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Reason for Termination: Study End
PROJECT OBJECTIVES: 1) to determine whether novel techniques used to hold hard clam seed successfully over the winter in Maine (November – April) can be applied effectively to other locations in the Northeast region; 2) to examine survival and growth of overwintered hard clam seed that are subsequently planted on farms at various locations in the Northeast region; 3) to compare field survival and usage carbohydrate reserves of hard clam seed overwintered using current (standard) methods vs. the new methods proposed here; 4) to assess the benefits of overwintering hard clam seed using the standard methods vs. the new methods proposed here; and 5) to measure temporal (year-to-year) variation in overwinter success.
ANTICIPATED BENEFITS:
Overwintering of hard clam (*Mercenaria mercenaria*) seed has been a source of continual, but unpredictable losses to the industry. These losses are largely in the sizes of seed that did not reach planting size by the early fall. Methods that have routinely been used to overwinter the soft-shell clam in Maine, if proven to be successful for the hard clam, would make a major improvement in hatchery profitability through enhanced survival rates, and reduced capital costs. The end-users will be those individuals who purchase or produce cultured hard clam seed for enhancing wild stocks (public aquaculture) or for private clam farmers. Losses of planted hard clam seed during the late fall and winter months in the Northeast U.S. can exceed 25% and can be even higher in more northern latitudes of Atlantic Canada. These first winter losses can be economically catastrophic since additional crop mortality is likely to occur before harvest two-three years later.

PRINCIPAL ACCOMPLISHMENTS: In the past two years we have overwintered 2 sizes of hard clam seed of 3 stocks (ME, NY and NJ) in field plots and in cages to compare these with high survival results reported in Maine. Seed from all locations were tested for disease levels and certified before transport. Environmental variables (DO, TPM, Chlorophyll) were generally similar at all sites in both years. Water temperatures during the first year were very similar in New York and New Jersey, with the exception of a very cold period in mid to late December in New Jersey during the first year that was not present at the New York or Maine site. The New Jersey site warmed more quickly in the spring than in New York. Water temperatures in Maine during the first year mirrored those in New York until late January when Maine water temperatures dropped below 0°C and remained there until early March when a warming trend developed. All sites were nearly 5°C warmer during the second year of the study, but overall Maine experienced the coldest temperatures, and as in the first year, New Jersey warmed sooner than the other two locations. In both years seed from New York and New Jersey placed in cages experienced heavy mortality (>95%) at all sites. In the first year seed from ME were planted in Maine and overwintered in cages in Maine, New York and New Jersey. NY seed were planted in New York and overwintered in cages in Maine, New York and New Jersey. NJ seed tested positive for Dermo and experience heavy mortality while purging the low level infections. We substituted NY seed for a comparison to the ME seed in NJ. Animals kept in field plots experienced slightly less mortality.

We had hoped to follow the original proposed seed movements in the second year, but the State of Maine would not allow importation and field studies of any out-of-state seed, even if it was tested and found to be disease free. We were able to maintain ME seed in Maine in both field and cages in Maine. ME seed were also held in cages in New York and New Jersey. NY and NJ seed were held in cages and the local strain was planted in each state. In the second year, when there were differences, smaller ME seed generally had higher mortality than larger seed. Overall, ME seed mortality was less than the NY or NJ seed when held at the southern sites. ME seed had higher mortality at the Maine and New Jersey sites than in the first year. In New York, ME seed had less mortality than in the first year. These data suggest that the ME seed line is better adapted to long overwintering periods than either of the two mid-Atlantic strains, but the temperature regimes in the Mid-Atlantic are less conducive to overwintering than the prolonged cold in Maine. Year to year differences can be large.

Surviving overwintered seed were planted in the spring and sampled in the fall. The plantings included the shells of the clams that experienced winter mortality, and thus counts of dead individuals at the end of the summer included a significant percentage of dead from the overwinter experiment. Few of the NY seed survived in NY or NJ during the first year, and ME seed were planted in NJ. Survival over the summer was poor and analysis of the dead suggests that most of the mortality was early after planting suggesting it is a continuation of the high levels of overwinter mortality. In the second year, NJ seed planted in NJ also had poor survival over the summer, and again counts of dead suggest this mortality took place early after planting. Survival of NY seed was so poor each year in New York that none were planted. Carbohydrate is the primary energy storage compound for small hard clams. Generally the ME seed started with higher carbohydrate content than either of the other two stocks. In the first year ME seed experienced a slow decline in carbohydrate content at all sites with the least loss at the New Jersey site. These seed at the New Jersey site still experienced higher mortality than the

same ME seed at the Maine site. The NY stock held in all three sites began to lose carbohydrate starting in January. Carbohydrate loss in these stocks was about the same in New York and Maine, and less in New Jersey. At all sites carbohydrate loss in the surviving clams was greater than the losses in the ME seed held at the same sites. In the second year ME and NY seed had similar beginning levels of carbohydrate and the NJ seed has about half of the other two. From October to January the NJ seed increased this storage product, and the ME seed at the New Jersey site maintained its high levels of carbohydrate until March-April. In the second year NY seed in New York, as in year 1 experienced a large drop in carbohydrate from deployment through the winter. The ME seed held in New York maintained high levels of carbohydrate during the first year, but during the second year these levels dropped rapidly and by January were lower than the native stock. In Maine the ME seed only lost small amounts of carbohydrate in the first winter, but during the second winter there was an abrupt decline from November to December and then remained relatively level as the clams experienced greater mortality than in the first winter. Thus mortality occurs almost always when carbohydrate reserves had dropped, but factors other than carbohydrate and predatory loss must be important because mortality can occur at times when carbohydrate reserves remain relatively high. The data suggest that the ME strains generally accumulate high levels of carbohydrate most winters, and may have a different physiological response to the level of carbohydrate reserves. Field planting of the seed, in almost all instances, yielded better survival than maintaining them in bags. The reasons for this are not apparent, but environmental conditions in the sediment are probably more stable than in the water column.

Impacts: Since seed generally survived the winter better when planted in the field it would be logical to develop field planting methods to maintain high densities with adequate protection. Because many of the seed are very small these protection methods should include a mechanism for early harvest in the spring. The ME seed appear to be physiologically better adapted for overwinter survival than either the NY or NJ strains. Further physiological and genetic studies should be conducted to determine the mechanism of this adaptation and if it can be transmitted to the other stocks.

Introduction:

In 2002, the aquaculture production of northern quahog, or hard clam, *Mercenaria mercenaria*, in the U.S. was estimated at $60.4 million (USDA 2006). Today, farming and public stock enhancement of hard clams in the Northeast region is the most economically important shellfish culture activity. One of the recurring problems that affects commercial production of farmed hard clams is losses due to winter mortality. Over winter losses in some Mid-Atlantic and Northeastern states, over and above losses due to predators, can exceed 50% (Damery 2000; Aldred et al. 2001; Ford 2001; Zarnoch and Schreibman 2008).

Hatcheries produce clam seed using various techniques to grow the animals to a size suitable for planting between 8-15 mm SL by late summer or early fall. Previous NRAC research on hard clam overwintering (Kraeuter et al. 1997) suggested the optimum would be for farmers grow quahog seed to sizes greater than 10 mm following the first year nursery. Due to time and logistic constraints both in the hatchery and nursery, significant quantities of seed do not reach this size. The remaining <10 mm seed are the most susceptible to overwintering mortality, and can be a significant portion of a growers annual production. There are at least three possibilities for small seed going into the winter. The safest and most expensive strategy is to ship these small clams to a more southern nursery where they will be maintained during the winter. This practice provides larger seed in the spring, but introduces the risk of transfer of diseases and parasites, and it is problematic to obtain necessary permits for such transfers in some states. Based on several years of experience, George Mathis (NJ) stated that this option is neither "practical nor reliable". The second option is to keep the seed in a hatchery through the winter, but this, too, is an expensive proposition because of the time and energy it takes to maintain large numbers of small seed. Growers who have attempted this found variable results not warranting its use (Sandra Macfarlane; Martin Byrnes;Personal Communications to Zarnoch). Instead, most individuals choose to overwinter the small seed using the same field techniques employed for larger seed, but significant and unpredictable mortality occurs in the field planted individuals (Leavitt, 2004; Ford 2001; Miron et al., 2005; Bricelj et al. 2007).

Most hard clam farmers in the Northeast, and communities that purchase seed for stock enhancement purposes (e.g., in MA, NY), place seed in protected nursery field plots or boxes (bottom culture) at high densities (ca. 3000-5000 m^{-2}) in the summer. These are then harvested in the fall and placed in growout plots at much lower densities (250-350 m^{-2}). Summer growth varies among years and sites, yielding small (< 10 mm) seed in some years. Placing small seed in nursery plots presents a large risk for farmers and communities. For example, Damery (2000) investigated mortality of hard clam seed held over the winter in cages and under netting in each of Cape Cod's municipal shellfish programs (10 communities). He reported that winter losses averaged 39% with a range from 10 to 60%, and recommended that towns conduct research to determine the most successful overwintering practices. Aldred et al. (2001) conducted overwintering trials comparing various methods including sediment filled boxes, ADPI bags, covered plots, and predator trapping. Mortality varied significantly across sites and with methodology; however, the most effective methodologies still yielded losses of ~50%. In the Mid-Atlantic many seed reach the 10 mm size for planting, but the remaining seed are overwintered using field planting under mesh, but the results are irregular and large mortalities are experienced.

These commonly encountered seed mortalities suggests that they cannot be mitigated using standard methodologies or that it is a result of physiological processes and/or disease.

Recently, Zarnoch and Schreibman (2008) have shown that loss in carbohydrate, associated with periods of low food availability during the spring when water temperatures rise from 5° to 12° C, caused significant clam seed mortality. Whether this is due to starvation or that physiological stress provides an entry to bacterial disease (Kraeuter and Castagna, 1984), or both, has yet to be determined. One technique that has proven to be effective in eastern Maine is holding the seed in mesh bags.

If overwintering techniques that worked well in eastern Maine can be applied successfully to cultured hard clam stocks in states south of Maine, hard clam farmers will be able to hold their seed in sites protected from natural events such as harsh winters, silting etc., and plant their seed in the spring when shell growth is faster (Jones et al. 1989; Jones et al. 1996). In a preliminary experiment ME hard clam seed were held in a manner similar to that described by Beal et al. (1995) for overwintering cultured individuals of *Mya arenaria*. Animals were sorted into two sizes added to 45 cm x 45 cm "soft" bags of nylon window screening at each of three densities of 7990, 11302, and 15510 individuals, and 3360, 6720, and 8960 individuals for small and large seed, respectively. Bags from each were added to overwintering containers constructed of vinyl-coated 14-guage wire mesh with a series of eight horizontal shelves. One bag containing clams from a single density was placed onto one shelf within each container except the bottommost. All containers were placed into a cement tank that received ambient, flowing seawater. Containers and bags of clams were removed from the tank and cleaned (sprayed with freshwater to remove silt) four times from November to May when a sample was taken from each bag, and the number of live and dead hard clams were recorded. Survival for the large and small clams was 99%, and there was no effect due density (Beal et al. 2009).

Studies by Pernet et al. (2006) Bricelj et al. (2007) and Zarnoch and Schreibman (2008) provide some information about the low temperature physiology in hard clams. When seawater temperatures fall below 5°C, active feeding ceases, and this places a metabolic burden on energy stores. If temperatures remain below this level for eight weeks or longer before water temperatures increase, significant mortality can occur in the spring when chlorophyll-*a* values are low (Zarnoch and Schreibman 2008). Laboratory experiments demonstrated a linear decrease in carbohydrates and significant mortality of clam seed (~7mm SL; obtained from MA) held at 1°C for ~12 weeks (Bricelj et al. 2007). Pernet et al. (2006) examined differences in lipid composition between wild and cultured hard clams during the winter at the northern distributional limit of this species near Neguac, New Brunswick, Canada. They found that phospholipids to sterol ratios, an indicator of membrane fluidity, in wild individuals increased 1.4- to 2.6-fold between August and October, immediately followed by a rapid decrease to initial values in December, whereas the ratio in the cultured individuals did not vary through time. In addition, oxygen consumption rates were approximately 33% lower in wild vs. selectively bred *M. mercenaria* suggesting there may be an energetic advantage in cold-adapted clams by reducing their energetic needs during overwintering. Determination of the levels of energy reserves (especially carbohydrates during and following overwintering is thus one key to understanding and predicting the site-specific success of overwintering under varying environmental conditions.

Materials and Methods:

a) Overwintering trials using local seed (November 2010 – April 2011; October 2011 - April 2012)

Within each state (Maine, New York, New Jersey), hard clam seed were obtained from a local (within-state) hatchery and divided into two size classes (Table 1). One kilogram of seed

from each size class was added separately to soft bags constructed of nylon window screening similar to that described by Beal et al. (2009). The soft bags were placed into rigid ADPI-like bags before adding one to each shelf in an overwintering cage (a modified lobster cage with 3 shelves; 2.54 cm square openings). Each cage (n = 5-14) held a single replicate of each size class (Completely Randomized Block Design). Cages were suspended from a permanent dock or wharf in New York and New Jersey, and in Maine held in flowing seawater in the building for the 6-month period. Due to seed availability and other factors this basic protocol was modified each year as indicated below (See *b*). At monthly intervals during each winter (Nov-April year 1; Oct-April year 2), the cages and all bags within each cage were cleared to remove mud/silt. Salinity, total particulate matter (TPM), percent particulate organic matter, chlorophyll-a, and temperature were measured at each site when bags of clams and cages were cleaned (n = 5 or 6 monthly measurements). Temperature was also monitored continuously at each site with HOBO temperature probes (Onset©, Bourne, MA). Each month the contents of three randomly stratified (large and small seed and each stock of seed) were sampled by taking one haphazard sample of 5g each. Animals were placed into bowls with ambient seawater, allowing sufficient time for live seed to begin siphoning, and then the numbers of live and dead clams were recorded. Clams that did not open in the time allotted were opened to determine if they were live or dead. In addition a sample of each of the lots was placed on ice and shipped to New York for carbohydrate analysis.

Prior to movement of the seed to the various locations, each size of hard clam seed (n = 30 from each size class) in each state was examined for the presence of disease organisms by standard histological techniques (Howard et al., 2004). In addition, samples were retained for bacteriological examination to determine levels of *Vibrionaceae* (Richards et al., 2005). Bacteria were analyzed twice in duplicate composites (100 animals) at the beginning and end of the overwintering period. Histological samples of 30 individuals of each size class were taken at the end of the overwintering period.

To compare overwinter results using the new vs. standard techniques, seeded field plots (2 m^2) (n = 5) using each of the two size classes of local clam seed (75/ft2 or ~808/m2) were established in November/December at farm sites in each state in both years. These plantings were also modified based on seed availability (see b below). Plots were covered with flexible, 6.4 mm aperture predator netting. In April 2009 and 2010, ten random benthic cores were removed from each plot to estimate densities of live and dead clams.

To determine whether clams that survive the novel overwintering trials (animals suspended in cages from docks/wharves in soft bags) will survive subsequent field planting conditions, in April 2010 and 2011 in each state lots of overwintered clams that had enough survivors to develop a plot at typical growout densities of 270/m^2 (or ~25/ft^2) from each size class in 2 m^2 were planted in protected field plots. These plots were harvested in the fall to estimate growth and survival (n = 10 random cores per plot).

b) Reciprocal experiments to overwinter hard clam seed (November 2010 – April 2011; October 2011 - April 2012)

These trials evaluated whether seed source affected overwinter survival. Seed of the same two sizes as above, originating/reared in each state were distributed to the other states (e.g., NJ will receive clams from NY source, and ME source, etc.), and deployed in the same manner as the local seed in overwintering cages as described above (n = 5-14) in October/November 2010 and 2011. Survival was estimated (as described above) in April 2011 and 2012. Seed was tested for disease and approved prior to transfers. In the first and second year Maine seed were

deployed at all sites, but a dearth of larger sizes prevented establishing all 5 replicates at each site (n=4). In the first year NJ seed began to die while being held for Dermo clearance, and a second group experienced the same mortality. NY seed were supplemented during this year and thus the experiment had NY and ME seed at all sites. In the second year, the State of Maine would not allow imports of any seed to be deployed in the field so no out-of-state seed were used in Maine, but in NY and NJ both the locally cultured seed and those from ME were deployed. Again there were not enough large ME seed to establish all bags.

c) Physiological condition

Triplicate subsamples of clams (ca. 25-100) from each strain and size class were collected monthly from the cages at all sites. Samples from New Jersey and Maine were shipped overnight on ice to New York for analyses. All samples were immediately frozen at -80°C until processed. For each clam, the tissue and shell were separated while partially defrosted and then dried to a constant mass at 60ºC. Dried tissue was stored at -80ºC. Carbohydrate analyses were conducted on the triplicate samples by pooling the dry tissue from 25-100 clams. Total carbohydrates were determined using a phenol-sulfuric acid method (Dubois et al. 1956) as per Zarnoch and Sclafani (2010).

Results:

a) Environmental Measurements

Water temperatures during the first year were very similar in New York and New Jersey, with the exception of a very cold period in mid to late December in New Jersey that was not present at the New York or Maine sites (Figure 1.). The New Jersey site warmed more quickly in the spring than in New York. Water temperatures in Maine during the first year mirrored those in New York until late January when Maine water temperatures dropped below 0°C and remained there until early March when a warming trend developed. All sites were nearly 5°C warmer during the second year of the study, but overall Maine experienced the coldest temperatures, and as in the first year, New Jersey warmed earlier than the other two locations. No temperatures of 0°C or below were recorded during the second year.

Mean total particulate matter (TPM), percent organic content of the seston (%OC), and chlorophyll-*a* (Chl-*a*) for each site are presented in Table 2. TPM generally ranged between 10 and 30 mg L^{-1} and was similar at all sites during 2010-2011. The New York site had significantly lower TPM than the Maine site and the New Jersey site had intermediate levels in 2011-2012. The %OC was similar at all sites in both years (Table 2). Chl-*a* levels in New York were similar to those in New Jersey in both years with the exception of a large bloom observed in February 2011 at the New York site. The Maine site had significantly lower chl-*a* than the southern sites (Table 2).

b) Mortality in overwintering cages

Seed from New York and New Jersey placed in cages experienced heavy mortality (>95%) at all sites and in both years (Figure 2). In the first year (2010-2011) seed from ME were planted in Maine and overwintered in cages in Maine, New York, and New Jersey. NY seed were planted in New York and overwintered in cages in Maine, New York and New Jersey. NJ seed tested positive for Dermo and experience heavy mortality while purging the low level infections. NY seed were substituted for a comparison to the ME seed in New Jersey using the overwintering cages. During the first year the New York and New Jersey sites exhibited significant first order effects (date, size class and strain) while the ME site showed a significant effect of date and strain but not size class. A significant date x strain interaction coupled with the significant date effect in mortality at all sites indicates the expected progression in mortality in

the NY strain relative to the ME strain (Table 3). The strong strain effect likely influenced the other significant interactions (Table 3). ME seed experienced low mortality in Maine (< 8%), intermediate levels in New Jersey (up to 46%), and high mortality in New York (up to 77%). Observed mortality in the ME seed at the New York and New Jersey sites was stable from November through January but then increased significantly from February through April as water temperatures increased. Greater mortality was observed in the ME small size class at the New York and New Jersey sites (Fig. 2). No size class effect was found in ME (Table 3). The NY seed held in New York and New Jersey during the first year experienced a significant increase in mortality from December to January as water temperatures decreased below 5°C. In Maine, significant mortality of the NY strain was observed within the first month of deployment. In the second year, the State of Maine would not allow importation and field studies of any out of state seed even if tested and found to be disease free. Therefore, the reciprocal transplant study was not performed in ME during 2011-2012. However, ME seed was maintained in Maine in both field plots and cages. ME seed were also held in cages in New York and New Jersey. NY and NJ seed were held in cages and the local strain was field planted in each state. During the second year all sites exhibited significant first order effects (date, size class and strain). As in the first year, a significant date x strain interaction was found which suggests greater progression in mortality of the NY and NJ strain relative to the ME strain (Table 3). A date by size effect was found in both Maine and New York likely due to the ME small size class having greater mortality than the ME large size class at each of these sites. The date x size effect was not significant in New Jersey (Table 3). In New York, the NY small and large size classes as well as the ME small size class experienced a significant increase (~50%) in mortality from January to February. The ME large seed had low mortality through January but experienced a large mortality (~50%) event through February and March. In New Jersey, observed mortality of the NJ small and large size class as well as the ME small size class increased throughout the study period. The ME large size class had low mortality throughout the winter period but higher mortality from February to March. In Maine, low mortality was observed in the ME large and small size classes until March when ~50% increase in mortality was observed in the ME small size class.

c) Physiological condition

Carbohydrate is the primary energy storage compound for small hard clams. Generally the ME seed started with higher carbohydrate content than either of the other two stocks (Fig. 3). In the first year ME stock had >40% higher carbohydrate content than the NY stock, and experienced a slow decline in content at all sites (Fig. 3). In New Jersey both strains experienced minimal loss of carbohydrates (-2.3%). In spite of this small loss, ME seed at the New Jersey site still experienced higher mortality than the same ME seed at the Maine site which experienced greater loss of carbohydrate (-19%). The NY stock held in New York and Maine showed a significant decline from December through February when mortality was increasing. Percent carbohydrate loss in these stocks was about the same in New York and Maine, and less in New Jersey. At all sites carbohydrate loss in the surviving clams was greater than the losses in the ME seed held at the same sites, but in both years, losses in NJ appeared to be less than the other sites. There were significant first order effects for date and strain, effects with respect to size (Table 4). There were also interactions between date and strain indicating the variation in carbohydrate loss rates. In New Jersey and Maine there were no interactions between size and strain indicating that at these sites, losses in the two seed sizes were similar.

In the second year ME and NY juveniles had carbohydrate levels over twice as high as the NJ juveniles (Fig. 3). From October to January the NJ strain increased this storage product, and the ME strain at New Jersey maintained its high levels of carbohydrate until March-April. The increase in NJ strain condition and maintenance of the high condition of the ME seed was probably due to the unusually high fall and winter temperatures. In the second year ME strain in New York, and the small NY strain experienced a large drop in carbohydrate from deployment throughout the winter. The losses in the small NY seed at this location were similar to the first year data, but the larger NY clams maintained its higher carbohydrate levels until March. The ME strain held in New York maintained high levels of carbohydrate during the first year, but during the second year these levels dropped rapidly and by January were lower than the native stock. In Maine the ME seed only lost small amounts of carbohydrate in the first winter, but during the second winter there was an abrupt decline from November to December and then remained relatively level as the clams experienced greater mortality than in the first winter. Results from ANOVA analysis were similar to the first year in that there were significant first order effects for date and strain, but not size. Because seed could not be imported into Maine there were no strain differences to be compared. The small size of the ME seed precluded carbohydrate analysis at all three sites. There were no interactions between date and size in New York or New Jersey.

There was a significant negative relationship between hard clam carbohydrate content and time (days) exposed to winter conditions within the cages for the NY strain clams at New York in both study years (Fig. 4A), the New York strain clams in Maine during 2010-2011, and the ME strain in Maine for both study years (Fig. 4B). There was no linear relationship found between time and carbohydrate content in ME seed in New Jersey for both years as well as the NY seed in New Jersey in the first year. In the second year, the NJ seed held in New Jersey experienced an increase in carbohydrate content (Fig. 3). ANCOVA was used to compare carbohydrate concentration through time for the ME and NY strains in Maine (Fig. 4B). The slope ($F = 18.74$; $p < 0.0001$) and intercepts ($F = 152.17$; $p < 0.005$) were found to be significantly different, indicating that the change in carbohydrate concentration though time was different for the two strains (i.e. greater loss in New York) and at a given time interval carbohydrates was higher for the ME strain. ANCOVA was also used to compare carbohydrate concentration through time for the NY strain held in New York (both years; Fig. 4A) and the NY strain held in Maine in 2010-2011(Fig. 4B). We found both the slope ($F = 5.26$; $p = 0.033$) and the intercept ($F = 39.85$; $p < 0.0001$) to be significantly different, indicating that the change in carbohydrate concentration through time was different for the two sites (i.e. greater loss in New York) and at a given time interval carbohydrates were lower at New York.

d) Mortality in field plots vs. overwintering cages

The mortality observed in field plots was lower than the mortality observed in cages for each strain and size class at all sites with the one exception of the ME large seed having less mortality in the cages during the second year (Table 5). A 2-way ANOVA was performed to compare mortality for the two seed sizes and culture methodologies for both years in New York and for the second year in New Jersey (Fig. 5). In New York, there was no effect of seed size on mortality but there was a significant effect of culture method in both years (Fig 5A and B). Mortality in the field plots was 51% and 66% in years 1 and 2, respectively as compared to > 95% in the overwintering cages (Table 5). Similarly, there was a significant difference in mortality in New Jersey when comparing field plots to overwinter cages during the second year (Fig 5C). There was also an effect of seed size in New Jersey as the NJ large seed experienced

significantly less mortality than the NJ small seed (54% vs. 86%) in the field plots. There was no difference in mortality between the 2 seed sizes held in overwintering cages in New Jersey. In Maine, the mortality observed in both the field plots and the overwintering cages was insignificant (<10%) during the first year. In the second year, mortality was higher for both culture methodologies with the small size class having lower mortality in the field plots while the large size class had lower mortality in the overwintering cages (Table 5).

Discussion:

The results of this study clearly demonstrate a genetic component to overwinter mortality as the ME strain had greater survival than the NY and NJ strains at all sites and in both study years. Both the ME large and small size classes exhibited low mortality through the winter months of November through February, while the NY and NJ strains experienced very high mortality, suggesting greater tolerance to low temperatures in the ME strain. Such traits may potentially be selected for in the development of aquaculture strains resistant to overwintering mortality. Genetic differences in overwinter mortality have been previously demonstrated in studies comparing selected and wild stocks in Atlantic Canada and New York (Bricelj et al. 2007; Pernet et al. 2006; Gionet et al 2009; Zarnoch and Sclafani 2010). However this is the first study to compare the overwinter mortality of different genetic stocks across a latitudinal gradient (Midatlantic US to eastern Maine) that encompasses the biogeographic range where hard clam overwintering mortality is problematic. In previous studies, cohorts of juvenile clams of the *notata* strain, selected for fast-growth, experienced significantly more mortality than stocks produced from wild broodstock, however, the exact physiological and genetic mechanism for the higher mortality in selected strains remains unclear. Pernet et al. (2006) has suggested that greater metabolic demand and a heterozygote deficit in selected clams may make them more vulnerable to overwintering stress. In contrast, Zarnoch and Sclafani (2010) did not find differences in metabolic demand between selected and non-selected strains, but did confirm greater use of carbohydrate reserves by the selected strain during the winter months. In the current study, the NY and NJ strains were *notata* variety, and produced from cultured broodstock selected for fast growth while the ME stock were produced from wild broodstock collected from eastern Maine. Therefore, it is not clear if the greater mortality observed in the NY and NJ strains as compared to the ME strain were a result of some negative physiological traits associated with selection for fast-growth, reduced tolerance to low temperatures, and/or their interaction. Future studies will need to examine overwinter mortality of aquacultured and wild strains within and among sites across a latitudinal gradient.

The timing of the observed mortalities during the overwinter period differed between NY/NJ strains and the ME strain which is likely due to differences in physiological condition, low temperature tolerance, as well as the variable temperature regimes that occurred over the two study years at each site. In the first year, New York and New Jersey experienced water temperatures below 5°C from early December until March. Significant mortalities of the NY strain were observed in both New York and New Jersey during the January sampling soon after water temperatures fell below 5°C (Fig. 2). This temperature change is significant since hard clams are known to significantly reduce metabolic activity at temperatures less than 5°C (Loosanoff 1939; Bricelj et al. 2007; Zarnoch and Sclafani 2010). In addition, the NY strain held in New York experienced a significant decrease in carbohydrate content from December through February coinciding with the mortality. While in New Jersey, the NY strain did not experience significant reductions in carbohydrate content, however, it started with a lower initial content, approximately 65 and 75 $\mu g\ mgDW^{-1}$ in New Jersey and New York/Maine, respectively

(Fig. 3). The NY strain transferred to Maine in the first year experienced significant mortality between November and December soon after being deployed in the cages. Similar to New York, there was a linear decline in carbohydrate content in the NY strain held in Maine during this time. The water temperature was 5°C at the time of deployment of the NY and ME strains in Maine and was < 0°C from January to March. These results corroborate previous findings where overwinter mortality is correlated with reductions in carbohydrate content (Bricelj et al. 2007; Zarnoch and Schreibman 2008).

The ME strain deployed in New York and New Jersey during the first study year experienced significant mortality (although much less than the NY strain) as water temperatures increased from February through April (Fig. 2) as compared to the NY strain which experienced increased mortality as temperatures decreased. In addition, the ME small size class had higher mortality than the large size class in both New York and New Jersey which explains the significant size effect in the ANOVA analyses (Table 3) since mortality was similar between the NY large and small size classes at all sites and in both years. In New York, the ME strain experienced the greatest increase in mortality from March to April when water temperatures were >5°C which was also where ME small experienced a significant decline in carbohydrate. Although, the ME large also experienced significant mortality from March to April it was not correlated with a decrease in carbohydrates. Instead the ME large had a reduction in carbohydrate content from December to February and then an increase through April. The ME strain held in New Jersey had relatively stable carbohydrate content through the study period. The ME strain held in Maine showed a slow linear decline in carbohydrate content even though no significant mortalities occurred during the study period. It is important to note, however, that the water temperature in ME was still <5°C at the end of the first study year. Therefore, in year 1 the ME strain in Maine was not exposed to the winter-spring transition, which has been correlated with significant mortality following a winter period where carbohydrates reserves are reduced (Zarnoch and Schreibman 2008; Zarnoch and Sclafani 2010).

The second study year was an unusually warm winter for all sites with temperatures approximately 5°C warmer than the first study year (Fig. 1). In New York the water temperature did not fall below 5°C until January which is also when the largest increase in mortality occurred during the winter months in the NY strain (Fig. 2). Unfortunately, due to changes in Maine regulations, which prevented importation of out of state shellfish, the NY or NJ strain clams could not be overwintered in ME during the second study year. The NY strain experienced a linear decline in carbohydrates from November to January when mortality was peaking. In New York, the ME small experienced mortality similar in magnitude (up to 93%) and timing to the NY strain while the ME large had low mortality through the winter with a ~50% increase from February to March. This mortality event correlated to a reduction in carbohydrate content (Fig 3). In New Jersey, the NJ strain (both large and small) experienced a significant mortality event soon after deployment and then mortality of the NJ strain and ME small increased from December to the end of the study period. As in New York, the ME large in New Jersey had low mortality through the winter months and then experienced a mortality event from February to March although the total mortality was lower in New Jersey (Table 5). In contrast to the NY strain, the NJ strain increased carbohydrate content despite high mortalities through the study period. Since water temperatures were mostly >5°C in New Jersey surviving clams were likely feeding through the winter. Similarly, clams held at 7°C in a laboratory experiment and fed cultured algae also increased their carbohydrate content despite significant mortality (Bricelj et al 2007). In Maine, the ME strain had an increase in mortality from February to March/April (Fig.

2). The timing of the mortality correlated with an increase in water temperature and a decrease in carbohydrate content in the ME large (Fig. 3).

The high mortalities observed in the NY and NJ strains at all sites appeared to be correlated with the decrease in water temperatures (fall-winter) while the mortalities observed in the ME strain were correlated with the increase in water temperatures during the winter-spring transition. This difference may be a result of the physiological condition of the different strains at the start of the experiments. ANCOVA analyses demonstrated that the NY strains had lower carbohydrate content on a given sample date as compared to the ME strain and the rate of loss from November to February was greater for the NY strains (Fig. 4). Zarnoch and Sclafani (2010) found the metabolic activity of selected and wild juvenile hard clams, estimated by the activity of the electron transport chain, was equally as high during the fall-winter transition as the winter-spring transition which led the authors to hypothesize that the fall-winter transition may be an important factor in overwintering mortality. The current study supports this hypothesis. Conversely, the ME strain appear to experience a slow linear decline in carbohydrate content during the winter months (although less significant change in New Jersey) and then experience significant mortality during the winter-spring transition which has been previously demonstrated as a time when significant mortality occurs in juvenile hard clams (Zarnoch and Schreibman 2008). Furthermore, the laboratory studies by Bricelj et al. (2007) suggest that clams exposed to 7°C are equally as challenged as clams exposed to 1°C based on the fact that both groups experience high mortalities. Therefore, the physiological condition of the clams when they are exposed to these stressful temperature transitions is likely a significant factor in determining the timing and magnitude of the mortality. For example in the current study, the NY and NJ strains had carbohydrate concentration between 61-102 µg $mgDW^{-1}$ at the start of the experiments and experienced significant mortality in the fall-winter transition. While the ME strain had a carbohydrate concentration of ~130 µg $mgDW^{-1}$ at the start of the experiments and experienced significant mortality during the winter-spring transition. Similarly, juvenile clams with carbohydrate concentrations >150 µg $mgDW^{-1}$ overwintered in Jamaica Bay, New York also experienced significant mortality during the winter-spring transition (Zarnoch and Schreibman 2008).

There was no significant difference in mortality of the ME strain held in cages or in field plots in Maine, however, survival of the NY and NJ strains in the field plots was higher than survival in the overwintering cages (Table 5; Fig. 5). There are several possible explanations for these results. First, the clams held in the cages within the water column may experience greater fluctuations in temperature than those buried in sediment. The ability of soils to buffer temperature fluxes and mitigate overwinter mortality of insects has been described extensively in the entomology literature (Turnock and Fields 2005 and reference within) but has not been documented in the marine bivalve literature. Temperature fluctuations that repeatedly shift clams from states of metabolic activity (>5°C) to metabolic inactivity (<5°C) are likely to being particularly stressful and are most likely to occur during the fall and spring seasonal transitions in New York and New Jersey. These types of temperature fluctuations did not occur in Maine during the first year when mortality was < 8% but did occur in the second year which was a warmer than average winter and resulted in much higher mortalities (17-56%) in both cages and field plots. A second potential explanation is that the clams in the field plots have access to more food resources than those in the overwintering cages. It is likely that the horizontal flux of food particles across the sediment surface is greater than the flux through the cages since the cages are likely to reduce horizontal flow and local resuspension of food particles occurs near the sediment

surface (Grizzle et al. 1992; Judge et al. 1992). Lastly, it is possible that hard clams require more energy to maintain valve closure in the cages when they do not have the force of sediments supporting valve closure. Further research comparing hard clam physiology in sediment and culture containers (i.e. cages, FLUPSYs) is needed to better understand the advantages and disadvantages of these culture methodologies to support growth and overwintering.

To synthesize the results of this study and the published literature on the overwinter mortality of juvenile hard clams a conceptual model (Fig. 6; adapted from Bricelj et al. 2007) is presented which highlights the specific temperature regimes that influence growth, physiological condition, and overwintering mortality during the first year of culture. During the growing season temperatures are adequate to support growth (Ansell 1968) and metabolizable energy intake is greater than maintenance metabolic demand (Bayne et al. 1999). The fall-winter and winter-spring transitions are periods where metabolic activity is increased (Zarnoch and Sclafani 2010) likely due to the seasonal temperature change, short-term temperature fluxes associated with local environmental conditions, and/or a lack of acclimation due to such change. Energy intake during these transitions may not meet energy demands due to low food availability during these periods (Zarnoch and Schreibman 2008) and/or culture conditions. During the winter period when water temperatures are <5°C metabolic activity is significantly reduced (Loosanoff 1939; Ansell 1964; Zarnoch and Sclafani 2010) and metabolic demands are met through carbohydrate reserves (Bricelj et al. 2007; Zarnoch and Schreibman 2008; Zarnoch and Sclafani 2010). Given these temperature regimes and their influence of on juvenile clam physiology two different scenarios are proposed that could result in significant overwinter mortality. First, if the physiological condition of the clam (i.e. low carbohydrate content) is poor at the end of the growing season the clams are likely to become challenged during the fall-winter transition and significant mortalities will be observed as water temperatures fall below 5°C. This scenario was demonstrated by the NY and NJ strains in the current study. Poor physiological condition at the end of the growing season may be a result of environmental variables (i.e. food availability), culture conditions (i.e. density, fouling), or both. Alternatively, the physiological condition of the clams may be excellent at the end of the growing season and the clams are able to use stored carbohydrate reserves through the fall-winter transition and the winter period. Mortality may then occur during the winter-spring transition as metabolic activity increases due to warming temperatures and carbohydrate reserves are reduced. This scenario was previously proposed by Zarnoch and Schreibman (2008). It was also demonstrated in the current study with the ME strain in New York and New Jersey during both years and in Maine during the second year.

Although, progress has been made on understanding the overwinter mortality of juvenile hard clams there is still a need to describe the physiological mechanisms at the molecular level (i.e. oxidative stress, membrane fluidity). In addition, it is still not clear how bacterial infection may be involved. The observations of Kraeuter and Castagna (1984) that a potential source of pathogenic bacteria is external to the clams (treatment with antibiotic in freshwater yielded the same level of mortality reduction as in ambient marine water) may be partly responsible is still an option once the clams become stressed. The proposed conceptual model (Fig. 6) will require additional study to adequately describe hard clam physiology during these temperature transitions, however, it does demonstrate the significance of these periods to the hard clam aquaculture industry. Growers should note that the fall-winter and winter-spring transitions are physiologically stressful periods and should maintain culture conditions that provide ample food resources and minimal handling. Basic research will be needed to further understand physiology during these stressful periods and applied research will be needed to identify culture

methodologies that minimize stress and declining physiological condition. This research will become more significant as warmer winters and longer fall-winter and winter-spring transitions become more common due to the effects of global climate change. Modeling efforts that integrate overwinter mortality in predicting the effects of global climate change on hard clam population dynamics will inform natural resource managers as well as the hard clam aquaculture industry. Lastly, the current study further supports previous reports of a genetic component making some stocks more susceptible to overwinter mortality. Therefore, genomic tools should be applied to to discover candidate genes important for overwintering performance and provide estimates of genotypic differentiation.

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Table 1. Mean shell lengths of the different strains and size classes used in the two study years. Significant differences in shell length within a column are indicated by different subscript letters.

Seed strain and size class	2010-2011 Shell length (mm) ±SE	2011-2012 Shell length (mm) ±SE
New York - Large	10.33^{a} (±0.12)	8.7^{a} (±0.12)
New York - Small	9.64^{b} (±0.1)	6.93^{b} (±0.12)
Maine - Large	10.27^{a} (±0.15)	9.95^{c} (±0.33)
Maine - Small	6.88^{c} (±0.06)	4.59^{d} (±0.09)
New Jersey - Large	-	8.74^{a} (±0.13)
New Jersey - Small	-	7.47^{b} (±0.12)

Table 2. Mean (±SE) total particulate matter (TPM; mg l^{-1}), percent organic content of the seston (%OC), and chlorophyll-*a* (Chl-*a;* µg l^{-1}) at the overwintering sites in NY, NJ, and ME during 2010-2011 (n = 5) and 2011-2012 (n = 6). Differences between sites are indicated by different letters within each column.

	2010-2011			2011-2012		
Site	**TPM**	**%OC**	**Chl-*a***	**TPM**	**%OC**	**Chl-*a***
NJ	21.44(3.83)	26.25(2.89)	5.21^{a}(0.65)	$12.59^{a,b}$(1.75)	24.87(2.02)	6.23^{a}(0.84)
NY	19.88(3.63)	22.51(2.2)	12.13^{a}(4.44)	10.34^{a}(1.62)	29.36(3.97)	$4.62^{a,b}$(1.01)
ME	14.49(5.62)	27.45(1.59)	1.54^{b}(0.23)	19.65^{b}(2.51)	26.16(1.17)	1.42^{b}(0.36)

Table 3. Analysis of variance on the arcsine transformed percent mortality of different strains and size classes of juvenile *M. mercenaria* overwintered in cages in New York, New Jersey and Maine during the winters of 2010-2011 and 2011-2012.

Source of Variation	SS	df	MS	F	Sig.
New York 2010-11					
Sample date	16.484	5	3.297	187.930	**<0.0001**
Size class	9.004	1	9.004	513.276	**<0.0001**
Strain (NY, ME)	0.698	1	0.698	39.795	**<0.0001**
Date x Size	0.147	5	0.029	1.678	0.144
Date x Strain	6.077	5	1.215	69.288	**<0.0001**
Size x Strain	0.481	1	0.481	27.422	**<0.0001**
Date x Size x Strain	0.376	5	0.075	4.286	**0.001**
New York 2011-12					
Sample date	24.241	6	4.040	273.885	**<0.0001**
Size class	1.073	1	1.073	72.768	**<0.0001**
Strain (NY, ME)	1.306	1	1.306	88.508	**<0.0001**
Date x Size	0.325	6	0.054	3.676	**0.002**
Date x Strain	1.749	6	0.292	19.763	**<0.0001**
Size x Strain	0.538	1	0.538	36.458	**<0.0001**
Date x Size x Strain	0.252	5	0.050	3.420	**0.007**
New Jersey 2010-11					
Sample date	8.249	5	1.650	80.001	**<0.0001**
Size class	11.274	1	11.247	546.684	**<0.0001**
Strain (NY, ME)	0.116	1	0.116	5.644	**0.019**
Date x Size	0.298	5	0.060	2.888	**<0.0001**
Date x Strain	5.413	4	1.353	65.616	**0.017**
Size x Strain	0.352	1	0.352	17.054	**<0.0001**
Date x Size x Strain	0.128	4	0.032	1.558	0.19
New Jersey 2011-12					
Sample date	3.371	6	0.562	24.489	**<0.0001**
Size class	0.987	1	0.987	42.999	**<0.0001**
Strain (NJ, ME)	3.210	1	3.210	139.920	**<0.0001**
Date x Size	0.161	6	0.027	1.172	0.331
Date x Strain	0.201	3	0.067	2.923	**0.040**
Size x Strain	0.003	1	0.003	0.116	0.734
Date x Size x Strain	0.080	3	0.027	1.157	0.332
Maine 2010-11					
Sample date	7.187	4	1.797	110.797	**<0.0001**
Size class	5.774	1	5.774	356.027	0.328
Strain (NY, ME)	0.016	1	0.016	0.969	**<0.0001**
Date x Size	0.013	4	0.003	0.204	0.936
Date x Strain	1.658	2	0.829	51.113	**<0.0001**
Size x Strain	0.001	1	0.001	0.051	0.822
Date x Size x Strain	0.010	1	0.010	0.631	0.429
Maine 2011-12					
Sample date	0.762	4	0.190	17.652	**<0.0001**
Size class	0.509	1	0.509	47.194	**<0.0001**

Table 4. Analysis of variance on the log transformed carbohydrate content of different strains and size classes of juvenile *M. mercenaria* overwintered in cages in New York, New Jersey and Maine during the winters of 2010-2011 and 2011-2012.

Source of Variation	SS	df	MS	F	Sig.
New York 2010-11					
Sample date	0.205	5	0.041	23.825	**<0.0001**
Size class	2.294×10^{-5}	1	$2.294X10^{-5}$	0.013	0.909
Strain (NY, ME)	0.798	1	0.798	464.106	**<0.0001**
Date x Size	0.057	5	0.011	6.638	**<0.0001**
Date x Strain	0.081	3	0.027	15.730	**<0.0001**
Size x Strain	8.193×10^{-5}	1	8.193×10^{-5}	0.048	0.828
Date x Size x Strain	0.002	3	0.001	0.302	0.823
New York 2011-12					
Sample date	0.218	6	0.36	21.412	**<0.0001**
Size class	4.377×10^{-8}	1	4.377×10^{-8}	<0.0001	0.996
Strain (NY, ME)	0.41	1	0.041	24.412	**<0.0001**
Date x Size	0.011	3	0.004	2.134	0.117
Date x Strain	0.022	3	0.007	4.475	**0.01**
Size x Strain	<0.0001	0			
Date x Size x Strain	<0.0001	0			
New Jersey 2010-11					
Sample date	0.037	5	0.007	5.33	**0.001**
Size class	0.001	1	0.001	0.623	0.435
Strain (NY, ME)	1.204	1	1.204	870.537	**<0.0001**
Date x Size	0.023	5	0.005	3.338	**0.013**
Date x Strain	0.01	3	0.003	2.351	0.086
Size x Strain	0.003	1	0.003	2.244	0.142
Date x Size x Strain	0.003	3	0.001	0.726	0.542
New Jersey 2011-12					
Sample date	0.141	6	0.024	5.5252	0.001
Size class	0.002	1	0.002	0.421	0.521
Strain (NJ, ME)	0.519	1	0.519	115.621	**<0.0001**
Date x Size	0.012	3	0.004	0.898	0.454
Date x Strain	0.048	3	0.016	3.557	**0.026**
Size x Strain	<0.0001	0			
Date x Size x Strain	<0.0001	0			
Maine 2010-11					
Sample date	0.133	5	0.027	15.632	**<0.0001**
Size class	0.006	1	0.006	3.399	0.072
Strain (NY, ME)	0.916	1	0.916	540.370	**<0.0001**
Date x Size	0.007	5	0.001	0.862	0.513
Date x Strain	0.037	3	0.012	7.323	**<0.0001**
Size x Strain	<0.0001	1	<0.0001	0.071	0.791
Date x Size x Strain	0.005	3	0.002	1.061	0.375
Maine 2011-12					
Sample date	0.072	5	0.014	20.982	**<0.0001**

Table 5. Percent mortality of juvenile *Mercenaria mercenaria* planted in the field and held in cages at final sampling in April 2011 and April 2012. NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

		Year 1					
		Maine		New York		New Jersey	
		Field	Cages	Field	Cages	Field	Cages
Year 1	**MES**	5	8		78		45
	MEL	5	8		60		22
	NYS		98	52	99		99
	NYL		98	48	99		99
		Year 2					
Year 2	**MES**	48	56		50		63
	MEL	28	17		50		24
	NYS			35	96		
	NYL			35	96		
	NJS					86	98
	NJL					55	82

Figure Legends:

Figure 1. Water temperature at three experimental sites: Beach Haven, New Jersey (NJ), Point Lookout, New York (NY) and Beals Island, Maine (ME) for the winter of 2010-2011 and 2011-2012.

Figure 2. Percent mortality of various stocks of juvenile *M. mercenaria* in small and large size classes observed in overwintering and reciprocal experiments using the new technology in New York during **A)** 2010-2011, **B)** 2011-2012, in New Jersey during **C)** 2010-2011, **D)** 2011-2012, and in Maine during **E)** 2010-2011, **F)** 2011-2012. The seed sizes and strains include: NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

Figure 3. Carbohydrate content of various stocks of juvenile *M. mercenaria* in small and large size classes observed in overwintering and reciprocal experiments using the new technology in New York during **A)** 2010-2011, **B)** 2011-2012, in New Jersey during **C)** 2010-2011, **D)** 2011-2012, and in Maine during **E)** 2010-2011, **F)** 2011-2012. The seed sizes and strains include: NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

Figure 4. The linear relationships between carbohydrate content and time under overwintering conditions of Maine (ME) and New York (NY) juvenile *M. mercenaria* strains and size classes (S = small; L = large) overwintered in cages in A) NY and B) ME during the winters of 2010-11 and 2011-12.

Figure 5. Comparison of overwinter mortality of juvenile *M. mercenaria* in cages and planted in field plots. All cages and plots were samples in March 2011 and March 2012. **A)** New York 2010-2011, **B)** New York 2011-2012, and **C)** New Jersey 2011-2012.

Figure 6. A conceptual model indicating the specific temperature regimes that influence growth, physiological condition, and overwintering mortality during the first year of culture (adapted from Bricelj et al. 2007).

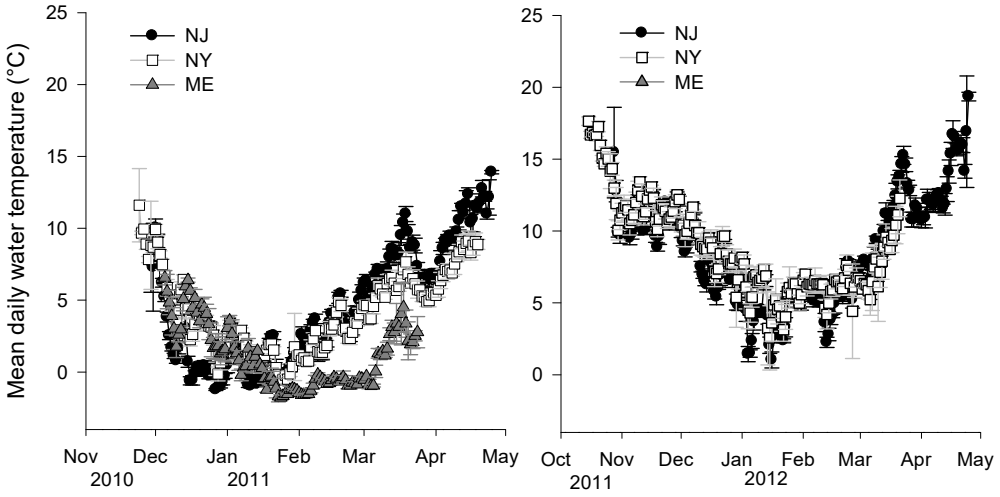


Figure 1. Water temperature at three experimental sites: Beach Haven, New Jersey (NJ), Point Lookout, New York (NY) and Beals Island, Maine (ME) for the winter of 2010-2011 and 2011-2012.

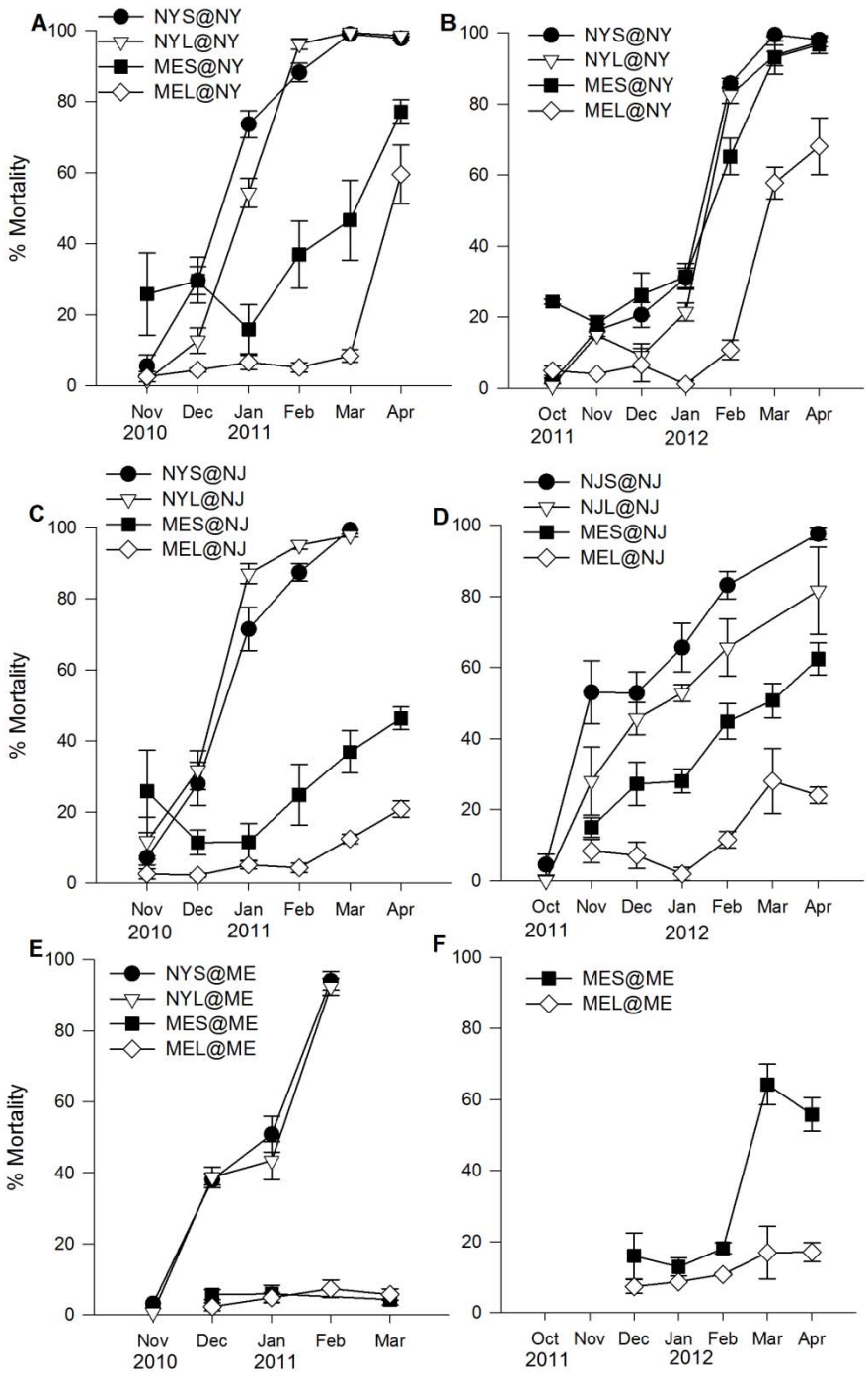


Figure 2. Percent mortality of various stocks of juvenile *M. mercenaria* in small and large size classes observed in overwintering and reciprocal experiments using the new technology in New York during **A)** 2010-2011, **B)** 2011-2012, in New Jersey during **C)** 2010-2011, **D)** 2011-2012, and in Maine during **E)** 2010-2011, **F)** 2011-2012. The seed sizes and strains include: NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

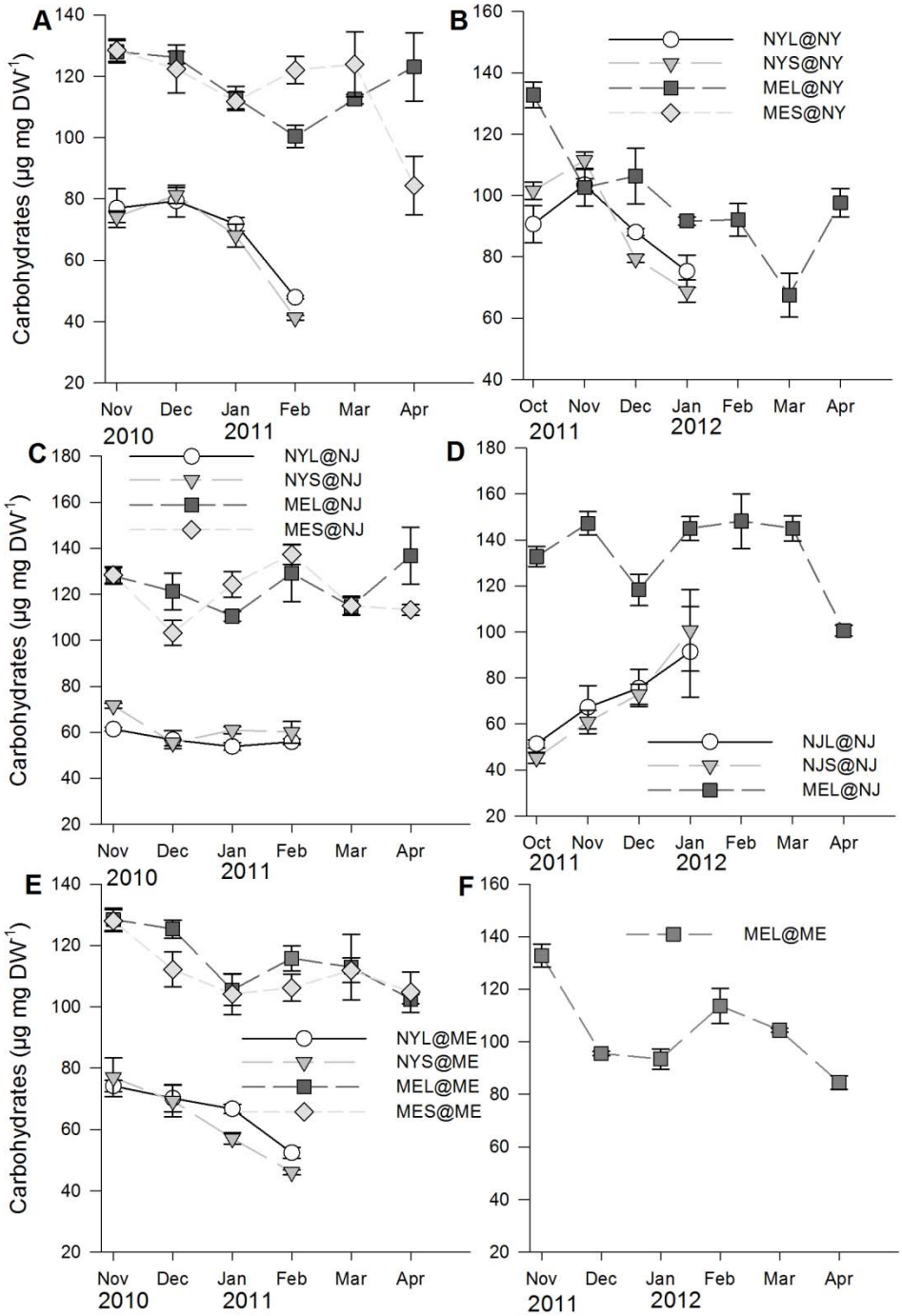


Figure 3. Carbohydrate content of various stocks of juvenile *M. mercenaria* in small and large size classes observed in overwintering and reciprocal experiments using the new technology in New York during **A)** 2010-2011, **B)** 2011-2012, in New Jersey during **C)** 2010-2011, **D)** 2011-2012, and in Maine during **E)** 2010-2011, **F)** 2011-2012. The seed sizes and strains include: NYS = New York strain and small size class; NYL = New York strain and large size class; NJS = New Jersey strain and small size class; NJL = New Jersey strain and large size class; MES = Maine strain and small size class; MEL = Maine strain and large size class.

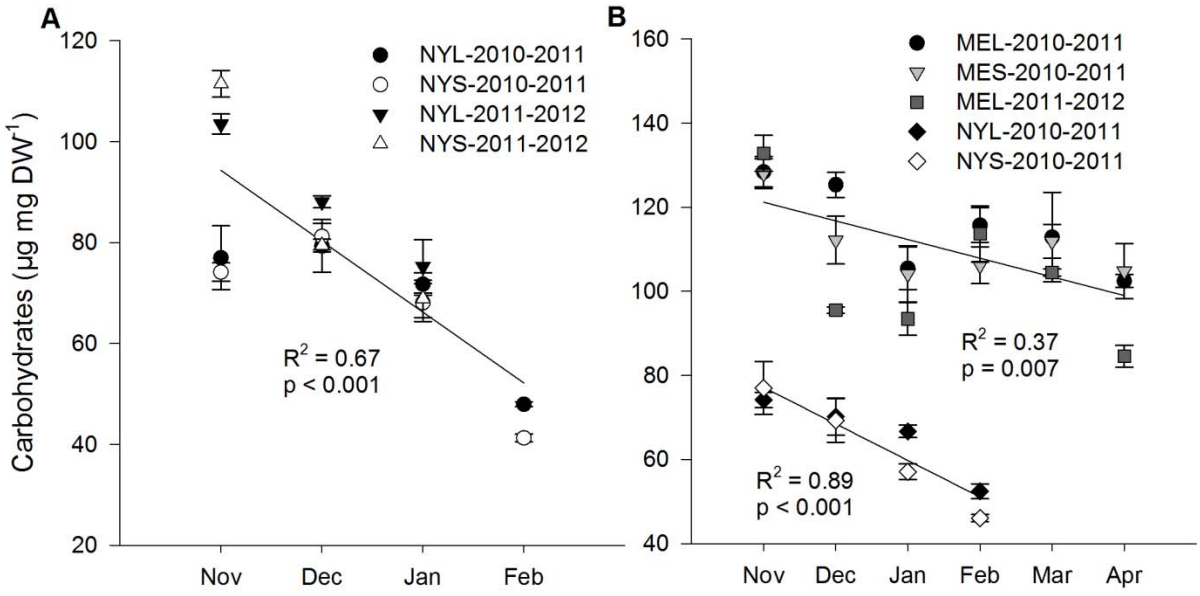
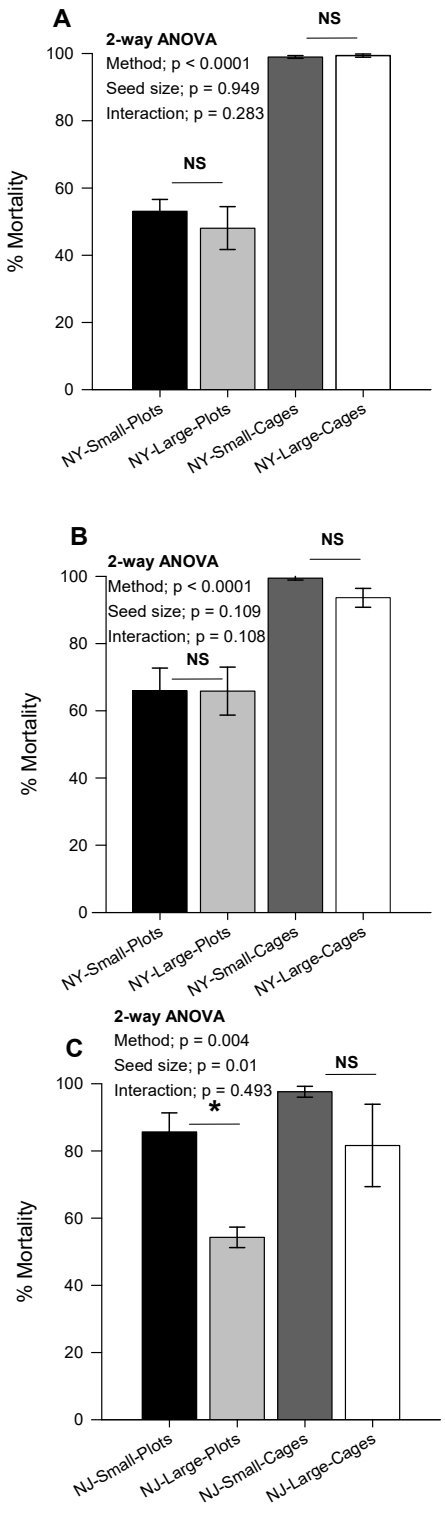


Figure 4. The linear relationships between carbohydrate content and time under overwintering conditions of Maine (ME) and New York (NY) juvenile *M. mercenaria* strains and size classes (S = small; L = large) overwintered in cages in A) NY and B) ME during the winters of 2010-11 and 2011-12.



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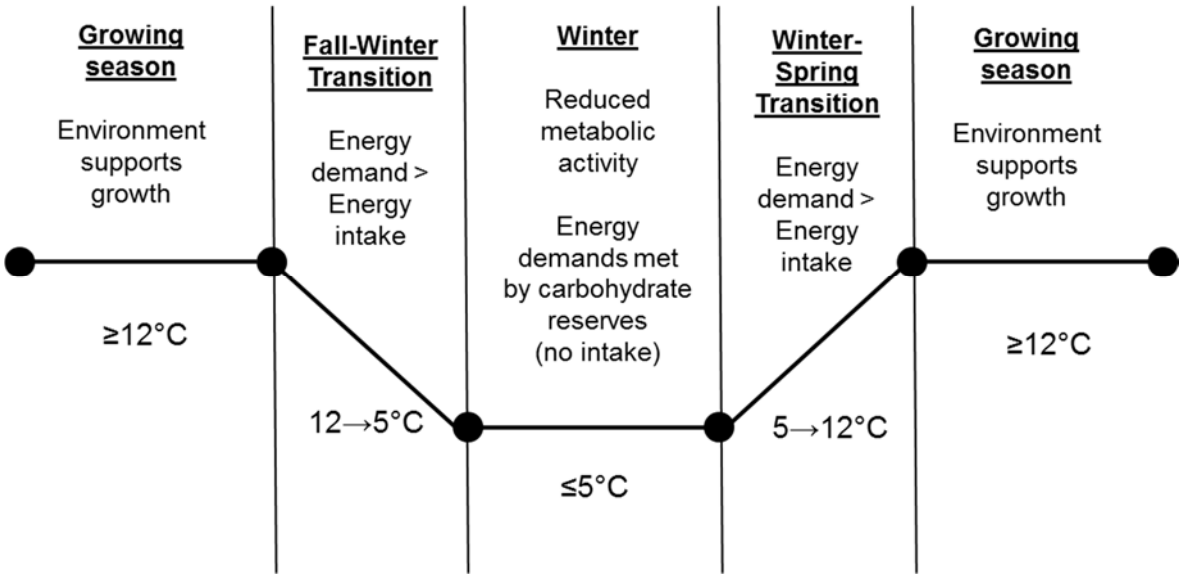

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