

RFA 4292

Large-scale, manipulative field tests involving cultured and wild juveniles of the soft-shell clam, *Mya arenaria* L.: Interactive effects of predator exclusion netting, aperture size, and planting area on seasonal growth and survival at two intertidal locations within the Hampton-Seabrook Estuary

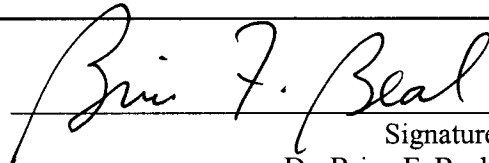
August 13, 2004

FINAL


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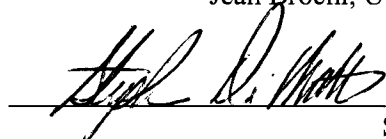
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A3 - Distribution List

Table 1. QAPP Distribution List

QAPP Recipient Name	Project Role	Organization	Telephone number and Email address
Brian Beal	Project Manager	Univ. of Maine at Machias	207-255-1314 bbeal@maine.edu
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Based on EPA-NE Worksheet #3

A4 - Project/Task Organization

The project manager for this study is Dr. Brian Beal of the University of Maine at Machias. Dr. Beal is responsible for maintaining and distributing the approved QA Project Plan, experimental and sampling designs, fieldwork, enumeration, clam measurement, data analysis, quality assurance, and filing interim and final reports with NHEP.

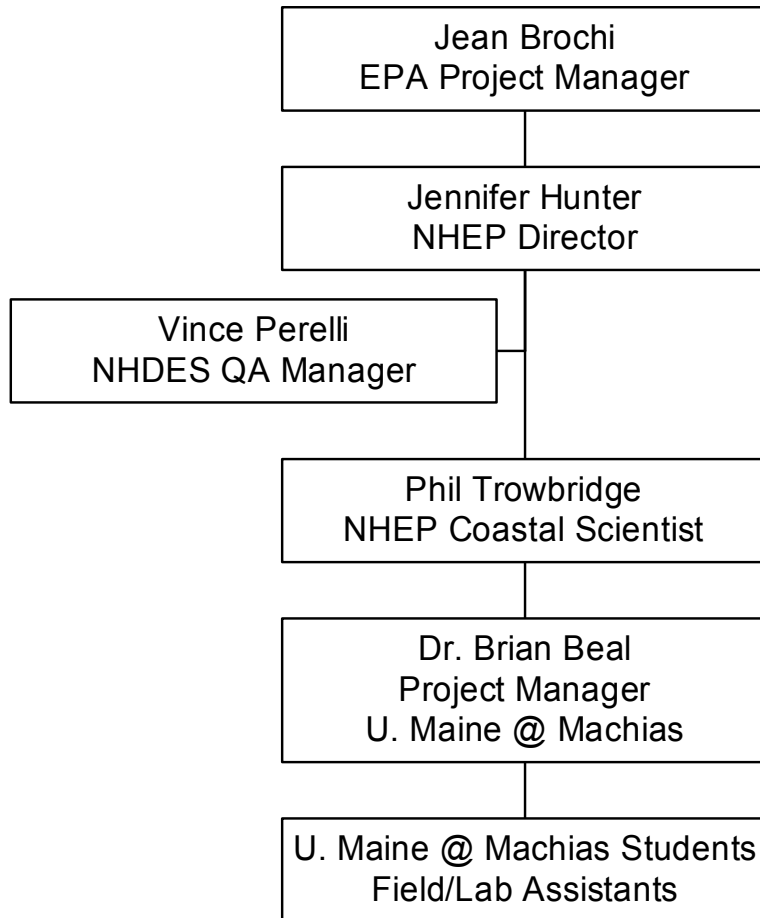
Micro Technologies of Richmond, Maine (Dr. Cem Giray is the laboratory contact) will perform disease testing for hematopoietic neoplasia on cultured juveniles of the soft-shell clam, *Mya arenaria* before bivalves are planted to the Hampton-Seabrook Estuary sites.

Dr. Beal is assisted with field and lab work by students from the University of Maine at Machias and staff from the Downeast Institute for Applied Marine Research and Education, Beals, Maine.

The principal data users will be personnel at the New Hampshire Estuaries Project and the New Hampshire Fish & Game Department.

The New Hampshire Estuaries Project is funding the study.

Figure 1: Organizational Summary



A5 - Problem Definition/Background

Soft-shell clams, *Mya arenaria* L., represent an important recreational fishery along the New Hampshire coast. During the fall 1998, over 900 clambers easily harvested their 9.5-liter limit when the Middle Ground flats in Hampton/Seabrook Harbor were opened (Varney, 1999). Since that time, clam abundance has dwindled and recent surveys of several Hampton/Seabrook clam flats suggest that the limiting factor for a sustainable fishery is poor juvenile survival. Despite apparent successful reproduction and larval settlement, the population of yearling clams (i.e., age 7-12 months and 26-50 mm shell length) is very low (NHEP, 2001).

Several factors may help to explain the paucity of small clams along these shores. These include: 1) predation by crustaceans such as green crabs and other invasive crustaceans, boring gastropods, fish, or nemerteans (Beal et al., 2001); 2) competition with other bivalves such as mussels (*Mytilus edulis* L.); 3) poor recruitment or slow growth at various tidal heights (Beal and Fegley, 1996); 4) disease (e.g., Brousseau and Baglivo, 1991); 5) winterkill due to ice scour or sea birds (Beal et al., 1995); 6) inorganic toxins such as heavy metals (White and Robertson, 1996); or 7) commercial or recreational shellfish harvesting (Beal and Vencile, 2001).

During the winter of 2001 and spring/early summer of 2002, the New Hampshire Estuaries Project commissioned a study to evaluate factors contributing to the mortalities of juvenile soft-shell clams in the Hampton-Seabrook Estuary. Results from two short-term field experiments at three intertidal sites demonstrated that mortality due to disease (specifically neoplasia), interspecific competition, and winterkill due to ice and storms was minimal. Clam losses associated with sediment scouring and predation exceeded 95% in some instances over the winter (November 2001 to March 2002; Beal [2002]). Similar losses at the same sites occurred during the period from March to July 2002, but in most cases, survival was enhanced by using protective mesh netting (6.4 mm aperture).

Among the limitations of those field tests were: 1) the use of small experimental units (6-inch plastic plant pots); 2) experiments were conducted once; 3) the use of a single mesh netting aperture size; and 4) no data were collected during times when seawater temperatures were seasonally greatest (i.e., July through September).

The New Hampshire Estuaries Project (NHEP) is funding projects to “enhance or restore shellfish resources in New Hampshire’s Estuaries.” Eligible projects included those that addressed soft-shell clam *Mya arenaria* populations in New Hampshire’s estuaries. The specific objectives of the funded study include a determination of the interactive effects of predator exclusion netting, mesh netting aperture size, and planting area on survival and growth of cultured and wild juveniles of the soft-shell clam during the fall and winter at the upper intertidal zone of one or more intertidal flats in the Hampton-Seabrook Estuary, and again during the spring through early fall at the same location(s).

For NHEP staff to assess the effectiveness of various restoration techniques, the experimental design will incorporate large experimental units (4, 8, 12, and 18 m² plots) that will bracket the size of units resource managers might use in large-scale enhancement projects.

The experimental design provides answers to the following questions:

- 1) What are the costs and benefits associated with enhancing intertidal areas with hatchery-reared individuals (ca. 8 mm shell length, SL)?
- 2) Does the use of netting across several planting areas and aperture sizes enhance clam survival compared with similar size areas that are planted but receive no netting?
- 3) Is it efficacious to use netting to create spatial refuges that protect small clams already in the sediments (or that are somehow attracted to netted areas)?
- 4) Does growth or survival of cultured and/or wild juveniles of the soft-shell clam vary with mesh aperture size?
- 5) What effects on growth and survival, if any, can be attributed to the actual size of the area seeded? Do clams respond “better” (i.e., faster growth and/or higher survival) when “edge effects” due to the size of the netted area are relatively minimal or maximal?
- 6) What time of year (spring vs. fall) is better to initiate clam enhancement programs?
- 7) Is the effectiveness of netted plots similar at different intertidal sites at the same tidal height?

A6 - Project/Task Description

The following work tasks have been specified for this project in the contract between the NHEP and UMM.

1. MEET WITH NHEP AND NHF&G STAFF

Before initiating field sampling or experiments, the project director will meet with NHEP, NHF&G, and other agency staff in Portsmouth NH to discuss the project and which locations on the clam flats should be used for field sampling and manipulative experiments.

2. OBTAIN PERMIT FROM NHF&G

Before initiating field sampling or experiments, the project director will obtain a scientific permit from NHF&G Region 3 to harvest soft-shell clams from the HSE.

3. PREPARE QUALITY ASSURANCE PROJECT PLAN

Before initiating field sampling or experiments, the project director will prepare a Quality Assurance Project Plan. This plan must be approved by Quality Assurance staff from NHDES and EPA Region I.

3a. EDUCATION AND OUTREACH

Before initiating field sampling or experiments, the project director and NHEP staff will develop a fact sheet about the project. Information from the fact sheet will be distributed starting September 1, 2004 through: NHF&G Salt water digest (edited in December/January); Stores selling licenses; Ferries to the flats; Division of Ports and Harbors / Harbormaster; September 2004 shellfish forum by GBCW; Wormers; Postings at the study location ("Clam Restoration Area"); and newspaper articles.

4. CONDUCT FIRST MANIPULATIVE FIELD EXPERIMENT (November 2004 – May 2005)

This test is designed to assess the interactive effects of plastic mesh netting (none vs. 4.2 mm vs. 6.4 mm aperture), presence of cultured juvenile clam seed (present vs. absent), and size of planted area (4 m², 8 m², 12 m², and 18 m²) on overwinter survival and growth of cultured and wild juveniles of *Mya arenaria* from November 2004 to May 2005.

5. CONDUCT SECOND MANIPULATIVE FIELD EXPERIMENT (May to November 2005)

This field test is designed to assess the interactive effects of plastic mesh netting (none vs. 4.2 mm vs. 6.4 mm aperture) and planting site on survival and growth of cultured and wild juveniles of *Mya arenaria* from May to November 2005.

6. CONDUCT DISEASE TESTING OF SOFT-SHELL CLAMS

Before any cultured clams from the Regional Shellfish Hatchery of the Downeast Institute are planted within the Hampton-Seabrook Estuary, animals will be tested for disease (hematopoietic neoplasia). Tests will be conducted prior to November 2004 and May 2005 by Micro Technologies of Richmond, Maine.

7. PERFORM STATISTICAL TESTS OF HYPOTHESES

The project director will test hypotheses concerning the relative importance of several factors influencing the fate and growth of soft-shell clam juveniles.

Major milestones for this project are summarized in the following table.

Table 2. Project Schedule Timeline

Activity	Dates (MM/DD/YYYY)		Product	Due Date
	Anticipated Date(s) of Initiation	Anticipated Date(s) of Completion		
Conduct first manipulative experiment	11/15/04	05/20/05	Data for study	5/20/05
Present interim findings to NHEP	06/01/05	06/30/05	Presentation to NHEP Management Committee and quarterly report	6/30/05
Conduct second manipulative experiment	05/15/05	11/15/05	Data for study	11/15/05
Present final report to NHEP	12/1/05	12/31/05	Final report to NHEP	12/31/05

A7 - Quality Objectives and Criteria

The overall goal of this project is to detect statistically significant differences in clam survival or growth between different treatments with nets, stocking density, and location. Statistically significant differences between treatments would give management clear information on the best treatments to use to enhance the clam populations. If no differences are observed, then the information provided by the project would be limited to anecdotal observations.

The statistical tests that will be applied are Analysis of Variance (ANOVA). A type I error rate (α – the probability of rejecting a null hypothesis that is true) of 0.05 will be used as a decision rule. Therefore, the ultimate data quality objective for the statistical component of this study is to be able to detect differences between treatments at the $p < 0.05$ level.

Operationally, the only event that could affect the statistical power of the experiment is the loss of experimental units due to scouring or ice. Statistical tests for the experiment can be run so long as 50% of the units in each treatment are retained. Therefore, a data quality objective of 50% completeness needs to be met for this project.

A8 - Special Training/Certification

Field assistants will be trained by the Project Manager prior to their conducting work on this project. The Project Manager will keep a record of the people who have been trained for the project in his files.

A9 - Documents and Records

The Project Manager will be responsible for maintaining the approved QA Project Plan and for distributing the latest version of the plan to all parties on the distribution list in section A3. A copy of the approved plan will be on file with the NHEP Coastal Scientist.

Quarterly interim reports and one final report will be produced for the NHEP. This report will be available to the public in hardcopy from the NHEP and its abstract (in text form only) will be included in the NHEP tracking database. See section C2 for the reporting schedule and details. The final report will be in the form of a manuscript that could be submitted to a marine ecology scientific journal for publication.

The Project Manager will be responsible for retaining field data sheets, electronic databases, final reports, and other records of this project for at least 5 years.

GROUP B: DATA GENERATION AND ACQUISITION

B1- Sampling Process Design (Experimental Design)

The work will begin in mid-November 2004 within the Estuary (sites to be chosen in consultation with Estuary Project members and other agency members). This test is designed to assess the interactive effects of plastic mesh netting (none vs. 4.2 mm vs. 6.4 mm aperture), presence of cultured juvenile clam seed (present vs. absent), and size of planted area (4 m², 8 m², 12 m², and 18 m²) on overwinter survival and growth of cultured and wild juveniles of *Mya arenaria* from November 2004 to May 2005. Sixty-four netted and 32 unnetted areas will be randomly assigned to a 12 x 8 matrix near the upper intertidal of the Willows Flat in the Hampton-Seabrook estuary during November 2004. Cultured juveniles (8-10 mm SL) from the Regional Shellfish Hatchery of the Downeast Institute for Applied Marine Research and Education (Beals, Maine) will be added to one-half of the protected (netted) and unprotected areas at a stocking density of 1,320/m². This size range is similar to that of wild clams on this flat during that time of year (Beal, 2002). The netting used will be the same that has been used in other experiments (Beal, 1994; Beal et al., 2001; Beal, 2002; Beal and Kraus, 2002) and in community stock enhancement programs in Maine and Massachusetts (Beal, pers. obs.). Two levels of aperture size of the plastic mesh netting will be used (4.2 mm and 6.4 mm) along with four levels of planting area (4, 8, 12, and 18m²). The other half of the netted areas and unprotected areas will receive no cultured seed to provide conditions that will allow resource managers to better understand the cost vs. benefits of purchasing cultured seed. No attempts will be made to adjust densities of wild seed.

Clams will be planted in four different size areas. Initially, seeded areas will be marked at each corner with a wooden lath. A piece of rope or heading twine will be tied to each corner lath so that the area between the laths is clearly delineated. Once clams have been distributed on the surface of the flat within the delineated area, a piece of pre-cut netting will be spread over the seeded area. To ensure that the netting remains in place, a furrow (30 cm deep) will be dug around the periphery of the seeded area. The edge of the net (which will be cut so that a 30 cm edge will exist around each side) will be pushed into the furrow and the sediment from the furrow back-filled into the furrow. This has been a successful way to keep nets in place in high-energy sites (Beal, pers. obs.). For example, the netting for a 4 m² plot (with a seeded area measuring 1

m x 4 m) will be pre-cut so that the actual dimensions are 1.3 m x 4.3 m. The area seeded will be 1 m x 4 m and the extra material (30 cm on each side) will be pushed into a 30 cm-deep furrow that will be dug immediately after the clams have been spread within the marked area. Sediments from the furrow will be placed back into the furrow to secure the netting. In muddy environments, styrofoam floats have been used to lift nets above the sediment surface during tidal inundation (see Beal and Kraus, 2002) to decrease sedimentation within the experimental area. This will not be necessary in the sandy sediments within the Hampton-Seabrook estuary (Beal, pers. obs). It is likely, however, that some nets will become completely choked with sediment and all the clams within the netted area suffer mortality due to either a gradual or short-term sediment event. The design proposed here will enable us to determine whether these events are related to the size of the planted area. The field site and nets will be checked regularly by the project director and NHEP staff. A field data sheet has been developed (Appendix) to record information about the status and condition of each plot. This information will be used to better understand the fate of the clams within each plot.

At the beginning of the experiment and before cultured seed are planted, a single benthic core (area = 0.018 m² to a depth of 15 cm) will be taken in the center of each of the 96 experimental areas. This will establish initial densities and size distribution of wild seed. At the end of the experiment, four benthic cores will be taken from each of the 96 experimental areas (N = 384). All samples will be washed through a sieve with a 2 mm aperture. All clams will be counted and measured (SL) to the nearest 0.1 mm using vernier calipers. It is possible to differentiate between wild and cultured clams as individuals within the latter group produce a distinct mark when they grow in the field (Beal et al., 1999). The design is not completely factorial because there can be no combination of treatments that incorporates net aperture size with an unnetted area.

A second manipulative field experiment will be conducted from May 2005 to November 2005. This field test is designed to assess the interactive effects of plastic mesh netting (none vs. 4.2 mm vs. 6.4 mm aperture) and planting site on survival and growth of cultured and wild juveniles of *Mya arenaria*. Cultured juveniles (8-10 mm SL) produced at the Downeast Institute (Beals, Maine) during the summer of 2004 and overwintered according to Beal et al. (1995) will be added to fifteen 18 m² experimental units (6 m x 3 m) at a stocking density of 1,320/m² at each of two upper intertidal sites at Willows Flat and another north of the Common Island Flat in the Hampton-Seabrook estuary. After spreading seed haphazardly within the 18 m² units, two-thirds (10) of them will be covered with plastic mesh netting. One half of the nets will be comprised of material with 4.2 mm apertures while the other half will have 6.4 mm apertures (Fig. 2, p.23). Nets will be held in place as described above.

At the beginning of the experiment and before cultured seed are planted, a single benthic core (area = 0.018 m² to a depth of 15 cm) will be taken in the center of each of the 30 experimental areas. This will establish initial densities and size distribution of wild seed. At the end of the experiment, seven benthic cores will be taken from each of the 30 experimental areas (N = 210). All samples will be washed through a sieve with a 2 mm aperture. All clams will be counted and measured (SL) to the nearest 0.1 mm using vernier calipers. This design is completely factorial.

The preceding paragraphs describe the plan for the experiment. The Project Manager is responsible for adjusting this plan as needed to handle any problems that arise. The Project Manager will consult with the NHEP Coastal Scientist if any major problems are encountered such that the experimental design needs to be changed.

B2 - Sampling Methods

Clams will be sampled by taking randomly placed cores (area = 0.018 m² to a depth of 15 cm) within each netted and unnetted area (as described in Section B1).

B3 - Sample Handling and Custody

Each benthic core will be sieved using a 2 mm aperture sieve. Animals will be frozen and then counted and measured at a later date.

B4 - Analytical Methods

Clams from each sample will be counted and measured in two linear dimensions to the nearest 0.1 mm using vernier calipers.

B5 - Quality Control

Quality control for this experiment will be accomplished through the verification procedures described in Section D2.

B6 - Instrument/Equipment Testing, Inspection, and Maintenance

There will not be any equipment used that requires inspection or maintenance.

B7 - Instrument/Equipment Calibration and Frequency

There will not be any instruments or equipment used that require calibration.

B8 - Inspection/Acceptance of Supplies and Consumables

There are no supplies and consumables that requires inspection.

B9 - Non-direct Measurements

No other data than those collected here are needed for project implementation.

B10 - Data Management

Data Recording Procedures: Field data will be recorded on standardized field data sheets. When completing these forms, the field staff will be sure that all entries are legible.

Data Entry Procedures: Data entry will be checked using two methods. First, the entire data set will be printed and checked against the entries in each data sheet by the Project Manager. Second, the Project Manager will use box-plots and other graphical tools (such as residual plots) to determine if there are outliers in the data set. If a potential outlier is discovered, the Project Manager will go back to the data sheet and then to the entered data and determine whether the outlier is a data-entry error or whether it was recorded as such on the data sheet.

Data Management: All data from the experiment will be maintained by the Project Manager. Data include mean estimates and 95% confidence intervals for natural densities, survival of experimental clams, and growth rates of experimental clams. Data will be stored electronically in spreadsheets or SAS datafiles. Management of electronic data and documents is described in Section A9.

Data Analysis: Data will be analyzed using ANOVA and a type I error rate of 0.05 will be used as a decision rule. Each hypothesis tested using ANOVA will be in the form of a rejection or a failure to reject a null hypothesis. This is consistent with data analysis in the field of marine ecology.

GROUP C: ASSESSMENT AND OVERSIGHT

C1 - Assessments and Response Actions

The Project Manager will evaluate the sample collection methodology both during and after the project. Unanticipated problems with the procedures will then be addressed to avoid difficulties during subsequent sampling efforts.

C2 - Reports to Management

Reports will be submitted to the NHEP according to the following schedule from the NHEP-UMM contract:

1. Interim report #1 on the project status plus an invoice for approved project costs – June 30, 2004 (two copies)
2. Interim report #2 on the project status plus an invoice for approved project costs – September 30, 2004 (two copies)
3. Interim report #3 on the project status plus an invoice for approved project costs – December 31, 2004 (two copies)
4. Interim report #4 on the project status plus an invoice for approved project costs – March 31, 2005 (two copies)
5. Interim report #5 on the project status plus an invoice for approved project costs – June 30, 2005 (two copies)
6. Interim report #6 on the project status plus an invoice for approved project costs – September 30, 2005 (two copies)
7. Final Report upon the completion of all tasks. The final report shall describe the interactive effects of predator exclusion netting, mesh netting aperture size, and planting area on survival and growth of cultured and wild juveniles of the soft-shell clam, *Mya arenaria* L., during the fall and winter 2004-2005 and during the summer and fall 2005 at the upper intertidal of an intertidal flat located within the Hampton-Seabrook Estuary. The report will include a description of the methodologies employed, a presentation and discussion of results, and conclusions. The final report should also present quantitative estimates of the contribution of each treatment to overall juvenile clam survival and growth - December 31, 2005 (five copies and one unbound original)

For information about where the data will be stored and the format of graphics and hardcopy reports, please see section A9.

GROUP D: DATA VALIDATION AND USABILITY

D1 - Data Review, Verification, and Validation

The Project Manager will be responsible for reviewing and verifying the data according to the procedures listed in Section D2.

D2 - Verification and Validation Methods

The process by which data will be verified will involve one or more of the following:

1. At the end of each field season, the project manager will evaluate whether the data quality objective for completeness stated in section A-7 of this plan was met.
2. The project manager will use the methods in Section B10 (Data Entry) to identify any anomalous entries or discrepancies in the database.
3. The project manager will discuss discrepancies or anomalies in the data with field assistances or the contract laboratory.
4. If discrepancies cannot be resolved, appropriate measures will be taken. These measures could include but are not limited to:
 - a. rejection and exclusion of data from reports with an explanation.
 - b. re-sampling the appropriate station.

D3 - Reconciliation with User Requirements

Any problems with the data analysis and interpretation will be reconciled by the Project Manager after consultation with New Hampshire Estuaries Program staff. The overall goal of the project is to detect statistically significant differences in clam growth or survival between different treatments. Therefore, regardless of the methods and whether the completeness data quality objective is met, the project will be considered a success if a statistically significant difference is detected. Limitations in the data will be clearly defined for potential end users in all reports produced.

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Appendix

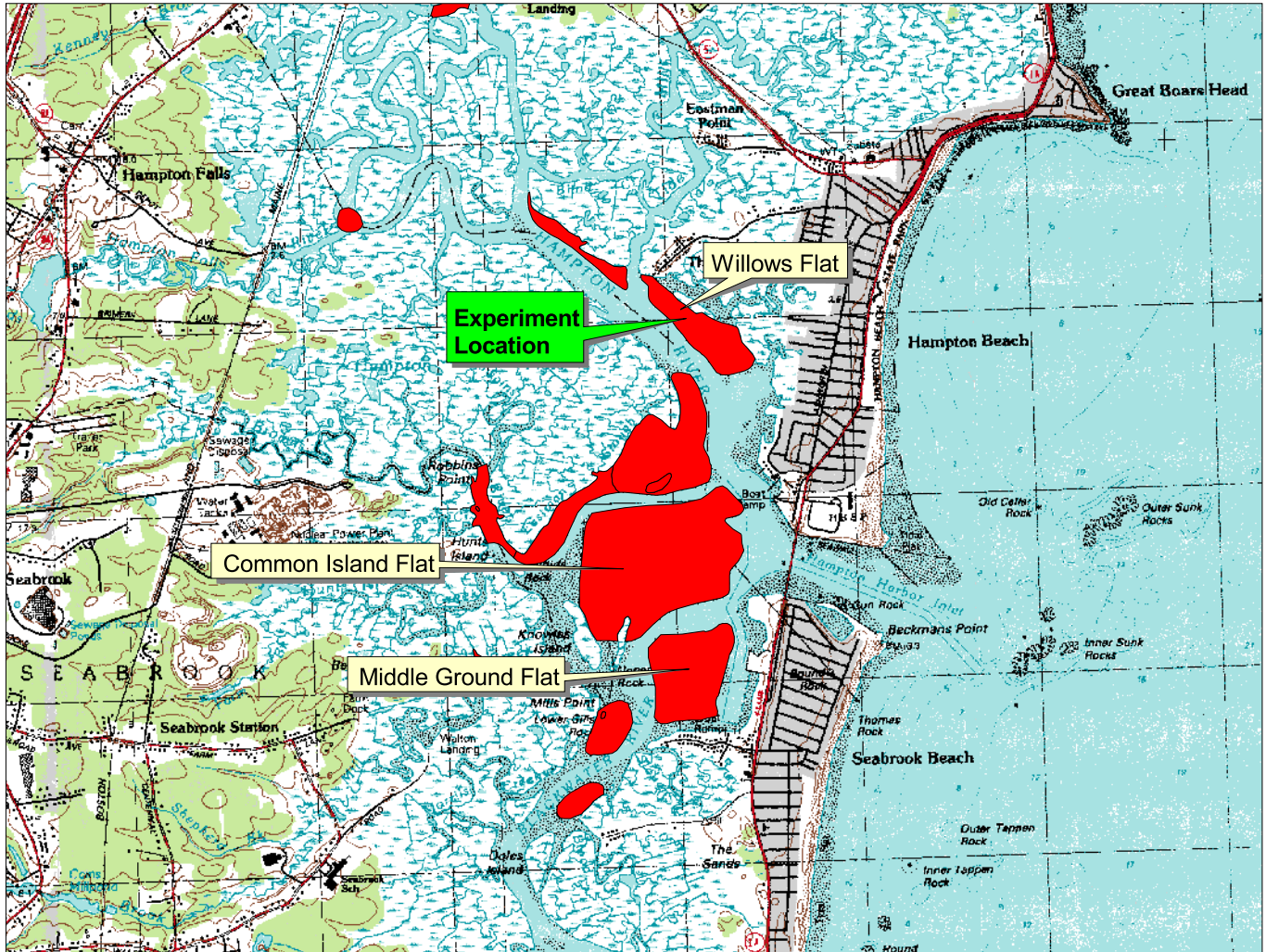
Data sheet for monitoring experimental plots

Matrix Position	Mesh type	Plot Size	Plot Condition
	1/8-inch	4 m2	Fine -- no problem
	1/4-inch	8 m2	Edges scoured
	none	12 m2	Net lifted
		18 m2	Net missing
			Heavy/light
			Sedimentation

Date:

Matrix Position	Mesh type	Plot Size	Plot Condition
1-1			
1-2			
1-3			
1-4			
1-5			
1-6			
.			
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.			
4-5			
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.			
8-12			

Location of Soft-Shell Clam Restoration Project Hampton-Seabrook Harbor (November 2004 to November 2005)



 Clam Resource Areas

