Project Completion Report

Subaward # Q169601 (year 1) & Q169901 (year 2)

Grant # 2002-38500-12056 (year 1); 2003-38500-13505 (year 2)

PROJECT CODE: 05-1 and 05-4

SUBCONTRACT/ACCOUNT NO: Q169601 & Q169901

PROJECT TITLE: Cross Breeding and Field Trials of Disease-Resistant Oysters

DATES OF WORK: June 15, 2006 to April 30, 2009

PARTICIPANTS:
Funded Participants
Paul Rawson, Project Coordinator, University of Maine
Scott Lindell, Marine Biological Laboratory
Ximing Guo, Haskin Shellfish Research Lab, Rutgers University
Roxanna Smolowitz, Roger Williams University
Steven Roberts, University of Washington
Inke Sunila, Bureau of Aquaculture, Connecticut Department of Agriculture
Richard C. Karney, Martha's Vineyard Shellfish Group

Cooperating, Non-funded Participants
Dale Leavitt, Roger Williams University
Bill Walton, Cape Cod Cooperative Extension
Tessa Getchis, University of Connecticut
Dana Morse, Maine Sea Grant
Christopher Davis, Pemaquid Oyster Co.

REASON FOR TERMINATION: End of Project

PROJECT OBJECTIVES:
The research conducted under this award, through a collaboration between the University of Maine, the Marine Biological Laboratory, the Connecticut Bureau of Aquaculture, and Rutgers University, addressed the following objectives:

1) Compare the growth and survival of disease resistant oyster lines and interline hybrids at grow-out sites in Maine, Massachusetts, Rhode Island and Connecticut via replicated common garden grow-out trials.

2) Place oyster lines demonstrating the highest yield at the end of the two-year grow-out trials in a repository, split between the University of Maine and the Marine Biological Laboratory, for future use by industry.

3) Report on project progress and results at regional and national meetings; post results in a timely manner on a project-specific website, and disseminate results to industry members via a Fact Sheet.

ANTICIPATED BENEFITS:

The ultimate goal of our research program is to provide commercial and public hatcheries with selected broodstock so that growers have access to oyster seed that perform optimally on their farm, and thereby improve the region's oyster production. Region-wide growers agree that the incidence of disease is the most serious impediment to increased production and revenue from cultured eastern oysters. The primary diseases of concern are MSX (and closely related SSO) and Dermo, caused by the protistan parasites *Haplosporidium nelsoni* and *Perkinsus marinus* respectively. The incidence of MSX and Dermo has increased in the past two decades and resulted in precipitous declines in the aquacultural production of oysters in some states. MSX and Dermo have historically been less of a problem for oyster culture operations in northern New England states. However, in this region, Roseaovarius Oyster Disease (ROD) causes stunting, uneven shell growth, and crop losses as high as 90% in some years. Our project was focused on testing and identifying oyster lines that demonstrate higher survival under the variety of disease-pressures and grow-out conditions experienced in the New England region.

Given that disease pressure on oyster culture operations in northeastern U.S. is likely to continue to increase, our project was designed to develop and test stocks of oysters with improved resistance to both protozoan (MSX and Dermo) and bacterial (ROD) diseases. We used a common-garden experimental approach to ask whether hybrid stocks produced by crossing the well established and commercially available Rutgers University New England Hybrid (NEH) and the University of Maine Flowers Select (UMFS) lines have better overall survival and yield when grown at sites affected by Dermo AND at sites impacted by ROD. We also tested the hypothesis that oyster stocks selected from local, natural environments in the New England area and that have survived heavy, annual, disease pressure from Dermo (EGP and Clinton lines) have improved survival, grow better and sustain higher yields than stock from more southerly climes (e.g., NEH). Thus, the results of our project will aid in the identification of the culture conditions under which oyster lines available to northeastern oyster farmers perform well, and support the continued development of oyster lines that perform well at a variety of sites in the region, particularly those sites where oyster diseases are endemic, and the development of lines of oysters that demonstrate combined resistance to multiple diseases.

PRINCIPAL ACCOMPLISHMENTS:

Objective 1 - Our NRAC-sponsored project sought to develop and identify high yield lines of eastern oysters that would benefit oyster farmers throughout the northeast. There

are two major impediments to increased yields for oyster farmers in this region. First, disease caused by both protistan and bacterial parasites can cause substantial crop losses and many farms contend with multiple and multiple types of diseases. While several currently available lines have putative resistance to one or two diseases, none have demonstrated a high degree of resistance to both bacterial and protistan pathogens. The second impediment to increased yields is poor growth, particularly in northern New England, which is often caused by less than optimal grow-out temperatures and conditions. Thus, we were keenly interested in monitoring the relative survival and growth potential of oysters from four primary oyster lines (NEH, UMFS, EGP and Clinton) and three groups of interline hybrids (UMFS x NEH F1 hybrids and F1 x parental line backcrosses) under a broad range of grow-out conditions. We specifically desired to test whether resistance to bacterial and protistan caused diseases could be obtained in hybrid lines while maintaining the high growth potential of the original lines used to generate the hybrids.

At grow-out sites in Maine where the UMFS line was developed, the UMFS and UMFS-BC lines generally had the highest survival. Similarly, at the Cape Shore site in New Jersey where the NEH line was developed, the NEH line had the highest survival. These results are similar to those we have observed in previous field trials and suggest that there has been some local adaptation among these lines either to disease pressure or environmental conditions. A key observation from the present study, however, is that increasing the genetic contribution from the NEH line among hybrid oysters results in a significantly higher survival rate at the Cape Shore site where Dermo is endemic (the UMFS line is naïve to Dermo). This effect was nearly additive, as the F1 line had a survival rate that was virtually the arithmetic average of the survival rate for the UMFS and NEH parental lines while the backcross groups had survival rates intermediate to that of the F1 and the respective parental lines. Unfortunately, we were not able to conduct examinations for disease on the oysters from the Cape Shore site so we cannot definitively determine whether Dermo was the cause of the mortality variation and whether among-line differences in survival are due to tolerance to parasite load or true resistance. Further, we did not see evidence of substantial ROD-challenge for the oysters grown at the Maine sites in our northern New England field trial. Thus it is unclear whether there is similar variation among the hybrid lines with respect to susceptibility or tolerance to the bacterial disease, ROD that is problematic in northern New England.

Our results, however, clearly show that at many sites oysters reached market size (75 mm) and that the hybrid lines grew faster than the parental lines. This is not surprising in that genetic analysis indicates the UMFS and NEH lines are relatively inbred (Guo; unpublished data) and so enhanced performance among hybrids likely results from a release from inbreeding depression. At the same time, our data suggest that hybridization has had no major impact on shell shape, at least when oysters are grown under cold-water conditions on the Damariscotta River in Maine. Although the NEH-BC line had a slightly better condition index than the UMFS and therefore had a denser meat at the time of sampling, the difference was subtle and unlikely to relate to differences in quality and marketability. Even so, future efforts should continue to monitor among-line variation in

shell shape and meat condition as well as other variables such as appearance and taste to ensure that only highly marketable lines are propagated for the northeast oyster industry.

In our Southern New England field trial we found that survival for the newly developed Clinton line and the two hybrid lines (F1 and NEH-BC) was often as good as or better than the survival for the NEH line that is typically preferred by the industry. Further, the similarity in survival rate for the Clinton and NEH lines indicates the potential for the development of local stocks using oysters which have survived repeated outbreaks of disease. As part of our Southern New England field trial we were able to monitor the patterns of disease prevalence and intensity through histological (MSX) and molecular (Dermo) analyses. The analyses we have completed to date have found no readily apparent correlation between the prevalence and intensity of MSX or Dermo and survival at the sites included in this trial. It is important to note that our experiment did not have a positive control (a naïve, wild type, susceptible strain), and with the exception of the EGP line, for which we have not data, all strains showed disease resistance. Thus, mortalities at these sites are not a direct result of infections; even when there was a high prevalence of MSX, such as seen in some Wellfleet oyster samples, plasmodia were often outside basement membranes or dead and phagocytosed by hemocytes. These are histological characteristics of MSX-resistant oysters. In addition, there were no signs of Dermo at our Connecticut site. Dermo occurs at this site but generally only becomes detectable after oysters have been in the field for at least three years (Sunila, unpublished data). Thus, our results are consistent with previous observations and bolster the conclusion that Dermo doesn't cause significant mortalities in Connecticut. Overall, we found no clear indication that disease pressure from MSX or Dermo was responsible for line-specific variation in survival. However, detailed observation of histological preparations indicates that parasites usually considered harmless to oysters can cause pathological lesions in some oyster strains introduced to new areas suggesting that such organisms act as opportunistic pathogens to oysters when the oysters are grown under sub-optimal conditions. Our results also argue for the continued maintenance of the primary lines, such as UMFS and NEH, to preserve their unique characteristics while capitalizing on the increased growth performance and yield afforded by interline crossing.

Objective 2 - The second objective of our project was to place oyster lines demonstrating the highest yield at the end of the two-year grow-out trials in a repository, split between the University of Maine and the Marine Biological Laboratory, for future use by industry. One weakness that has stymied many selective breeding programs in agriculture, in general, and aquaculture, in particular, is the lack of institutional commitment for maintaining stocks and making them available to industry. We have recovered the lines from our project and are maintaining them at the Maine Oyster Broodstock Development Program Hatchery at the Darling Marine Center and at the Marine Biological Laboratory, two of the region's most established marine labs. This 'repository' will continue to be maintained to provide the lines for our future research efforts, for the safe-keeping of the lines and, perhaps more importantly, so the lines can be made available for use by commercial and public hatcheries involved in aquaculture production and public restoration.

Objective 3 - We proposed to report on the progress of our project at regional and national meetings, to post results in a timely manner on a project-specific website, and to disseminate results to industry members via a Fact Sheet. As indicated in the appendix, below, we have met the first of these expectations through the delivery of at least 5 presentations at regional and national meetings and are currently in the process of placing project updates on the Maine Oyster Broodstock Development Program's website. We have recently completed and submitted to the NRAC office a Fact Sheet on the history of interline hybridization in the development of eastern oyster stocks along with an update of our project results that we expect will be available to the industry within a few weeks.

IMPACTS:

- The results of our project confirm the value of local selected and locally adapted lines.
- Our project has supported the continued production and testing of interline hybrids with potential for combined resistance to multiple diseases.
- Our study provides indication of relative conditions under which available lines and their crosses are likely to perform best.

RECOMMENDED FOLLOW-UP ACTIVITIES:

Our results argue for the continued maintenance and propagation of currently available oyster lines, such as UMFS and NEH, to preserve their unique characteristics while capitalizing on the increased growth performance and yield afforded by interline crossing. Future oyster broodstock development efforts should continue to monitor among-line variation in shell shape and meat condition in addition to growth, survival and disease incidence, to ensure that highly marketable lines are propagated for the northeast oyster industry.

SUPPORT:

YEAR	NRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1	$131,462	$48,747	$29,907		$3,930	$82,584	$214,046
2	$117,969	$50,273	$22,144		$3,930	$76,347	$194,316
TOTAL	$249,431	$99,020	$52,051		$7,860	$158,931	$408,362

PUBLICATIONS AND MANUSCRIPTS:

None to report

PAPERS PRESENTED:

Rawson, P., C. Davis, B. Barber, B. Hawes, and S. Feindel. OYSTER BROODSTOCK DEVELOPMENT IN MAINE: A COOPERATIVE EFFORT BETWEEN MAINE'S OYSTER INDUSTRY AND THE UNIVERSITY OF MAINE. Annual Meeting of the National Shellfisheries Association, San Antonio, TX, February 2007**.**

Rawson, P.D., X. Guo and S. Lindell. CROSS-BREEDING AND FIELD TRIALS FOR DISEASE RESISTANT OYSTERS. National Shellfisheries Association Annual Meeting, Providence RI, April 2008.

Rawson, P.D., X. Guo and S. Lindell. CROSS-BREEDING AND FIELD TRIALS FOR DISEASE RESISTANT OYSTERS. Northeast Aquaculture Conference and Exhibition (NACE), Portland ME, December 2008References Used – Get text from files.

Guo, X., P. Rawson, S. Lindell, and I. Sunila. CROSS-BREEDING FOR IMPROVED DISEASE RESISTANCE IN EASTERN OYSTERS, *CRASSOSTREA VIRGINICA*. National Shellfisheries Association Annual Meeting, Savannah, GA. March 2009.

Project Completion Report

Cross-breeding and Field Trials of Disease-Resistant Eastern Oysters

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PART II
TECHNICAL ANALYSIS AND SUMMARY

SIGNATURE PAGE

PROJECT CODE: 05-1 and 05-4

SUBCONTRACT/ACOUNT NO: Q169601 (year 1) & Q169901 (year 2)

PROJECT GRANT NUMBER: 2002-38500-12056 (year 1); 2003-38500-13505 (year 2)

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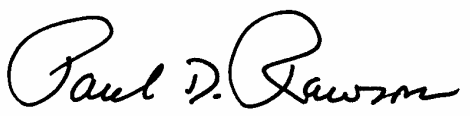

Project Coordinator

July 9, 2009
Date

PROJECT FINAL TECHNICAL REPORT

Cross-breeding and Field Trials of Disease-Resistant Eastern Oysters

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SUBCONTRACT/ACOUNT NO: Q169601 (year 1) & Q169901 (year 2)

PROJECT GRANT NUMBER: 2002-38500-12056 (year 1); 2003-38500-13505 (year 2)

DATES OF WORK: June 15, 2006 to July 15, 2008 (This was the grant period - due to the design of our field trials, monitoring of lines and disease testing continued through December of 2008 and April of 2009, respectively.)

PARTICIPANTS:

Funded Participants

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The research conducted under this award, through a collaboration between the University of Maine, the Marine Biological Laboratory, the Connecticut Bureau of Aquaculture, and Rutgers University, addressed the following objectives:

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METHODS AND PROCEDURES:

Objective 1

The ultimate goal of our research program is to provide commercial and public hatcheries with selected broodstock so that growers have access to oyster seed that perform optimally on their farm, and thereby improve the region's oyster production. Region-wide growers agree that the incidence of disease is the most serious impediment to increased production and revenue from cultured eastern oysters. The primary diseases of concern are MSX (and closely related SSO) and Dermo, caused by the protistan parasites *Haplosporidium nelsoni* and *Perkinsus marinus* respectively. The incidence of MSX and Dermo has increased in the past two decades and resulted in precipitous declines in the aquacultural production of oysters in some states. MSX and Dermo have historically been less of a problem for oyster culture operations in northern New England states. However, in this region, Roseaovarius Oyster Disease (ROD) causes stunting, uneven shell growth, and crop losses as high as 90% in some years (Davis et al. 1997). Thus, our project primarily focused on identifying oyster lines and interline hybrids that demonstrate higher survival under the variety of disease-pressures and grow-out conditions experienced in the New England region.

The project consisted of two overlapping field trials. For these field trials we created a series of pure and hybrid lines involving well-established commercially viable Rutgers University's NEH and University of Maine's UMFS lines. In addition, we deployed and tested two strains of local oysters from Edgartown Great Pond, MA (EGP line) and Clinton, CT, (Clinton line) which were developed from oysters that have survived heavy, annual disease pressure at New England latitudes. The following table briefly outlines the stocks deployed in our two field trials and their anticipated benefit.

Stocks deployed in northern and southern New England field trials

NEH - control with demonstrated MSX and Dermo resistance

UMFS - best northern line with ROD resistance and cold tolerance.

F1 (NEH x UMFS) -hybrid line with potential ROD and MSX/Dermo resistance

UMFS-BC (F1 x UMFS) - untested backcross with potential for better growth and survival

NEH-BC (F1 x NEH) - untested backcross with potential for better growth and survival

EGP - putative Dermo disease resistance, local MA strain.

Clinton - putative MSX resistance, local CT strain.

Because of hatchery limitations, not all lines were produced at a single hatchery. Instead, oyster seed from the NEH, UMFS, UMFS x NEH F1 and UMFSxNEH backcross lines were produced at the University of Maine's Oyster Broodstock Program (OBP) hatchery, while EGP and Clinton seed were produced at the Martha's Vineyard Shellfish Group (MVSG) and Noank Aquaculture Cooperative hatcheries in Massachusetts and Connecticut, respectively. Similarly, because the grow-out space at industry partners' lease sites is generally limited not all locations received all seven lines.

The two field trials we conducted in this project had separate but complementary goals. In the past, we observed that lines resistant to ROD had high mortality when subjected to Dermo and MSX and lines resistant to Dermo and MSX had poor survival at sites where ROD is problematic. Thus, in one field trial in northern New England we were primarily interested in testing the potential gains from cross-breeding between lines that are putatively resistant to ROD and Dermo. We deployed NEH, UMFS, and F1 hybrid and first generation backcross lines (UMFS/NEH F1 x NEH=NEH-BC and UMFS/NEH F1 x UMFS = UMFS-BC) at grow-out sites in Maine and at a control site in New Jersey in order to determine their resistance to Dermo and provide comparisons to the previous field trials conducted by Guo at this site. In the second field trial in southern New England we focused on the relative performance of local stocks and thus compared the performance of the "local" EGP and Clinton stocks to NEH derived (NEH, F1 and NEH-BC) oysters.

Northern New England Field Trial

Conditioning and spawning - Fifty oysters from the NEH, UMFS and UMFS x F1 hybrid lines were obtained and placed in quarantine at the Darling Marine Center/OBP hatchery in March of 2007. The oyster broods were acclimated to 22-24°C and fed a mixed diet of *Isochrysis galbana*, *Tetraselmis* sp., *Pavlova lutheri*, and *Chaetoceros muelleri*, provided through a pulse-feeding system. Animals were strip-spawned to

allow us to equalize each egg and sperm concentrations among parents and lines and to maximize the number of parents contributing to each line and enabled us to spawn all 50 animals from each of the three groups in synchrony so that the outcrosses could be performed and the eggs fertilized within a reasonable amount of time. For the strip spawning procedure, broodstock were shucked and immediately sexed by microscopically examining a small biopsy of gonad tissue at 40X magnification. Gonads were then scored with a disposable scalpel (changed after each oyster to prevent cross-contamination) and eggs were teased out of the gonad into 1 L cups containing filtered (1 µm) seawater at 23-24°C. Sperm was collected "dry" in a microcentrifuge tube and stored on ice until all animals were stripped. Egg suspensions were washed through a 150 µm sieve to eliminate debris and retained on a 20 µm sieve where they were briefly rinsed with filtered seawater and then combined into 20 L buckets. A dilute sperm suspension was made from each line and used to fertilize the egg buckets at an approximate ratio of 100 sperm per egg. Reciprocal hybrid lines (UMFS x NEH F1, UMFS-BC and NEH-BC) were produced by dividing egg suspensions from each of the contributing lines separately and fertilizing with sperm from the appropriate line. Reciprocal hybrids were kept separate until they developed into D-stage larvae and were then combined in equal numbers. Eggs were stocked into 300 L conical tanks at densities of 40-50 eggs•ml^{-1} and thinned to densities of 10 larvae•ml^{-1} at the time of first drain-down the following day.

Larval, post-set, and nursery culture - Larvae were raised at 24°C at a salinity of 27-30 ppt and fed a mixed diet of *Isochrysis galbana, Pavlova lutheri, Pavlova sp*. (CCMP459), and *Thallasiosira pseudonana* (strain 3H). Tanks were drained down every other day. Larvae were gradually thinned to density of 1-2 individuals•ml^{-1} mL prior to setting but size culling was kept to a minimum. Two tanks of each line were maintained to serve as a back-up in case of culture failure in one of the tanks. Upon becoming competent, larvae were placed into screened (180 µm) floating wooden tray culture units with microcultch. These bins were rinsed daily and tanks were cleaned and refilled with filtered seawater every other day. Post-set animals were pulse-fed a mixed diet of live algae. Bins were washed every four days and graded as needed (while maintaining the smallest healthy grade).

When the animals reached a size of 1-2 mm they were placed in a recirculating upweller tank. Every 1-2 d the tank water was replaced with 1 µm filtered seawater and the animals were pulse-fed a mixture of live algae (algae species listed previously in addition to *Tetraselmis chui, Chaetocerous meulerii,* and *Rhodomonas* sp.) and algae paste (Reed Mariculture, Campbell, CA, Shellfish diet 1800). While oysters of this size are normally put into a system where they feed off natural phytoplankton, our protocol of maintaining the seed in a filtered sea-water system and feeding cultured algae was included as an added safeguard against potential disease transfer from raw Damariscotta River water to sites beyond the river. Ford and Borrero (2001) found that cohorts maintained inside a hatchery experienced no ROD and that ROD occurrence was site-related rather than traceable to a particular broodstock or hatchery.

Upon reaching a size of 3-4 mm, oyster seed from each line was placed inside three replicate window-screen mesh "inserts" at a density of 1000 seed per insert. These mesh inserts were placed inside the typical ADPI bag system used for surface culture of oysters at each site. Replicates were identified by inclusion of a color and number-coded cattle tag in the bag (NASCO, Modesto ,CA). Three replicate inserts from each of the five lines were deployed at the Rutgers University Haskins Shellfish Laboratory's Cape Shore site, Pemaquid Oyster Company's and Glidden Point Oyster Company's lease sites on the Damariscotta River, Maine and at the Baggaduce River Oyster Company's lease site on the Baggaduce River, Maine (4 sites x 5 lines x 3 replicates = 60 bags or 60,000 animals total for field trial 1).

Field Monitoring - The performance (mortality and size) of oysters in all deployed bags was assessed every 2-3 months during two growing seasons (2007 and 2008). The measuring protocol consisted of recording the total wet-packed volume, haphazard sampling of individual animals and measuring the shell height (distance from the umbo to outer growing edge) of 50 or more animals while separating out mortalities, using an electronic hanging balance (Best Weight HS-15) to record total bag weights and an OHaus Navigator™ field balance to record individual weights. Animals that were cemented together ("doubles") were assumed to be growing abnormally and excluded from the sample. An Allegro™ handheld computer with attached digital calipers/ balance was used to record all data. The number of mortalities from the sample was tallied and general comments on the seed batch noted. Occasionally it was necessary to cull contaminating organisms (e.g. mussels) or dead shell, if mortalities were significant. Bags were thinned by reducing the oyster volume by half when necessary with the other half going back to the cooperating grower's crop. Final grow-out densities were approximately 125-300 oysters per bag depending on the site. Oysters were recovered from the Maine grow-out sites in mid-December of 2007 and overwintered in the hatchery at the Darling Marine Center. They were redeployed in May of 2008 for the second season.

Southern New England Field Trial - Methods

In southern New England, we planted five lines of oysters; three lines were identical to those grown in Maine and New Jersey – NEH, NEHxUMFS F1, and NEH-BC. The other two lines were selected from oyster populations local to two of our grow-out sites, Edgartown Great Pond (EGP) and Clinton, CT where natural and persistent disease pressures have been measured. Hereafter, these two additional lines will be referred to as Clinton and EGP.

Conditioning and spawning - Fifty oysters from the Clinton, CT site were obtained and conditioned in the Noank Cooperative Hatchery in Clinton, CT. Similarly, fifty oysters from the Edgartown Great Pond site were obtained and conditioned in the Martha's Vineyard Shellfish Group Hatchery in Vineyard Haven, MA. Standard oyster breeding and larval rearing protocols, as described for the northern New England field trial, were followed.

Larval, post-set, and nursery culture - Larvae were raised at 24°C at a salinity of 27-30 ppt and fed a mixed diet of *Isochrysis galbana, Pavlova lutheri, Pavlova sp*. (CCMP459), and *Thallasiosira pseudonana* (line 3H). Tanks were drained down every other day. Larvae were gradually thinned to density of 1-2 individuals•ml^{-1} mL prior to setting but size culling was kept to a minimum. Two tanks of each line were maintained to serve as a back-up in case of culture failure in one of the tanks. Seed oysters from the NEH, NEHxUMFS F1, NEH-BC and Clinton lines arrived via overnight shipment from Maine and Connecticut on June 19, 2007. The seed was counted, packed and shipped and planted in Wellfleet that day. Seed was distributed and planted in Rhode Island and Connecticut on June 20, 2007, and to Edgartown Great Pond on Martha's Vineyard on June 21, 2007. Seed oysters belonging to the EGP line arrived from MVSG on July 17, 2007 a month later than most the other seed due to problems at the nursery upweller site and natural food abundance.

Delivered seed were 3-4 mm in size and were placed inside window screen mesh "inserts" for each site at a density of 1200 seed per insert. Three replicate inserts from each of the five lines were deployed at each site; the Cedar Island Marina in Clinton CT, Winnapaug Pond in Westerly RI, Edgartown Great Pond in Martha's Vineyard, and Wellfleet Bay, MA (4 sites x 5 lines x 3 replicates = 60 bags or 60,000 animals total). These mesh inserts were placed inside typical ADPI bags used for surface culture of oysters at Edgartown Great Pond, and in bag and racks setups in Westerly, RI (subtidal) and Wellfleet, MA (intertidal). Custom-made hanging trays suspended under a dock were used in Clinton, CT (subtidal) which supported mesh bags. Replicates were identified by inclusion of a color and number-coded tag in the bag. The four sites are hereafter referred to as Connecticut, Rhode Island, Martha's Vineyard and Wellfleet.

Field Monitoring - The performance (mortality and size) of oysters in all deployed bags was assessed every 6 to 8 weeks during two growing seasons (2007 and 2008). The measuring protocol consisted of (1) recording the total wet-packed volume, (2) haphazard sampling of individual animals and measuring the shell height (distance from the umbo to outer growing edge) of 30 or more animals, (3) separating and counting mortalities, and (4) recording total bag weights via an electronic hanging balance (Best Weight HS-15). Animals that were cemented together ("doubles") were assumed to be growing abnormally and excluded from the sample. Occasionally it was necessary to cull contaminating organisms (e.g. slipper shells) or dead shell, if mortalities were significant. Bags were thinned by reducing the oyster volume by half when necessary with the other half going back to the cooperating grower's crop. The first assessment of mortality (November 2007) was coincident with the last sampling of the first season; up until that time we could not reliably distinguish live from dead oysters as they were still small. Final grow-out densities were approximately 125-300 oysters per bag depending on the site. Oysters were over-wintered at each field site or nearby in deeper water and redeployed in April of 2008 for the second season.

Disease Testing (Southern New England Field Sites Only) - Approximately 30 oysters from each of the five lines deployed at the southern New England sites were sampled in the fall of 2007 and 2008 disease testing to estimate the prevalence and intensity of MSX, SSO, ROD and Dermo infection in each stock. The disease sampling and testing work was coordinated by Lindell and conducted by Sunila (histological analyses) and Roberts (molecular analyses). Initially oysters were processed for histological examination using standard methods (Howard et al. 2004). A 4-mm cross-section was excised from each oyster and fixed in 10% formalin in seawater. The samples were then sent to be processed by Massachusetts Histology Services to produce a 5µm Hematoxylin-eosin stained slide. Slides were "read" (diagnosed) for infectious agents, such as lesions caused by viruses, bacteria, prokaryotes (Chlamydia, Mycoplams and Rickettsia), ciliates, gregarines, and trematodes., and haplosporidian parasites MSX (*H. nelsoni*) and SSO (*H. costale*). Haplosporidian infections were staged as initial, intermediate, advanced, and terminal to allow prognosis of survival time. Slides were diagnosed for inflammatory responses (acute and chronic), degenerations (inclusions, vacuolization), ceroidosis), cell and tissue death (necrosis, apoptosis), growth derangements (hyperplasia, metaplasia), hemodynamic and fluid derangements (edema, hemorrhage), and neoplasia (benign or malignant) according to Sunila (1998) to assess not only pathological lesions caused by parasites, but also the general health of the oysters. In addition, DNA was isolated from gill tissue from each oyster sampled for disease testing using standard methods (e.g.10% Chelex). The DNA was used in QPCR assays for quantifying the level of *P. marinus* (Dermo) infection. The QPCR assay for *P. marinus* has recently been validated by comparison of RFTM and QPCR assay results on samples from a common set of oysters (DeFaveri et al. 2009). Using a dual-labeled probe approach, this assay accurately detects from one to several thousand *P. marinus* organisms present in oyster tissues. The assays were conducted by the Roberts Lab and included standard curves with known amounts of *P. marinus* and proper positive and negative reaction controls. The prevalence of pathogens and lesions were compared using a graphical approach.

Statistical Analyses for Both Field Trials - Periodic measures of line performance were taken throughout the experiment at all locations. Tracking line performance through time can facilitate identifying critical time points at which adjustments in husbandry methods may improve line performance. Such an analysis may also highlight time points at which genetic selection should be focused for improved performance. This is particularly true with respect to mortality where regular, periodic monitoring of mortality may help to identify time periods when mortality is acute, perhaps due to disease. In this project report, we present graphical analysis of cumulative mortality for each line at each location as a function of sampling period and where appropriate we can correlate dramatic changes in mortality with disease prevalence.

However, the focus of our study is on the relative performance of each line over a typical production cycle. Thus, we present a graphical analysis of relative performance for each line at each location for production characteristics including total cumulative mortality, final shell height, final wet weight and adjusted yield, where yield is estimated as [(1-cumulative mortality) * (final weight) * 1000] for each replicate bag at each site. This

measure provides an estimate of the final biomass harvested for each line per 1000 oysters deployed at the start of the experiment.

Statistical analyses of differences in line performance were conducted via analysis of variance (ANOVA). Because culture conditions were not standardized between locations and due to the complete loss of the UMFS line at the Cape Shore site, and EGP line at the Wellfleet and Connecticut sites (which would create an imbalanced design with missing cells), we relied on a single factor ANOVA testing for line effects on a site by site basis. In each case, we considered line to be a fixed effect because we are interested only in direct comparisons among the lines deployed in our study. Each ANOVA was run using the General Linear Model routine in SYSTAT. When a significant line effect was detected we conducted post-hoc pairwise comparisons between lines with a Bonferroni correction. For some analyses in the Northern New England field trial, we used both a Lillefors test and an examination of the distribution of residuals to determine the appropriateness of the ANOVA model. For total cumulative mortality and adjusted yield the Lillifors test were both significant and the residuals non-randomly distributed. We used an arcsine transformation for total cumulative mortality and a logarithmic transformation for adjusted yield in order to improve the fit of the ANOVA models for these two variables.

The primary goal of selective breeding is to produce lines that show highest performance across multiple sites and uniform performance at each site. To assess uniformity of performance we examined the coefficient of variation in shell growth among individual oysters in each replicate bag for each line at each site. To assess the performance of lines across sites we compared the marginal means for each line with performance at each site receiving equal weighting.

One concern for a broodstock program focused primarily on fast growth is the resulting lines may have enhanced shell growth but poor quality meat or shape which, in turn, could result in an eventual loss in market value. We used several methods to assess the meat quality and shape of oysters from each of the lines grown at the Pemaquid Oyster Co. culture site on the Damariscotta River. First, we simply compared meat-to-shell ratios to how consistently meat growth corresponds to shell growth for each line. A second approach compared the shell height (SH) to width (SW), and shell height to shell inflation (SI) ratios (see Figure 1). This approach attempted to determine the relative cupping of the shell. Since deeply cupped oysters usually contain more meat, they are more desirable in the market place while a stock that is losing its cup would be undesirable. We also determined the condition index (Crosby and Gale,

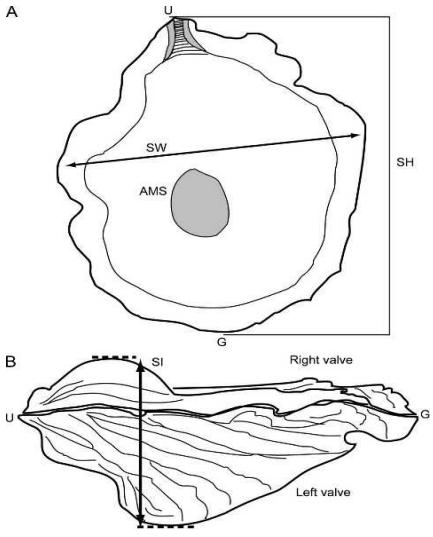

Figure 1. Shell measurements used to estimate shape and degree of cupping (as per Harding,2007).

1990) by measuring individual live weights, sacrificing the oyster and separating all tissue from the shell and then determining the shell weight, and the dry meat weight. Condition index typically compares the dry meat weight of an oyster to the internal cavity volume. However, Lawrence and Scott (1982) have pointed out that the density of oyster meats have a density of approximately 1 $g \bullet cm^{-3}$, and internal cavity volume can be estimated by subtracting the weight of the shell valves in air from the total live weight of an oyster in air. Thus, condition index for each of 30 oysters from the UMFS, NEH, F1, and UMFS and NEH backcross lines was estimated as:

CI = dry soft tissue weight (g) x 1000 / [valve weight (g) - total live weight (g)]

The shell height, dry weight, condition index, and the shell dimension ratios were statistically analyzed using nested analysis of variance (ANOVA) with oyster line as the main effect and replicate bag within line as a nested effect using the General Linear Model option in SYSTAT.

RESULTS AND DISCUSSION

Northern New England Field Trial Results

There were dramatic differences in mortality among lines at all four sites in the northern New England field trial (Fig. 2). At the Cape Shore "control" site, where Dermo is endemic, we observed an extremely high rate of mortality for both the UMFS and UMFS-BC backcross lines from the outset of the experiment. Mortality for both these lines exceeded 60% within three months of deployment. For the UMFS line mortality was 100% by the end of the first winter and though the mortality rate for the UMFS backcross line declined, cumulative mortality exceeded 80% by the end of two grow-out seasons. These results are consistent with previous studies that concluded the UMFS line has no demonstrated resistance to Dermo, although we did not explicitly test for Dermo prevalence among oysters deployed at Cape Shore as part of this experiment. In contrast, although there was substantial mortality for the NEH, NEH backcross and F1 lines at the Cape Shore site, the mortality rate was consistent across time periods. The high rate of morality for the UMFS line at Cape Shore has been observed in previous field trials at this site that inlcuded the UMFS line. Our trial is the first to include both an UMFS x NEH F1 and UMFS backcross lines and our results suggest that there may be an additive effect for mortality at the Cape Shore site; with an increase in the contribution from the UMFS line there is an associated and almost linear increase in the level of mortality at every time point in our experiment. The lone exception to this observation is the F1 lines for which mortality is only marginally higher than the mortality observed for the NEH backcross line.

Cumulative mortality was much lower at the three Maine sites where these same lines were deployed. Overall, mortality was the lowest at the Baggaduce Oyster Co. site on the Baggaduce River where mortality did not set in until late in the first season and principally among NEH oysters. Mortality for the NEH line at this site was nearly 30%

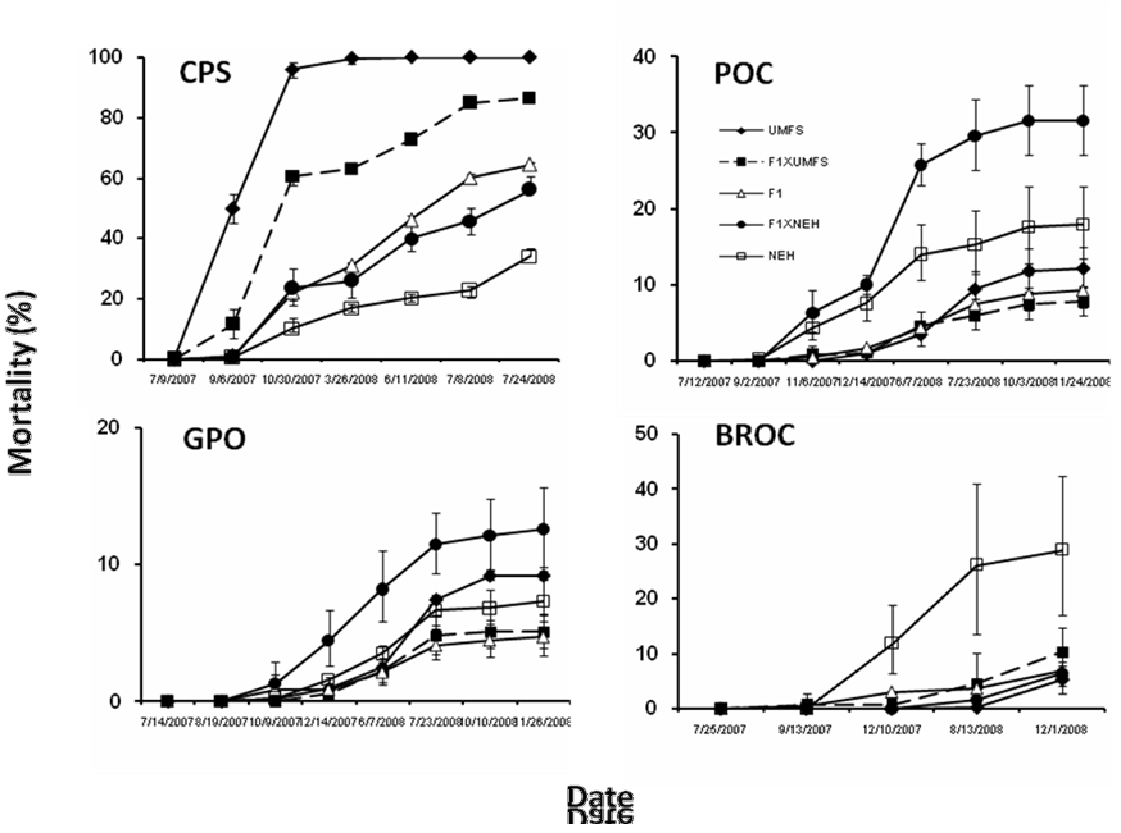

Figure 2. Cumulative mortality among the UMFS (closed triangles), NEH (open square), UMFS x NEH F1 hybrid (open triangle), F1 x UMFS backcross (closed squares) and F1 x NEH backcross (closed diamonds) lines at the Cape Shore (CPS), Pemaquid Oyster (POC), Glidden Point (GPO) and Baggaduce River (BROC) test sites. Symbols represent the mean cumulative mortality among three replicate bags while error bars indicate the mean ± standard error.

by the end of the field trail even though mortality for the other four lines did not exceed 10%. Mortality among oysters deployed at both the Glidden Point Oyster Co. and Pemaquid Oyster Co. sites was relatively constant over time. The only notable exception is a sharp increase in mortality for the NEH backcross line at the Pemaquid Oyster site during the overwintering period between seasons. At both of these sites, mortality for the F1 and UMFS backcross lines was the lowest at all sampling periods with relatively higher mortality for the UMFS, NEH and NEH backcross lines.

Final cumulative mortality estimates were examined both graphically and through ANOVA (Fig. 3). As discussed above, there were dramatic differences in the overall morality rate, for all lines combined, among sites. Mortality was highest at the Cape Shore site and lowest at the Glidden Point, Maine site. ANOVA indicated that there were line specific differences in mortality at three of the four sites in this field trial. At the Cape Shore site, the NEH line had the lowest total cumulative mortality while the F1 and NEH backcross lines had intermediate and statistically indistinguishable total mortality and the UMFS and UMFS backcross lines had the highest mortality. This pattern was

somewhat reversed at the Pemaquid Oyster Co. site where the UMFS, UMFS backcross and F1 lines had roughly equivalent and low mortality while the NEH backcross and NEH lines had significantly higher mortality. The single factor ANOVA for mortality at the Glidden Point site suggested that there was a significant effect of line. However, post-hoc tests failed to detect significant pair-wise differences in mortality at this site. Finally, there was no evidence for line-specific mortality at the Baggaduce River Oyster Co. site.

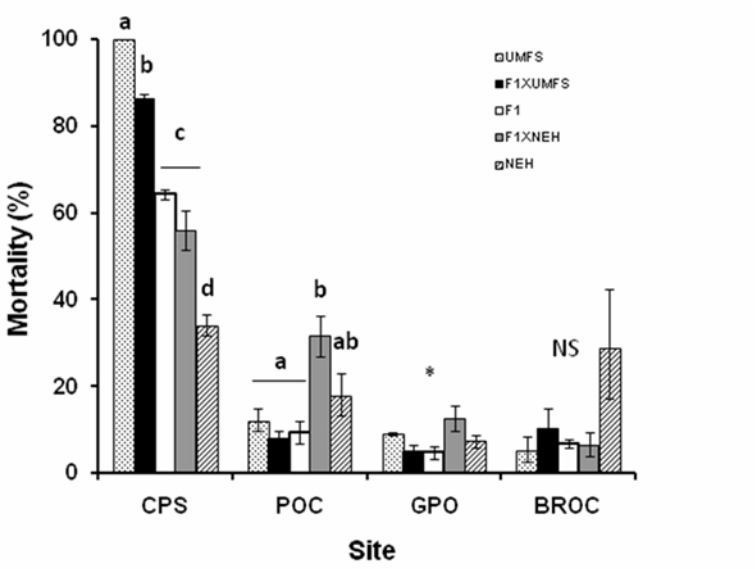

Figure 3. Final cumulative mortality among three replicate bags for each line deployed at the Cape Shore, Pemaquid Oyster, Glidden Point and Baggaduce River sites. Error bars indicate the mean ± standard error for each line at each site. Letters above each bar indicate which means were significantly different from one another within a site as determined by *post-hoc* Bonferroni pairwise comparisons. NS – no significant differences among means; * - ANOVA indicated significant line effects but no significant differences detected in post-hoc comparisons.

We observed line and site-specific effects on two measures of growth, shell height and wet weight (Figure 4). In general, the oysters at the Cape Shore site were at least 33% smaller (with respect to both shell height and wet weight) compared to oysters at the Maine sites at the end of the experiment. There were no significant differences in growth among lines at the Cape Shore site with the exception of the UMFS line which had lowest growth simply by virtue of the fact that all UMFS oysters had perished by the end of the experiment. The highest growth was obtained among oysters deployed at the Pemaquid Oyster Co. site. In general, the UMFS, UMFS backcross and F1 lines outperformed the NEH backcross and NEH lines with respect to growth at the POC site. Growth was slightly lower for the oysters held at the Glidden Point Oyster Co. site relative to those held at the Pemaquid Oyster Co. site. At GPO, there was no evidence of line-specific variance in growth with respect to shell height, although the F1 line had significantly higher weight than the NEH line after two seasons of growth. Growth at the Baggaduce River Oyster Co. was intermediate to that observed at the two other Maine sites and the Cape Shore site and there were no significant line effects on growth at this site.

Given the line and site-specific differences in mortality and growth presented above, it is not surprising that we also observed dramatic differences in adjusted yield (Fig. 4; bottom). The lowest adjusted yield was obtained at the Cape Shore site where yield for all

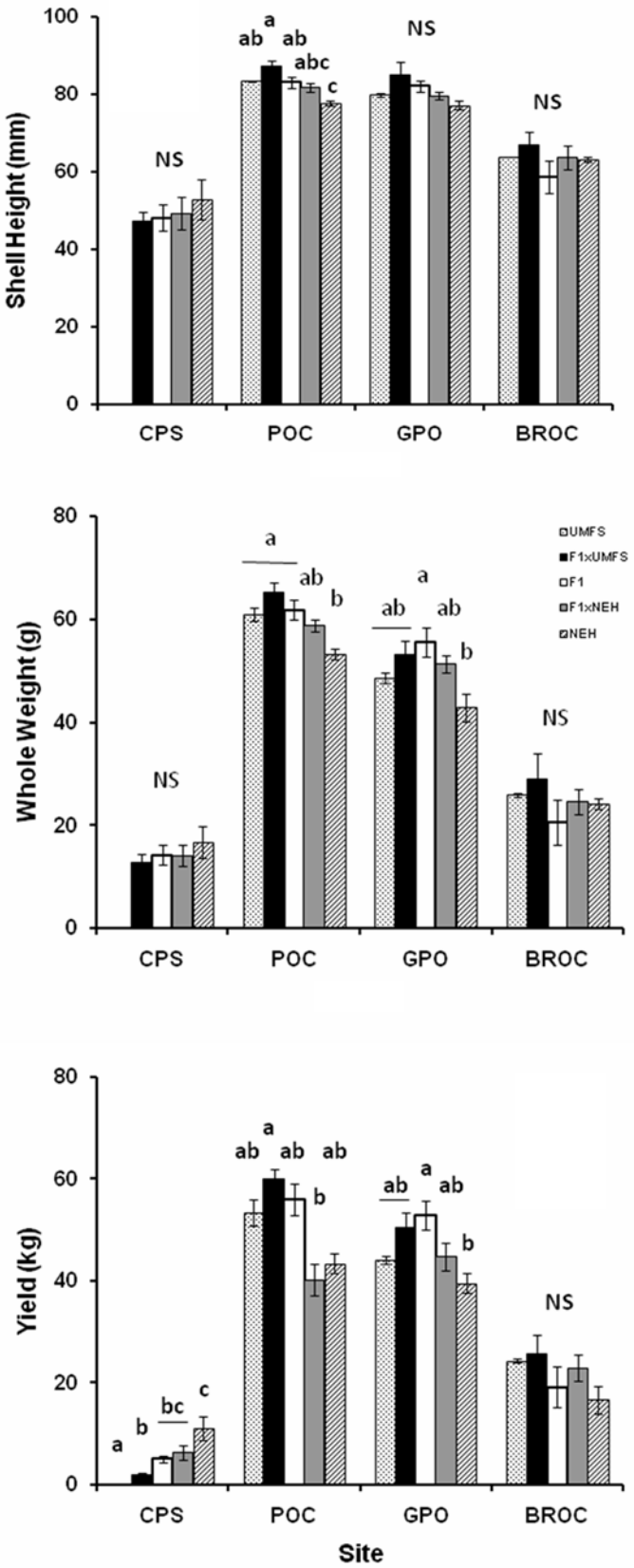

Figure 4. Mean shell height (top), wet weight (middle), and yield (bottom) among three replicate bags for each line deployed at the Cape Shore, Pemaquid Oyster, Glidden Point and Baggaduce River sites. Error bars indicate the mean shell height, wet weight, or yield, respectively, ± standard error for each line at each site. Letters above each bar indicate which means were significantly different from one another within a site as determined by *post-hoc* Bonferroni pairwise comparisons. NS – no significant differences among means; * - ANOVA indicated significant line effects but no significant differences detected in post-hoc comparisons.

five lines was less than 10kg. In contrast, yield at both the Pemaquid Oyster Co and Glidden Point Oyster Co sites exceeded 40 kg for all lines. There were significant differences in adjusted yield among lines at these three sites. At Cape Shore yield was closely associated with differences in survival; yield for the NEH line was higher than the NEH backcross and F1 hybrid lines while the UMFS line and UMFS backcross line had the poorest yield. In contrast, yield at the Pemaquid Oyster Co and Glidden Point Oyster Co. sites was highest for lines with a large UMFS genetic contribution. At Pemaquid Oyster Co site, the UMFS backcross line had the highest yield followed by the UMFS and F1 hybrid line while at Glidden Point the F1 line slightly outperformed the UMFS backcross and UMFS lines. Similar line-specific variation in yield was observed at the Baggaduce River Oyster Co site, although the differences there were not statistically significant. At all three Maine sites the NEH line provided the lowest yield and line-specific differences in yield appeared to be most strongly influenced by growth.

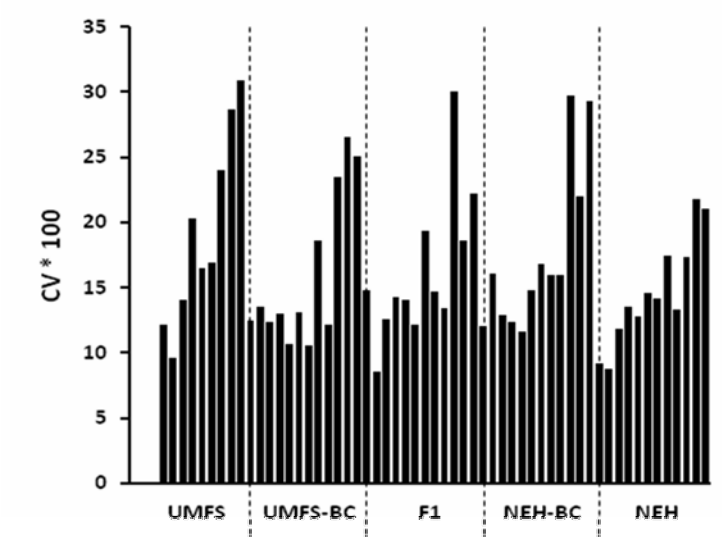

Figure. 5. Coefficient of variation (CV) for shell height among individual oysters in each replicate at each site. Estimates for each line are arranged by site with Cape Shore on the left followed by the Pemaquid Oyster Co site, the Glidden Point Oyster Co. site and Baggaduce River Oyster Co. site.

One goal of selective breeding programs is to create cultivars with uniform performance under the variety of conditions where they are grown. This goal can be hard to achieve with aquaculture species for which it is often difficult to standardize culture conditions. We assessed whether there were line-specific differences in phenotypic variation by examining the coefficient of variation for shell height in each replicate bag for each line at all four sites (Fig. 5). Because individual replicate bags can experience very different microenvironments at any given location, we analyzed the level of variation in growth as a function of the mean (coefficient of varation) at the replicate level. One expectation of such an analysis is that variation should be lower for the replicates of the parental lines (UMFS and NEH) if these two lines have lower underlying genetic variation for growth traits (i.e., show signs of inbreeding as has been suggested by genetic analysis). Graphical analysis of the coefficients of variation suggests that standardized variance was roughly 60-70% higher for all lines at the Baggaduce River Oyster Co. site. In contrast, there were only small differences in the level of within replicate variance among lines. Thus, the parental lines did not exhibit more uniform growth than the hybrid lines in our

experiment. However, we cannot address whether they exhibit more or less uniform growth when compared to non-selected lines.

Southern New England Field Trial Results

Large differences in cumulative mortality of all oyster lines across sites were observed (Fig. 6). The EGP line consistently performed the worst at all sites except on Martha's Vineyard, where this line is endemic and had the lowest mortality. Higher mortality for the EGP line can also be attributed to a later deployment date than other lines and thus culminating in a shorter first growing season and smaller size at the onset of winter. At the Wellfleet and Connecticut sites 100% mortality was recorded for the EGP line. Excluding the EGP line, all the lines had similar mortality across sites except for the Clinton line which suffered 5-10% higher mortality at all sites except Rhode Island and the NEH line which had 15% higher mortality at the Rhode Island site.

Site specific mean mortality for combined lines was highest in Rhode Island and lowest at Martha's Vineyard. However, the abnormally high mortality attributed to the EGP line

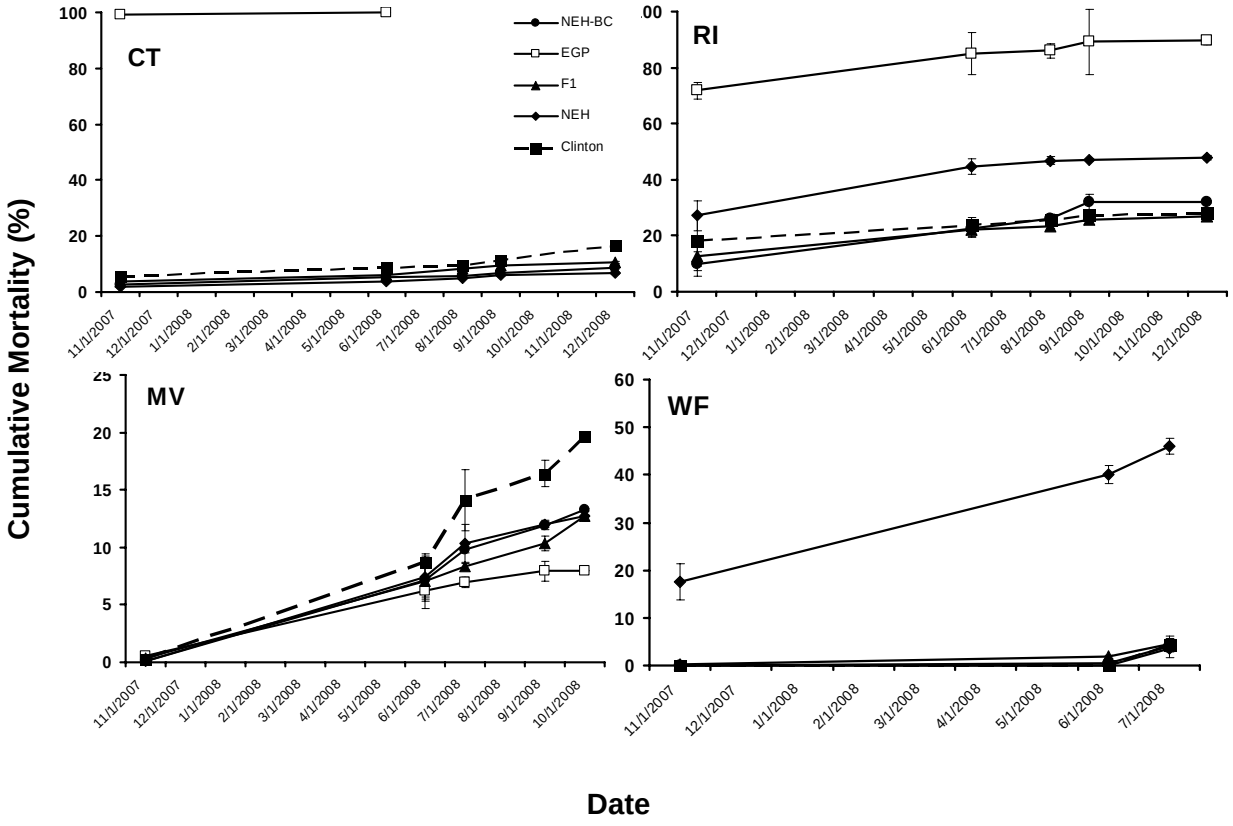

Figure 6. Cumulative mortality among the F1 x NEH Backcross (NEH-BC), EGP, NEH x UMF (F1), NEH and Clinton lines deployed at Connecticut (CT), Rhode Island (RI), Martha's Vineyard (MV), and Wellfleet (WF) sites. Symbols represent the mean cumulative mortality among three replicate bags while error bars indicate the mean ± standard error.

skewed the mean mortality at all sites. Removing the EGP data decreased mean mortality at Rhode Island from 44.9% to 33.7%, from 33.0% to 16.2% at Wellfleet and 28.5% to 10.7% in Connecticut respectively.

Mortality remained relatively constant (between 5-10%) at our Martha's Vineyard site for all five lines over-wintered from November, 2007 until June 2008. Second year sampling cumulative mortality diverge among lines. The Clinton line suffered the worst with over twice the cumulative mortality of the best surviving EGP line. The NEH-BC, NEH and F1 lines all suffered intermediate cumulative mortality. At the Rhode Island site over 70% of the EGP line had died by the time of November 2007 mortality count. The NEH line had lost over 25% by November 2007, with the remaining lines losing less than 20%. The rate of mortality remained relatively constant across lines, and therefore mortality in the first season played a substantial role in final cumulative mortality.

Final cumulative mortality estimates (Fig. 7) were examined using ANOVA. No significant differences were detected in final cumulative mortality at the Martha's Vineyard site, although there was ~2.5 fold higher mortality for the Clinton line relative to the EGP line. This lack of significance for this difference is likely driven by the high variance and low power associated with an ANOVA based on three replicates for each line and we suggest that a significant difference would likely have been detected at the Martha's Vineyard site with greater sampling. Significant differences were detected for the EGP line at both Wellfleet and Connecticut, where they suffered 100% mortality. No significant differences were detected in final cumulative mortality for the remaining lines.

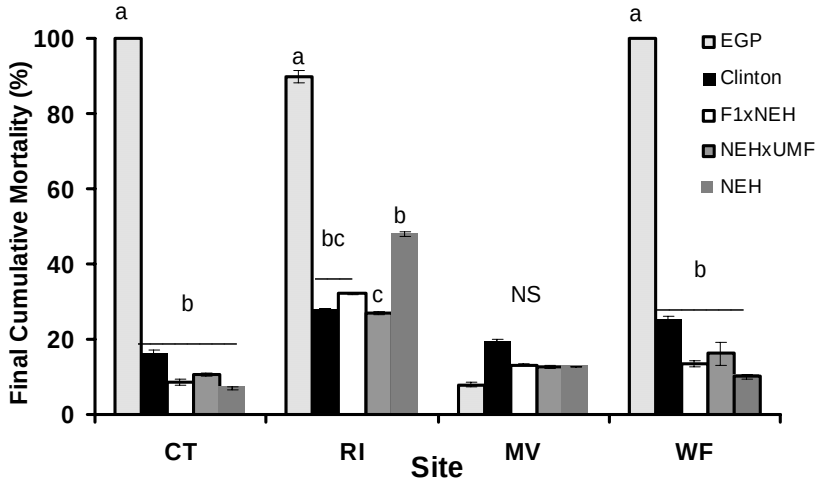

Figure 7. Final cumulative mortality among three replicate bags for each line deployed at Connecticut, Rhode Island, Martha's Vineyard, and Wellfleet sites. The height of the bars indicates the mean cumulative mortality among three replicate bags for each line while the error bar indicates the mean ± standard error. Letters above each bar indicate which lines were significantly different from one another. Non-significant differences are represented by NS.

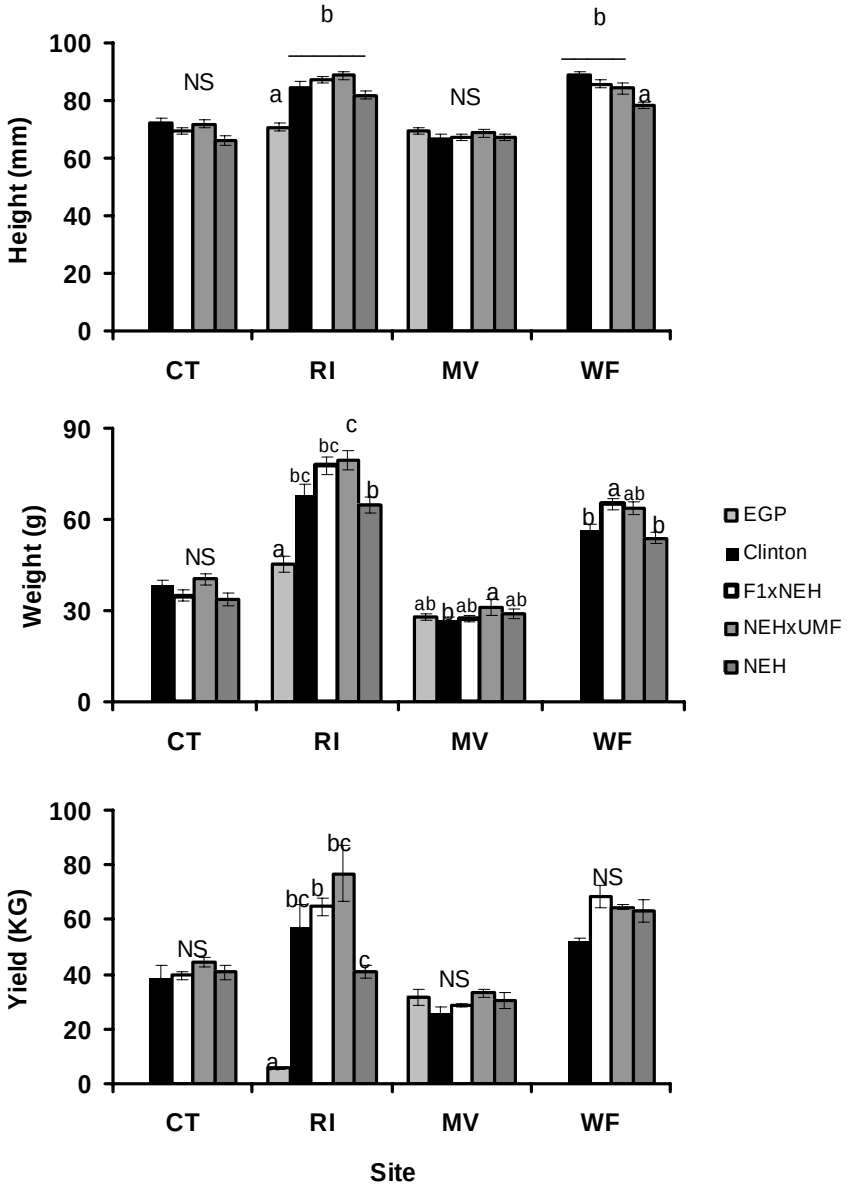

Figure 8. Mean final shell height (top), wet weight (middle) and yield (bottom) at the end of two grow-out seasons for oysters from the NEH, F1, NEH-BC, Clinton and EGP lines at Connecticut (CT), Rhode Island (RI), Martha's Vineyard (MV), and Wellfleet (WF) sites. The height of the bars indicates the mean shell height, weight or yield, respectively, among three replicate bags for each line while the error bar indicates the mean ± standard error. Letters above each bar indicate means that were significantly different from one another within a site as determined by *post-hoc* Bonferroni pairwise comparisons. NS – no significant differences among means.

Oyster mean growth across all lines was consistently better in terms of live weight and shell height at the Rhode Island site (Fig. 8). However, oysters grown at the Rhode Island site were not significantly larger than Wellfleet grown oysters. Rhode Island and Wellfleet grown oysters were significantly larger than Connecticut and Martha's Vineyard oysters in both growth parameters. Line and site-specific effects were observed for both shell height and live weight of oysters. Oysters at commercial grow-out sites (Wellfleet and Rhode Island) were generally 20% longer and 50% heavier than those grown at non-commercial grow-out sites (Connecticut and Martha's Vineyard). No significant difference was detected in shell height among lines at Martha's Vineyard; however, a significant weight effect was detected between the F1 (NEHxUMFS) and Clinton lines. No significant differences in shell height and live weight were detected among lines deployed in Connecticut. Shell height of the NEH line was significantly smaller than the other lines in Wellfleet. Oyster live weight of the Clinton and NEH lines was also significantly different from the F1xNEH line in Wellfleet. The EGP line in Rhode Island was significantly smaller (in both shell height and live weight) than other lines. This could be explained by later deployment and shorter first field season than other lines. The NEH line was significantly different from the NEHxUMF F1 line in live weight. Graphical examination of the coefficient of variation for shell height as a function of site and line (Fig. 9) suggests that growth was most uniform for the NEH-BC line and most variable for the Clinton line regardless of site while growth for all lines was more uniform at the Martha's Vineyard and Connecticut sites than at the Rhode Island and Wellfleet sites.

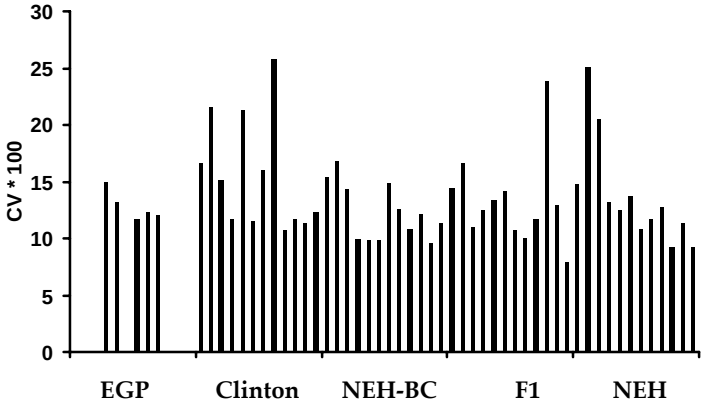

Figure 9. Coefficient of variation for shell height among individual oysters in each of three replicates for each line at each site. Estimates for each line are arranged by site with Rhode Island on the left followed by Wellfleet, Connecticut and Martha's Vineyard sites.

Disease Testing: Southern New England Sites

No MSX was detected at either the Martha's Vineyard site or the Rhode Island site while the highest MSX prevalence was observed at the Wellfleet site (Fig. 10). The most heavily infected line was the NEHxUMF F1 line followed by Clinton, NEH-BC and NEH. MSX infection at the Connecticut site was substantially lower than Wellfleet. The highest MSX infection at the Connecticut site was observed in the Clinton line and the lowest in the NEH line. At both sites where MSX was recorded NEH had the lowest infection rate which is consistent with previous observations that this line demonstrates both Dermo and MSX resistance. These were the two sites which recorded 100%

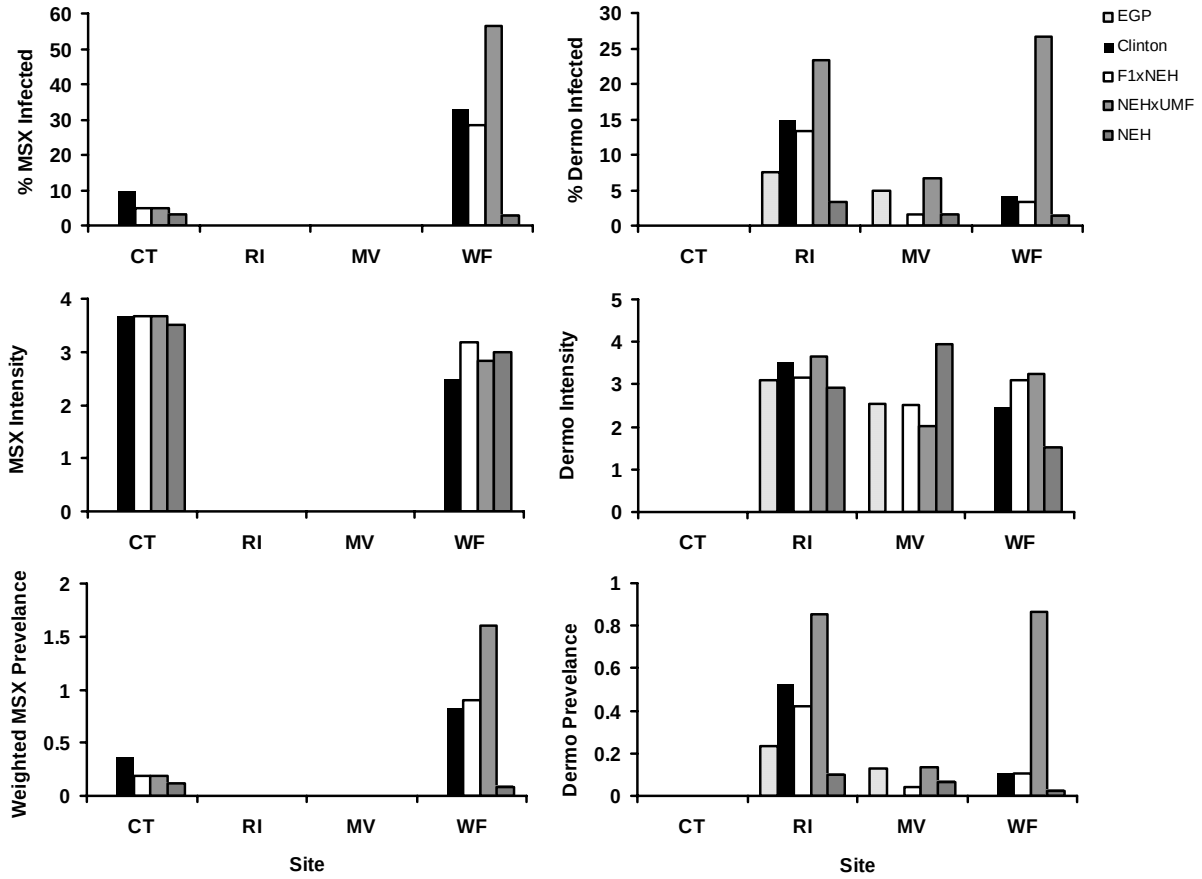

Figure 10. Patterns of infection for MSX (left) and Dermo (right) at the southern New England field sites. The percentage of oysters in the EGP, Clinton, NEH-BC, F1 and NEH lines with signs of infection at each site are shown in the top panels while the intensity of MSX and Dermo infections are shown in the middle panels. The intensity of MSX infections was classified into stages as follows; 1: initial (infection limited to gill epithelium), 2: intermediate (few isolated foci in the digestive diverticulum besides the epithelium), 3: advanced (plasmodia dispersed throughout the tissues), 4: terminal (profuse parasitemia in all tissues). The intensity of Dermo infections was determined by QPCR as outlined in DeFaveri et al. (2009). The weighted prevalence of MSX and Dermo in each line at each site is presented in the bottom panels with the prevalence of MSX infections estimated as the sum of MSX stages divided by the number of oysters infected by MSX, and the prevalence of Dermo infections estimated as the sum of Makin scores divided by the number of oysters infected by Dermo.

mortality for the EGP line and therefore no EGP samples were available for MSX analysis. Mean MSX intensity across lines was higher at the Connecticut site, however, this was based on a total of 14 infected oysters and no line had more than 6 oysters from any individual line were found to be infected with MSX. Mean MSX intensity was lower for the Wellfleet grown oysters relative to those grown in Connecticut due to relatively low prevalence but high incidence (a total of 76 MSX infected oysters were observed at Wellfleet). At the Wellfleet site, the highest mean MSX intensity was for the NEH line and the Clinton line had the lowest mean MSX intensity.

No Dermo was detected at Connecticut site. The highest Dermo prevalence was observed at the Rhode Island site where 12% of all oysters tested had some stage of Dermo infection. The most heavily infected line was NEHxUMF F1 which had infection rates twice the other strains in Rhode Island, Martha's Vineyard and Wellfleet. The average level of Dermo infection at the Martha's Vinyard site was substantially lower than at the Rhode Island and Wellfleet sites while the mean Dermo intensity across all lines was highest at the Rhode Island site. The highest mean infection stage was 4 (on the Makin scale) for the NEH line grown in Martha's Vineyard, even though this line has high survival at the Cape Shore site where Dermo is endemic. The NEHxUMF F1 had the highest mean Dermo infection rate in Rhode Island and Wellfleet

Shell Shape & Oyster Condition Analysis

The average shell height, wet weight (not shown) and dry weight for subset of oysters we used in our analysis of shell shape and condition were highly similar to that seen in the larger sample presented earlier in this report (see Figs. 4 and 11). Shell height was significantly smaller for the NEH relative to the other four lines while the UMFS and UMFS-BC lines had the fastest shell growth. Differences in shell growth correspond closely with differences in meat growth. However, the variation in meat weight among individuals within replicates at the Pemaquid site substantial and the differences among lines was not statistically significant.

Height-to-shell inflation ratios were compared to height-to-width ratios to investigate variation in shape among oysters of the five lines in this project (Fig. 12). Large height to width ratios are indicative of oysters that are growing faster along the height axis than the width axis and are "long" while low values indicate oysters that are "broader". On the other hand, large height to shell inflation ratios occur when oysters are relatively flat with no cupping and little internal cavity and meat while

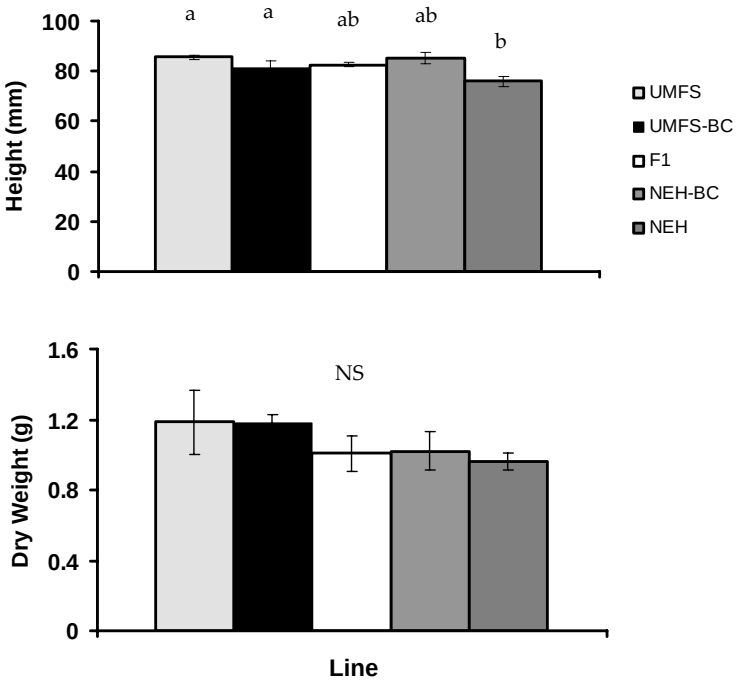

Figure 11. Mean shell height and dry weight for a subset of oysters sampled from the Pemaquid Oyster Co. site on the Damariscotta River, ME. The mean shell height and dry weight is indicated by the bar height and error bars indicate ± standard error and letters above each bar indicate means that are statistically significant from one another.

low ratios < 2 are expected when there is deep cupping. The majority of the oysters at the Pemaquid Oyster Co. site were found to have intermediate values for both ratios; the height-to-width ratios were between 1 and 2 and height-to-inflation ratios between 3 and 5 for nearly all individuals. Three exceptions to this pattern included one outlier from the NEH line that had a relatively low shell height to inflation ratio, one outlier from the NEH line with a relatively large shell height to width ratio, and one individual from the UMFS-BC line with a large shell height to inflation ratio. Thus, there is little evidence for line-specific variation in shape and cupping. An ANOVA indicated that there was no statistically significant among line difference in the mean shell height to width ratio or in the shell height to shell inflation ratio.

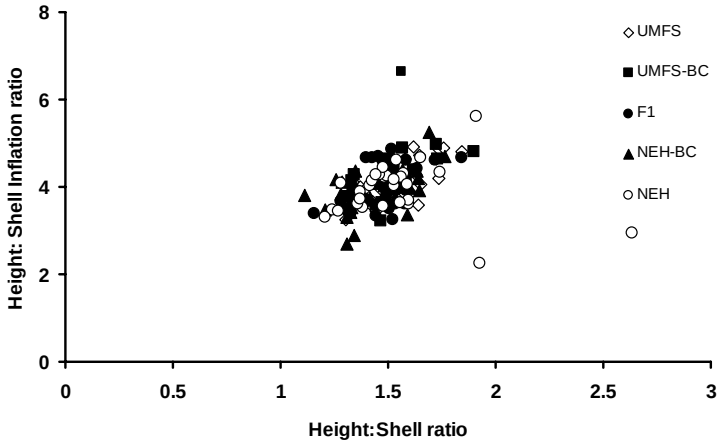

Figure 12. Graphical analysis of shell shape comparing the height-shell ratio and the height-shell inflation ratio for 30 individual oysters from the UMFS, UMFS-BC, F1, NEH-BC, and NEH lines after two seasons of growth at the Pemaquid Oyster Co. site.

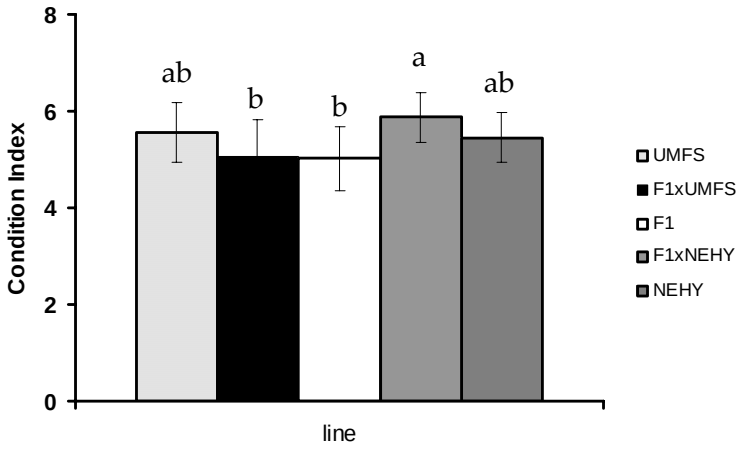

Figure 13. Mean condition index ± one standard deviation for 30 oysters from the UMFS, UMFS-BC, F1, NEH-BC and NEH lines after two seasons of growth at the Pemaquid Oyster Co. Letters above each bar indicates which means were found to be statistically different from one another at an experimentwise P-value of 0.05.

There were subtle but statistically significant differences in the condition index among the five lines grown at the Pemaquid Oyster Co. site. The NEH-BC line had the highest average condition index while the UMFS-BC and F1 lines had statistically lower condition index values (Fig. 13). These results suggest that the NEH-BC line tends to have higher meat weights relative to the size of the internal shell cavity formed by shell growth in each individual. Condition index is a widely used measurement for

examining the nutritive status of bivalves (Crosby and Gale 1990). This method has primarily been used to look at oysters before and after spawning or oysters grown at different sites to see whether there are seasonal or site-specific variation in the lipid and protein content of oysters. However, in this project, condition index was used to determine if there was a difference in the quality of oysters from different lines that were grown under common garden conditions and sampled at the same time. The condition index showed that the NEH-BC stock had a higher overall quality than the other lines even though this line does not produce more meat, on average (see Fig. 11). This is surprising because it seem to be the opposite of what the shell height and dry meat weight data indicates. Since the condition index compares shell volume to dry meat weight, we conclude that the NEH-BC line simply filled more of its shell volume with meat than the other hybrid lines.

Summary

Our NRAC-sponsored project sought to develop and identify high yield lines of eastern oysters that would benefit oyster farmers throughout the northeast. There are two major impediments to increased yields for oyster farmers in this region. First, disease caused by both protistan and bacterial parasites can cause substantial crop losses and many farms contend with multiple and multiple types of diseases. While several currently available lines have putative resistance to one or two diseases, none have demonstrated a high degree of resistance to both bacterial and protistan pathogens. The second impediment to increased yields is poor growth, particularly in northern New England, which is often caused by less than optimal grow-out temperatures and conditions. Thus, we were keenly interested in monitoring the relative survival and growth potential of oysters from four primary oyster lines (NEH, UMFS, EGP and Clinton) and three groups of interline hybrids (UMFS x NEH F1 hybrids and F1 x parental line backcrosses) under a broad range of grow-out conditions. We specifically desired to test whether resistance to bacterial and protistan caused diseases could be obtained in hybrid lines while maintaining the high growth potential of the original lines used to generate the hybrids.

At grow-out sites in Maine where the UMFS line was developed, the UMFS and UMFS-BC lines generally had the highest survival. Similarly, at the Cape Shore site in New Jersey where the NEH line was developed, the NEH line had the highest survival. These results are similar to those we have observed in previous field trials and suggest that there has been some local adaptation among these lines either to disease pressure or environmental conditions. A key observation from the present study, however, is that increasing the genetic contribution from the NEH line among hybrid oysters results in a significantly higher survival rate at the Cape Shore site where Dermo is endemic (the UMFS line is naïve to Dermo). This effect was nearly additive, as the F1 line had a survival rate that was virtually the arithmetic average of the survival rate for the UMFS and NEH parental lines while the backcross groups had survival rates intermediate to that of the F1 and the respective parental lines. Unfortunately, we were not able to conduct examinations for disease on the oysters from the Cape Shore site so we cannot definitively determine whether Dermo was the cause of the mortality variation and

whether among-line differences in survival are due to tolerance to parasite load or true resistance. Further, we did not see evidence of substantial ROD-challenge for the oysters grown at the Maine sites in our northern New England field trial. Thus it is unclear whether there is similar variation among the hybrid lines with respect to susceptibility or tolerance to the bacterial disease, ROD that is problematic in northern New England.

Our results, however, clearly show that at many sites oysters reached market size (75 mm) and that the hybrid lines grew faster than the parental lines. This is not surprising in that genetic analysis indicates the UMFS and NEH lines are relatively inbred (Guo; unpublished data) and so enhanced performance among hybrids likely results from a release from inbreeding depression. At the same time, our data suggest that hybridization has had no major impact on shell shape, at least when oysters are grown under cold-water conditions on the Damariscotta River in Maine. Although the NEH-BC line had a slightly better condition index than the UMFS and therefore had a denser meat at the time of sampling, the difference was subtle and unlikely to relate to differences in quality and marketability. Even so, future efforts should continue to monitor among-line variation in shell shape and meat condition as well as other variables such as appearance and taste to ensure that only highly marketable lines are propagated for the northeast oyster industry.

In our Southern New England field trial we found that survival for the newly developed Clinton line and the two hybrid lines (F1 and NEH-BC) was often as good as or better than the survival for the NEH line that is typically preferred by the industry. Further, the similarity in survival rate for the Clinton and NEH lines indicates the potential for the development of local stocks using oysters which have survived repeated outbreaks of disease. As part of our Southern New England field trial we were able to monitor the patterns of disease prevalence and intensity through histological (MSX) and molecular (Dermo) analyses. The analyses we have completed to date have found no readily apparent correlation between the prevalence and intensity of MSX or Dermo and survival at the sites included in this trial. It is important to note that our experiment did not have a positive control (a naïve, wild type, susceptible strain), and with the exception of the EGP line, for which we have not data, all strains showed disease resistance. Thus, mortalities at these sites are not a direct result of infections; even when there was a high prevalence of MSX, such as seen in some Wellfleet oyster samples, plasmodia were often outside basement membranes or dead and phagocytosed by hemocytes. These are histological characteristics of MSX-resistant oysters. In addition, there were no signs of Dermo at our Connecticut site. Dermo occurs at this site but generally only becomes detectable after oysters have been in the field for at least three years (Sunila, unpublished data). Thus, our results are consistent with previous observations and bolster the conclusion that Dermo doesn’t cause significant mortalities in Connecticut. Overall, we found no clear indication that disease pressure from MSX or Dermo was responsible for line-specific variation in survival. However, detailed observation of histological preparations indicates that parasites usually considered harmless to oysters can cause pathological lesions in some oyster strains introduced to new areas suggesting that such organisms act as opportunistic pathogens to oysters when the oysters are grown under sub-optimal conditions. Our results also argue for the continued maintenance of the primary lines, such as UMFS and

NEH, to preserve their unique characteristics while capitalizing on the increased growth performance and yield afforded by interline crossing.

Objective 2

The second objective of our project was to place oyster lines demonstrating the highest yield at the end of the two-year grow-out trials in a repository, split between the University of Maine and the Marine Biological Laboratory, for future use by industry. One weakness that has stymied many selective breeding programs in agriculture, in general, and aquaculture, in particular, is the lack of institutional commitment for maintaining stocks and making them available to industry. We have recovered the lines from our project and are maintaining them at the Maine Oyster Broodstock Development Program Hatchery at the Darling Marine Center and at the Marine Biological Laboratory, two of the region's most established marine labs. This 'repository' will continue to be maintained to provide the lines for our future research efforts, for the safe-keeping of the lines and, perhaps more importantly, so the lines can be made available for use by commercial and public hatcheries involved in aquaculture production and public restoration.

Objective 3

We proposed to report on the progress of our project at regional and national meetings, to post results in a timely manner on a project-specific website, and to disseminate results to industry members via a Fact Sheet. As indicated in the appendix, below, we have met the first of these expectations through the delivery of at least 5 presentations at regional and national meetings and are currently in the process of placing project updates on the Maine Oyster Broodstock Development Program's website. We have recently completed and submitted to the NRAC office a Fact Sheet on the history of interline hybridization in the development of eastern oyster stocks along with an update of our project results that we expect will be available to the industry within a few weeks.

CONCLUSIONS

- Hybrid vigor between the UMFS and NEH lines was observed at nearly all sites in both field trials.
- Even though hybrid lines did not have the highest survival they often had highest yields, ostensibly due to improved growth.
- Our results suggest there are additive effects on survival among UMFS x NEH hybrid lines grown at Dermo endemic site.
- The new, local Clinton line has performance as good as or better than the industry standard NEH line at southern New England sites.
- We found little variation in shell shape or meat condition among NEH, UMFS lines and their hybrids.

Our results also argue for the continued maintenance of the primary lines, such as UMFS and NEH, to preserve their unique characteristics while capitalizing on the increased growth performance and yield afforded by interline crossing.

IMPACTS

- The results of our project confirm the value of local selected and locally adapted lines.
- Our project has supported the continued production and testing of interline hybrids with potential for combined resistance to multiple diseases.
- Our study provides indication of relative conditions under which available lines and their crosses are likely to perform best.

SUPPORT

Total Project Costs (2 years): $408,362
Total Support from NRAC (2 years): $249,431
Matching Funds: Total: $158,931
University of Maine: $83,451
Rutgers University: $15,569
Connecticut Bureau of Aquaculture: $7,860
Maine Oyster Growers Working Group (Industry): $10,008
Connecticut, Rhode Island and Massachusetts Oyster Growers: $42,043

PUBLICATIONS IN PRINT
None to Report

PAPERS PRESENTED

Rawson, P., C. Davis, B. Barber, B. Hawes, and S. Feindel. OYSTER BROODSTOCK DEVELOPMENT IN MAINE: A COOPERATIVE EFFORT BETWEEN MAINE'S OYSTER INDUSTRY AND THE UNIVERSITY OF MAINE. Annual Meeting of the National Shellfisheries Association, San Antonio, TX, February 2007**.**

Rawson, P.D., X. Guo and S. Lindell. CROSS-BREEDING AND FIELD TRIALS FOR DISEASE RESISTANT OYSTERS. National Shellfisheries Association Annual Meeting, Providence RI, April 2008.

Rawson, P.D., X. Guo and S. Lindell. CROSS-BREEDING AND FIELD TRIALS FOR DISEASE RESISTANT OYSTERS. Northeast Aquaculture Conference and Exhibition (NACE), Portland ME, December 2008References Used – Get text from files.

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