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Mortality of first-year cultured northern quahogs, *Mercenaria mercenaria*, through thermal decline: Impacts of low temperature, the rate of temperature decrease and dietary 20:5n-3 and 22:6n-3

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A B S T R A C T

In a preliminary investigation, first-year cultured northern quahogs, *Mercenaria mercenaria* (Linnaeus, 1758), were administered a matrix of dietary highly unsaturated n-3 fatty acids, 20:5n-3 and 22:6n-3 to observe their effect on homeoviscous adaptation to low temperature. The quahogs were subjected to high magnitude thermal fluctuations of the natural, declining temperature regime of a temperate estuary during the approach to winter. Inverse correlations emerged between mortality and dietary abundance of 20:5n-3 during the upper range of the temperature decline (18°–12 °C), and 22:6n-3 during the lower range (12°–6 °C), demonstrating their unique roles in supporting homeoviscous adaptation within each respective phase of thermal decline. High survival of a group whose diet was devoid of these n-3 fatty acids was associated with high molar % increases of endogenic non-methylene-interrupted fatty acids, 22:2Δ7,13 and 22:2Δ7,15. Conversely in the current investigation, similarly grouped juvenile quahogs all experienced *reductions* in incorporated 22:2Δ7,13 and 22:2Δ7,15 when subjected to a mild temperature decrease of 0.2 °C per day, suggesting that high magnitude thermal fluctuation is a signal for the synthesis of non-methylene-interrupted fatty acids. The unfavorable circumstance of this group of quahogs deprived of dietary 20:5n-3 and 22:6n-3 was exacerbated by diminishing incorporation of these compounds and suffered the earliest and highest overall mortality of all dietary treatments. High early-phase mortality of this same group was associated with high levels of previously incorporated 22:6n-3, considered to be homeoviscously unfavorable at the upper thermal range above 12 °C, and this direct relationship fell neatly within the 95% confidence interval of a linear regression model established in the preliminary study.

Statement of relevance: Results of this supplemental experiment provide novel correlations among thermal decline, diet and mortality of juvenile northern quahogs, distinct from the preliminary investigation cited in this paper. This work distinguishes the effects of absolute temperature decline and rapid decline on mortality of juvenile northern quahogs. This work also demonstrates the requirement of high magnitude thermal decline to signal the endogenic biosynthesis of EPA, DHA and 22:2 NMIs in juvenile northern quahogs in homeoviscous adaptation to falling temperature. Results of this work can be applied to tailor supplemental diets of northern quahog seed to prepare for forecasts of rapid thermal decline. Such timely dietary supplementation may reduce loss of bivalve seed which might improve the industries annual production and profitability.

1. Introduction

The approach of harsh winters in American northeast estuaries challenge native and genetically selected strains of cultured northern quahogs, *Mercenaria mercenaria*, as they cope with physiological changes associated with decreasing temperature. First year juveniles may be particularly vulnerable as thermal conduction expedites the rate of temperature change through their smallness (Witherington and Ehrhart, 1989). Loss of cultured livestock during the overwinter period is a testament to their trials and poses a significant threat to the bivalve aquaculture industry (Bricelj et al., 2007).

Prior investigations have demonstrated that juvenile northern quahogs remodel cellular membrane lipids to maintain fluidity through decreasing temperature (Pernet et al., 2006). Acclimation to low temperature favors incorporation of long chain polyunsaturated fatty acids which provide cell membrane fluidity while maintaining a high degree of phospholipid bilayer packing order (Russell and Nichols, 1999). Increasing cell membrane unsaturation can be tracked through the unsaturation index which is greatly influenced by the fatty acids 20:5n-3 (EPA) and 22:6n-3 (DHA) due to their high degree of unsaturation, 5 and 6 double bonds, respectively. Juvenile and adult quahogs feeding from the natural environment have demonstrated

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increased incorporation of these two fatty acids as autumn temperature progressively declines (Pernet et al., 2006; Parent et al., 2008); EPA is favored early during the temperature decline and DHA at the lower thermal range. This observed sequential trend of incorporation facilitated high survival of an experimental set of juvenile quahogs during the harsh temperature profile of the 2003/2004 fall/winter/spring season in Eastern Canada (Pernet et al., 2006; Fabrice Pernet pers. Comm.).

A novel preliminary investigation (Portilla et al., 2015) of juvenile quahogs in the fall of 2011 confirmed that controlled dietary abundance of EPA and DHA have distinct impacts on the early- and late-phase survival. Results indicate that groups fed substantial dietary EPA (Groups C and D) displayed negligible mortality during the temperature decline at the higher thermal range above 12 °C and the group fed abundant DHA (Group B) enjoyed lowest mortality below 12 °C. Alarmingly high rates of mortality occurred at lower temperatures in all groups that were not richly provided DHA. Nearly all mortality in these groups, ranging from 48% to 93% of total initial sample pools, occurred within the 2 week period. The timing of these mortalities was associated with two significant thermal events that occurred contemporaneously: the rapid temperature decline from 14.5 °C to 7.9 °C over 5 days, and the crossing of the approximate threshold of 12 °C from early- to late-phase. It is not clear to which factor these mortalities should be attributed or to each what extent.

A supplemental experiment, which is the subject of this paper, was designed in part to discern the degree to which each of these two factors should be attributed to the mortality events. The experiment described herein was designed to provide a mild, even temperature decrease. The study took place over 65 days between 18 °C and 6 °C, the temperature below which quahogs do not feed (Grizzle et al., 2001).

An additional component of this investigation stems from the high early-phase survival of a group of quahogs whose diet was devoid of both EPA and DHA. High survival of this group strayed considerably from the rubric established in the preliminary investigation correlating high dietary EPA with low early-phase mortality. A prior association was reported in a review of homeoviscous adaptation in *M. mercenaria* (Portilla and Branco, in press) regarding the functional similarity of EPA and the 22-carbon diene non-methylene-interrupted (22:2 NMI) fatty acids, 22:2Δ7,13 and 22:2Δ7,15 in homeoviscous adaptation. These irregularly unsaturated fatty acids were not present in any of the cultured diets and, in general, are not manufactured in marine phytoplankton; rather they must be biosynthesized de novo by quahogs. In the preliminary investigation elevated 22:2 NMI incorporation was observed in this group's lipid profile, the highest among all experimental groups, indicating that prolific biosynthesis of these compounds occurred in response to rapid thermal fluctuations in the absence of dietary EPA and DHA. This experiment will enable a comparison of that biosynthetic response to one solicited by a mild, even temperature decrease.

2. Methods

2.1. Experimental apparatus

The three experimental diets (A, B and C) contained a matrix of DHA and EPA abundance described in Table 1. The experimental quahogs were provided a mild temperature decrease averaging 0.2 °C day^{-1} for 65 days, the 20-year mean number of pre-winter days between 18 °C and 5 °C in Town of Islip waters of Great South Bay, New York. The temperature was stepped down 1.0 °C every 5 days using a Delta Star chiller and NEMA 4× Digital Temperature Controller from Aqualogic, Inc. Temperature was logged once before each feeding twice daily.

Table 1
Matrix of dietary docosahexanoic acid (DHA) and eicosapentanoic acid (EPA) available in three experimental groups, A, B, and C, of northern quahogs of the aquaculturally selected notata variety of *M. mercenaria*. Target dietary abundances of EPA and DHA are high, low and none.

FA/Diet	A	B	C
20:5n3 (EPA)	None	Low	High
22:6n3 (DHA)	None	High	Low

Each dietary treatment was administered in triplicate, each isolated in a separate system receiving the same diet and subject to the same controlled temperature regime. Each subgroup (1, 2 and 3) of each group (A, B and C) began with 300 first-year juvenile quahogs of the selected notata variety, mean shell length (MSL) 8.16 mm ± 0.72. One additional subgroup (control) was established for each dietary treatment in an isothermal system at 19 °C ± 1 °C with 300 individuals. They otherwise received the same dietary and water quality treatment as the experimental subgroups without thermal fluctuation. The juvenile quahogs were provided by the Town of Islip Shellfish Culture Facility, New York.

All subgroups (1, 2, 3 and control) of 300 quahogs were maintained in 16-L well-aerated vessels providing favorable density for high survival (Kraeuter et al., 1998). Aeration provided substantial circulation within these vessels that maintained suspension of the cultured algae which served as their diets. In order to assess clearance rates, feedings were administered twice daily. Cell counts were performed at each feeding using a Neubauer hemocytometer with 0.1 mm deep counting chamber. Average clearance rates in each system (sub-group) were calculated as algal cells removed from suspension per day per quahog. Water changes were provided at a rate of 50% per feeding, twice a day, for a total of 75% per day to minimize potential effects of poor water quality. Change water was thermally pre-conditioned before it was integrated into the systems. The thermally controlled system containing all these experimental subgroups was insulated on all sides, top and bottom to minimize the effects of incidental thermal fluctuation within the laboratory. Mortality was determined by gaping valves and recorded daily at the time of first feeding at which time all expired individuals were removed, counted and shell length measured.

Intensive sampling occurred each 13 days which accounted for a resolution of 6 data points during the 65 days in the experimental period. On these dates the shell lengths of sixty randomly selected individuals per sub-group were measured to monitor changes in mean shell length (MSL) over time. Also, soft tissue biomass from 12 randomly selected individuals from each subgroup was procured, offering triplicate fatty acid data for each dietary treatment. Biomass samples were stored in methylene dichloride at −20 °C (Parrish, 2009) until lipids were extracted and esterified.

2.2. Experimental diets

The three groups (A–C) were provided monocultures of three different algal species common to the field of bivalve aquaculture. These dietary treatments were administered to all subgroups (1, 2, 3 and control) equally. Stock cultures of these species were acquired from the Town of Islip Shellfish Culture Facility, East Islip, NY, and the National Atmospheric and Oceanic Administration (NOAA) Fisheries, Northeast Fisheries Science Center, Milford Laboratory, CT. The dietary treatments conformed to those of the preliminary investigation for comparison of results. The Group A diet was a monoculture of *Dunaliella tertiolecta* (Butcher, 1959), devoid of both DHA and EPA and no fatty acids longer than 18 carbons; Group B was a monoculture of *Isochrysis galbana* (Parke), low in EPA and high in DHA; Group C was a monoculture of *Thalassiosira weissflogii* (Grunow) G.Fryxell & Hasle, high in EPA and low in DHA (Table 1). All dietary fatty acids were verified through gas chromatography (GC) (Table 2).

The algae were cultured in 20 L carboys and illuminated under continuous light with actinic blue T5HO bulbs and harvested during the logarithmic growth phase to achieve consistent nutritional profiles. For any given week two sets of cultures were harvested for daily feedings, and a third set was retired. This third set was cleansed,