Influence of temperature and food availability on the biochemical composition and mortality of juvenile Mercenaria mercenaria (L.) during the over-winter period

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Abstract

Over-winter mortality of juvenile aquacultured northern quahogs (=hard clams), Mercenaria mercenaria, is a significant problem for the Mid-Atlantic and Northeast aquaculturists. Although protecting seed from predators improves survival, significant mortalities still frequently exceed 50%. The mortality has been attributed to severe winter temperatures, however, this suggestion has yet to be systematically investigated. We hypothesize that extended periods of low water temperatures (<5 °C) will result in reduced hard clam pumping, and a subsequent increase in the use of energy stores for metabolism. This would then lead to demise in physiological condition and cause mortality in the spring when water temperatures increase, food levels are low and metabolic demand is high. In this study, juvenile aquacultured hard clams were planted at two sites in Jamaica Bay, New York during the fall in the years of 2001, 2002, and 2004, to investigate the magnitude and mechanisms of action of over-winter mortality through the winter and spring. Measurements of temperature, chlorophyll-\(a\), and total clam biochemical composition were conducted to identify any correlations with over-winter mortality. The field data indicate that a mild winter (2001 – 2002) results in negligible mortality. Similarly, a severe winter (2004 – 2005) followed by a spring in which the rise in water temperature coincides with high food (chlorophyll-\(a\)) levels also results in low mortality. In contrast, significant mortality (up to 0.99% per day) occurs in the spring following a severe winter (2002 – 2003), at a time when water temperatures are rising but food levels are low (Chl-\(a\) < 3 µg L\(^{-1}\)). During this period a rapid decline of carbohydrate content is observed, suggesting the use of energy reserves to maintain metabolic activity. Mortality is associated with carbohydrate levels below 10% of the tissue dry weight. Therefore, it appears that significant mortality of juvenile aquacultured hard clams occurs when phytoplankton abundance is low as water temperature is increasing during the spring. It would be more appropriate to refer to this phenomena as a winter–spring mortality.

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Keywords: Mercenaria mercenaria; Over-winter mortality; Carbohydrate content; Jamaica bay; Winter–spring mortality

1. Introduction

Northern quahogs, Mercenaria mercenaria, also known as hard clams, are an important natural resource that has supported a commercial fishery along the Atlantic coast of North America for over 100 years (Belding, 1912). Declines in wild populations have led to increased aquaculture production in the United States and Canada. US Clam aquaculture resulted in a farm gate value of $50,076,000 in 1998 (U.S.D.A., 1998). Clearly, hard clam aquaculture is an important industry for the east coast of the United States. However, the growth of this industry is impeded by high over-winter mortalities of first-year cultured clams. This mortality has frequently exceeded 50% of a population of cultured clams in the Mid-Atlantic US through Canada (Ford, 2001; Zarnoch, 2006). Losses of this magnitude can be devastating to a hard clam aquaculturist since the
purchase of hard clam seed is one of the largest capital costs in the industry. Aquaculture production is not economically feasible if less than 50% of the purchased clam seed does not make it to market (Kraeuter, 2005).

Over-winter mortality of hard clams has been attributed to the effect of extreme cold weather on clams that are exposed at low tide or that are in shallow water (Dow and Wallace 1951; Haven and Andrews 1957; Greene and Becker 1977; Bower 1992), however, this hypothesis has not been systematically investigated. Mature hard clams will cease pumping and their valves will largely remain closed at and below 5 °C (Loosanoff, 1939; Ansell, 1964). At less than 3 °C the clams will remain closed completely for periods up to 18 days (Loosanoff, 1939). During this time the clams reduce their metabolism to a level where the respiratory rate is reduced to 5% the normal requirement. According to Ansell and Lander (1967), the clams catabolize stored nutrient substrates under these conditions for maintenance of their metabolic energy balance. The mature clams examined in this study used 16% of their total organic production (for one year), as determined by a reduction in carbohydrate stores and dry weight, through the course of a single winter season. However, mature clams do not experience the same large-scale mortality events as the aquacultured juveniles. Although a considerable amount of knowledge is available on the physiological ecology of hard clams (see review by Grizzle et al., 2001), there is a lack of understanding of the energy requirements and the physiological mechanisms associated with over-winter stress. Thus, a detailed analysis is needed of the utilization of endogenous reserves by juvenile hard clams in relation to the low temperatures during winter months.

Endogenous energy stores are an important component of the total metabolizable energy in marine organisms (Lucas, 1996). Lipid and carbohydrate reserves are most important during early development in clams particularly before and after larval settlement. Approximately 40 days after settlement, an ontogenetic transition occurs to a carbohydrate-protein metabolism (Mann and Gallagher, 1984). In mature clams, seasonal changes occur in carbohydrates and proteins in relation to the gametogenic cycle (Ansell and Lander, 1967). In contrast to other bivalves such as Argopecten irradians (Barber and Blake, 1981; Epp et al., 1988) and Mytilus edulis (Bayne, 1976), hard clams rely more on the immediate environment for resources needed for gametogenic activity and less on nutrient stores (Ansell and Loosmore, 1963; Bricelj and Malouf, 1980; Eversole, 2001). Juvenile scallops rely heavily on protein stores in the adductor muscle during over-winter stress (Sundet and Vahl, 1981; Epp et al., 1988). The role of energy reserves during the winter months in juvenile hard clams has not been studied. Determining the composition of carbohydrates, lipids and proteins before winter, during, and after would provide an understanding of the utilization patterns and quantity of these substrates. Developmental and seasonal metabolic cycles are a reflection of the complex interactions between food availability, temperature, growth and reproductive activity (Gabbott, 1983). Corroborating this biochemical data with other physiological indices and environmental parameters will help elucidate these complex interactions.

\[ Q_{10} \] is an index defined as a ratio of a rate function (i.e., feeding rate) at one temperature to that 10 °C higher or lower. Complete acclimation is indicated by a \( Q_{10} = 1 \), as observed in M. edulis (Bayne et al., 1977). Hard clams do not fully acclimate their feeding rate to temperature, as indicated by a \( Q_{10} > 1 \) (Hamwi, 1969; Hibbert, 1977; Doering and Oviatt, 1986). This \( Q_{10} \) indicates that hard clams use a response strategy that involves both the ability to exploit short-term favorable conditions (in relation to temperature and food availability) but also leaves them vulnerable to unfavorable conditions. Ansell and Sivadas (1973) found that the bivalve Donax vittatus shows an exaggerated increase in metabolic rate in response to temperature increases within its normal physiological range. This metabolism leaves D. vittatus vulnerable to prolonged stress because the animal is dependent on limited energy reserves to maintain metabolic rate when the food collected is inadequate to support this metabolism. This would be particularly severe during the spring months when temperatures rise and reserves are at a low due to depletion over the winter. Ansell and Sivadas (1973) suggested that this mechanism is responsible for mass mortalities of Donax during the spring months.

The current study investigates the relationships among environmental parameters (i.e., water temperature and food availability), physiological condition, and mortality of juvenile aquacultured hard clams in Jamaica Bay, New York, U.S during three separate winters.

2. Materials and methods

2.1. Experimental sites and clam sampling

The field sites are located in Jamaica Bay which is an urban estuary, approximately 4000 ha, located at the southwestern end of Long Island and thus it is part of the Hudson River estuary (Franz and Tanacredi, 1993). The two field sites were at Floyd Bennett Field (FBF; N 40°36.334’ W 073°53.137’) and at Dead Horse Bay (DHB; N 40°35.227’ W 73°54.195’). The depth of the FBF site at mean low water is approximately 0.76 m. The depth of the DHB site at mean low water is approximately 0.61 m. Analysis of the gross sediment type using the methods outlined by Holme and McIntyre (1984) indicates that the two study sites differ, the sediment at FBF is coarser, containing larger particles than the sediment at DHB. Both sites sustain a natural population of M. mercenaria (personal observation, 2001). Studies were conducted during the winters of 2001–2002, 2002–2003, and 2004–2005.

Wooden boxes with the dimensions 30 cm × 30 cm × 15 cm were constructed from 19 mm plywood with a closed bottom and open top and then were covered with 6 mm plastic mesh after stocking to exclude predators and retain the clams. All live clams were measured to determine shell length (anterior to posterior). A daily mortality rate \( - \) was calculated directly from samples. A daily mortality rate \( - \) indicates that hard clams use a response strategy that involves both the ability to exploit short-term favorable conditions (in relation to temperature and food availability) but also leaves them vulnerable to unfavorable conditions. Ansell and Sivadas (1973) found that the bivalve Donax vittatus shows an exaggerated increase in metabolic rate in response to temperature increases within its normal physiological range. This metabolism leaves D. vittatus vulnerable to prolonged stress because the animal is dependent on limited energy reserves to maintain metabolic rate when the food collected is inadequate to support this metabolism. This would be particularly severe during the spring months when temperatures rise and reserves are at a low due to depletion over the winter. Ansell and Sivadas (1973) suggested that this mechanism is responsible for mass mortalities of Donax during the spring months.

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Wooden boxes with the dimensions 30 cm × 30 cm × 15 cm were constructed from 19 mm plywood with a closed bottom and open top and then were covered with 6 mm plastic mesh after stocking to exclude predators and retain the clams. Each box was filled with sediment from the site (DHB or FBF) and stocked with 30 juvenile M. mercenaria var. notata with a mean shell length of 9.8 mm ± 0.9 mm (SD). All clams used in this study are referred to as juveniles since hard clams do not achieve sexual maturity until they have a shell length of 20 to 35 mm (Eversole, 2001). All boxes were numbered and deployed at mean low water (MLW) during a spring tide in November when the water temperature was 12 °C. Chlorophyll-a analysis was conducted on three replicate samples of 500 ml using an acetone extraction method (Parsons et al., 1984).

The sampling began in November of each year and was conducted bi-weekly through June. Live clams as well as dead ones (defined as “gaping” or “clapper”) were counted and percent cumulative mortality (% MO = dead clams/total clams) was calculated directly from samples. A daily mortality rate for each sampling period (Patrick et al., 2006) was calculated as: [(initial number − final number/initial number− initial number)/2]/number of days × 100. All live clams were measured to determine shell length (anterior to posterior).
Whole live clams were opened and the tissue was carefully removed. The wet tissue weight and shell weight were determined separately. The tissue and shell samples were then placed into a drying oven at 60 °C for >48 hr then reweighed to obtain the dry weight of each sample. The dried tissue was stored at ~20 °C until used in biochemical analyses. A condition index was calculated as a ratio of dry tissue weight to shell length×1000. The condition index is a measure of the metabolic condition of a bivalve and is related to the quantity of glycogen stored (Mann, 1978; Lucas and Beninger 1985).

2.2. Biochemical analyses

Biochemical analyses were conducted on pooled dry tissue from all live clams within a box to determine gross carbohydrate, protein, and lipid content. The dried tissue samples were ground to a fine powder using a mortar and pestle. Subsamples of this powder were then used for each biochemical assay. Therefore, the reported variance is methodological rather than variability among boxes. The determination of carbohydrate content was determined in triplicate using the phenol-sulfuric acid method of Dubois et al. (1956). A commercial kit (Pierce Biotechnology, IL.) of the coomassie blue method (Bradford, 1976) was used to determine protein content in triplicate. A calibration curve was constructed using bovine serum albumin as a standard. Total lipid content was estimated gravimetrically with three replicates ( Folch et al., 1957). The results of all biochemical analyses are expressed in μg of carbohydrate, protein, and lipid in each mg of dried clam tissue.

2.3. Statistical analyses

Statistical analyses employed a one-way analysis of variance to test all differences in shell length, dry tissue weight, and condition index. Normality was assured using the Kolmogorov–Smirnov statistic and adequate transformations were performed when necessary. Post-hoc comparisons were made using the Tukey HSD test. To test for differences in biochemical content among samples in a given year, the Kruskal–Wallis test was employed. To test for differences in biochemical content between two specific groups the Mann–Whitney test was employed (Zar, 1999). Statistical tests were performed with SPSS® version 11.5 and figures were created with SigmaPlot 8.0 (SPSS Inc., Chicago, IL).

3. Results


During the winter of 2001–2002 water temperatures were below 5 °C for two weeks at DHB and for four weeks at FBF during January (Fig. 1). Water temperature remained between 6 °C and 10 °C until March. There was no mortality observed at either site until March (Fig. 1). Thereafter, daily mortality rate (in the spring) was low ranging from 0.06% to 0.2%. Data collection at FBF ceased in May 2002 due to vandalism of the boxes.

Chlorophyll-α was greater than 20 μg L⁻¹ throughout the winter (Fig. 2). A phytoplankton bloom was observed during January and February with a peak value of 56 μg L⁻¹ observed at DHB and a peak value of 69 μg L⁻¹ observed at FBF. The quantity of chlorophyll-α began to decline during March and with the lowest values measured in May. The minimum observed value at DHB was 2 μg L⁻¹ and 4 μg L⁻¹ at FBF. These low values occurred when the water temperatures were approximately 14 °C at both sites. A summer bloom was observed at DHB during June as indicated by rising chlorophyll-α values up to 55 μg L⁻¹.

There was no significant (ANOVA; p > 0.05) change in condition index, shell length, and tissue dry weight at either site throughout the winter, when compared to initial measurements made prior to the winter (Fig. 3). However, an increase in these parameters was observed during April and continued to increase through the remaining sampling period. The increase in condition index was first noted at DHB in early April. Shell length and tissue dry weight significantly increased simultaneously at FBF and DHB. Growth (an increase in shell or somatic tissue) began when water temperatures were 12 °C and greater. The initial observed growth increase occurred when chlorophyll-α were at the lowest observed values (2 μg L⁻¹ at DHB and 4 μg L⁻¹ at FBF). Clams at DHB increased in shell length from 14.78 mm to 19.00 mm and in tissue dry weight from 47.86 mg to 107.10 mg. This is a growth rate of 1.1 mm week⁻¹ in shell length and 14.8 mg week⁻¹ in tissue dry weight (Table 1).

There were no significant differences observed in tissue dry weight or condition index through the winter, however the biochemical composition of the clams varied significantly during the sampling period (Fig. 4). This was most evident in the changes in protein content as it decreased through the winter and spring. The decrease was not statistically significant at FBF (Mann–Whitney; p = 0.063), however at DHB the decreases were statistically significant (Mann–Whitney; p ≤ 0.05). The first significant decrease was noted in December at the time the water temperature fell below 5 °C. The protein content fell from an initial value of 480 μg mgDW⁻¹ to 439 μg mgDW⁻¹ and remained significantly lower than the initial value through the sampling period. A minimum value of 319 μg mgDW⁻¹ was observed in April at the same time chlorophyll-α values were at a minimum (2 μg L⁻¹ at DHB and 4 μg L⁻¹ at FBF). The protein content then increased as the summer bloom occurred.

The carbohydrate content of the clam tissue did not significantly differ from the initial measured value at FBF throughout the sampling period. A minimum value of 319 μg mgDW⁻¹ was observed in April at the same time chlorophyll-α values were at a minimum (2 μg L⁻¹ at DHB and 4 μg L⁻¹ at FBF). The protein content then increased as the summer bloom occurred.

![Fig. 1. Daily mortality rate of hard clams, *M. mercenaria*, and water temperature observed at Floyd Bennett Field (FBF) and Dead Horse Bay (DHB) during the winter of 2001–2002.](image)

![Fig. 2. Chlorophyll-α and water temperature measured at Floyd Bennett Field (FBF) and Dead Horse Bay (DHB) during the winter of 2001–2002.](image)

![Fig. 3. Chlorophyll-α and water temperature measured at Floyd Bennett Field (FBF) and Dead Horse Bay (DHB) during the winter of 2001–2002.](image)
period. At DHB, significant increases in carbohydrates occurred in January, February, April, and June. The observed changes seemed to fluctuate and all observed increases occurred at a time when chlorophyll-α values were high. However the exception to this observation is in April when chlorophyll-α and protein content were at a minimum.

The lipid content of the clam tissue differed significantly from the initial value on two sampling dates at FBF. In December it decreased significantly and in April it increased significantly. The lipid content of clams at DHB also varied with observed decreases in January and increases occurring in February and June.


During the winter of 2002–2003 water temperatures at DHB and FBF were below 5 °C for fourteen consecutive weeks (Fig. 5). Mortality, up to 0.49% daily, was found throughout the winter at both sites and was first noted when water temperature fell below 5 °C. However, daily mortality rate increased up to 0.99% at DHB and up to 0.63% at FBF in the spring as the water temperature rose above 5 °C (Fig. 5).

The chlorophyll-α content of the seston was between 15 and 20 µg L⁻¹ at both sites through most of the winter (Fig. 6). FBF had slightly higher values than DHB throughout the sampling period. The winter–spring bloom peaked at the end of January but continued through the beginning of March. However, chlorophyll-α values drastically decreased at the end of March and continued to decrease through May reaching minimum values of 0.9 and 1.6 µg L⁻¹ at DHB and FBF respectively. The decrease in chlorophyll-α during the March to June period coincided with the increase in temperature (Fig. 5). This also coincided with the greatest mortalities observed in the samples.

Table 1
Temperature and clam growth rate of shell length and tissue dry weight during the final four weeks (May–June) of experimental periods at Dead Horse Bay (DHB) and Floyd Bennett Field (FBF)

<table>
<thead>
<tr>
<th>Year and site</th>
<th>Growth in shell length (mm week⁻¹)</th>
<th>Growth in tissue dry weight (mg week⁻¹)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2002 — DHB</td>
<td>1.1</td>
<td>14.8</td>
<td>17–22</td>
</tr>
<tr>
<td>2003 — DHB</td>
<td>0.19</td>
<td>0.8</td>
<td>13–14</td>
</tr>
<tr>
<td>2003 — FBF</td>
<td>0.43</td>
<td>2.1</td>
<td>14–15</td>
</tr>
<tr>
<td>2005 — DHB</td>
<td>0.63</td>
<td>6.8</td>
<td>15–21</td>
</tr>
<tr>
<td>2005 — FBF</td>
<td>1.0</td>
<td>9.0</td>
<td>15–21</td>
</tr>
</tbody>
</table>

No data was collected in May and June at FBF in 2002.

Fig. 4. Weight specific content (µg substrate mgDW⁻¹) of proteins, carbohydrates, and lipids in tissues of hard clams, M. mercenaria, at Floyd Bennett Field (FBF) and Dead Horse Bay (DHB) during the winter of 2001–2002. Values are means±SE.

Fig. 5. Daily mortality rate of hard clams, M. mercenaria, and water temperature observed at Floyd Bennett Field (FBF) and Dead Horse Bay (DHB) during the winter of 2002–2003.
Measurements of condition index, shell length and tissue dry weight of clams at DHB were lower than the initial value during the winter and early spring, but the difference was not statistically significant. No significant increase in these values was observed in the late spring at DHB. An increase in condition index and tissue dry weight was observed at FBF in May. At FBF, condition index, shell length and, tissue dry weight increased throughout the last four weeks of the sampling period (Fig. 7). Growth of shell length was 0.43 mm week\(^{-1}\) and growth of tissue dry weight was 2.1 mg week\(^{-1}\) (Table 1).

There were relatively few changes observed in condition index, shell length, and tissue dry weight, compared to the significant differences in the biochemical composition, particularly carbohydrate content, of the clams during this period. The protein content (Fig. 8) of the clam tissue remained relatively stable throughout the winter at both sites (initial value of 442 µg mg\(\text{DW}^{-1}\) up to 480 µg mg\(\text{DW}^{-1}\)). An increase in protein content was observed at both sites on the final two sampling dates increasing to a maximum value of 558 µg mg\(\text{DW}^{-1}\) and 545 µg mg\(\text{DW}^{-1}\) at DHB and FBF respectively. Carbohydrate content differed significantly (Mann–Whitney; \(p = 0.02\)) at the beginning of March falling from an initial value of 442 µg mg\(\text{DW}^{-1}\) to 149 µg mg\(\text{DW}^{-1}\). This pattern continued as carbohydrates decreased 63% at DHB from the initial value to 72 µg mg\(\text{DW}^{-1}\) on 23 April. The carbohydrates in clams at FBF also decreased in the spring, however not quite as dramatically. A significant decrease of 48% of the initial value was observed on 10 April when levels dropped to 101 µg mg\(\text{DW}^{-1}\) (Mann–Whitney; \(p = 0.05\)). The decrease in carbohydrate content coincided with low chlorophyll-a levels and when water temperatures were rising above 5 °C. The lipid content of the clam tissue was initially 76 µg mg\(\text{DW}^{-1}\) and increased during the spring up to maximum observed values of 95 µg mg\(\text{DW}^{-1}\) at FBF and 90 µg mg\(\text{DW}^{-1}\) at DHB.

### 3.3. Winter 2004–2005

The winter of 2004–2005 was similar to 2002–2003 in that water temperatures were below 5 °C for sixteen weeks. Water temperature fell below 5 °C at the end of December and did not increase above 5 °C until April (Fig. 9). Mortalities were low throughout the winter samples as well as in the spring. The largest observed daily mortality rate was measured on 6 June at both sites with 0.55% and 0.32% at DHB and FBF respectively (Fig. 9).

Chlorophyll-a values at both sites were low (<5 µg L\(^{-1}\)) through November and December. The maximum values measured occurred at the end of March during the winter–spring bloom (Fig. 10). The highest value at FBF was 64 µg L\(^{-1}\), nearly double the highest value of
36 µg L$^{-1}$ at DHB. The chlorophyll-α decreased during May to a low of 3.7 µg L$^{-1}$ at DHB and 11 µg L$^{-1}$ at FBF; water temperature this time was approximately 15 °C at both sites. Chlorophyll-α was higher at FBF on most sampling dates.

There was no significant difference in condition index, shell length, and tissue dry weight through the winter; however, changes were observed in the last three samples which included May and June (Fig. 11). During the last four weeks of the sampling period significant growth occurred at both sites (Table 1). However, clams at FBF grew faster, 1.02 mm week$^{-1}$ in shell length and 8.98 mg week$^{-1}$ in tissue dry weight compared to 0.62 mm week$^{-1}$ in shell length and 6.75 mg week$^{-1}$ in tissue dry weight at DHB.

The biochemical content of clams during the winter of 2004–2005 did not undergo any decreases as seen in previous years (Fig. 12). Protein content in clams fluctuated through November and December as water temperature began to fall and chlorophyll-α levels were low. The increase in protein content in March at both sites coincided with the winter–spring phytoplankton bloom observed. Water temperature was >5 °C at this time. Protein content at FBF reached a maximum of 528 µg mgDW$^{-1}$ and 496 µg mgDW$^{-1}$ at DHB during this bloom. On 24 May, low protein levels were observed (372 µg mgDW$^{-1}$ at FBF and 384 µg mgDW$^{-1}$ at DHB). This decrease in protein content occurred when water temperature was 15 °C and chlorophyll-α was at the lowest observed value during the spring. The carbohydrate values did not change significantly from November through February. At DHB, a significant decrease was observed on 24 March, dropping from
the initial value of 180 µg mgDW\(^{-1}\) to 160 µg mgDW\(^{-1}\). An increase in carbohydrate content was observed at both sites on the final sample in June increasing to approximately 190 µg mgDW\(^{-1}\). The lipid content of the clams followed the same seasonal pattern of the protein content, increasing in March during the winter–spring bloom, declining in April and May, and increasing again during the last four weeks of the sampling period.

4. Discussion

4.1. Mortality, temperature, and chlorophyll-a

This work is the first field study to monitor the survival of juvenile hard clams from November to June for three winters and to correlate mortality with water temperature fluctuations, food availability and physiological analyses. Previous investigators that experienced over-winter mortality in field populations conducted their sampling in spring or summer following the winter (Elderidge et al., 1976; Walker and Humphrey, 1984). Kraeuter and Castagna (1984), however, observed significant over-winter mortality of juvenile hard clams (size range of 2–10 mm shell height) in land-based nursery systems. A majority of the mortality seemed to occur in the spring as water temperatures approached 10 °C. The timing of this mortality corroborates well with the mortality events experienced during our field studies.

In the same study, Kraeuter and Castagna (1984) isolated a Vibrio species from dead clams and hypothesized that the mortality might have been due to a bacterial infection. However, they could not induce mortality when they exposed healthy clams to Vibrio isolates or to dead and dying clams. Therefore, the mechanism for the mortality remained unclear. Our field studies suggest a relationship between mortality and food availability in the spring.

The majority of deaths observed during our field studies of 2002–2003 occurred in the spring as water temperatures increased to 10 °C, following a winter where water temperatures remained less than 5 °C for at least 14 consecutive weeks. The increase in water temperature occurred at the same time when the winter–spring bloom of phytoplankton had waned and the summer bloom had not yet started. Therefore, food in the form of a winter–spring bloom was available to the clams (2002–2003) when they could not utilize it; due to the fact clams cease feeding at temperatures below 5 °C (Loosanoff, 1939; Zarnoch, 2006). Unfortunately, when the temperature did increase to 10 °C (a temperature where the clams would actively feed), the bloom had been reduced. Therefore, high food availability is important as the clams become active with increasing temperatures after winter dormancy. In our study of winter of 2001–2002, mild air temperatures caused the water temperature to be between 6 and 10 °C during most of the winter–spring bloom. The water temperature was 12 °C when the winter–spring bloom began to dissipate. Interestingly, little mortality was observed in the spring, regardless of the low food availability. In the winter of 2004–2005, the cold air temperatures resulted in water temperatures to remain below 5 °C for sixteen weeks. In 2004–2005, the winter–spring bloom occurred at the end of March and did not dissipate until May. The high food availability as water temperatures increased to 10 °C led to increased survival of the clams. Therefore, when contrasting 2002–2003, where significant mortality occurred, with the 2001–2002 and 2003–2004 where little mortality occurred, the critical role of food availability during the increase in temperature from 5 to 10 °C is observed. As seen in the winter of 2001–2002, the amount of available food is not as critical when the water temperature is above 12 °C.

4.2. Condition index, shell length, and tissue dry weight

Changes in classical measures of growth, such as condition index, shell length, and tissue dry weight are affected by changes in feeding rate (Bayne and Newell, 1983). At temperatures less than 5 °C, hard clams cease feeding (Loosanoff, 1939; Zarnoch, 2006) and thus no increase in growth would be observed. Growth may occur at temperatures above 7 °C with maximum growth occurring between 20° and 24 °C (Ansell, 1968).

In our field studies, changes in growth parameters occurred only in the spring and early summer when water temperatures were greater than 12 °C. Condition index was the most sensitive parameter studied to indicate statistically significant increases in growth. Condition index was the first to indicate a statistically significant increase in growth or it would do so simultaneously with increases in tissue dry weight and/or shell length. Therefore, condition index is the most useful indication of growth in juvenile hard clams. However, neither condition index or tissue dry weight are good indications of a significant decrease in somatic tissue. This was demonstrated in the 2002–2003, when mortality was high and there was low phytoplankton abundance. Therefore, the use of more sensitive indices such as ash free dry weight or biochemical assays is advised when investigating negative potential decreases in clam physiological status.

When examining the different growth rates observed during May and June through the study years, it is evident that growth is influenced by temperature and food availability. The shell growth rates we observed were comparable to other published reports. Grizzle et al. (2001) reviewed published shell growth rates for juvenile clams fed natural seston and found a mean growth rate of 0.74 mm week\(^{-1}\). This is consistent with the combined mean growth rate of 0.67 mm week\(^{-1}\) from both field sites through our study.

4.3. Biochemical analysis

The analyses conducted as part of this research provide unique data, unavailable until now, on the biochemical content on juvenile hard clams. The only published data on the biochemical composition of hard clams was research conducted on adults (Ansell and Lander, 1967). There is an inherent problem in assuming that the biochemical content of adult bivalves would be similar for juveniles since the adults utilize energy reserves for gametogenesis (Gabbott, 1975). Therefore, this work provides novel data on the seasonal changes in biochemical
content unrelated to gametogenesis. Additionally, this research highlights the importance of simultaneously measuring tissue biochemical content and chlorophyll-a.

We believed that a decrease in biochemical stores would be found throughout the winter season, based on our assumption that clams rely on stored energy to support metabolism when water temperatures causes them to cease feeding. This would seem likely as metabolic energy demands must be met from endogenous reserves during periods of starvation (Bayne and Newell, 1983). We further hypothesized that protein, as seen in juvenile bay scallops (Epp et al., 1988), and carbohydrates, as seen in mature clams (Ansell and Lander, 1967), would be utilized during this period. The results show that there were few statistically significant decreases in biochemical stores during the winter; instead all significant changes occurred in the spring as water temperature rose above 5 °C. Observed decreases in biochemical composition were not only related to changes in temperature but to food availability, as well.

Our investigation of protein, carbohydrate, and lipid content of juvenile clams during the study years, with simultaneous measurement of temperature and chlorophyll-a, has led to the identification of three physiological responses that relate metabolic flux to over-winter mortality. The contribution of lipids to the entire biochemical body content of juvenile clams is small, ranging from 5% to 10%. When changes (increases or decreases) in lipid content were observed over the study years they mirrored changes in protein content. Therefore, since lipid content plays such a minimal role in the physiological energetics of juvenile hard clams during the over-winter period and since any observed changes over the years of study mirror protein fluxes, lipids will not be discussed in detail here.

The first identified physiological response is a reduction in metabolic rate to ≤5% the normal metabolic rate when the water temperature is below 5 °C (Ansell and Lander, 1967) and juvenile hard clams do not show any reduction in biochemical content. Metabolic rate, as measured by oxygen consumption, is not at all influenced by the quantity of food available during this low temperature period (Zarnoch, 2006). Apparently, this dormancy is highly efficient so that insignificant quantities of energy reserves are used indicated by the absence of significant decreases for all winters studied. Other bivalve species found in temperate waters do not show the same ability to conserve energy. M. edulis and C. edule lose 0.5% to 1.0% of their body weight per day during the winter (Bayne and Newell, 1983).

First year A. irradians lose 63–66% of the stored protein in their adductor muscle during winters in New York waters (Epp et al., 1988). Hard clams are different from these bivalves, in that they do not store significant amounts of energy reserves since most metabolic demands (including somatic and gonadal growth) are met by freshly assimilated material (Ansell and Loosmore, 1963). Thus, they have adapted a strategy to conserve energy reserves during periods when they are not feeding. Additionally, this research highlights the importance of simultaneously measuring tissue biochemical content and chlorophyll-a.

Another important physiological response identified in this research is related to water temperatures between 5° and 12 °C. The patterns of endogenous energy utilization in juvenile hard clams are quite dynamic in this temperature range. These patterns are also highly dependent on the amount of food available to the clams. Increases in temperature cause increases in the metabolic rate in hard clams. This is an advantageous strategy during periods of abundant food as the clams can better exploit the available resources. However, if an external food supply is not available, or is too low to meet the increase in clam metabolic rate, clams must utilize endogenous energy reserves. As we have seen, this is a particularly severe problem for juvenile hard clams since they do not store significant quantities of reserves.

The winter of 2002–2003 showed a relationship between decreasing carbohydrate reserves and increasing mortality. This was also correlated with water temperatures that were between 5° and 12 °C and with low food availability. During this period, hard clams at DHB and FBF lost approximately 63% and 48% of their carbohydrate content, respectively. This resulted in carbohydrate levels of 7.2% and 10% of the clams’ total dry weight at DHB and FBF. Observations during the winters of 2002–2003 demonstrate that increases in water temperature above 5 °C leads to clams becoming metabolically active and thus must utilize endogenous energy reserves to support the high rate of metabolism.

Another bivalve that follows a similar metabolic pattern in relation to temperature is D. vittatus (Ansell and Sivadas, 1973). Donax will increase its metabolic rate with increases in temperature and will maintain this metabolic rate even under condition of no or little food by utilizing energy reserves. Initially, carbohydrates will be utilized followed by rapid catabolism of protein. The authors suggested this was the mechanism responsible for the mass mortalities of Donax that occur in the spring. The adaptive significance of maintaining a high metabolic rate even under conditions of low food in juvenile hard clams may be related to a need to grow large enough so that they would not be quite as vulnerable to predators. Kraeuter (2001) discussed this concept in an extensive review of all the predators of hard clams. He suggested that mortalities due to predation decrease when clams reach 20–25 mm in shell length.

Increased metabolic rate in response to increased temperature facilitates the use of energy reserves under conditions of low food. In the winter of 2004–2005, the increase in water temperature coincided with the winter–spring bloom; therefore the abundant food supply was sufficient to support the clams’ high metabolism. During this period there was no loss of carbohydrate or protein reserves. Thus, a metabolic imbalance will only occur when water temperatures are between 5° and 12 °C and external food supply is not sufficient to support metabolism.

The results obtained from the winters of 2002–2003 demonstrate that significant mortalities occur when carbohydrate content falls to levels at or below 10% the clams’ total dry weight. A minimum threshold level of total carbohydrates has been associated with summer mortality events of Crassostrea gigas in Japan and France (Mori, 1979; Patrick et al., 2006). In these oysters, the summer mortality occurs at a time when metabolic demand is high due to gonad maturation and when carbohydrate content falls below 10%. It is now believed that a similar threshold exists for hard clams during physiologically stressful periods.
The third physiological response identified in this study dealt with the relationship of growth to temperatures greater than 12 °C. As discussed earlier, growth may occur when water temperatures are above 7 °C (Ansell, 1968). However, in our study growth was not observed until water temperatures were above 12 °C, with maximum growth occurring between 15° and 20 °C. Analysis of the changes in biochemical composition revealed a decrease in protein content when temperatures are above 12 °C and chlorophyll-α values are low. This is evident during the winter of 2001–2002 when a significant decrease in protein content occurred in the spring at the same time that food levels were minimal. The clams, nevertheless, were experiencing significant growth. Another example of this phenomenon occurred in the winter of 2004–2005. During this year the winter–spring bloom occurred later than normal and persisted into the spring when water temperatures had increased. However, food availability was low and water temperatures were still increasing at the end of the winter–spring bloom and before the beginning of the summer bloom (end of May). We observed a decrease in protein content but continued growth in the clams. In both these examples, tissue protein content increased when food levels eventually increased during the summer bloom. In all instances where temperatures were above 12 °C and food levels were high, there was no decrease observed in any biochemical substrate. An explanation for this decrease in proteins, observed during periods of growth, could be protein turnover. This is the continuous degradation and replacement of cellular proteins (Hawkins and Day, 1996). If protein macromolecules were being broken down into amino acids and nucleotides during this proposed physiological response to temperature and food availability (Mandelstam, 1960), the Bradford protein assay used in these analyses would not detect these smaller molecules (Kruger, 1996). This could explain the lower measured protein values. However, it would still suggest that the clams are using a protein-based metabolism under these specific environmental conditions. The role of protein turnover and its related metabolic costs during growth should be further examined in hard clams.

Over-winter mortality of juvenile hard clams appears to be related to a complex of interactions of temperature dynamics and food availability and that is closely linked to the physiological condition of the organism. Mortality is greatest when water temperature increases to greater than 5 °C and there is little food available to support an increase in metabolic rate. Therefore, the mortality is not occurring “over-winter” and thus should be referred to as winter–spring mortality. This winter–spring mortality is associated with a decrease in carbohydrate content to a level below 10% the tissue weight. There is also a clear disparity between our two field sites, as mortality was consistently greater at DHB than at FBF. The quantity of carbohydrates in clams prior to winter may be important in determining their ability to survive. It appears as though a greater carbohydrate content entering the winter would allow a clam to withstand a period of low food and high metabolic rate longer than a clam with low carbohydrate content entering the winter. However, juvenile clams do not appear to store significant quantities of carbohydrates as they instead allocate it to somatic growth (Kraueter, personal communication 2005; Zarnoch, personal observation, 2006).

Jamaica Bay is a highly productive estuary due to excessive nutrient loadings and thus its primary productivity is generally higher than other estuaries. This is interesting in relation to winter–spring mortality because its high productivity aids in supporting clam metabolism during physiologically stressful periods (as outlined above). Thus, our research may underestimate the magnitude of winter–spring mortality because in other estuaries, where primary productivity is limited by nutrients, winter–spring mortality may be more severe.

The quality of the food available to the clams is another important consideration in addition to quantity. For example, Great South Bay, New York often experiences blooms of picoplankton (<1 to 4 µm) that hard clams do not efficiently feed upon. Apparently, this is due to poor absorption efficiency of these cells due to indigestible cell walls (Bass et al., 1990) or short residence time in the gut of the hard clams (Bricelj et al., 1984). If this type of phytoplankton assemblage were to dominate at a time when temperatures are rising between 5° and 12 °C, the juvenile clams would have to use energy reserves to support metabolism and this could lead to mortality as demonstrated in this study. Poor food quality has already been suggested as a mechanism that is reducing hard clam reproductive effort in certain locations in Great South Bay (Newell et al., 2003).

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