Hampshire and Massachusetts and the provinces of New Brunswick and Nova Scotia sites will be selected to represent two posited extremes of marine health. One site should represent clean reference conditions free of known contamination. A second site should be an area known to receive an active discharge of contaminants.

Since mussel growth and mussel body burdens are affected by many different variables, each site must meet certain minimum criteria to control variability and enable a statistically valid comparison. Other variables, less easily controlled in the site selection process should be annotated upon the initial site visit so that they may be incorporated into the statistical design.

Station Standards

- Sites must be subtidal. Mussel growth is known to vary with tidal elevation.
- Sites must be adjacent to the mainland (excepting Machias Seal, no offshore islands). Water quality varies from offshore to nearshore due to upwelling, terrigenous sources and currents. We are attempting to compare landside anthropogenic factors with natural factors.
- Natural indigenous subtidal mussels in the 50-60 cm shell length range must be present so that caged mussels can be deployed adjacent to them.

Station Preferences

- Circulation should be roughly understood in relation to "upstream" factors influencing growth and/or heavy metal accumulation. Reference sites should be located at least one mile from or upstream of known sources of contamination such as outfalls, population centers, etc. Conversely, "dirty" sites should be located within one quarter mile of an active contamination source. Stations under the influence of offshore upwelling should be avoided as upwelling is known to affect growth and body burdens.
- Wave exposure affects mussel condition. Sites should be located in areas not constantly subjected to heavy swells and wind.
- Freshwater inflow should be minimal with salinity above 27 parts per thousand.
- Turbidity due to sediment resuspension or landside discharges should be minimal in both clean and dirty sites. This requirement probably eliminates large tidal flats exposed to turbulent conditions.
- Vandalism potential should be balanced with ease of access for collection.

Station field sheets (Appendix A) will be filled out to qualify various site variables potentially affecting results.

The location of mussel collection and deployment sites should be precisely located on a 1:40,000 nautical chart or 1:24,500 topographic map in order for others to reoccupy the site in the future. Latitude and longitude should be given to the nearest second. In addition, a detailed narrative description should be included on the Field Sheet.

Deployment and Collection of Mussels

Caged mussels will be collected from the "clean" reference sites within each jurisdiction and deployed according to a variation on the Standard Operating Procedure for Deploying Mussels developed by the U.S. Environmental Protection Agency's Environmental Research Laboratory in Narragansett, Rhode Island.

Initially, all mussels within the Pilot Project were to come from the same stock; however, because biological contamination is a threat in its own right to the Gulf, this option was discarded. Furthermore, permits are required for transborder shipment of live specimens at both the state, provincial, and federal levels. Each State and Province is therefore now responsible for supplying its own "clean" mussels.

Blue mussels, Mytilus edulis, are collected from locations previously determined to be "clean" with respect to contaminants. Collection of sub-tidal mussels may be completed using a small scallop dredge towed behind a boat or clam rake in shallow areas. Mussels are collected within 4 days of deployment to minimize the effects of holding.

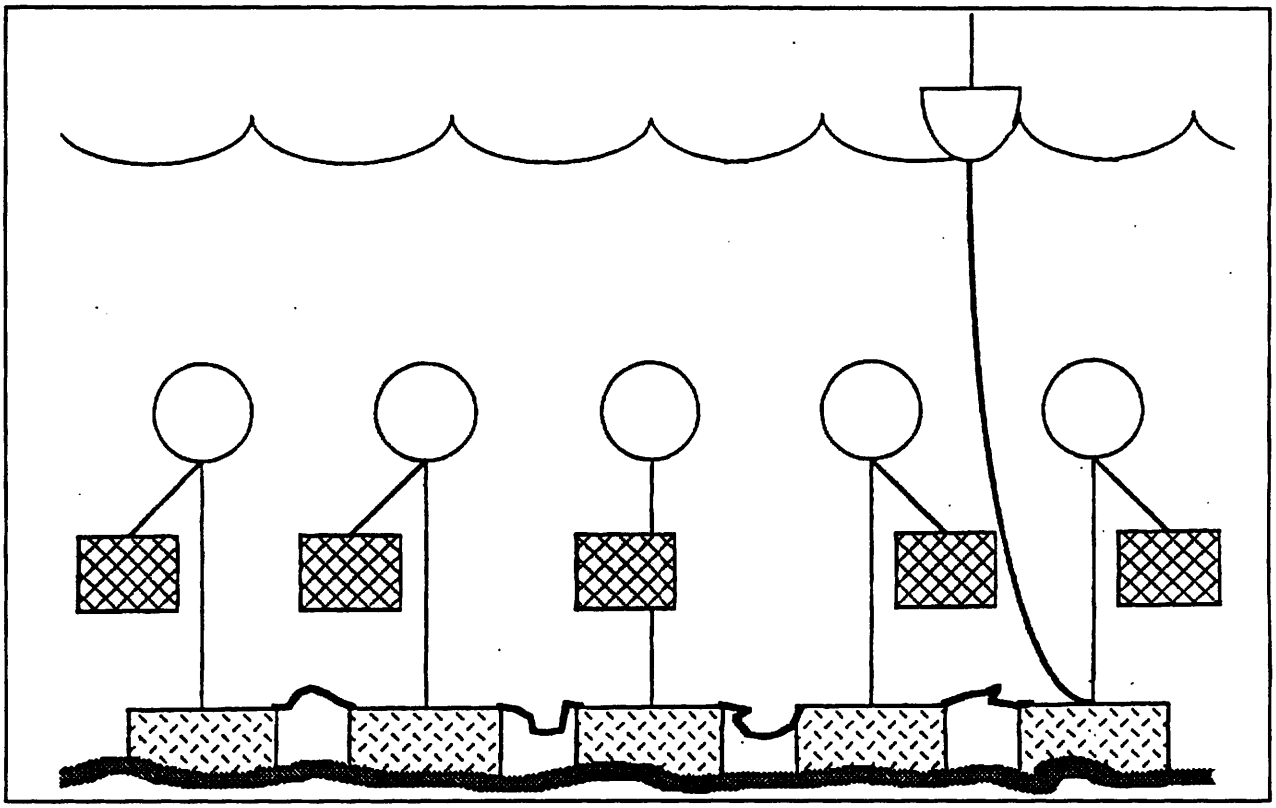
After collection, mussels are cleaned of all external growth and accumulations, returned to the laboratory and maintained in running ambient unfiltered seawater until deployment. These organisms are sorted to obtain a uniform shell length (usually 5-6 cm) to minimize possible differences in age. Typical field deployments include enough mussels at each station for both chemical analyses and biological measurements. For statistical purposes, each field station treatment includes three replicate composite samples. (Note: U.S.E.P.A. recommends a minimum of four replicates yet we have insufficient funds to meet this recommendation) Each replicate consists of a small polyethylene basket (obtained commercially or fabricated in-house) containing 25 mussels.

A subset of the mussels returned to the lab are further sorted to obtain a very narrow shell length range ($\pm$ 1mm) for shell growth determinations. These mussels are subsequently individually numbered with a diamond engraving bit, the shell length of each measured to the nearest 0.1 mm with a vernier caliper and the length recorded. A total of 15 marked mussels are included in each replicate basket. Upon collection, the length of each mussel is measured and recorded to determine growth during the deployment period. Because this is a non-destructive process these mussels also can be used for additional chemical or biological measurements. The remaining mussels (10) are frozen for chemical analyses or distributed to other researchers for additional testing.

Each station consists of a series of satellite (i.e. replicate) moorings (usually a concrete block) connected together with line in a trawl (Figure 1). Each individual mooring consists of a sub-surface float suspended 1 meter above the bottom to which the mussel baskets are attached. Field deployments can be completed using either diver-tended or surface-tended

stations. The advantage of employing divers is that possible vandalism from surface boats is reduced, however, this approach is more costly, time consuming, often impractical and dangerous in severely contaminated areas. The second approach is to use a sub-surface buoy system accessible from the surface by a surface float attached to the last mooring in the trawl. This type of system is both inexpensive and easy to maintain from a small boat or possibly by wading.

Figure 1
Example of Cage Suspension and Deployment System



Timing/Season of Field Trials

Deployment for 28 days will be done such that retrieval of caged and collection of indigenous mussels will occur near the end of the summer peak water temperature (in mid-coast Maine this is around the end of August first of September). Indigenous mussels should be collected the same day as retrieval of the caged mussels.

Purging

The issue of whether or not body burden results should reflect tissues alone versus tissues and gut content (i.e. sediment) remains problematic. Resolution is necessary before the animals are collected. Purging is not necessarily a very well controlled process when reingestion of purged materials is possible. On the other hand, if we are interested in mussels for their integrative capacity over time, by not purging there is the potential of having the data reflect short term turbidity events coincident with the retrieval period and therefore not reflective of the integrated environmental history. If we are interested in comparing our results with the NOAA Mussel Watch program, it is important to recognize that NOAA does not purge but rather collects aluminum and iron to determine whether the samples are sediment laden. On the other hand, NOAA does not normalize its data to Al or Fe content therefore making it difficult to compare data regardless. Purging is not desirable if we are interested in the trophic transfer of contaminants (also a human health issue) however contaminants held on sediment in the gut may not be biologically available whereas contaminants in tissues certainly are.

The decision will necessarily be somewhat arbitrary. Our proposal for discussion is that suspended caged mussels are to be analyzed without purging. In situ mussels, however, should be brought back to the laboratory where they will be purged for 36 hours in "clean" unfiltered ambient sea water. The mussels will be held suspended in the midwater section of aquaria on polyethylene screens such that pseudofeces and solids will precipitate to the bottom and not be available for reingestion.

Laboratory Analyses

Chemical Analyses

After each mussel within each replicate (r=3) composite (n=15) are cleaned of external growth, byssal threads, drained and individually weighed, their shells are measured for length, width, and height. Meats are to be removed and stored frozen as three (3) replicate composite samples. Arrangements will be made for scheduled shipment to the Maine Department of Environmental Protection Laboratory where analyses will be conducted (see Laboratory Services below).

Each blue-mussel tissue composite will be thawed and blended in an acid washed glass blender. A 10 gram aliquot will be digested in 8N HNO_3, dried and muffled at 450°C. The digestion is repeated until all white residue dissolves in 5% HNO_3. Furnace atomic absorption spectrophotometry will be used to analyze the mussel digestions (AOPC, 1980) for Cd, Cr,

Cu, Fe, Ni, Pb, and Zn. Mercury analyses will be according to EPA (1983) methods. U.S. E.P.A. standard trace metals in fish will be used for a recovery standard in a tissue matrix.

Contract Laboratory

Unless strong objections are voiced, heavy metal analyses will be conducted by the Maine Department of Environmental Protection Laboratory. Using the Maine Department of Environmental Protection Laboratory has some distinct administrative advantages since the funds for the Pilot Project were awarded through the Maine Coastal Program. Funds can easily be transferred directly to the Laboratory without the need for contract bidding, review, negotiation, and setting up a separate account, consuming time at an untimely point in the project. Certainly, however, this alone is not reason enough. Technical competence is imperative. The Laboratory has experience analyzing mussels for heavy metals in its conduct of Maine's Mussel Watch Program and the Laboratory already participates with NOAA's QA/QC Program. Analytical costs are more competitive than the private sector or federal laboratories capable of tissue metal analysis. Furthermore, by having one laboratory conduct all the analyses, differences between laboratory performance is eliminated as a confounding variable.

It should be noted, however, that this arrangement is <u>for the purposes of this Pilot Project only</u>. Future decisions regarding analyses will be on a case by case basis. As Gulfwatch grows, more laboratories will have participated in NOAA's QA/QC program and thus be able to respond to new analysis requests.

<u>Decision Points</u>

A frequent criticism of monitoring programs in general is that they are conducted in the absence of clear objectives. By stating and testing the above four hypotheses, the Pilot Project will address this common problem. This, however, still remains one step short of linking science with management. Knowing how the data and information will be used by management is necessary. For the Pilot Project, four arbitrary (4) decision points or action levels have been identified.

- Any contaminant level exceeding U.S.F.D.A., Canadian, W.H.O. or other regulatory health standard will be immediately reported to the jurisdiction from which the value was collected in order for that jurisdiction to take any necessary and appropriate action.

- Heavy metal levels equal to or exceeding two standard deviations from the mean ($\geq \bar{X} + 2s$) metal concentration of the control population will signify these stations as anomalous.

- Exceedance of either of the above decision point should initiate verification measures. If the elevated value is verified, investigation into probable cause for the anomaly or health risk should be initiated. Results should be reported to the Council within one year.

- If anthropogenic sources are identified, a remediation plan should be developed and scheduled for implementation by the appropriate jurisdiction. A report to the Council should be prepared for discussion of implementation.

Data Management

Data collected by the Pilot Project will be entered into the Gulf of Maine Data Management System. The Data Management System will provide statistical analyses for data interpretation and project evaluation. Graphics for incorporation into the Pilot Project Final Report will also be produced by the Data Management System.

Public Participation

Although public information and participation was identified as a key component of the Monitoring Program, the Pilot Subcommittee decided that commencement of a citizen's participation component of the pilot project would, at this stage, be counterproductive for several reasons. First, given the time constraints and limited budget, coordinating and training the public was seen as too large an undertaking to successfully accomplish at this juncture. Second, it was felt that involving the public in the pilot program when changes are likely to be made as a result of the project may damage long term credibility and ability to establish a public participation component. Rather, we decided to focus on completion of the pilot program and recommend areas of public involvement after the pilot has been properly evaluated.

Management Liason

Liason with the Council and thus to the Governors and Premiers will take place through the Monitoring Committee of the Council Working Group. The Council will be queried on the most appropriate reporting and information exchange. Through collaboration, management actions will be developed based on decision points identified by the Pilot Project.

Budget

Laboratory Services (76 samples @ $500)		$38,000
Travel	In-kind	$4,000
Personnel		
Coordination (2 staff days per jursd'n @ $500)	In-kind	$5,000
Field (6 staff field days per Jurisdiction @ $400)	In-kind	$12,000
Project oversight and final report (Co-chairs)	In-kind	$16,000
Supplies and Equipment (cages, calipers, etc.)	In-kind	$1,300
Shipping, printing, telephone, misc.	In-kind	$2,000
Total Cost		$78,300

Of the $78,300, $38,000 (48%) is actual cash the remaining $40,300 (52%) in-kind services and contributed material provided by the states, provinces and federal governments.

Schedule

Draft workplan for Pilot Project	April 15, 1991
Final workplan for Pilot Accepted	May 15, 1991
In-field trial with field crews	June 19-21 1991
Sampling, deployment of cages, and collection	July-September 1991
Laboratory analyses	Fall 1991
Draft Pilot Project Final Report	February 1992
Pilot Project Final Report	March 1992

Responsibilities

The Gulf of Maine Council on the Marine Environment will contribute $38,000 US for laboratory analyses, logistical support, and final report preparation and printing.

The U.S. Environmental Protection Agency will provide mussel cages to each jurisdiction and supply the protocol for proper cage deployment. The Agency will also oversee the collection of mussel growth measurement.

The Canadian Wildlife Service will assess the presence of Mytilus edulis on Machias Seal Island for possible future inclusion in Mussel Watch.

The U.S. National Oceanic and Atmospheric Administration will make participation in their Interlaboratory Quality Assurance/Quality Control Program available to each jurisdiction. NOAA will also be responsible for providing cytopathology preparation and/or archiving protocols.

Environment Canada will be responsible for coordination of the Pilot Project with the Canadian Mussel Watch Program. Environment Canada will also be responsible for analysis of select organic chemicals if sufficient funds become available for those analyses.

Maine Department of Environmental Protection will be responsible for laboratory analyses of heavy metals.

Each State and Province will be responsible for selecting stations and conducting the field portion of the project according to the approved workplan and timetable. Mooring gear (float, line and block), boats, travel funds, and miscellaneous supplies will be supplied by each jurisdiction.

Review Comment

The Gulfwatch Pilot Project Subcommittee invites your comments at any time during the life of this pilot. Our intent is to be flexible and responsive to newfound information. We welcome comments which will help us improve our final product and will attempt to incorporate suggestions. Please call either co-chair (Judith Pederson at (617) 727-9530 or John Sowles at (207) 289-7779) of the Gulfwatch Pilot Project with your comments, concerns, and questions.

Gulfwatch - Pilot Project Subcommittee

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APPENDIX A

DATA FORMS

GULFWATCH
PILOT PROJECT
1991
STATION DESCRIPTION FORM

Station Name (Permanent unique name used to identify this station):

Station Location Information

State/Province:	**Town**:

Waterbody Name (Be specific using the name of the cove, bay, point etc.):

Detailed narrative of access: **Boat** ☐ **Auto** ☐

Example: Sample area is reached off Landing Road in Boothbay. 50 feet south of telephone pole # 32-5, enter a path leading east to the shore. Walk about 5 minutes to come out on the shore next to an old granite pier. Sample area begins at the base of the pier and runs south to the outermost tip of Jellison Point.

Station Description

Station Category: Clean- ☐ Contaminated- ☐

Bottom: Ledge- ☐ Boulder- ☐ Cobble- ☐ Gravel- ☐ Sand- ☐ Mud- ☐

Tidal Range (Meters):

Distance from nearest river (km): **River Name:**
(measured as a boat would have to travel)

River Drainage Area: 10 km2- ☐ 100 km2- ☐ 1000 km2- ☐ 10,000km2- ☐

Distance from nearest human population center (km):

Human Population Size:

Distance from nearest pollution source (km):
Pollution Type: Municipal ☐ Industrial ☐
Agricultural ☐ Mixed ☐

Morphormetry of sample area: **Enclosed embayment-** (opening < 45° arc) ☐
Semi-enclosed embayment- (45° - 135° arc) ☐
Open - (opening > 135°) ☐

Predominant Wave Effect (compass direction from which most wave action comes) :
Onshore ☐ Offshore ☐ Longshore ☐

Additional Notes (Is this area closed to shellfish harvest? Are there unique hydrographic conditions? Is there anything which may affect data interpretation?) :

GULFWATCH
PILOT PROJECT
1991
CAGED MUSSEL FORM

DEPLOYMENT DATA

Station Name:
Date:
Time:

Temperature:
Salinity:
Field Crew:

Number	Length	Height	Width	Weight

Number	Length	Height	Width	Weight

Number	Length	Height	Width	Weight

Additional Observations:
(Turbidity, Phytoplankton blooms, Oil sheens, any unusual observations which may help interpret anomalous results)

GULFWATCH
PILOT PROJECT
1991
CAGED MUSSEL FORM

RETRIEVAL DATA

Station Name:

Date:

Time:

Temperature:

Salinity:

Field Crew:

Number	Length	Height	Width	Weight	Number	Length	Height	Width	Weight	Number	Length	Height	Width	Weight

Additional Observations:
(Turbidity, Phytoplankton blooms, Oil sheens, any unusual observations which may help interpret anomalous results)

GULFWATCH
PILOT PROJECT
1991
INDIGENOUS MUSSEL
CONDITION INDEX FORM

Station Name:
Date:
Time:

Temperature:
Salinity:
Field Crew:

Number	Length	Height	Width	Weight	Condition

Number	Length	Height	Width	Weight	Condition

Additional Observations:
(Turbidity, Phytoplankton blooms, Oil sheens, any unusual observations which may help interpret anomalous results)

ADDENDUM

TO METHODS

MEMORANDUM

DATE: July 22, 1991

TO: Mussel Watch Pilot Field Crew

FROM: John Sowles , Co-Chair

SUBJECT: Final instructions for field work

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Some **significant changes** in our plans have occurred since our meeting. First, Bill Robinson of the New England Aquarium in Boston provided us with some very helpful comments based on his own experience with caged mussels. We have extended the deployment period to 60 days from the original 28 because it appears that metal body burdens take at least that long to reflect ambient conditions. This should not affect the front end of the Pilot but will defer the final collection by about a month. It will, however, also increase the risk of vandalism and loss by storms. Second, we will be placing more mussels in the cages than originally planned so that Environment Canada may run organic analyses on a subsample. Third, we will be calculating a condition index on a subsample of the group. Thus, instead of collecting only length measurements, you are asked to also collect width and height. I will measure dry weights of the meats after we shuck the animals for analysis.

By now, you should have a clear understanding of the goals of the pilot project in order to resolve your own site specific problems while remaining more or less compatible with the overall project methods. Stations should have been selected according to the criteria set forth in the Gulfwatch Mussel Pilot Project workplan. The following is an attempt to answer a few lingering questions and hopefully clarify some details.

As you read through these instructions, please make notes in the margins where further clarification or comments are needed. These instructions will be refined for next year's participants. Please do the same on the field forms and if possible suggest improvements.

1.) **Site verification**

Deployment site suitability must be confirmed by a visit or prior knowledge. 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.

2.) **Collection of transplant stock**

A day or two before you intend to deploy the transplanted mussels, collect enough mussels from a site known to be uncontaminated for deployment at both clean and dirty sites. You should gather at least 50 mussels per replicate per treatment, which comes to 300 mussels. (Not to worry, we are <u>not</u> marking and measuring that many!) Their size should be in the 50 - 60 mm length range. This allows for some mortality. Place the mussels into a clean (washed and rinsed) cooler on a bed of ice covered with seaweed. Do not let melt water build up so that the mussels can siphon. This will kill them quite effectively! Transport them to the lab without delay where they will be marked.

3.) **Marking, measuring and handling**

In the lab, only <u>**25 of the 50 mussels per replicate are to be marked**</u> on the side of the shell using a high speed engraving tool (Dremel type or similar) with a diamond engraving bit. Number each mussel with a unique identifier. It is strongly recommended that, if you have never marked a mussel before using this tool, you practice on some mussels which you can afford to sacrifice. Care must be given to go deep enough to be able to read the numbers after two months in the ocean yet not so deep as to penetrate the shell and injure or kill the animal.

For each numbered mussel, length, width and height (see attached) should be measured to the nearest 0.1mm using a vernier caliper and recorded on the deployment field sheet next to the mussel number (see 5. below). Originally, we were to record only length, however, we are now asking you to also record width and height. As each replicate composite is completed, it should be returned to the ice in a discrete batch. You may be able to

place the composite directly in its cage and I think probably two cages would fit into a large cooler. The important thing to remember is that we must be aware of what mussels are in what cages so that growth can be ascribed to the proper cage system (treatment)! Although the ice and seaweed bed is preferable because the opportunity for contamination is minimal, mussels can only be held for 1 or 2 days and still remain in good health. If you are not able to deploy the mussels within two days, alternatively, the mussels could be placed into a filtered seawater aquarium whose source of water is "clean." I prefer that they go on ice however, to eliminate contamination by the aquarium water source.

4.) **Deployment**

The mussels may now be taken on ice to their respective sites for deployment. If they have not already been placed in their cages at this point, do so, but be sure to record on the appropriate form the number of each marked mussel going into each cage. Remember that 25 marked and 25 unmarked mussels go into each cage for a total of 50. There are three cages per treatment. The baskets are taken out to their site and moored one meter off the bottom using an arrangement similar to the one discussed at our meeting (attachment) with ample plastic "quick ties." Be absolutely sure that the cages will always be underwater (below low low tide, not just below mean low tide). To minimize drag and hence risk of the system being washed away by currents, storms, and tide, the surface area of the floatation should be minimal. It is best to test your system's buoyancy so that there is sufficient floatation to buoy the full cages and line with a little extra to account for fouling organisms and growth over the next two month but not so much that there will be too much drag from the mooring gear. Unless you are familiar with your site, you may want to experiment a bit beforehand.

Whether to have a surface float or not is up to you. A diver must deploy and retrieve a system without a surface float but the system is generally safer from vandalism. On the

otherhand, it may be difficult to locate the submerged system and it definitely is more labor intensive. If you choose to moor the cages off existing buoys, be aware that mooring chains may move with the tides and resuspend soft sediments thereby violating another of our criteria that the water be free of excessive sediment turbidity. Similarly, pilings may contain creosote and result in anomalous organic analyses. While this may be reflective of dirty sites, it may not be a desirable effect in clean sites. Simply take these factors into consideration as best you can when selecting the cage mooring system.

5.) **Forms**

Station Description Form

You should fill out only one form for each treatment since the indigenous mussel site and the caged mussel sites should be in close enough proximity to have the same features. If they do not, then the criteria are not being met. It behooves us to be as detailed as possible when describing access since others totally unfamiliar with the site may want to reoccupy this station in the future.

Under Morphormetry, what we are after is the "protectedness" or "enclosedness" of the sample area. We chose to measure this by degrees on the compass that the shoreline is open to the ocean. For example, a straight beach have an arc of 180^{o} and a cove with a narrow mouth might have an arc of less than 45^{o}.

Under Exposure we are asking for a subjective evaluation of from what direction most of the storm surge is coming.

Finally, under this section, we will need a map or chart with a scale of 1:40,000 or better which shows the location of your stations, both indigenous and caged mussels for each of you treatments.

Deployment, Retrieval, and Indigenous Mussel Forms

On these sheets, there are three measurement forms to record mussel number, length, width and height, one for each of the three replicates. All measurements should be metric. **(Co-chair Confession:** I am not sure these forms are designed for

ease of use since I have not actually used them. Please feel free to use your own creativity and help me design a better version. The important point is that all the information be there. Send me your ideas and I should be able to redo the best version before you do your final measurements.)

NOTE: Please maintain current duplicate forms so that if one set is damaged or lost, there will always exist a backup copy at all stages of the data collection process.

6.) **Collection and Retrieval**

Caged mussels should be retrieved and indigenous mussels collected within one or two days of each other. You should be able to do it on the same day, however. The collection date should be about 60 days after initial deployment but at least three days after any storms or other events which might cause bottom sediment to resuspend or river sediments to be introduced to the water column.

Indigenous mussels will also be collected in replicate samples. The way to do this is to sample 40 mussels from each of three discrete areas of the subtidal zone which ideally lie within a 50 meter section of shore. In any event, the three should "represent" one zone of water quality and environmental conditions. Subtidal is defined for the purposes of this study as below the low low water line. Check tide tables and operate accordingly. I collect during the window of one half hour around low tide and am sure to collect in water at least arms (meter) length deep. In the Maritimes, that may not be enough.

Collected mussels (both caged and indigenous) should be placed in their respective labeled containers without water. Environment Canada uses solvent rinsed quart mason jars with a tin foil liner on the lid. These are then placed in ice filled coolers for transport back to the lab.

7.) **Final measurement, draining and freezing**

Once all living marked mussels and 25 indigenous mussels have been measured for length, width, and height to the nearest

0.1mm, the mussels must be prepared for storage and analysis. The mussels must be drained of free sea water before they are frozen. With an acid washed stainless steel blade, pry open the valves to release any remaining free sea water. Be sure not to cut into the soft tissues. (Actually, there should be little water remaining in those that have set for several hours in their jars on ice.)

Once drained, the mussels are now ready for freezing. Place the mussels in their respective labeled jars and freeze in a standard freezer. At this point, you may now contact me (John Sowles) at the Maine Department of Environmental Protection (MDEP) to arrange a date and packing instructions for shipment or pickup. Since the MDEP does not have sufficient freezer space for all samples at the same time, you may have to hold them for several weeks.

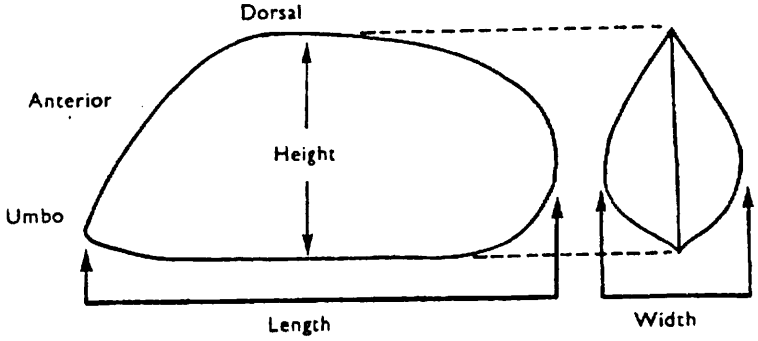
8.) Finally, if there are outstanding questions, concerns or corrections, please call one of us for clarification (assuming we know the answer) or at least to enable Gulf wide standardization. Thanks and good luck.

John Sowles Office (207) 289-7779 (or in the case of a government shutdown, my home number is (207) 846-4714)
Judy Pederson Office (617) 727-9530 (ext, 413)

SUGGESTED CONDITION INDEX

$$CI = \frac{SO}{LE \times WI \times HE}$$

where: SO = soft tissue dry weight
LE = length
WI = width
HE = height
(following Seed 1968)



Text-fig. 3. Terminology of the mussel shell.

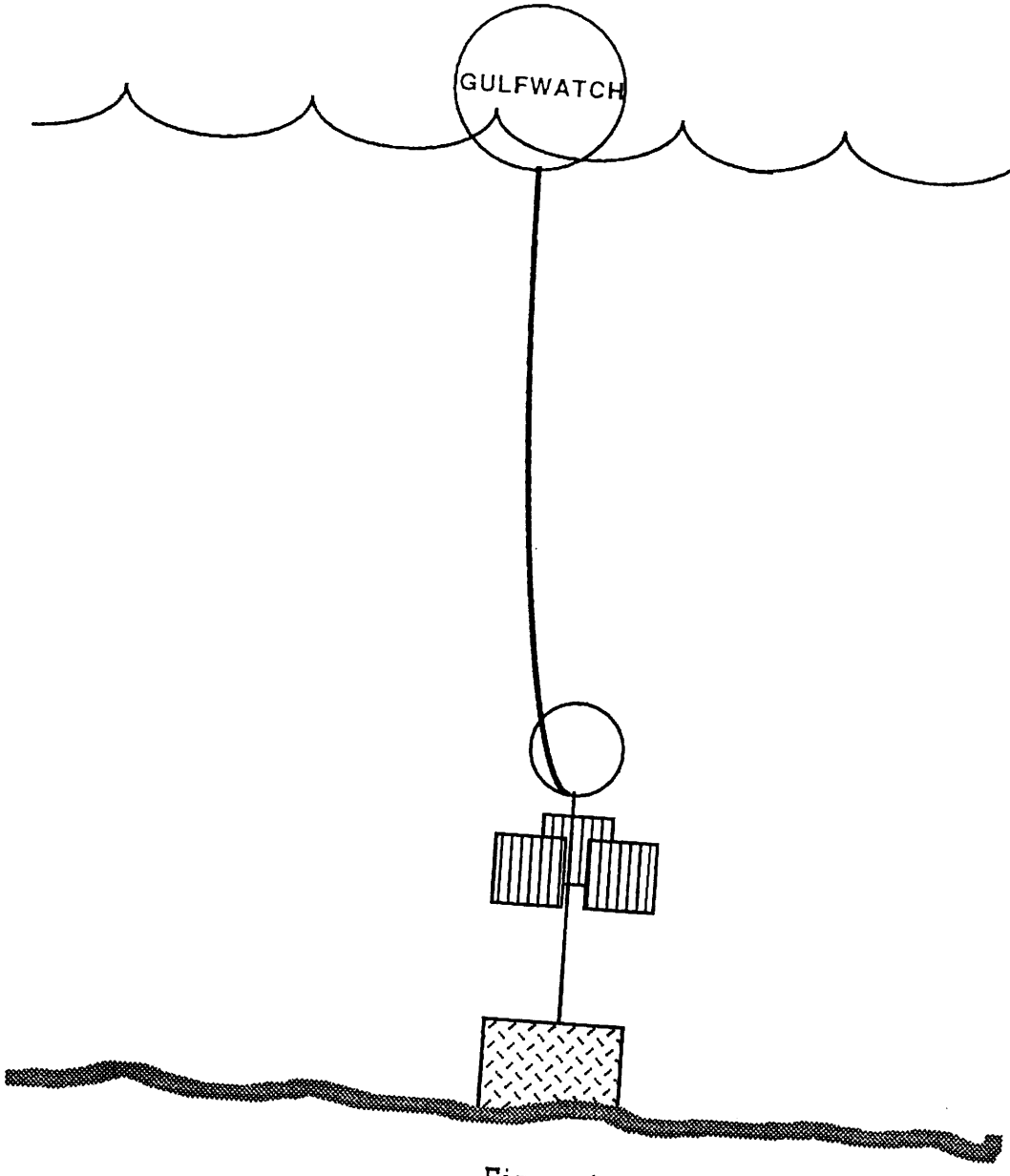


Figure 1

GULF OF MAINE PROJECT
Mussel Exposure System

Variables

1. anchors--2 concrete blocks is normally adequate. Those placed in extremely high energy areas (ie. New Brunswick) may need to increase the weight.

2. Line--5/8" polypropylene line or 5/16" polypropylene encased steel cable. (1/4" may be used for subsurface buoys).

3. Station marker buoys-- (Coast Guard approved) subsurface floats-- to support mussel cages: 30 cm. dia. orange plastic floats or 8" trawl float.

4. Cages--test tube baskets given. These should be lashed at each corner after mussels are inserted, with pull ties that are not removeable without cutting.

   Cages can be set on the large line with plastic pull ties or the line can be run through the cages.