STUDIES ON THE PHYSIOLOGICAL BASIS FOR OVER WINTER MORTALITY IN JUVENILE AQUACULTURED HARD CLAMS, *MERCENARIA MERCENARIA* (LINNAEUS, 1758)

by

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Abstract

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Co-Advisers – Martin P. Schreibman and Jennifer Basil

Over-winter mortality of juvenile aquacultured hard clams is a significant problem for the Mid-Atlantic and Northeast aquaculturists. Although protecting seed from predators improves survival, significant mortalities still frequently exceed 50%. This is often attributed to the severe temperatures of winter; however, this suggestion has yet to be systematically investigated. We hypothesize that extended periods of low water temperatures (<5ºC) will result in reduced hard clam pumping, and thus an increase in the use of energy stores for metabolism. This would then lead to an insult 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 (SL=10mm) were planted at two sites in Jamaica Bay, New York during the fall of each year between 2001-2004, to investigate the magnitude of over-winter mortality through the winter and spring. Measurements of temperature, chlorophyll-*a* and clam biochemical composition were conducted to identify any correlations with over-winter mortality. Concurrent controlled laboratory experiments (using recirculating aquaculture systems) studied the effect of varying temperatures and food regimes in relation to clam metabolic processes. The field

data indicate that a mild winter results in negligible mortality. Similarly, a severe winter followed by a spring in which the rise in water temperature coincides with high food levels also results in low mortality. In contrast, significant mortality (up to 45% per sample) occurs in the spring following a severe winter, at a time when water temperatures are rising but food levels are low ($< 3 \mu g\ L^{-1}$). 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, significant mortality of juvenile aquacultured hard clams occurs when phytoplankton abundance is low as water temperature is increasing during the spring. A seemingly unrelated mortality occurred in the laboratory experiments and appears to be associated with significant loss of endogenous protein reserves.

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INTRODUCTION

The hard clam, *Mercenaria mercenaria,* is an infaunal bivalve commonlyfound in coastal embayments from the Gulf of Maine to Florida and along the Gulf Coast of the United States. They have also been introduced to the Pacific coast of North America, Puerto Rico, England, France, and Taiwan (Chew, 2001). Hard clams inhabit bays, estuaries, and other protected areas where temperatures are between 2ºC and 28ºC and salinities range from 17 to 32 ppt. (Castagna and Chanley, 1973; Kraeuter and Castagna, 1977). Large populations of this suspension feeder reside in shallow, subtidal flats, but are also found intertidally as well at depths up to 15m. They prefer a sandy or soft bottom containing shell fragments, but can also be found in seagrass beds (Pratt, 1953; Pratt and Campbell, 1956; Wells, 1957).

Hard clams are a high demand food source and have sustained a commercial fishery dating back to the turn of the century. In fact, Belding (1912) believed people of European descent ate hard clams for as long as they have occupied North America. During the present century, northern states have produced far more hard clams than southern states. This was likely due to the tremendous production from New York's Great South Bay and New Jersey's coastal bays. However, Florida's production has grown considerably since the 1980's. Total US hard clam landings have declined through the Twentieth Century, in 1997 yields from Maine to Florida were about half of what they were in 1950 (Kraeuter, 2005). A large part of this decline was due to production declines in New York and New Jersey. In 1976, peak landings were recorded in New York, with 750,000 bushels harvested worth an estimated 14.6 million dollars. However, overfishing has decimated natural clam beds thus impeding recruitment by the removal of

sexually mature animals and leaving few productive beds. In addition, pollution has impacted the industry through the degradation of water quality, especially bacteriological factors, that have resulted in the closure of productive clam beds to fishing. In 2000, approximately 190,000 bushels of clams were harvested from New York. Forty-six percent of this harvest was the result of clam transplants from uncertified waters in Raritan Bay, NY to the certified waters off Long Island for depuration. In March 2003, the New York State Department of Environmental Conservation (NYSDEC) closed down the transplant program because Raritan Bay clams were infected with the disease "Quahog Parasite Unknown", (Barnes et al., 2004). Although, this parasite has not proven to be harmful to humans, it is believed that it can be transmitted to wild populations of hard clams at the depuration areas of eastern Long Island. This clam bed closure will likely increase pressure on natural resource managers and legislators to find alternative strategies for clam harvests.

In an effort to alleviate pressure on natural stocks and to increase commercial harvest, a number of hard clam aquaculture programs have been developed in the US. These programs play an integral role in US and foreign economies. Production of "seed", or juvenile clams, has been shown to be a successful management tool for local government "put-and-take" programs, as well as for commercial grow-out (Malouf, 1989). The so-called "put and take" programs involve local governments funding the reseeding of clam beds in order to help support the fishing community, economy, and environment. Although clam aquaculture practices vary with location along the coast, the Northeast region uses similar production techniques with the common goal of producing a legal size clam (1" shell [hinge] height in NY) in as short a time frame as possible.

Commercial production of hard clams typically occurs in three phases. The first phase is the hatchery component, beginning in early spring and continuing through May, during which adult broodstock hard clams are conditioned to prepare for spawning by increasing temperature and food levels. The adults are then induced to spawn through thermal shock (sharp increase in temperature) and addition of a gamete suspension. The gametes are mixed and the resulting larvae are then cultured in the hatchery until they reach 2mm in shell length (measure of anterior to posterior). At this time the clams are then moved to the nursery phase, which uses natural bay water either in land based upwelling/downwelling systems or floating (*in situ*) upwelling systems (FLUPSY). The nursery phase may last only one to two months or culture in these systems can continue through the growth season (June through August). The field grow-out phase involves culturing the now larger clams (sizes vary) in racks or bags out in the bay for the duration of the growing season. This typically begins in early summer and continues through the fall season. In the last step of production, the juvenile clams are planted in the fall on the bottom of the bay with or without predator protection, until they reach market size.

One significant problem encountered by shellfish aquaculturists is the mortality of juvenile clams during their first winter in the field. Mortalities ranging from 5 to 100 % have been observed by shellfish aquaculturists (Aldred et al. 2001, Ford, 2001, Walker and Humphrey 1984, Flagg and Malouf 1983, Elderidge et al. 1976) when they sample the planted populations during the following summer growing season. Predation has accounted for most of this mortality. There are a number of predators that prey on shellfish; the species that seem to be the most devastating in Long Island waters include the starfish, *Asterias vulgaris,* the whelk, *Buscyon sp.*, and several species of crabs. The

data available on the significance of these predators have on clam seed are reviewed by Kraeuter (2001). In order to avoid predation many aquaculturists have adopted techniques to protect the seed that include the use of gravel and shell aggregates, mesh bag systems, crab trapping, and mesh covered plots (Kraeuter and Castagna, 1985). Although, this increases labor and production costs it also significantly reduces predation. However, there is evidence of significant mortality of clam seed even when the animals are under protected conditions (Aldred et al. 2001,Walker and Humphrey 1984, Elderidge et al. 1976).

Aldred et al., (2001) conducted a comprehensive over-wintering experiment that tested the feasibility and efficacy of a number of methods for the extended culture of clams. One method used wooden boxes (measuring 2 ft. x 2 ft.) filled with sediment, stocked with 200 clams and covered with a ¼" mesh screen on one surface. The boxes were deployed during August and October of 1999 and were sampled in July 2000. The result of these efforts was survival as low as 47%.

Such large-scale mortalities, often seen from the Mid-Atlantic US through Canada, appear to be related 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). Mature hard clams will cease pumping and their valves will largely remain closed at and below 5ºC (Loosanoff, 1939; Ansell, 1964). At less than 3ºC 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. Under these conditions, the clams are catabolizing stored nutrient substrates for maintenance of their

metabolic energy balance (Ansell and Lander, 1967). These studies were conducted on wild mature hard clams, which 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 cold temperature stress and mortality of juvenile aquacultured hard clams.

In an attempt to identify the possible factors involved in this mortality and improve survival, Kraeuter et al. (1997) initiated a comprehensive study that incorporated shellfish aquaculture groups from New York, New Jersey, and Massachusetts. The variables manipulated at or before the fall deployment of clam seed included: nutrition manipulation during a conditioning period (starved [no food], ambient food only, or ambient augmented with cultured algae); temperature at deployment (12º or 14°C); sediment type (sand or mud); and clam size (6, 8, 10, 12 mm average shell width). Size was the only variable that correlated with mortality. Larger clams survived better than the smaller clams. This result is consistent with the theory that larger clams would have greater energy stores (carbohydrates, proteins, and lipids) thus decreasing mortality.

Ansell and Lander (1967) demonstrated that mature hard clams use energy stores during the winter months, as determined by a reduction in carbohydrate stores and dry weight. They reported that mature clams used 16% of their total organic production (for one year) through the course of a single winter season. Kraeuter et al. (1997) did not follow the physiological condition of the hard clams throughout the winter period and the environmental variables related to their condition. Thus, a detailed analysis of the

utilization of endogenous reserves by juvenile hard clams in relation to the low temperatures during winter months is needed.

Kraeuter et al. (1997) suggest that providing supplemental feed prior to planting in the fall, thereby increasing endogenous reserves, does not increase survival during the winter. This suggests that the quantity of energy stores going into the winter may not be as significant a factor as perhaps the length of time the clam must rely on these stores. However, Ansell and Lander (1967) demonstrated that endogenous reserves are depleted during the course of a winter season. This may be dependent on the severity of the winter and how long the temperature is below the 5ºC threshold.

Kraeuter has also indicated that when supplemental feedings were performed prior to deployment, only a small increase in glycogen reserves was observed and the amount stored was capped because clams use this resource for growth (personal communication, 2003). Adult hard clams also store only small amounts of reserves and thus gonadal development occurs sporadically in response to the external food supply (Ansell and Loosmore, 1963). This is in contrast to other bivalves such as *Argopecten irradians* (Barber and Blake, 1981) and *Mytilus edulis* (Bayne, 1976) in which gonadal development takes place largely at the expense of endogenous reserves stored in somatic tissues (i.e., adductor muscle) during periods of high food availability.

There may good reason to further investigate the observations of Kraeuter and his associates (1997) that supplemental feedings do not show any increase in survival of over-wintered hard clams. Aquaculturists observe low mortalities in their field cultures during the fall when clam seed is still in racks or other culture units. We believe that this mortality is a result of clams being challenged by being in a high-density culture situation

while subjected to falling temperatures and low food availability, both typical of fall conditions. The clams will then enter the winter dormancy in a compromised state. To our knowledge this hypothesis has not been investigated. Therefore, further study is warranted to understand the relationship of the clams' physiological condition prior to field planting in the fall with the incidence of over-winter mortality, and to specifically determine if supplemental feeding can decrease the chance of planting physiologically compromised clams.

In addition to playing an important structural role, endogenous energy stores are also 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). However, 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). Aside from the work of Ansell and Lander (1967), the role of energy reserves during the winter months in hard clams has not been studied. Most research on other bivalves describes the use of these substrates in relation to the gametogenic cycle. Juvenile scallops rely heavily on protein stores in the adductor muscle during over-winter stress (Epp et al., 1988; Sundet and Vahl, 1981). 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 and environmental indices will help elucidate these complex interactions.

Three response strategies have been outlined in marine invertebrates for the maintenance of energy balance as influenced by temperature (Newell and Branch, 1980). "Response type I" is an adjustment of feeding rate and metabolic energy expenditure in response to environmental temperature change. In "response type II", there is adjustment of feeding rate, but no compensation of metabolic energy expenditure in response to environmental temperature change. "Response type III", on the other hand, involves no adjustment of feeding rate, but there is compensation of metabolic energy expenditure in response to environmental temperature change. Hard clams do not fully acclimate their feeding rate to temperature, as indicated by a $Q_{10} > 1$ (Hibbert, 1977; Doering and Oviatt, 1986; Hamwi, 1969). Q_{10} is an index defined as a ratio of a rate function (i.e., feeding rate) at one temperature to that at 10ºC lower. Complete acclimation is indicated by a $Q_{10} = 1$, as observed in *Mytilus edulis* (Bayne et al., 1977). This Q_{10} indicates that hard clams use "response type II" to environmental temperature change. This response strategy 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* exhibits "response type II": it 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 enable normal metabolic rate to be maintained 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. In order to test the hypothesis that increased temperature after a long, cold period would result in higher metabolic costs (feeding and respiring), which are too burdensome for the animal when food availability is inadequate; both physiological parameters and food availability must be studied simultaneously.

Oxygen consumption is typically used as a measure of metabolic changes associated with constant temperature adaptation. It can also be an indirect measure of the amount of heat aerobically dissipated by an organism through the oxidation of energy substrates. In addition, oxygen consumption can be used in conjunction with other measures of catabolism (ammonia excretion and carbon dioxide production) to generate catabolic indices and to identify the substrate catabolized and the magnitude of utilization for respiration. Monitoring oxygen consumption in clams during winter conditions may be fruitless because they are either living anaerobically or, at most, respiring at a significantly reduced rate. However, when the temperature increases in the spring, their response can be measured by this metabolic rate. This is a period of particular interest since the clams should increase their feeding rate with increasing temperature. In addition, hard clams do not fully acclimate oxygen consumption rate (VO_2) to

temperature as indicated by a $Q_{10} > 1$ (Hibbert, 1977; Hamwi, 1969). Hard clams show a marked increase in VO_2 (as well as feeding rate; noted above) with increasing temperature between 10ºC and 26ºC. In a study in which juvenile clams (10-15mm SL) were exposed to ambient seston in a temperature controlled, flow-though system, both VO_2 and feeding rate (measured as food clearance) increased with temperature. The increase in feeding rate was greater than VO_2, resulting in a net energy gain between 12ºC and 27ºC (Grizzle et al., 2001). However, there is no data that describe how hard clams would respond to an increase in temperature and an inadequate food supply.

In addition to respiration, excretion is also an important component in the energy budget that should be studied during periods of stress. Respiration and excretion along with fecal production are principal loss terms in the energy budget; therefore their evaluation is critical during periods of negative scope for growth. The ratio of oxygen consumed to nitrogen excreted (O/N) is a physiological index of nutritive stress in bivalves (Bayne, 1973; Barber and Blake, 1985; Garcia-Esquivel et al., 2001). Bayne (1973) suggested that O/N ratios below 30 are indicative of nutritive stress and catabolism of endogenous substrates in marine bivalves. On the other hand, Mayzaud (1973) found O/N ratios as low as 7 when the substrate is exclusively protein, the theoretical minimum in marine copepods. Measurement of the O/N ratio should provide an understanding of the physiological condition of hard clams during their response to increased temperature after prolonged low temperature acclimation.

Another assay commonly used in bivalve studies to link metabolic activity with nutritive stress is the activity and condition of the digestive tubules in the digestive gland. These tubules are responsible for intracellular digestion. They are comprised of two

types of cells: (1) digestive or secretory-absorptive, which cycle between tall (25µm), columnar cells and short (6µm), cuboidal cells and, (2) basophil cells (Eble, 2001). Digestive cells will cycle depending on their physiological condition. This cycle has been divided up into four types (Robinson and Langton, 1980): Type 1 is normal, Type 2 is absorptive, Type 3 is disintegrating, and Type 4 is reconstituting. Under conditions where there is a continuous food supply, there should be a high frequency of "Type 1" and "Type 2" cells. When food is limited or feeding has ceased, there should be a high frequency of "Type 3" cells (Robinson et al., 1981). Histological analysis of the digestive gland could thus provide information of the feeding activity of hard clams.

Mass mortality events observed in bivalve populations are often attributed to disease. Researchers at the Haskin Shellfish Research Laboratory have isolated *Vibrio* from dying clams that were held in a nursery system over-winter. However, when infected clams, or their homogenates, were introduced to healthy clams, it failed to cause mortality (Ford, 2001). In order to further investigate whether disease (bacterial or other infections) is relevant to over-winter mortality, studies should be conducted on the immune function of clams subjected to physiologically stressful conditions. Phagocytosis is described as the principal defense mechanism in the immune system of bivalves and there is ample evidence that molluscan hemocytes avidly ingest foreign materials (Foley and Cheng, 1975; Ford, 2001). Monitoring immunocompetence, in terms of phagocytosis, during stressful periods would provide an indication of its connectedness to metabolism and physiological condition. In addition, observations of the immunocompetence of hard clams at the subcelluar level may be useful. In several bivalve species subjected to various stressors, lysosomes, an organelle of hemocytes,

was the site of the earliest stress response (Harding et al., 2004). Investigating lysosome membrane integrity as a biochemical indicator of stress is quite common in bivalve studies (Drynda et al., 1998; Hauton et al., 1998). It has also been used in monitoring levels of xenobiotics in marine coastal environments (Castro et al., 2004).

Sediment characteristics can significantly affect the growth of hard clams. Several studies cite that growth in hard clams is slower in mud sediments compared to sand (see review: Grizzle et al., 2000). Pratt and Campbell (1956) discussed three potential mechanisms that could explain this relationship. Hard clams held in aquaria in muddy sediments remained near the sediment surfaces and maintained an open burrow. In contrast, hard clams in sandy sediment often remained completely covered, pumping water through the sediment. They demonstrated that reduced permeability of mud affects burrowing and feeding behavior. A second potential mechanism is that muddy sediments are easily entrained in the feeding currents and thus must be expelled as pseudofeces, which is energy depriving. The third hypothesis presented is that muddy sediments may be hypoxic or anoxic at times and contain toxic substances such as hydrogen sulfide. This may negatively impact the clams as they pump. These three hypotheses are credible mechanisms for affecting the condition of hard clams and thus, may play a role in over winter mortality.

Over-winter mortality of juvenile aquacultured hard clams remains a major obstacle for the success of clam aquaculture and research on this issue is imperative to the industry. In order to identify the parameter, or synergistic effect of several parameters, responsible for this observed mortality a quantitative understanding of the environmental factors (i.e., temperature, food availability, and sediment type) must be

obtained using multifactor experimental approaches in both field and laboratory studies. In addition, an understanding of the physiological effects of temperature at the level of the whole organism should be determined, to integrate the various physiological functions into an index of energy balance (Bayne et al., 1983).

We propose that extended periods of severely cold temperatures will result in the use of endogenous energy stores and this will result in the mortality of challenged clams in the spring when water temperatures rise, food levels are low and metabolic costs are high. Thus, the current study will identify and correlate clam over-winter mortality with environmental and physiological mechanisms through a series of field and laboratory experiments with the intent of determining the underlying mechanism. This will be accomplished by:

1) Determining the magnitude of over-winter mortality of juvenile (shell length = 10mm), aquacultured hard clams exposed to ambient winter conditions at two field locations in Jamaica Bay, New York.
2) Investigating correlations between food availability and over-winter mortality.
3) Determining the main energy reserves and the patterns of utilization by hard clams during late fall, winter, and spring months.
4) Understanding the effects of temperature on the physiological mechanisms and concurrent metabolic response of juvenile aquacultured hard clams.

This will enable us to:

1) Increase our knowledge of the physiological ecology of hard clams in the juvenile phase of their ontogeny and provide data that may be incorporated into models of hard clam population dynamics.
2) Provide data that will be useful to aquaculturists and natural resource managers in determining the efficiency and profitability of their seeding protocols.

MATERIALS AND METHODS

The scope of this study encompasses four successive years of field study at two locations in Jamaica Bay, NY and controlled laboratory experiments using recirculating aquaculture systems. All field studies followed a general protocol with some specific differences in each year of study.

General Protocol:

The field sites are located in Jamaica Bay, which is a 10,000 square acre urban estuary located at the southwestern end of Long Island and thus it is part of the Hudson River estuary (Franz and Tanacredi, 1993). It is also a significant component of Gateway National Recreation Area, a major urban United States National Park. The two field sites were at Floyd Bennett Field (FBF) at the mouth of Mill Basin (N40º36.334'W073º53.137') and at Dead Horse Bay (DHB), adjacent to Gateway Marina (N40º35.227'W073º54.195').

The depth of the FBF site at mean low water is approximately 0.76m. The depth of the DHB site at mean low water is approximately 0.61m. Analysis of the gross sediment type using the methods outlined by Holme and McIntyre (1984) indicates that the two study sites differ in composition. The sediment at FBF is coarser, containing larger particles than the sediment at DHB. Both sites sustain a natural population of *Mercenaria mercenaria* (personal observation, 2001). The clams used for the study were derived from animals produced at AREAC (2001-2002) with broodstock obtained from the Frank M. Flowers and Sons shellfish hatchery (Oyster Bay, NY) or were directly obtained from either the Frank M. Flowers and Sons shellfish hatchery or Cornell Cooperative Extension Marine Center (Southold, NY). Each box was stocked with 30

juvenile *Mercenaria mercenaria* var. *notata* with a mean shell length of 9.8 mm ± 0.9 mm (SD). A stocking density of thirty clams per box was chosen to avoid density-dependent stress (Castagna, 2001) and to provide a large enough sample for significant condition data (Kraeuter et al., 1997). Plywood boxes with the dimensions 1' x 1' x 6" were constructed with a closed bottom and open top, which was covered with ¼" Vexar® mesh after stocking to exclude predators and retain the clams. The boxes were set up on an anchored trotline to facilitate sampling and to prevent box movement during possible storm events. The trotline was set up parallel to the shoreline. All boxes were numbered and deployed at mean low water (MLW) during a spring tide in November when the bay water temperature consistently read 12°C. At this time, an initial sample of ≥25 clams per site was taken for assessment of baseline data on morphometry and biochemical composition. In addition, water quality parameters such as water temperature, salinity, and dissolved oxygen were determined at each site. Atmospheric conditions, such as air temperature, wind speed and direction was also recorded. Water samples (two liters) were taken initially and on each subsequent sampling date to measure chlorophyll *–a.* Water samples were collected near the benthos in an amber glass bottle and kept on ice until analysis. Chlorophyll-*a* analysis was conducted on three replicate samples of 500 ml using an acetone extraction method (Parsons et al., 1984). Absorbance was measured using a Hach® 2010 spectrophotometer. Water samples were studied microscopically on an irregular basis to determine species composition. The typical pattern of phytoplankton abundance in this estuary is an winter-spring bloom of a diatom-dominated assemblage followed by a summer bloom of a flagellate dominated assemblage (Franz and Tanacredi,

1993). This is also typical of other Northeast bays such as Narragansett Bay (Pratt, 1965).

Every two weeks after deployment boxes were selected using a random digit table (Zar, 1999) and sampled from each site at MLW. The use of the random digit table addresses sampling bias and potential inshore/offshore and upstream/downstream influences. The clams were collected from the boxes by sieving the entire contents of the box at the field site through a 2mm sieve. In addition, water and atmospheric parameters were measured as described above. The clams were kept in sea water and stored at ambient temperature of the bay until analysis (< 24 hours). The sampling began in November of each year and was conducted bi-weekly through June.

Clams collected from the field sites were brought back to the laboratory for condition and biochemical analysis. Live clams as well as dead ones (defined as "gaping" or "clapper") were counted and percent cumulative mortality (% MO) was calculated directly from samples as:

% MO = (dead clams/total clams) X 100

All clams were measured with calipers to determine shell length (anterior to posterior), shell width (from the umbo to the outside edge) and shell height (measured perpendicular to the hinge ligament). Whole live clams were weighed with an electronic balance (0.0001g), carefully opened and all the tissue removed. The wet tissue weight and wet shell weight were determined separately. The tissue and shell samples were then placed into a drying oven (Thelco model 26) at 60°C for ≥ 48 hrs then reweighed to obtain the dry weight of each sample. The dried tissue was then placed in a polypropylene vial and stored in a dessicator at -20°C until used in biochemical analyses. A "condition index"

was calculated as a ratio of dry tissue weight to shell length x 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).

Biochemical analyses were conducted on pooled dry tissue from all live clams within a box to determine carbohydrate, protein, and lipid content. The tissue samples were ground to a fine powder using a mortar and pestle. Sub-samples of this powder were then used for each biochemical assay.

The determination of carbohydrate (glycogen is the most prominent carbohydrate stored in marine bivalves {Gabbott, 1975}) content was determined in triplicate using the phenol – sulfuric acid method of Dubois et al. (1956). A 3.0-7.0 mg portion of tissue was placed in a test tube with 10 ml of distilled water and then homogenized. A 1.0ml sample was taken form this solution and diluted to a final volume of 2ml. A 50µl aliquot of 80% phenol reagent was added to the tube and vortexed. A 5 ml aliquot of 95% sulfuric acid was then rapidly added to the tube. The sample was allowed to stand at room temperature for 30 minutes. The absorbance of the sample was read at 490nm on a Hach® 2010 spectrophotometer. A calibration curve was created using glucose.

A commercial kit (Pierce Biotechnology, Rockford, IL.) of the coomassie blue method of Bradford (1976) was used to determine protein content in triplicate. Portions of dried tissue (4.0-10.0mg) were placed in a test tube with 10 ml of 0.1N NaOH, homogenized, and left overnight at room temperature. A 50µl aliquot of this solution was then placed in a test tube and 1.5 ml of the Coomassie Plus™ protein assay reagent was added to the tube and vortexed. The absorbance of the sample was measured at

595nm on a Hach® 2010 spectrophotometer. A calibration curve was constructed using bovine serum albumin as a standard.

Total lipid content was estimated, with three replicates, using the method of Folch et al. (1957). A portion of dried tissue (9.0 – 50.0mg) was placed in a test tube with 5 ml of 2:1 (v/v) chloroform-methanol mixture and was left to stand for one hour. The solution was made bi-phasic by adding 1ml of distilled water (20% of chloroform-methanol mixture) and vortexed. The mixture was centrifuged at 3000 rpm for five minutes to further separate the two phases. The upper phase was typically 40% and the lower phase 60% of the total volume of the system. A 2ml sample of the lower phase (chloroform with lipids) was placed on a previously combusted and weighed aluminum weigh boat. The weigh boat was then placed on a clean surface in the fume hood with a glass plate above the weigh boat to prevent dust contamination. After the chloroform had evaporated, the weigh boat was placed into an oven at 110ºC for 30 minutes. The weigh boat was then placed in a dessicator until cool and weighed. Total lipids were calculated by subtracting the initial weight of the weigh boat from the weight of the weigh boat plus lipids and then multiplying by the proportion of chloroform in the original system and divide that product by the weight of the tissue used in the sample. A blank was created by mixing 5 ml of 2:1 (v/v) chloroform-methanol solution and 1 ml of distilled water and then measuring the volume of chloroform (lower layer), which yields the proportion of chloroform in the original system.

Field studies 2001-2003

There were fifteen boxes deployed at FBF and at DHB during the winters of 2001-2002 and 2002-2003. The mean shell length of the clams used was 9.8 mm ±0.9mm (SD). One box was sampled from each site bi-weekly from November to June.

Field study 2003-2004; Reciprocal sediment transplant experiment

Seventy boxes were deployed at each site. Thirty-five boxes were filled with sediment from the DHB site and thirty-five boxes were filled with sediment from the FBF site. Therefore, each site had seventy boxes, thirty-five of which were filled with sediment from the opposite site. The number of boxes was increased in this study in order to increase replicates as well to determine if the mortality was higher at the DHB site due to a sediment or site effect. The clams in this study had a mean shell length of 6.6mm ±0.9mm (SD). This smaller clam was the only size available in New York in November 2003. The intended sampling schedule was biweekly with two boxes of each sediment type to be sampled from each site. However, a storm destroyed many of the boxes one week after deployment. Therefore, sampling had to be reduced to once a month in order to collect samples through the November to June period.

Field Study 2004-2005

Fifty boxes were deployed at each of the two sites. There was no continuation of the reciprocal sediment exchange. Three replicate boxes were sampled biweekly from each site from November to June. Five remaining boxes were used to assess cumulative mortality (sampling at the end of experiment). In addition, the Floyd Bennett Field location was moved further into Mill Basin Creek approximately 100 yards west of the old site. This move was prompted by US Army Corp of Engineer construction at FBF.

The clams had a mean shell length of 10mm ±1.0mm (SD).

Laboratory Studies 2005-2006

In the fall of 2005, a laboratory experiment was conducted to test the effect of clam physiological condition prior to winter and the effect of food availability and temperature changes on clam survival in the spring. Approximately, 1,500 clams [10mm±1.0mm (SD)] obtained from the Frank M. Flowers and Sons shellfish hatchery were brought into the laboratory and placed in ¼" mesh ADPI® bags and then divided into two independent recirculating aquaculture systems (RAS). Each RAS consisted of a Styrofoam insulated 90-gallon fiberglass trough tank (84"x22"x12"), a trickling bio-filter (20 gallons) with bio-ball media, 1/10 hp magnetic drive pump, and an in-line chilling unit. The clams in each tank received a different feeding regime. The high condition regime was fed 3.0% dry weight of algae per wet weight of hard clams per day (Bolton, 1982; Coutteau, 1994). The low condition regime was fed 1.0% dry weight of algae per wet weight of hard clams per day. Feedings were delivered in three batches per day and consisted of live algae including *Chaetoceros neogracile, Isochrysis galbana* (T-Iso) and *Tetraselmis chui* (Ply-429) cultured in F/2 media. All algae species were strains obtained from the NOAA- Milford collection. A commercial paste (Reed Mariculture, CA) was also used to augment the live algae. This paste was a mixed species of *T-Iso, Pavlova, Thalassiosira* and *Tetraselmis*. Temperature during this conditioning period was 16-18ºC. After one week, twenty-five clams from each treatment was sampled and processed for analysis of condition index and biochemical content using the methods described earlier. Although, the condition index significantly increased in both groups, there was no significant difference between groups. The low condition treatment was

then fed 0.5% dry weight of algae per wet weight of hard clams per day and the high condition treatment was fed 2% dry weight of algae per wet weight of hard clams per day. After one week, another sample of clams was taken and, again, no difference in condition index was observed. The same feeding regime was maintained for a third week and a significant difference was observed between the two groups.

The RAS's were then fitted with ¼" plywood to create twelve 1sq. ft. boxes which were six inches deep. Each box was then filled with FBF sediment that had been previously dried and then rinsed before adding to the RAS. The boxes in each RAS were numbered and then randomly assigned a treatment group, so that each RAS had six boxes representing high condition groups and six boxes representing low condition groups. Each box was then stocked with sixty clams of the corresponding treatment group. The water temperature which had been 16ºC at the time of stocking was then decreased one degree per day until 4ºC. The RAS were maintained at this temperature for four weeks. During this time and throughout the experimental period water quality analyses were conducted three times a week on both tanks. Salinity, pH, dissolved oxygen, ammonia, nitrite, nitrate, alkalinity and calcium were maintained so they were well within hard clams tolerable range (Epifanio and Srna, 1975). The quantity of algal feed given to each RAS was 3% dry weight of algae per wet weight of hard clams per day; however, this was reduced as it became apparent that the clams reduced their pumping rate. Instead, daily algal cell counts were conducted using an improved Neubauer hemocytometer to ensure a concentration of >150 cells μl^{-1} (Walne, 1970) throughout out the experimental period. This cell concentration equated to a chlorophyll-*a* content of >5.0 $\mu g\ L^{-1}$.

Boxes representing high condition and low condition were sampled from each RAS after four weeks at 4ºC. This left eight boxes remaining in each RAS, four of which was high condition and four was low condition. Up to this point both RAS had been treated identically in terms of temperature and food. In order to identify the relationship of clam condition and food availability in the spring, the temperature in each RAS was raised one degree per day until it reached 14ºC and a disparity was established between the RAS in terms of how much food was given to them on a daily basis. One RAS was fed a low food treatment consisting of 0.5% dry weight of algae per wet weight of hard clams per day and the other RAS was fed a high food treatment consisting of 3% dry weight of algae per wet weight of hard clams per day. Each RAS was kept at 14ºC for two days and then the remaining boxes (n = 8) were sampled.

On each sampling date, percent mortality per box was calculated. Sixty clams were sampled from each box. Twenty to twenty-five clams were used to determine dry weight, condition index and biochemical parameters as described in the field experiments. An additional nine clams of both high condition index and low condition index were used to measure oxygen consumption and nitrogen excretion. These clams were then sacrificed and hemolymph was collected from the adductor sinuses as well as from the pericardial cavity. Approximately 100µl of hemolymph was collected and placed in a 1.5 ml microcentrifuge tube and kept on ice to prevent cell aggregations until analysis. Five of the clams used for hemolymph analysis were fixed in Bouin's fixing solution under vacuum and later processed for histological analysis. Five moribund clam samples were also fixed for histological analysis. These analyses were conducted on clams representing the high and low conditions initially that were taken at the start of the

experiment for baseline data and then again at 4ºC. When the water temperature was increased to 14ºC and disparity was created between the tanks in terms of food availability, the remaining live samples from each treatment were taken for analysis. The groups of clams studied included low condition clams with low food available, low condition with high food available, high condition with low food available, and high condition clams with high food available.

Oxygen consumption by the clams was measured using a polarographic oxygen electrode unit (Qubit Systems, Canada) and Logger Pro 3 software (Vernier Software and Technology, OR, USA). The incubation sea water (20ml of 0.45 µm-filtered SW, 27 ppt) was added to the chamber with a 10 ml pipette. Zero calibration was obtained with the addition of sodium dithionite ($Na_2S_2O_4$) and oxygen saturation was achieved by continuous aeration of the experimental water in a separate container. The incubation chamber was kept at a constant temperature (4±1ºC or 14±1ºC) by running the analyses in a cold room. Blank corrections were determined from the slope of the meter reading prior to introducing the clams. Once the meter's oxygen consumption was obtained, three clams were placed into the experimental chamber on a small sieve to avoid any contact with the stir bar. Oxygen measurements were taken 100 times per minute for sixty minutes. Three runs were conducted on three groups of clams on each sampling day. This was the general procedure for all treatments. Oxygen consumption rate was estimated from the corrected slope of the oxygen probe. Oxygen consumption was measured in mg L^{-1} and then transformed into the universal units of ml L^{-1} for dissolved oxygen by multiplying by 1 ml/1.42903mg. Oxygen consumption was converted to atomic units for use in an O: N ratio.

Nitrogen excretion was determined by placing three clams each in 20ml of filtered (0.45µm) sea water in three scintillation vials previously washed with 10% HCl and dried. The vials were capped and wrapped in Parafilm® and placed in the RAS at 4±1ºC or 14±1ºC. Incubations lasted 3.0 -3.5 hours. At the end of this period, the sea water was filtered through a 0.45 µm GF/F glass fiber filter. Three 20ml samples were transferred to 125 ml Erlenmeyer flasks and analyzed for ammonium concentration, according to Solorzano (1969). A calibration curve was constructed on each sampling date using ammonium sulfate as the standard. Ammonium excretion (µg-atom N/liter) was converted to atomic units for use in an O: N ratio.

The immunological competence was determined by measuring phagocytosis and lysosome membrane integrity in samples of hemolymph. A yeast suspension was prepared by mixing 0.025g of *Saccharomyces cerevisiae* in 5 ml of sea water (0.45µm filtered, 27 ppt). Clam hemolymph (discussed above) was extracted using a tuberculin syringe with a 27-gauge needle. A 20µl aliquot of hemolymph was mixed with 20µl yeast suspension and 20µl of filtered sea water. Three replicates of 20µl of the resulting assay were transferred to a glass slide and incubated in darkness in a damp chamber for 60 minutes at room temperature (Witt, 2003). Phagocytic cells were identified as granular hemocytes containing visible yeast particles within the cytoplasm and having cytoplasmic extensions (fixed phagocytes) or cells with amoebocyte morphology (free phagocyte). The number of phagocytosed yeast particles within the first 30 granulocytes was recorded.

Lysosome membrane integrity was investigated using the Neutral Red Assay (Witt, 2003; Harding et al., 2004). Neutral red is a cationic probe. It is also a weak base and as

with other weak bases (e.g., chloroquine), it has been shown to accumulate in lysosomes as a result of the pH difference between the lysosome and the cytoplasm and to differential permeability of the membrane for protonated and unprotonated molecules (Andrew et al., 1997). The degree of lysosome membrane integrity is measured by the rate at which the reagent permeates through the lysosome membrane. The end point of the assay was determined when 50% of the cells lose dye to the cytoplasm (Hauton et al., 2000). This is manifested by diffuse pink stained cytoplasm in comparison to red stained vacuoles. Neutral red assay reagent was prepared by mixing 5µl of neutral red stock (0.5g neutral red in 100ml of absolute alcohol) in 2.5 ml of filtered sea water (0.45µm filtered, 27 ppt). A 20µl aliquot of hemolymph was mixed with 20µl of sea water (0.45µm filtered, 27 ppt) and 20µl of neutral red assay reagent. Next, three replicate samples of 20µl of this assay mixture was transferred to a glass slide and incubated for 15 minutes to allow dye loading of acidic vacuoles. Slides were examined every 15 minutes to measure progression of the assay.

Analysis of the digestive gland was conducted using standard histological techniques (Presnell and Schreibman, 1997). In brief, five samples from each treatment were fixed in Bouin's solution, embedded in polyfin and sectioned transversely at 7µm. Sections were mounted on glass slides and stained using Masson's trichrome stain. The digestive tubules were given a score of 1 to 4 based on tubule histology (see Introduction). Scores from replicate clams from each treatment and the frequency distribution was calculated for each temperature tested.

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

Kolmogorow-Smirnov statistic. 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 conducted using SPSS® version 11.5. All references in the text to statistical significance are at a probability level of $P \leq 0.05$, unless otherwise indicated.

RESULTS

Winter 2001-2002

During the winter of 2001-2002 **water temperatures** were below 5ºC for two weeks at DHB and for four weeks at FBF during January (Fig. 1). Water temperature remained between 6ºC -10ºC until March. There was no **mortality** observed at either site until March (Fig. 1). Mortality in the collected boxes was low, ranging from 3% to 11.5% in the spring. Data collection at FBF ceased in May 2002 due to vandalism of the boxes.

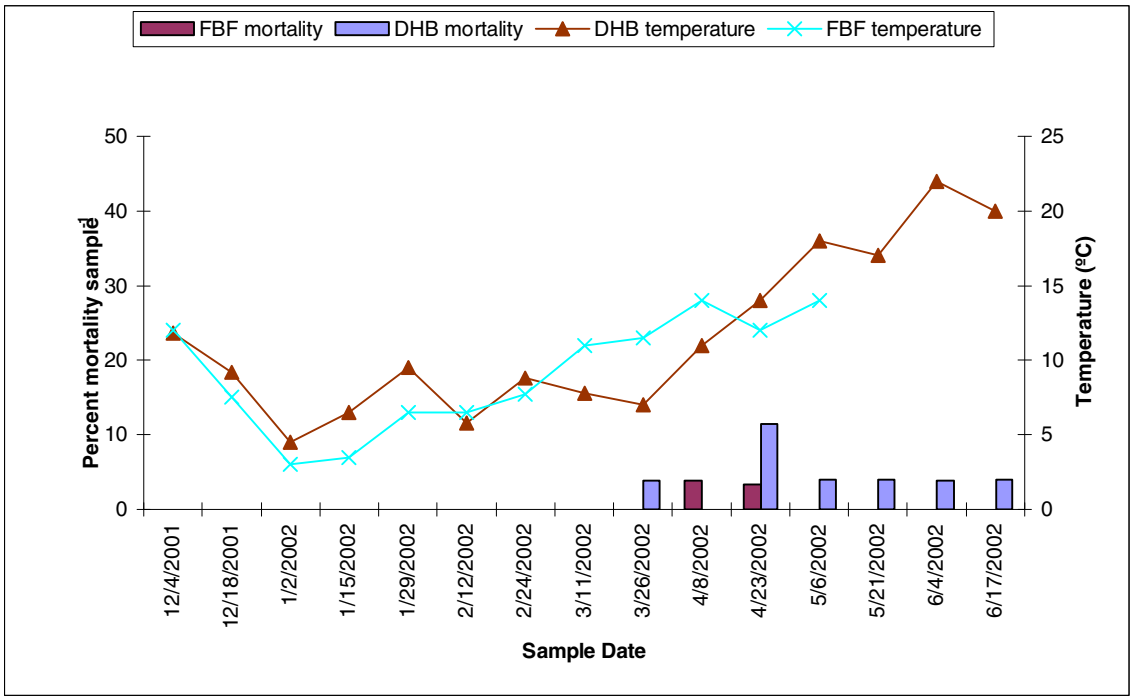

Fig. 1. Percent mortality and water temperature observed at Floyd Bennett Field and Dead Horse Bay during the winter of 2001-2002.

Chlorophyll-*a* was greater than 20µg 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. A winter-spring

bloom of this proportion is typical in Jamaica Bay. The quantity of chlorophyll-*a* 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 14ºC at both sites. A summer bloom was observed at DHB during June as indicated by rising chlorophyll-*a* values up to 55 µg L^{-1}.

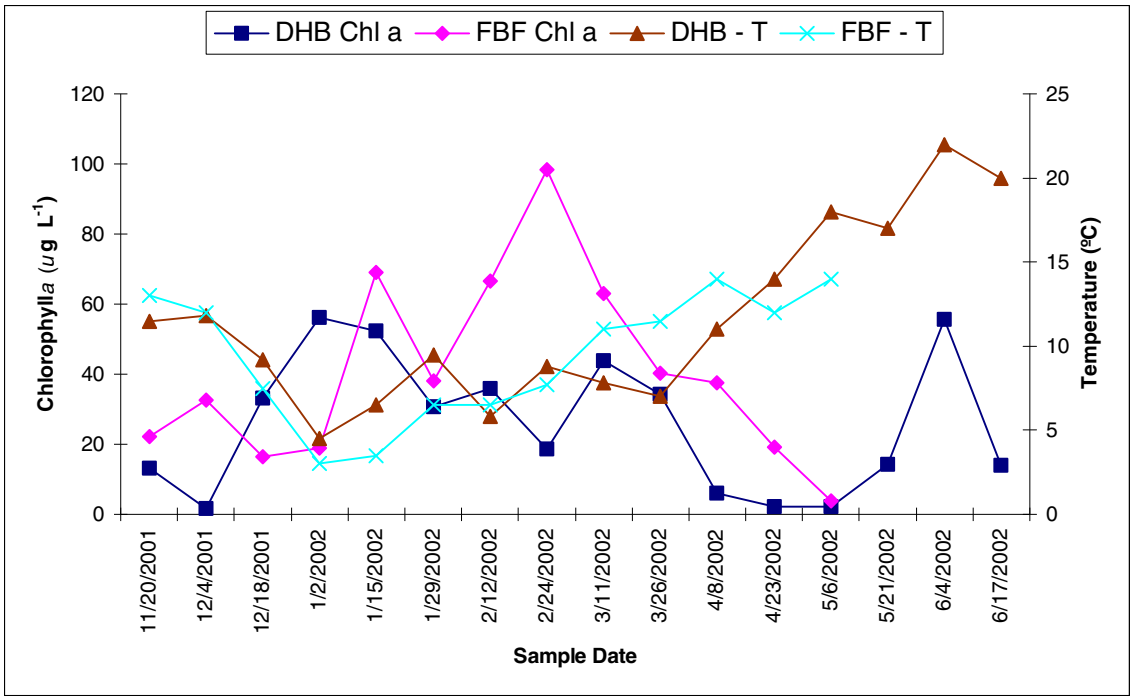

Fig. 2. Chlorophyll-*a* and water temperature measured at Dead Horse Bay and Floyd Bennett Field during the winter of 2001-2002. Chlorophyll-*a* was not measured at FBF after 9 May 2002.

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 (Tables 1 & 2). 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 12ºC and greater. The initial observed growth increase occurred when chlorophyll-*a* were at the lowest observed values (2 µg L^{-1} at DHB and 4 µg L^{-1} at FBF). Growth due to the summer bloom and warm water at DHB was remarkable as clams 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 1.1mm $week^{-1}$ in shell length and 14.8 mg $week^{-1}$ in tissue dry weight (Table 13).

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. 3). 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; $P \leq 0.05$). The first significant decrease was noted in December at the time the water temperature fell below 5ºC. The protein content fell from an initial value of 480µg $mgDW^{-1}$ to 439µg $mgDW^{-1}$ and remained significantly lower than the initial value through the sampling period. A minimum value of 319µg $mgDW^{-1}$ was observed in April at the same time chlorophyll-*a* values were also 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. 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-*a* values were high. However the exception to this observation is in April when chlorophyll-*a* 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.

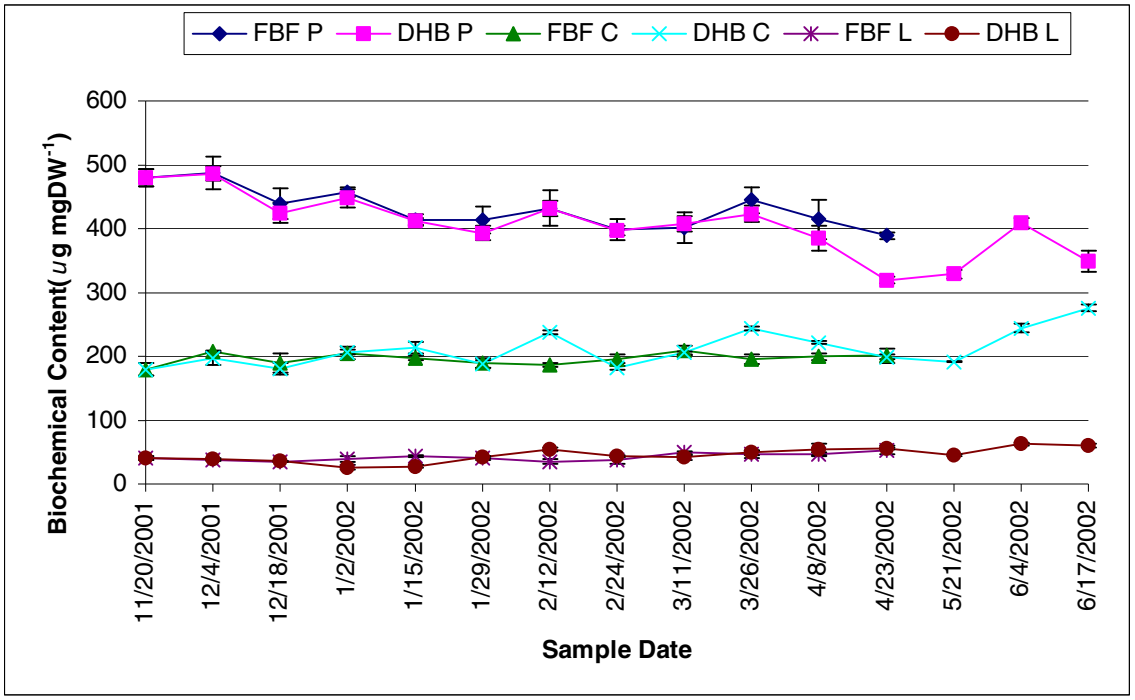

Fig. 3. Changes in biochemical content including protein, carbohydrates and lipids in clams at Dead Horse Bay and Floyd Bennett Field during the winter of 2001-2002.

Winter 2002-2003

The winter of 2002-2003 was colder than 2001-2002 with **water temperatures** at DHB and FBF below 5ºC for fourteen weeks (Fig. 4). **Mortality,** up to 16% in sampled boxes, was noted throughout the winter at both sites and was first noted when water temperature fell below 5ºC. However, mortality increased up to 43% at DHB and up to 30% at FBF in the spring as the water temperature rose above 10ºC (Fig. 4). There was some variability among samples as three spring samples (out of eight) contained only 10% mortalities.

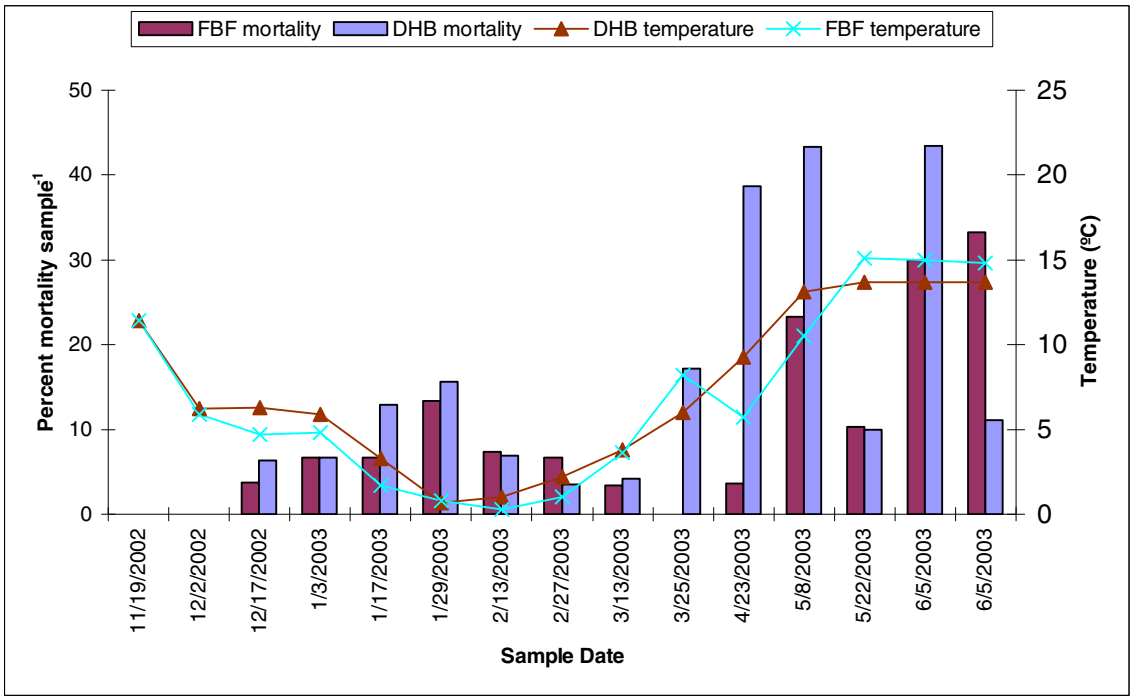

Fig. 4. Percent mortality and water temperature observed at Floyd Bennett Field and Dead Horse Bay during the winter of 2002-2003.

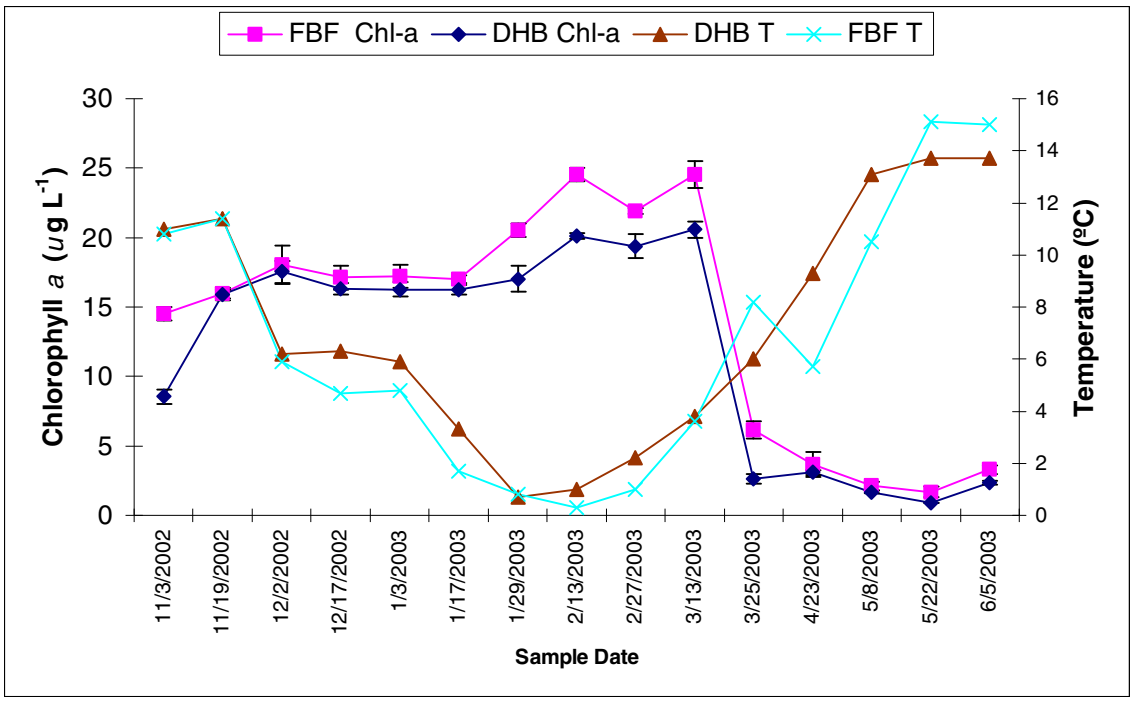

Fig. 5. Chlorophyll-*a* and water temperature measured at Dead Horse Bay and Floyd Bennett Field during the winter of 2002-2003

The **chlorophyll-*a*** content of the seston was between 15 and 20µg L^{-1} at both sites through most of the winter (Fig. 5). 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-*a* 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-*a* 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.

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 (Table 3). No significant increase in these

values was observed in the late spring at DHB (Table 3). 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 (Table 4). Growth of shell length was 0.43mm $week^{-1}$ and growth of tissue dry weight was 2.1mg $week^{-1}$ (Table 13).

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. 6) of the clam tissue remained relatively stable throughout the winter at both sites (initial value of 442µg $mgDW^{-1}$ up to 480µg $mgDW^{-1}$). An increase in protein content was observed at both sites on the final two sampling dates increasing to a maximum value of 558µg $mgDW^{-1}$ and 545µg $mgDW^{-1}$ at DHB and FBF respectively. Carbohydrate content differed significantly (Mann-Whitney; P=0.02) at the beginning of March the carbohydrate content fell from an initial value of 195µg $mgDW^{-1}$ to 149µg $mgDW^{-1}$. This pattern continued as carbohydrates decreased 63% at DHB from the initial value to 72µg $mgDW^{-1}$ 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 101µg $mgDW^{-1}$ (Mann-Whitney; P=0.05). The decrease in carbohydrate content coincided with low chlorophyll-*a* levels and when water temperatures were rising above 5ºC. The lipid content of the clam tissue was initially 76µg $mgDW^{-1}$ and increased during the spring up to maximum observed values of 95µg $mgDW^{-1}$ at FBF and 90µg $mgDW^{-1}$ at DHB.

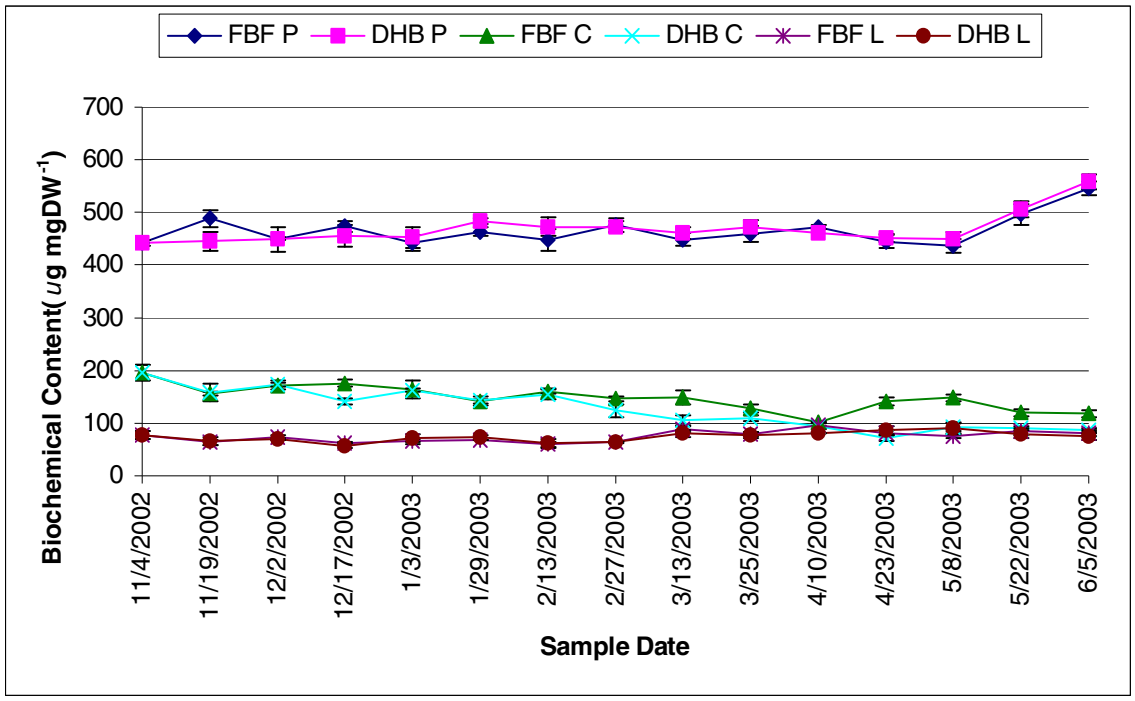

Fig. 6. Changes in biochemical content including protein, carbohydrates and lipids in clams at Dead Horse Bay and Floyd Bennett Field during the winter of 2002-2003.

Winter 2003-2004

During the winter of 2003-2004 **water temperatures** were below 5ºC for approximately eight weeks at FBF and six weeks at DHB (Fig. 7&8). The water temperature began to rise at the end of March at both sites. **Mortality** was similar (t-test; $P > 0.05$) at both sites and in both sediment types from November through March ranging from 5% to 23% per sample at FBF and from 5% to 35% at DHB. However, mortality increased significantly at both sites (although greater at DHB) during the final three sampling dates and was similar in sediment types at each site. Mortalities over the last three sampling dates at FBF in the sand sediment (from FBF) averaged 49%. Mortalities in the mud sediment (from DHB) at FBF averaged 47%. Mortalities occurring over the last three sampling dates were greater at DHB, 88% in the mud sediment and 71% in the sand sediment. There was a marked increase in mortalities at both sites when the water temperatures increased to 10ºC.

Chlorophyll-*a* values were low ($< 3 \mu g\ L^{-1}$) at both sites when water temperature decreased from 12ºC to 6ºC in November and December (Fig. 9). The chlorophyll-*a* values peaked during early March at the time of the winter-spring bloom. At this time chlorophyll-*a* was significantly greater at FBF ($100 \mu g\ L^{-1}$) than DHB ($33 \mu g\ L^{-1}$). Chlorophyll-*a* then decreased in the spring reaching a low of $8 \mu g\ L^{-1}$ at both sites. Lowest spring chlorophyll-*a* values coincided with the largest number of mortalities.

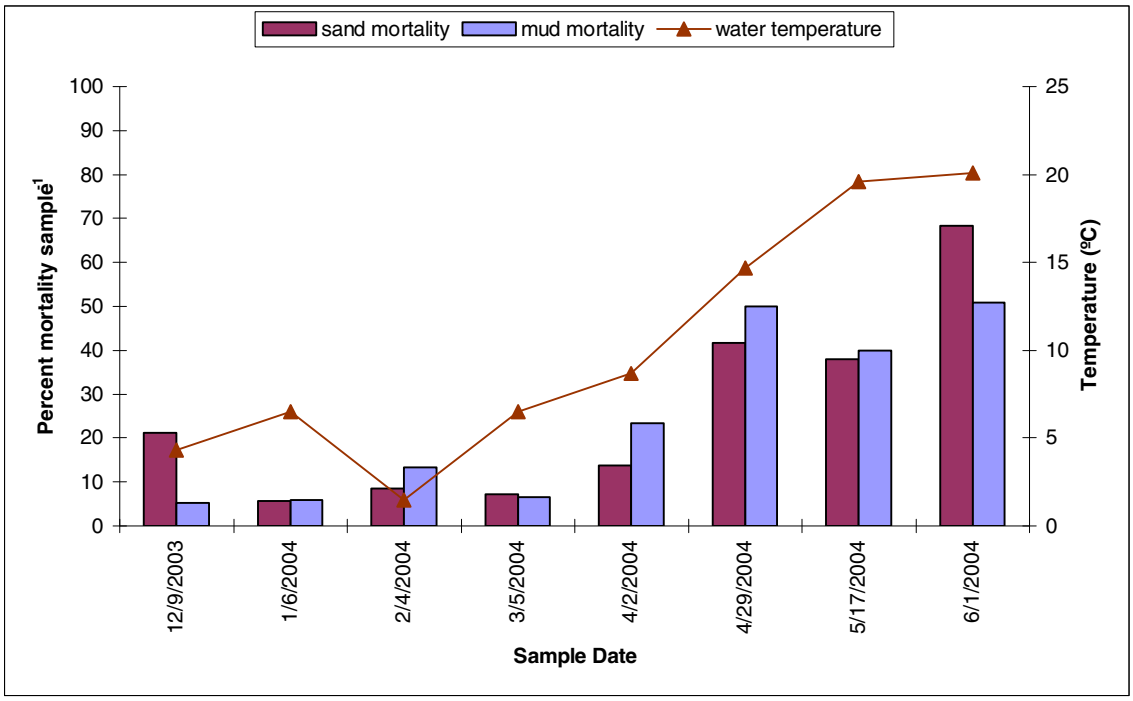

Fig. 7. Percent mortality in sand and mud boxes and water temperature observed at Floyd Bennett Field during the winter of 2003-2004.

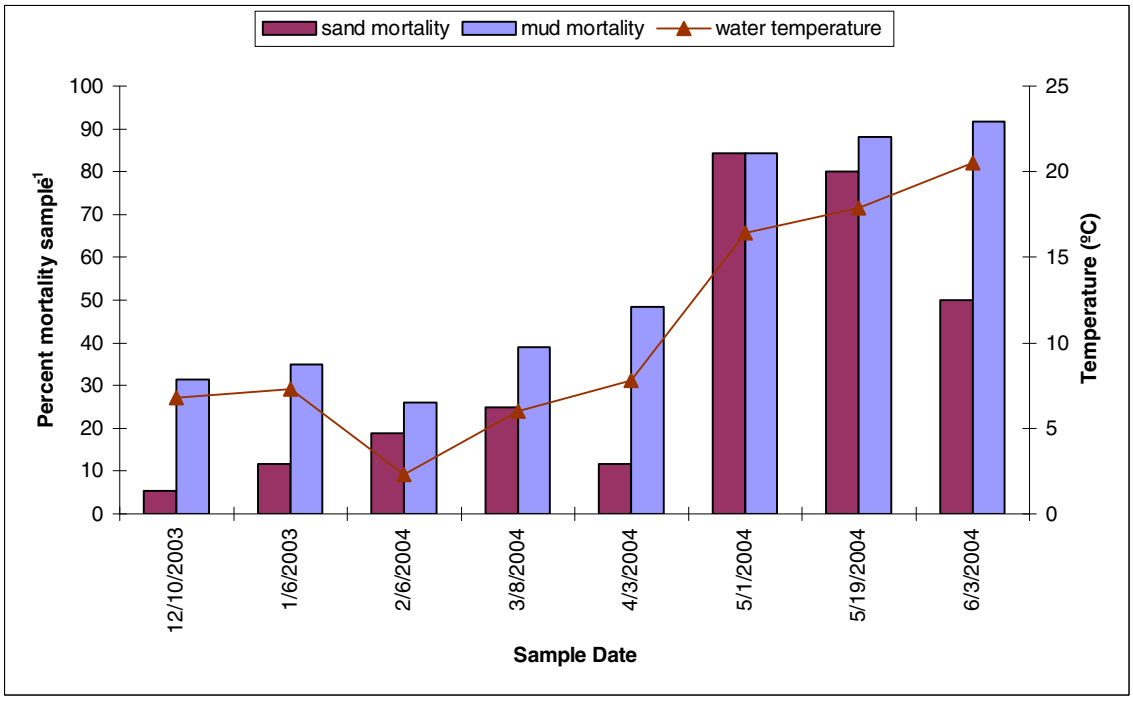

Fig. 8. Percent mortality in sand (FBF sediment) and mud (DHB sediment) boxes and water temperature observed at Dead Horse Bay during the winter of 2003-2004.

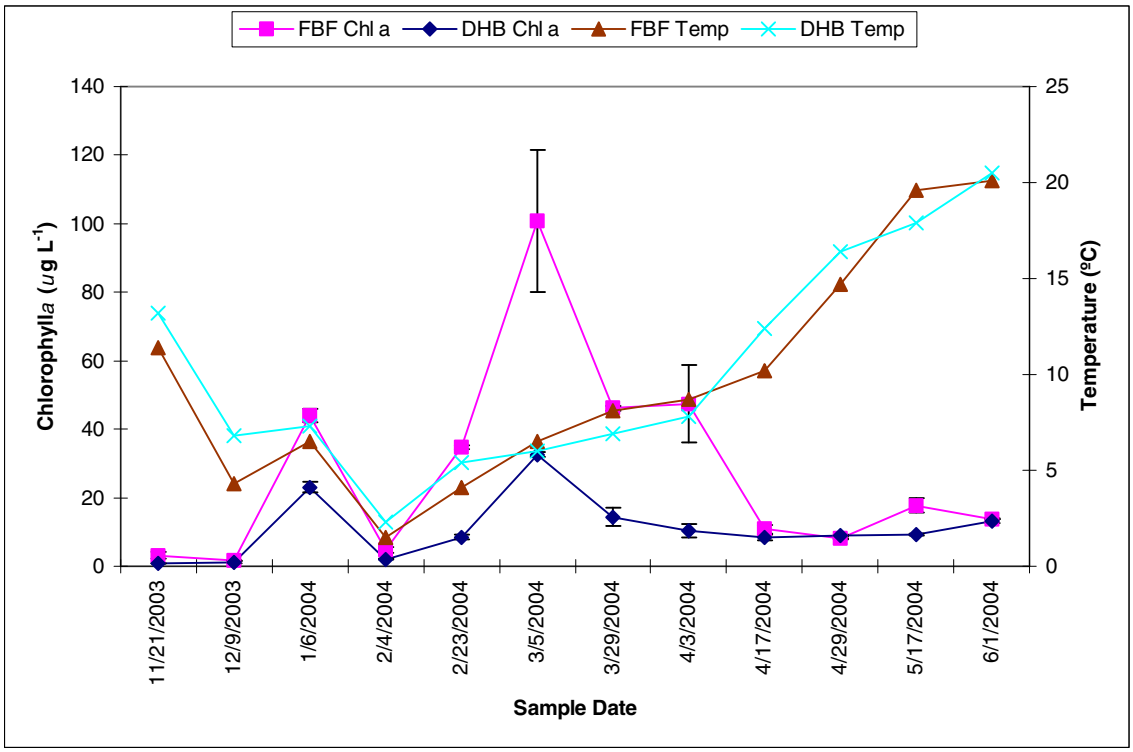

Fig. 9. Chlorophyll-*a* and water temperature measured at Dead Horse Bay and Floyd Bennett Field during the winter of 2003-2004.

Clams planted in sand sediment at DHB were not statistically different in **condition index**, **shell length**, and **tissue dry weight** through the winter. However, condition index and tissue dry weight increased significantly at the May sampling. In June, all three parameters were significantly higher than the initial values (Table 5). Clams planted in the mud sediment at DHB showed a similar trend, with the exception of shell length, which did not increase through the sampling period (Table 6). Clams planted in sand sediment at FBF showed no significant changes in condition index, shell length, and tissue dry weight until 29 April, when condition index increased. All three parameters increased significantly during the May and June samplings (Table 7). Clams planted in mud sediment at FBF increased significantly in all three parameters at the May

and June samplings (Table 8). Growth was greater at FBF than at DHB during the last four weeks of sampling (Table 13).

The **biochemical composition** of clams did not differ between sediment types at either site (Figs 10 & 11). The protein content of the clams at both sites increased from an initial value of 363µg $mgDW^{-1}$ to 465µg $mgDW^{-1}$ and 470µg $mgDW^{-1}$ at FBF and DHB (both sand and mud) respectively in early December. The protein increased again in early March to 571µg $mgDW^{-1}$ and 588µg $mgDW^{-1}$ at FBF in the mud and sand sediment respectively. Protein also increased during early March at DHB, up to 583µg $mgDW^{-1}$ in the mud sediment and to 578µg $mgDW^{-1}$ in the sand sediment. This significant increase in the protein content of all clam samples coincides with the maximum availability of food as measured in chlorophyll-*a*. Water temperature was approximately 6ºC at both sites at this time. However, as the water temperature increased and the chlorophyll-*a* values decreased, protein content also decreased. In addition, the carbohydrate content of clams under all conditions (site/sediment) also decreased as the water temperature increased and chlorophyll-*a* decreased. However, a statistically significant decrease in carbohydrate content occurred in February at DHB and in March at FBF when chlorophyll-*a* values were high. The initial carbohydrate value of 112µg $mgDW^{-1}$ decreased to approximately 90µg $mgDW^{-1}$ at FBF and 85µg $mgDW^{-1}$ at DHB and continued to decrease reaching lows in April of 74µg $mgDW^{-1}$ in the mud and 70µg $mgDW^{-1}$ in the sand. The clams at DHB experienced a more severe decrease in carbohydrate content, with values falling in April to 58µg $mgDW^{-1}$ in the mud sediment and 71µg $mgDW^{-1}$ in the sand sediment. The decrease in carbohydrate content in all clams coincides with the high mortalities observed during the spring of 2004. An increase

in carbohydrate content occurred during the May and June samplings among all clams regardless of site or sediment type. Lipid content of all clams was 25µg $mgDW^{-1}$ in November and remained relatively constant through the winter. Increases were observed on the May and June samplings up to 30µg $mgDW^{-1}$.

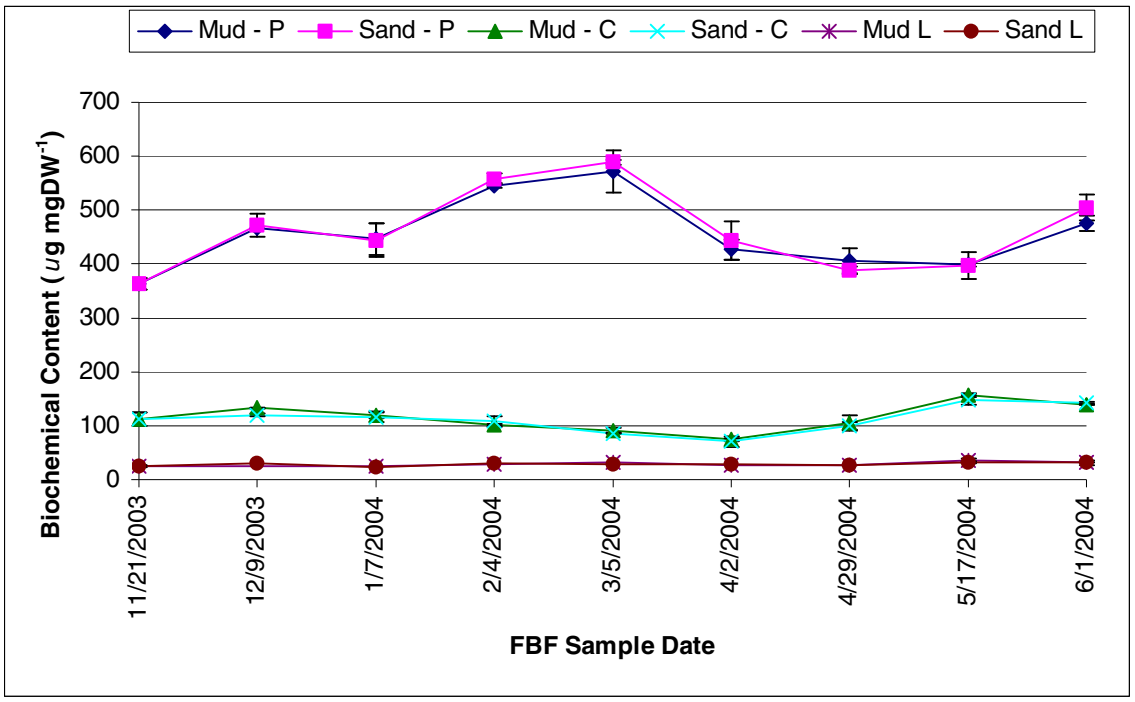

Fig 10. Changes in biochemical content including protein, carbohydrates and lipids in clams planted in mud sediment and sand sediment at Floyd Bennett Field during the winter of 2002-2003.

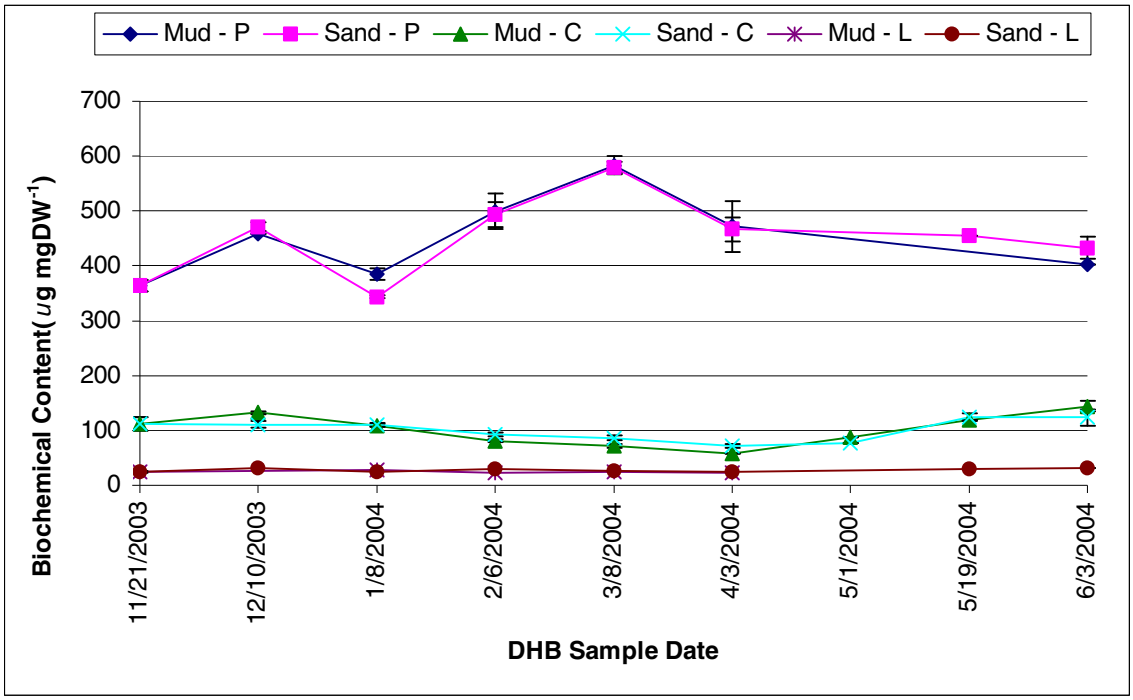

Fig 11. Changes in biochemical content including protein, carbohydrates and lipids in clams planted in mud sediment and sand sediment at Dead Horse Bay during the winter of 2002-2003.

Winter 2004-2005

The winter of 2004-2005 was similar to 2002-2003 in that **water temperatures** were below 5ºC for sixteen weeks. Water temperature fell below 5ºC at the end of December and did not increase above 5ºC until April (Fig. 12). Mortalities were low (1-2%) throughout the winter samples. Mortalities were also relatively low in the spring. The largest observed mortality in a sample occurred on 6 June at both sites with 13% and 15% at DHB and FBF respectively (Fig. 12).

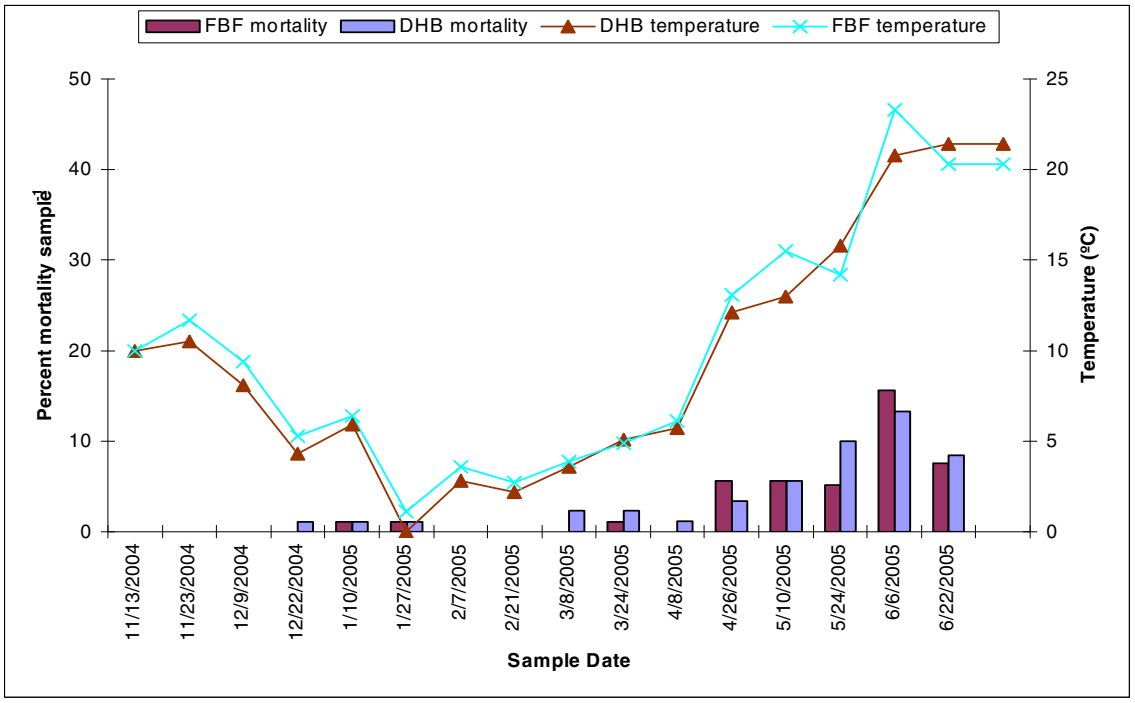

Fig. 12. Percent mortality and water temperature observed at Floyd Bennett Field and Dead Horse Bay during the winter of 2004-2005.

Chlorophyll-*a* values at both sites were low (< 5µg L^{-1}) through November and December. The maximum values measured occurred at the end of March during the winter-spring bloom (Fig 13). The dominant phytoplankton species associated with this bloom was the diatom *Chaetoceros didymus*. The highest value at FBF was 64µg L^{-1};

nearly double the highest value of 36µg L^{-1} at DHB. The chlorophyll-*a* decreased during May to a low of 3.7µg L^{-1} at DHB and 11µg L^{-1} at FBF; water temperature this time was approximately 15ºC at both sites. Chlorophyll-*a* increased through June, most likely a result of the summer bloom. Chlorophyll-*a* was higher at FBF on most sampling dates.

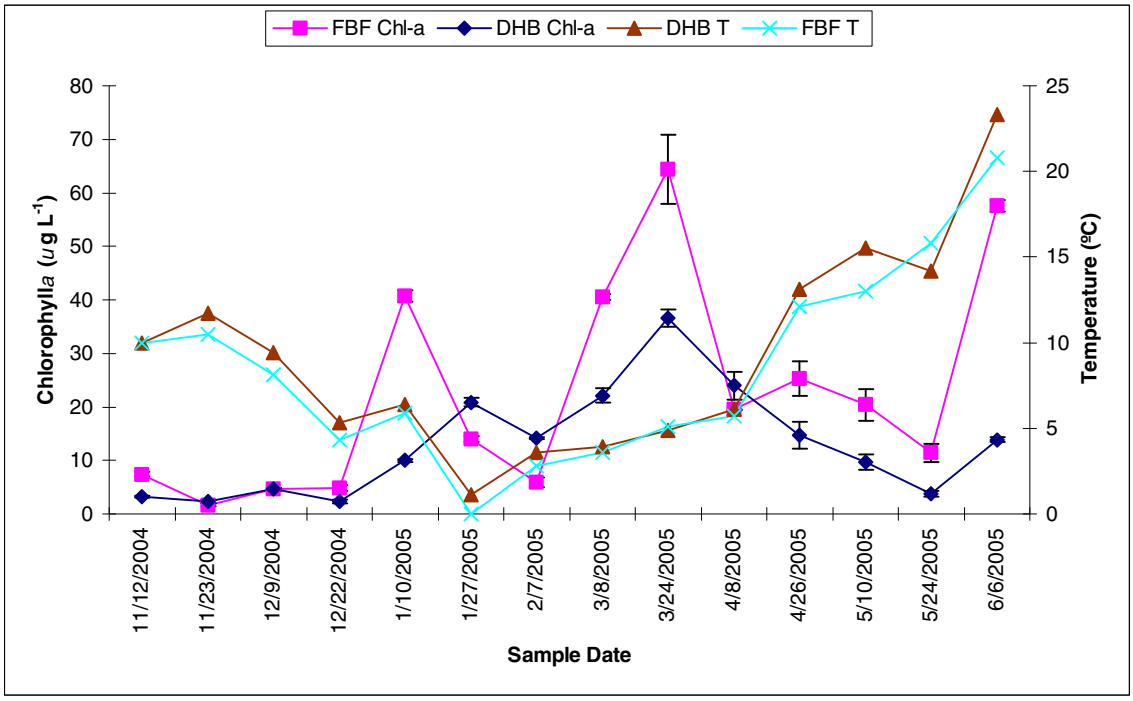

Fig. 13. Chlorophyll-*a* and water temperature measured at Dead Horse Bay and Floyd Bennett Field during the winter of 2004-2005.

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. These statistically significant increases occurred simultaneously in all three parameters (Tables 9 &10). During the last four weeks of the sampling period growth was impressive at both sites (Table 13). 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. 14). Protein content in clams fluctuated through November and December as water temperature began to fall and chlorophyll-*a* levels were low. The increase in protein content in March at both sites coincided with the winter-spring phytoplankton bloom observed. Water temperature was >5ºC at this time. Protein content at FBF reached a maximum of 528µg mgDW^{-1} and 496µg mgDW^{-1} at DHB during this bloom. On 24 May, low protein levels were observed (372µg mgDW^{-1} at FBF and 384µg mgDW^{-1} at DHB). This decrease in protein content occurred when water temperature was 15ºC and chlorophyll-*a* 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 180µg mgDW^{-1} to 160µg mgDW^{-1}. An increase in carbohydrate content was observed at both sites on the final sample in June increasing to approximately 190µg 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.

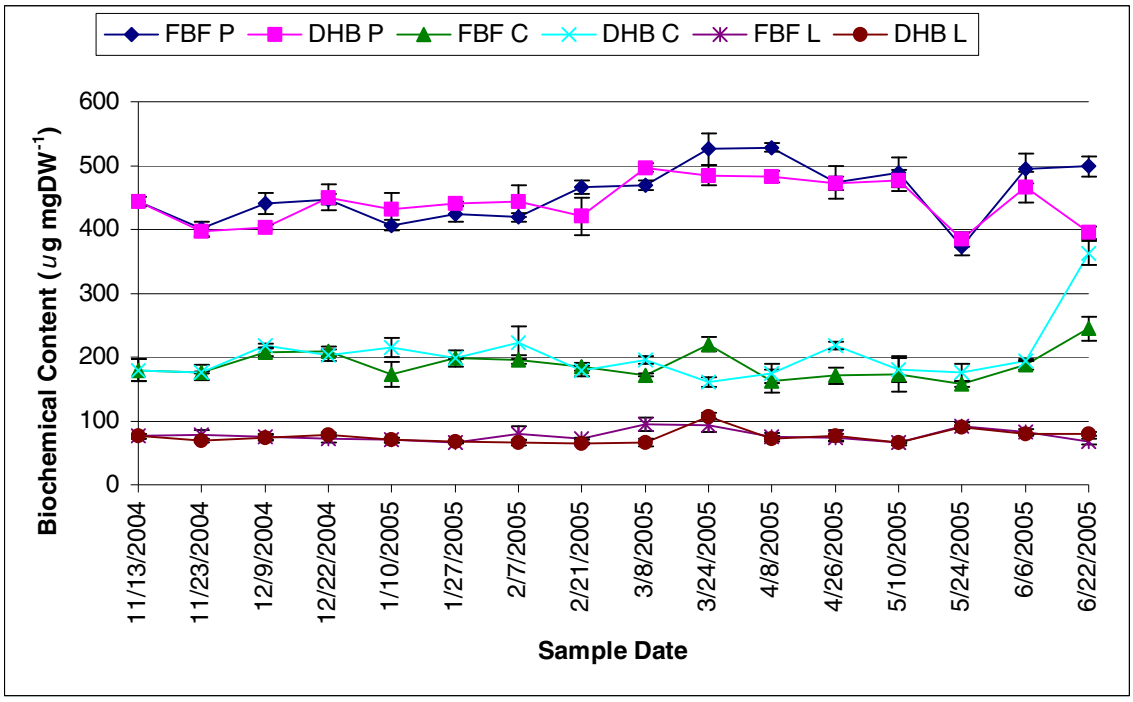

Fig. 14. Changes in biochemical content including protein, carbohydrates and lipids in clams at Dead Horse Bay and Floyd Bennett Field during the winter of 2002-2003.

Laboratory Experiment 2005-2006

Prior to the start of the laboratory experiment, clams were fed different amounts of micro-algae so as to produce two statistically significant groups (as determined by ANOVA), one with high (condition index = 1.7) the other with low (condition index = 1.3) condition indices (see **Materials and Methods**).

There was a direct correlation between the physiological parameters and temperature change through the laboratory experiment (Figs 15-19). The high condition clams had greater rate of oxygen consumption (0.45 ml^{-1} h^{-1} ind^{-1}) than the low condition clams (0.22 ml^{-1} h^{-1} ind^{-1}) at 14ºC. At 4ºC oxygen consumption was near zero. Oxygen consumption increased when the water temperature was returned to 14ºC. During this increase in temperature (4ºC to 14ºC) the food treatment was initiated. The groups formed were **high** conditon clams treated with **high** food; **high** condition clams with **low** food; **low** condition clams with **low** food; and **low** condition clams with **high** food. However, VO_2 did not increase to the same levels seen prior to the winter treatment. During this simulated spring, the low condition clams consumed more oxygen than the high condition clams. There was no difference in oxygen consumption between food treatments in either high or low condition clams (Fig 15). The ammonium excretion rate was similar in both the high and low condition treatments at the initial 14ºC and at 4ºC. Ammonium excretion decreased with the decrease in temperature. During the simulated spring, ammonium excretion rates were higher than other previously measured values in all treatments (Fig 17).

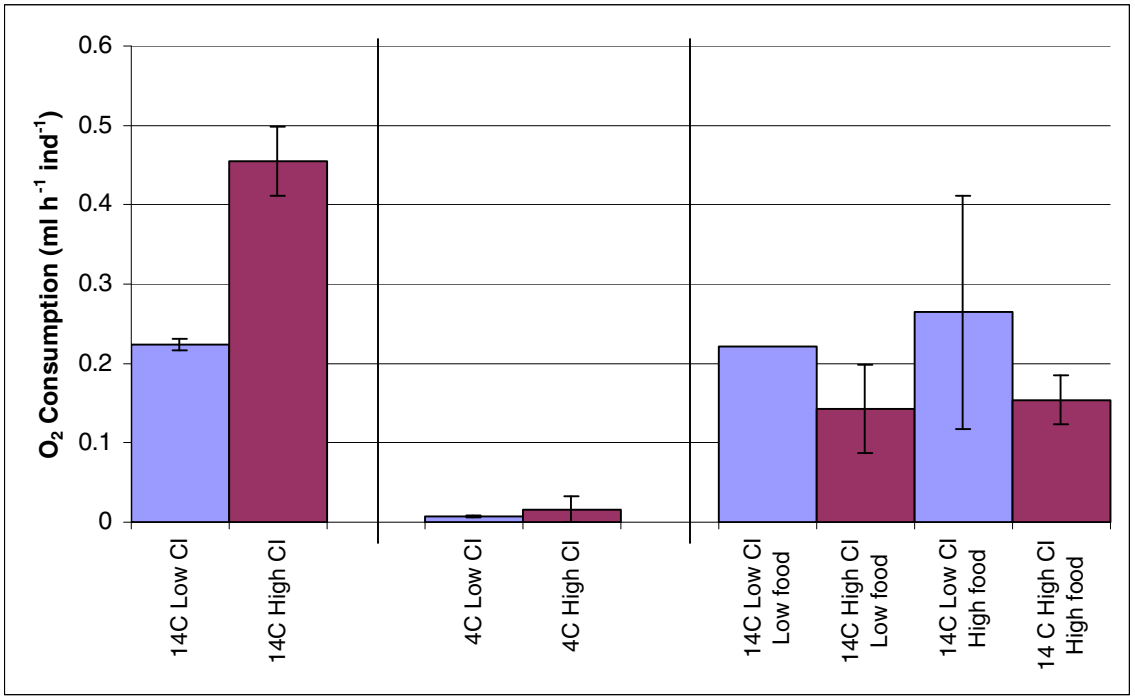

Fig 15. Oxygen consumption of high condition and low condition clams in a laboratory experiment under varying temperatures and food regimes.

High condition clams excreted in excess of 50% more ammonium than low condition clams. There was no difference in ammonium excretion rate between low condition clams fed high and low levels of food. High condition clams in the low food treatment excreted in excess of 50% more ammonium than the high condition clams in the high food treatment.

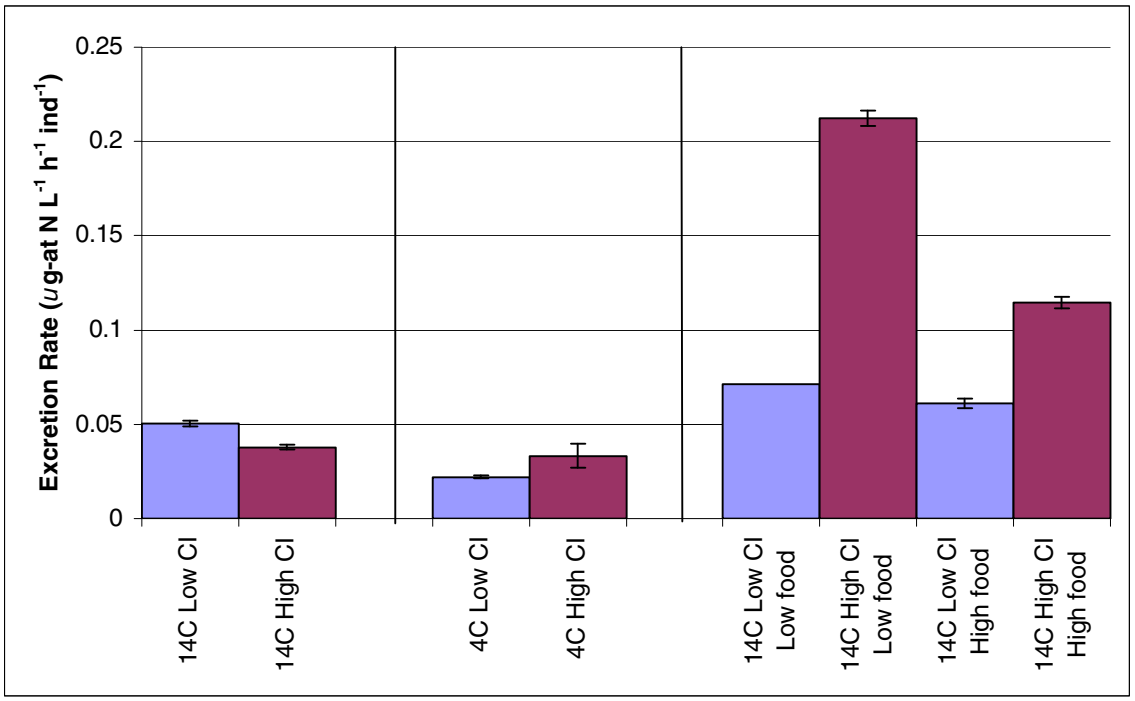

Fig. 16. Ammonium excretion of high condition and low condition clams in a laboratory experiment under varying temperatures and food regimes.

The results of the atomic ratio of oxygen consumed to ammonia-nitrogen excreted show that the high condition clams initially were using mainly a carbohydrate-protein based metabolism as indicated by an O: N of >20 (Fig. 17). In all other measurements the O: N is well below 7, which is indicative of high protein catabolism. These results mirror the ammonium excretion results.

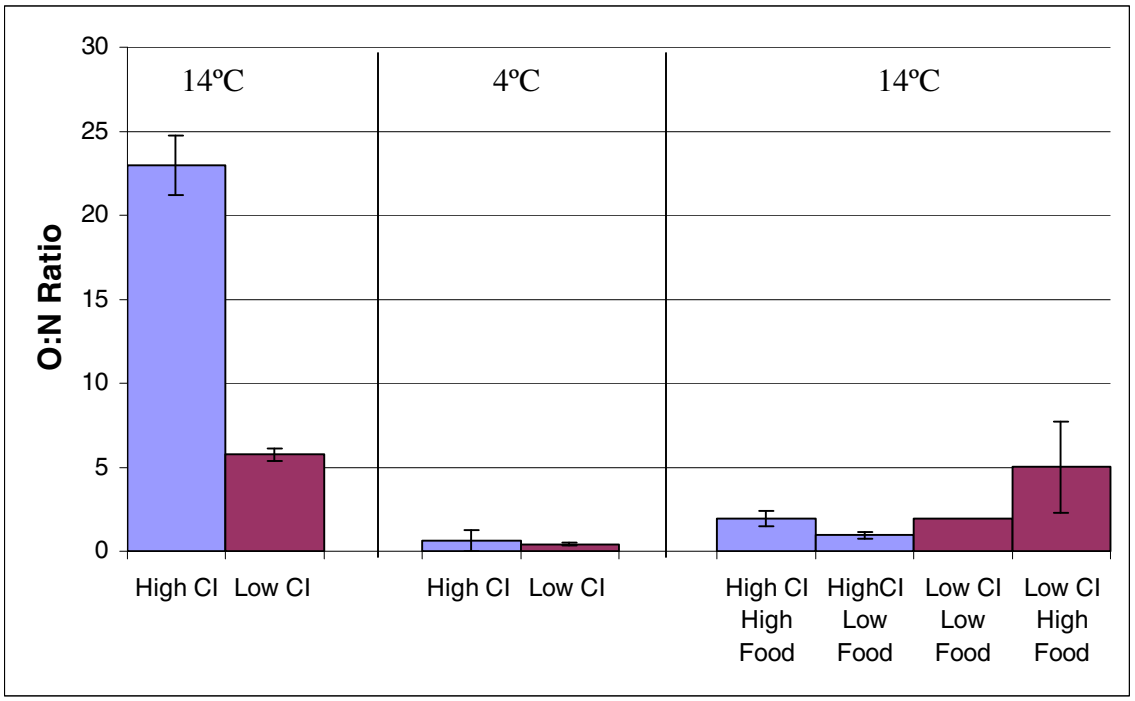

Figure 17. The O: N ratio of juvenile hard clams of high and low condition under varying temperatures and food regimes.

The phagocytosis assay demonstrated a higher level of immunocompetence in the high condition clams as compared to the low condition clams. As shown in Fig. 18, 57% of the observed hemocytes had ingested a yeast cell in the high condition clams as compared to the 40% in low condition clams at 14ºC. At 4ºC, phagocytosis was equal between the two groups. Then when the water temperature was increased back up to 14ºC, the difference between the groups was re-established. On each sampling date and under all treatments the results of the neutral red assay was a retention time of 120 minutes. Therefore, no changes were observed in lysosome membrane integrity.

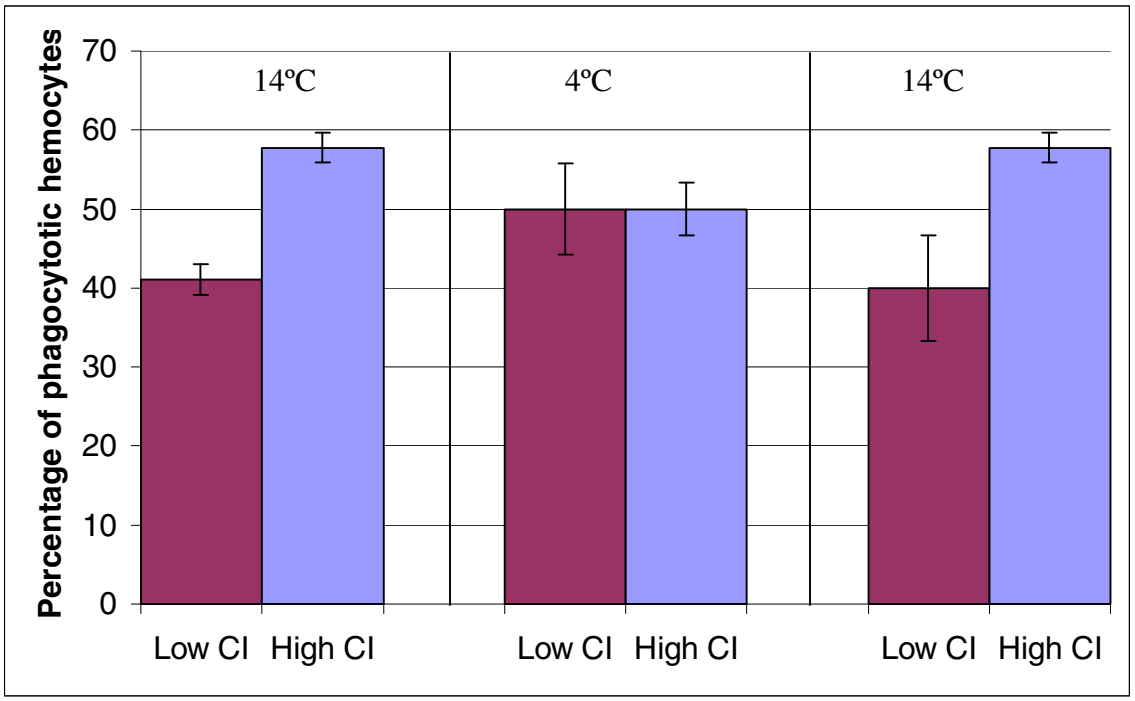

Fig. 18. Percentage of phagocytosis of yeast cells by low condition and high condition juvenile hard clam hemocytes at varying temperatures and food regimes.

Histological analysis of clam digestive tubules during the laboratory experiment also showed a clear relationship to temperature. There was no difference observed in tubule structure in either high or low condition clams at the three temperatures. Therefore, the two groups are pooled in the frequency distribution shown in Fig. 19. However, the high condition clams sampled at the initial 14ºC had a higher frequency of "type 2" digestive tubules. Since this tubule type, along with "type 1" tubules is indicative of a normal, healthy feeding clam, it was deemed appropriate to pool the condition types. Tubule type 1 can be seen in Fig. 20a, as the tubules have a clear lumen with a defined epithelial structure. In addition, the digestive cells are columnar. Approximately 90% of the tubules analyzed at 4ºC were "type 3" indicative of nutritive stress. These tubules are shown in Fig. 20b, the photograph shows that the basophil cells

are disorganized and the digestive cells are disintegrating into the middle of the lumen. The clam tubules analyzed during the spring simulation had the greatest frequency of "type 1" tubules (Fig. 20c) similar to what was originally observed at the start of the experiment.

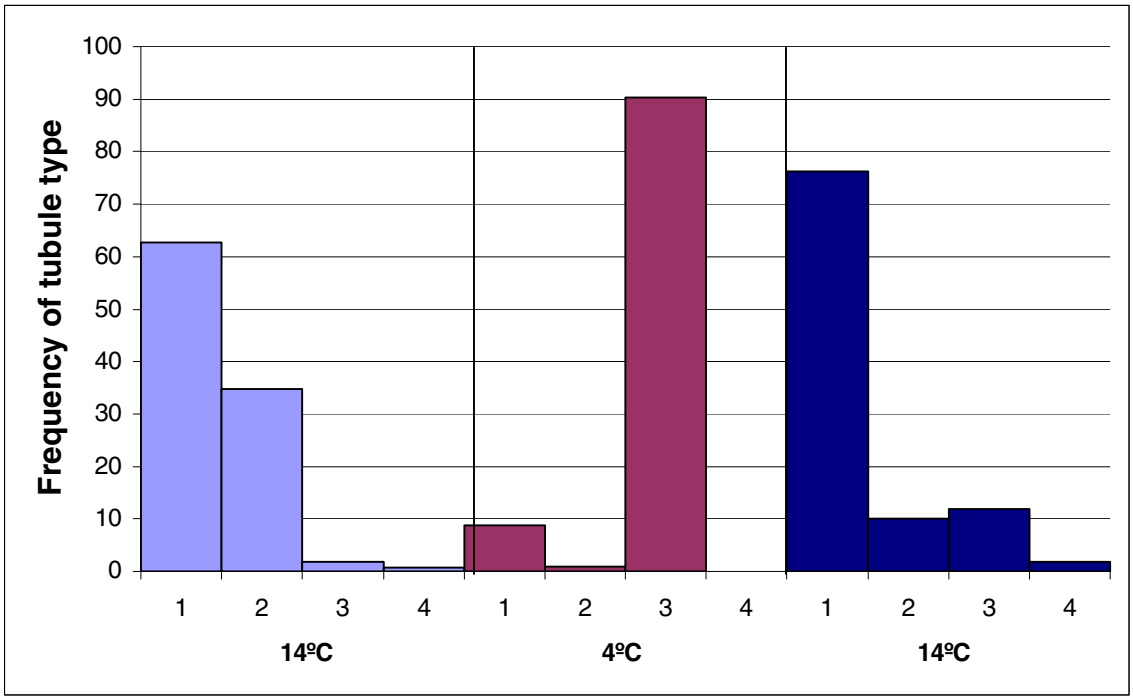

Fig. 19. Frequency distribution of clam tubule types in a laboratory experiment at three different temperatures (see **Introduction** for a description of tubules).

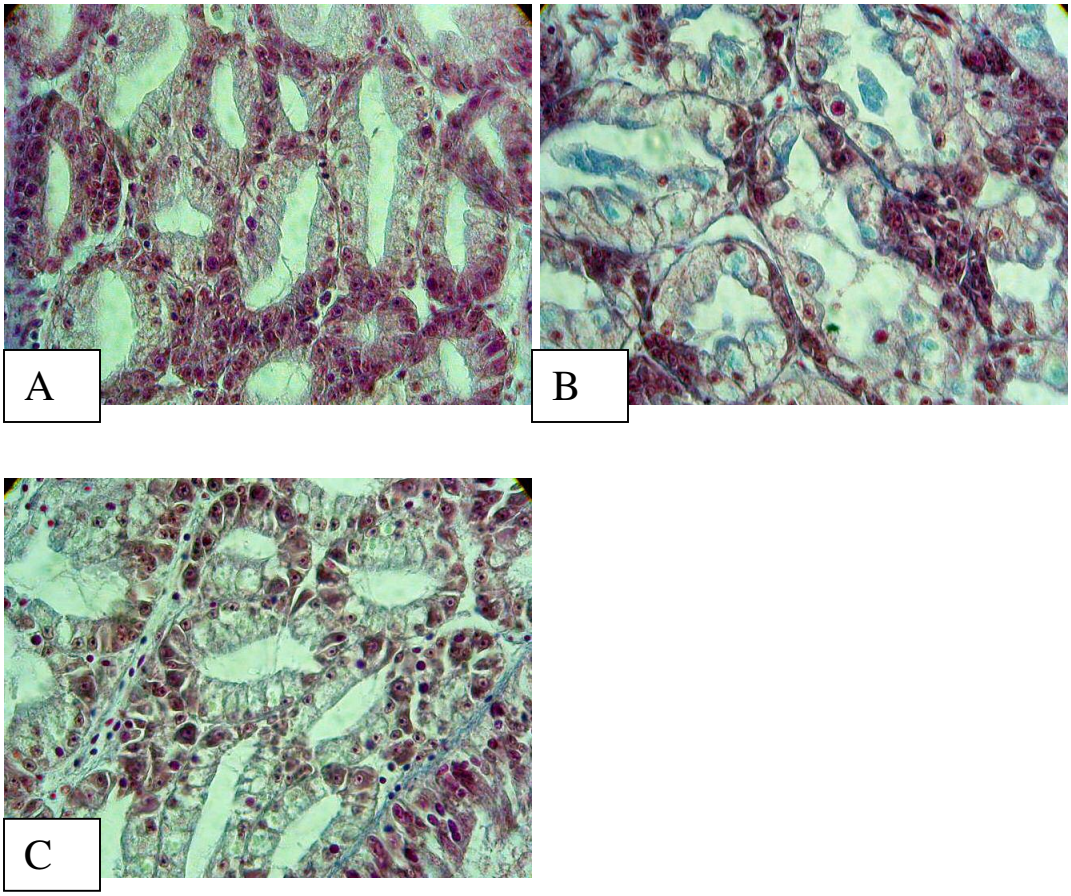

Fig 20a-c. Photomicrographs of A) Normal "type 1" tubules during the initial 14ºC treatment; B) Disintegrating "type 3" tubules during the 4ºC treatment; C) Normal "type 1" tubules during the final 14ºC treatment. 270x

The **condition index** of high and low condition clams was statistically significant (ANOVA; $P<0.01$) at 4ºC for the high condition clams when compared to the initial value. No other differences were observed. In addition, no differences were detected between **shell length** and **tissue dry weight** in all treatments (Tables 11 & 12). However, significance was not calculated for the low condition clams under the low food treatment (Table 12) due to a small sample size. There is a clear decrease in condition index and tissue dry weight in this treatment.

Protein, carbohydrate, and lipid levels all decreased in clams sampled at 4ºC, with protein decreasing the most significantly (21%) down to 390µg $mgDW^{-1}$. The **biochemical content** of the clams changed considerably under each experimental manipulation (Figs. 19 & 20). The high condition clams started with a protein content of 495µg $mgDW^{-1}$, carbohydrate content of 155µg $mgDW^{-1}$, and a lipid content of 89µg $mgDW^{-1}$ (Fig. 21). All three of these parameters decreased in clams sampled at 4ºC, with the protein decreasing most significantly (21%) down to 390µg $mgDW^{-1}$. When returning the temperature to 14ºC from 4ºC clams increased in protein content in both high and low food treatments to 440µg $mgDW^{-1}$ and 430µg $mgDW^{-1}$ respectively. However, the carbohydrate content of the low food treatment decreased by 60% down to 44µg $mgDW^{-1}$. The carbohydrate content of clams in the high food treatment did not change. Lipid content fell approximately 25% in both food treatments during the simulated spring.

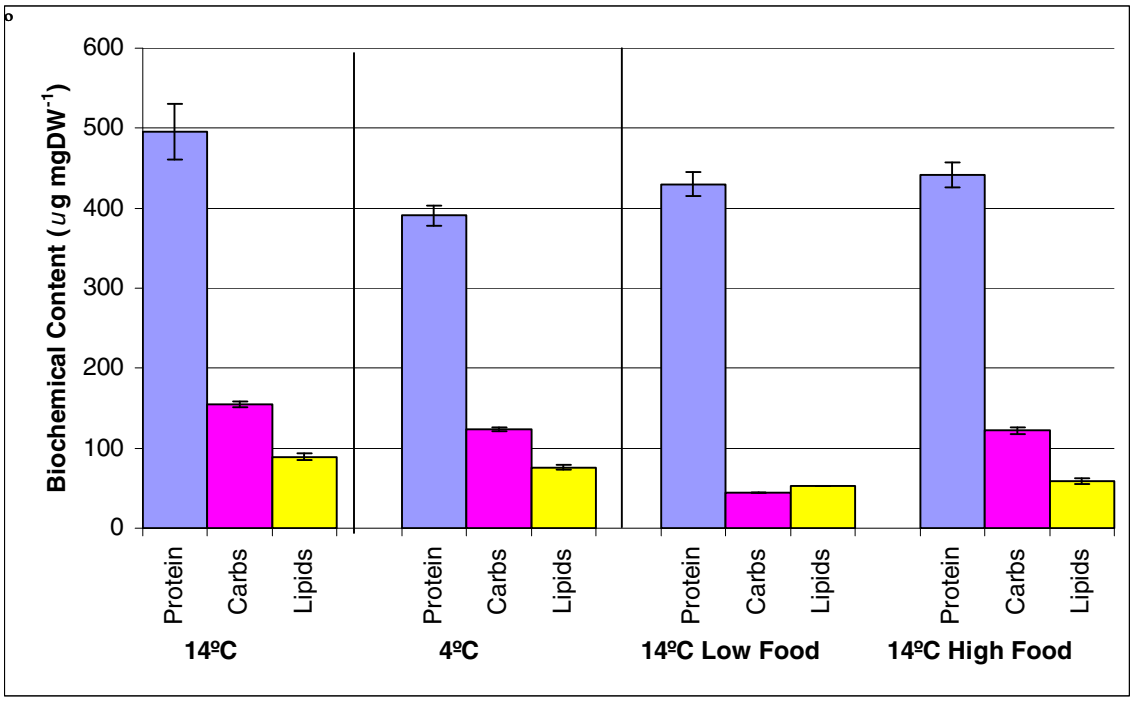

Fig. 21. Biochemical content of high condition clams in a laboratory experiment at different temperatures and food regimes.

At the start of the experiment, the low condition clams had a protein content of 483µg mgDW^{-1}, a carbohydrate content of 116µg mgDW^{-1}, and a lipid content of 87µg mgDW^{-1} (Fig. 22). All three components decreased significantly in clams sampled at 4ºC, with protein content deceasing 21% down to 379µg mgDW^{-1}. In the simulated spring, protein content increased to 410µg mgDW^{-1} in the high food treatment. The low food treatment continued to decrease to 276µg mgDW^{-1}. The carbohydrate content decreased significantly during the simulated spring to 56µg mgDW^{-1} and 33µg mgDW^{-1} in the high and low food treatments respectively. Lipid content was approximately 55µg mgDW^{-1} in both food treatments at the final sampling.

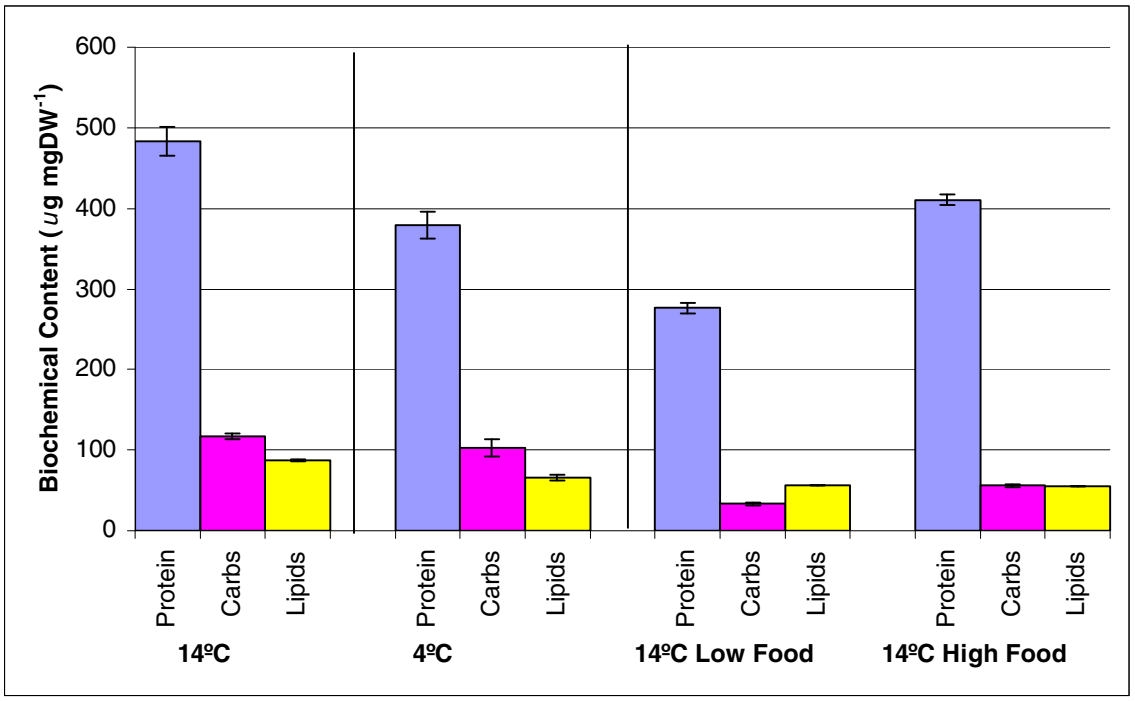

Fig. 22. Biochemical content of low condition clams in a laboratory experiment at different temperatures and food regimes.

The mortality observed in the final sampling of the laboratory experiment following temperature and food manipulation was extremely high (Fig. 23). Mortality was equal between all treatments except the high condition and high food treatment, which had significantly higher survival than the low condition with low food. High condition index and high food was also the only group in which carbohydrate content was not observed to fall below 100µg $mgDW^{-1}$.

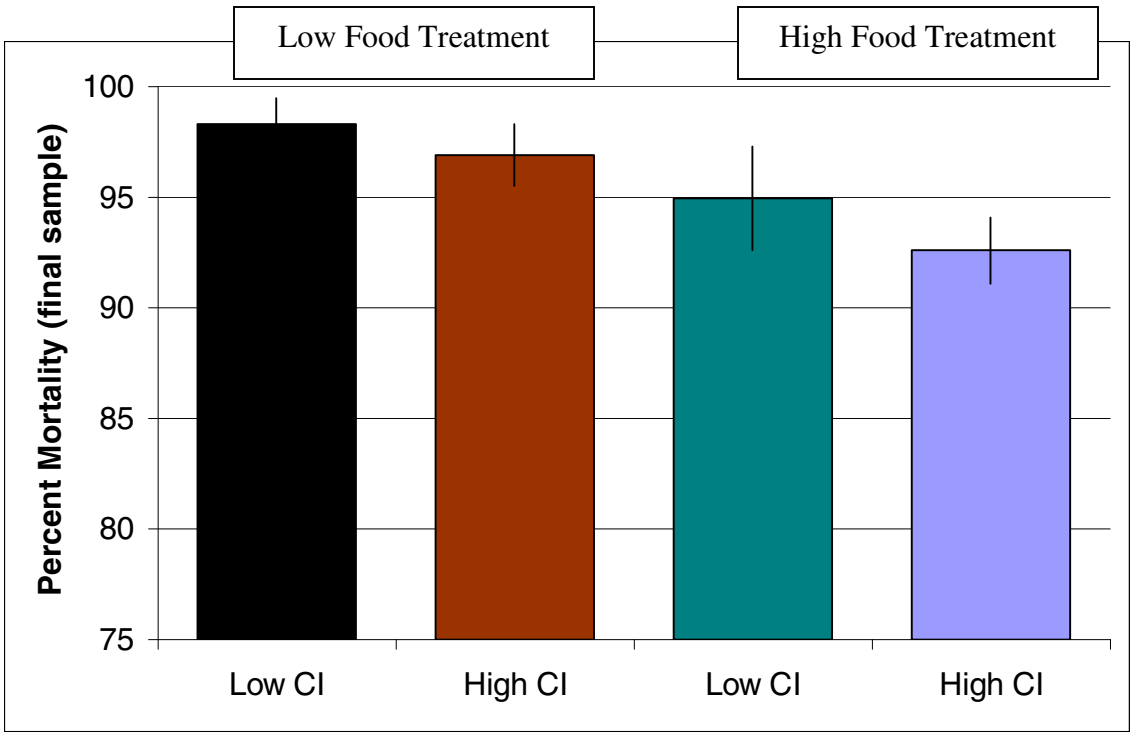

Fig. 23. Percent mortality observed at the final sampling of all treatments.

DISCUSSION

Mortality, Temperature, and Chlorophyll-*a*

This work is the first field study to monitor the survival of juvenile hard clams every two weeks from November to June for four consecutive years and to correlate mortality with temperature fluctuations, food availability and physiological analyses. Previous investigators (Aldred et al. 2001; Walker and Humphrey, 1984; Elderidge et al., 1976) that experienced over-winter mortality in field populations conducted their sampling in spring or summer following the winter. Kraeuter and Castagna (1984), however, observed significant over-winter mortality of juvenile hard clams (size range of 2-10mm shell height) in land-based nursery systems (flow through systems typically used in the nursery phase of aquaculture production). A majority of the mortality seemed to occur in the spring as water temperatures approached 10ºC. The timing of this mortality corroborates well with the mortality events experienced during our field studies.

In the same study, Kraeuter and Castagana (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. The authors didn't study mortality in relation to any other ecological parameters. Our field studies show a clear relationship between mortality and food availability in the spring.

The majority of deaths observed during the field studies of 2002-2003 and 2003-2004 occurred in the spring as water temperatures increased to 10ºC, following a winter where water temperatures remained less than 5ºC for at least eight 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 5ºC (Loosanoff, 1939; Zarnoch, this manuscript). Unfortunately, when the temperature did increase to 10ºC (a temperature where the clams would actively feed), the bloom had been reduced. The disappearance of the winter-spring bloom is likely to be a result of zooplankton grazing, as blooms of zooplankton closely follow phytoplankton blooms in Jamaica Bay (David R. Franz, personal communication, 2003). 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 6-10ºC during most of the winter-spring bloom. The water temperature was 12ºC 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 5ºC for sixteen weeks. In 2004-2005, the winter-spring bloom occurred at the end of March and did not dissipate until May. In previous years, the winter-spring bloom occurred in late February or early March and had dissipated in April. The high food availability as water temperatures increased to 10ºC lead to increased survival of the clams. Therefore, when contrasting the two years where significant mortality occurred with the two years where little mortality occurred, the critical role of food availability during the increase in temperature from 5 to 10ºC is observed. As seen in the winter of 2001-2002, the amount of available food is not critical

when the water temperature is above 12ºC. This would seem logical since mortality is not observed in aquaculture systems (see **Introduction**) during the summer grow-out phase when chlorophyll-*a* values are low (Malinowski and Siddall, 1989).

We also observed that mortality during the winter of 2003-2004 was nearly double the amount observed during the winter of 2002-2003. This is likely due to the fact that smaller sized clams (SL = 6.6mm) were used in the 2003-2004 experiment. In the only other study that investigated over-winter mortality of clam seed, the major finding was that larger clams (≥10mm) survived better than smaller clams (Kraeuter et al., 1997). In our studies, the winter-spring bloom was over when the water temperature started to increase during the winters of 2002-2003 and 2003-2004. However, the lowest chlorophyll-*a* value observed during 2003-2004 was 8µg L^{-1} while the lowest chlorophyll-*a* observed during 2002-2003 was 0.9µg L^{-1}. This suggests that the smaller clams may need more food to sustain their metabolic activity or that the quality of the food differed between these two years. Thus, the differential effect of varying food quality and quantity may play a role in over-winter mortality. In a study of the growth of juvenile hard clams, a 1.5-2.0% food ration of a 50/50 mixture of *Isochrysis galbana* and *Chaetoceros gracilis* was sufficient to support growth (Coutteau et al., 1994). In a separate study, Wikfors et al. (2001), found that 1.0 –2.0% rations of *Tetraselmis* was insufficient for growth and that growth could only be achieved with rations of 5.0-10.0%. The hypothesis of varying food quality in addition to the already demonstrated effect of food quantity should be investigated further in relation to over-winter mortality.

The use of two different sites in these studies led to findings that should be useful to the aquaculture industry. Mortality was greater at Dead Horse Bay (DHB) than at Floyd

Bennett Field (FBF) during the winters of 2002-2003. The reciprocal sediment exchange in 2003-2004 was conducted to determine if this was an effect of site or sediment type. The results from this experiment demonstrated that there was greater mortality at DHB. However, there was no difference in the number of mortalities between the two sediment types, thus, indicating disparity between the quality of the field sites in terms of sustaining juvenile hard clams through the experimental period. One explanation for this disparity is that chlorophyll-*a* was consistently higher at FBF than at DHB through all four years of monitoring. In addition, the sediment type differences between the two sites are likely due to differences in the rate of water flow over the benthos (Holme and McIntyre, 1984). A site with low flow would promote the settlement of finer particles (DHB) and a site with high flow (FBF) would be characterized by coarse sediments. The rate of water flow would also indicate the flow of food particles being brought to the clams, so that a high flow site would bring more food to the clams. Therefore, FBF had a higher flow available and a greater rate of food delivered to the clams, possibly contributing the lower mortality observed. Aquaculturists should consider this type of disparity between sites when choosing locations to over-winter clams. Site selection should also be a concern of natural resource managers when planting hard clam seed for restoration purposes, as the success of the effort may be influenced by over-winter mortality.

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 5ºC, hard clams cease feeding (Loosanoff, 1939; Zarnoch, this manuscript) and thus no increase in growth would be observed. Growth may occur at temperatures above 7ºC with maximum growth occurring between 20º to 24ºC (Ansell, 1968).

In our field studies, changes in growth parameters occurred only in the spring and early summer when water temperatures were greater than 12ºC. Condition index was the most sensitive parameter studied to indicate statistically significant increases in growth. As seen in tables 1-10, condition index was always first to show 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 would be the most useful measure to indicate growth in studies on juvenile hard clams. However, neither condition index or tissue dry weight was capable of indicating a significant decrease in condition (decrease in somatic tissue). This is demonstrated in the 2002-2003 and 2003-2004 experiments, when mortality was high and nutritive stress was evident. Therefore, the use of more sensitive assays such as ash free dry weight or biochemical indices would be advisable when investigating negative changes in clam condition.

The clams studied in the winter of 2002-2003 and winter 2004-2005 were similar in shell length (9.0-10.0 mm) at the start of the experiments in November. However, there were other differences, as the clams from 2004-2004 had a greater tissue dry weight (ANOVA; $P=0.01$) and thus a greater condition index (ANOVA; $P<0.001$) than the

clams from 2002-2003. This may have been caused by varying ecological conditions (such as temperature and chlorophyll-*a*) or by bio-fouling on the field culture equipment prior to deployment in the boxes in 2002-2003. In this instance, the clams were held in pearl nets suspended in the water column at the Dead Horse Bay site prior to being planted in the sediment filled boxes. During this time organisms such as sea squirts (*Molgula sp.*) and macroalgae set on the nets thus reducing the water flow to the clams and competed with the clams for food. The role that condition prior to winter plays in over-winter mortality was investigated in the controlled laboratory experiments of this study (see: **Materials and Methods**).

When examining the different growth rates observed during May and June throughout the four 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 0.74mm week^{-1}. This is consistent with the combined mean growth rate of 0.78mm week^{-1} from both field sites through our four years of study. There was, however, a site difference as clams at FBF typically had higher growth rates than clams at DHB (Table 13); this may be due to the differences discussed above (see: Mortality, Temperature, and Chlorophyll-*a*).

During the sediment reciprocal transfer experiment of 2003-2004, we found that sediment type or the interaction of sediment type and site did result in differences in growth rate at DHB. Clams in the sand sediment (FBF) grew much faster in terms of shell and tissue growth than clams in the mud sediment. Our observations are consistent with other clam transplant studies. Belding (1912), Pratt (1953) and Grizzle and Morin

(1989) have also reported decreased growth rates in mud sediment as compared to sand sediments. These authors noted shell growth inhibition of 6% to 24% in mud sediments, compared to the 30% shell growth inhibition we noted. However, no significant difference in shell growth rate between sediment types was found at FBF during the winter of 2003-2004, thus, indicating that the interaction of site and sediment type has a much greater effect than just sediment type alone.

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 biochemical content and chlorophyll-*a*.

It was our hypothesis 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). It was 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 no statistically significant decreases in biochemical stores during the winter, instead all changes occurred in the spring as water temperature rose above 5ºC. The observed decreases in biochemical composition were not only related to changes in temperature but to food availability, as well.

Our study of protein, carbohydrate, and lipid content of juvenile clams during the winters of 2001-2005, with simultaneous measurement of temperature and chlorophyll-*a,*

has led to the identification of three physiological responses (summarized in Fig. 24) 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 four years of study they mirrored changes in protein content. This would be logical since any degradation of protein-based tissue would be associated with the degradation of membranes. In addition, membrane production would be associated with increases in somatic tissue. Therefore, since lipid content plays such a minimal role in the physiological energetics of juvenile hard clams and since any observed changes over the four 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 5ºC and juvenile hard clams do not show any reduction in biochemical content. Measurement of metabolic rates such as oxygen consumption and nitrogen excretion at 4ºC during the laboratory experiments demonstrate this reduction in metabolic activity (see: Laboratory experiment). Metabolic rate is not at all influenced by the quantity of food available during this low temperature period. Apparently, this dormancy is highly efficient so that insignificant quantities of energy reserves are used. Therefore, this is a response where no loss of endogenous energy reserves is observed as indicated by the absence of significant decreases in energy stores for all the winters we studied. Other bivalve species found in temperate waters do not show the same ability to conserve energy. *M. edulis* and *C. edule* lose between 0.5% to 1.0% of the 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., 1989). 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). Therefore, they must adapt a strategy to conserve energy reserves during periods when they are not feeding.

There was a second important physiological response identified in this research that is related to water temperatures between 5º and 12ºC. 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. Water temperature increased to 7ºC during early March in the winter of 2003-2004; this coincided with the peak of the winter-spring bloom. At this time the hard clams significantly increased their protein content from an initial value of 363µg $mgDW^{-1}$ to approximately 580µg $mgDW^{-1}$, at both sites. The increase in protein content was not translated into somatic growth, probably due to the temperatures being below 12ºC (not warm enough for growth). This high rate of protein synthesis was supported by the high food available at the time due to the winter-spring bloom.

This highlights the response strategy of hard clams to temperature (Newell, 1980). Increases in temperature cause increases in metabolic rates. 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.

During the period of protein synthesis, there is a concomitant decrease in carbohydrate content in the clams. This is likely due to the high metabolic demands of maintaining feeding rates and to mobilizing nutrients for protein synthesis. As the winter-spring bloom waned during the winter of 2003-2004, the temperature remained between 5º to 12ºC. At the same time when food availability was at its lowest, a highly significant decrease in carbohydrate content was observed at both sites (a 48% drop at DHB and 37% at FBF). These decreases resulted in carbohydrate contents of approximately 5.8% and 7.1 %, respectively, of the clams' total dry weight. In addition, protein content decreased from the maximum observed value in March to values of approximately 400µg $mgDW^{-1}$. The observed decline in these biochemical substrates occurred at the same time high mortality was observed in the boxes, suggesting a relationship between mortality and the significant losses of carbohydrates and protein.

The winter of 2002-2003 also showed a relationship between decreasing carbohydrate reserves and increasing mortality. This was also correlated with water temperatures that were between 5º and 12ºC and with low food available. 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. From our observations during the winters of 2002-2004, it is apparent that as water temperatures increase above 5ºC clams become metabolically active and 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 *Donax vittatus* (Ansell and Sivadas, 1973). *Donax* will increase its metabolic rate with

increase 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 suggests that mortalities due to predation decrease when clams reach 20-25mm 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 supporting 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 5º and 12ºC and external food supply is not sufficient to support metabolism. This will then lead to the use of energy reserves.

The results obtained from the winters of 2002-2003 and 2003-2004 demonstrate that significant mortalities occur when carbohydrate content falls to levels at or below 10% the clams' total dry weight. A 10% tissue weight threshold of 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 believed that a similar threshold exists for hard clams during physiologically stressful periods.

Carbohydrate metabolism may also explain why sexually mature hard clams do not experience significant over-winter mortality. In some bivalves, (i.e., *M. edulis*) sexually mature animals have specialized connective tissue cells that provide a means for sequestering carbohydrates (glycogen) and proteins for gametogenesis. It is possible that the development of these cells during sexual maturation increases the quantity of carbohydrates maintained in clams and thus extends the amount of carbohydrates that can be utilized before reaching the 10% threshold. In order for this hypothesis to hold true, differential allocation of resources must occur in adults and juveniles. Theoretically, juveniles would turn all assimilated resources into growth, while sexually mature clams would allocate resources between growth and storage cells used for gametogenesis. In a study by Peterson and Fegley (1986), a difference in growth was observed between juveniles and adults during November to January in a North Carolina estuary. The juveniles showed considerable growth, while no growth was observed in adults. The authors suggested that this might be due to sexually mature clams partitioning resources into storage in preparation for gametogenesis. However, they could not eliminate the possibility that juveniles use an alternative food source (i.e., dissolved organic material) not available to the adults. Therefore, further research is necessary to quantify the capacity of carbohydrate storage in juvenile and sexually mature clams in order to understand the significance of the differential partitioning of resources.

The third physiological response identified in this study dealt with the relationship of growth to temperatures greater than 12ºC. As discussed earlier, growth may occur

when water temperatures are above 7ºC. However, in our study growth was not observed until water temperatures were above 12ºC, with maximum growth occurring between 15º-20ºC. It was puzzling to observe that growth occurred at these temperatures regardless of how much food was available. However, when we analyzed the changes in biochemical composition through each sampling year a pattern of protein utilization was identified with temperatures above 12ºC. Apparently, endogenous protein will be used to support growth when the external food supply cannot. 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, are 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 increased during the summer bloom. In all instances where temperatures were above 12ºC and food levels were high, there was no decrease observed in any biochemical substrate, suggesting that the external food supply was supporting growth.

Another particularly interesting aspect to this identified physiological response is that carbohydrates do not decrease. Apparently, there is a difference in metabolic demand at temperatures of 5º to 12ºC and greater than 12ºC. We would have anticipated a decrease in carbohydrate content if metabolic demand was as great at the higher

temperatures as it is at the lower temperatures during periods of low food availability. Therefore, it appears that the role of carbohydrates in juvenile clams is solely to support metabolism. As the clams mature, carbohydrates will also contribute to gametogenesis. Protein is used to support somatic growth (when temperatures permit) in juvenile clams during periods of low food. The use of proteins during periods of starvation has been reported in immature Iceland scallops (Sundet and Vahl, 1981) and immature bay scallops (Epp et al., 1988). These observations are consistent with our findings.

An alternative explanation to the decrease in proteins observed during periods of growth could be due to protein turnover. This is defined as the continuous degradation and replacement of cellular proteins (Hawkins and Day, 1996). If protein macromolecules were being broken down into amino acids and nucleotides (Mandelstam, 1960) during this proposed physiological response to temperature and food availability, the Bradford (1976) protein assay used in these analyses would not detect these smaller molecules (Kruger, 1996). This could explain the lower observed protein values. However, this would still indicate that the clams are using a protein-based metabolism and thus would corroborate well with our hypothesis (Fig. 24).

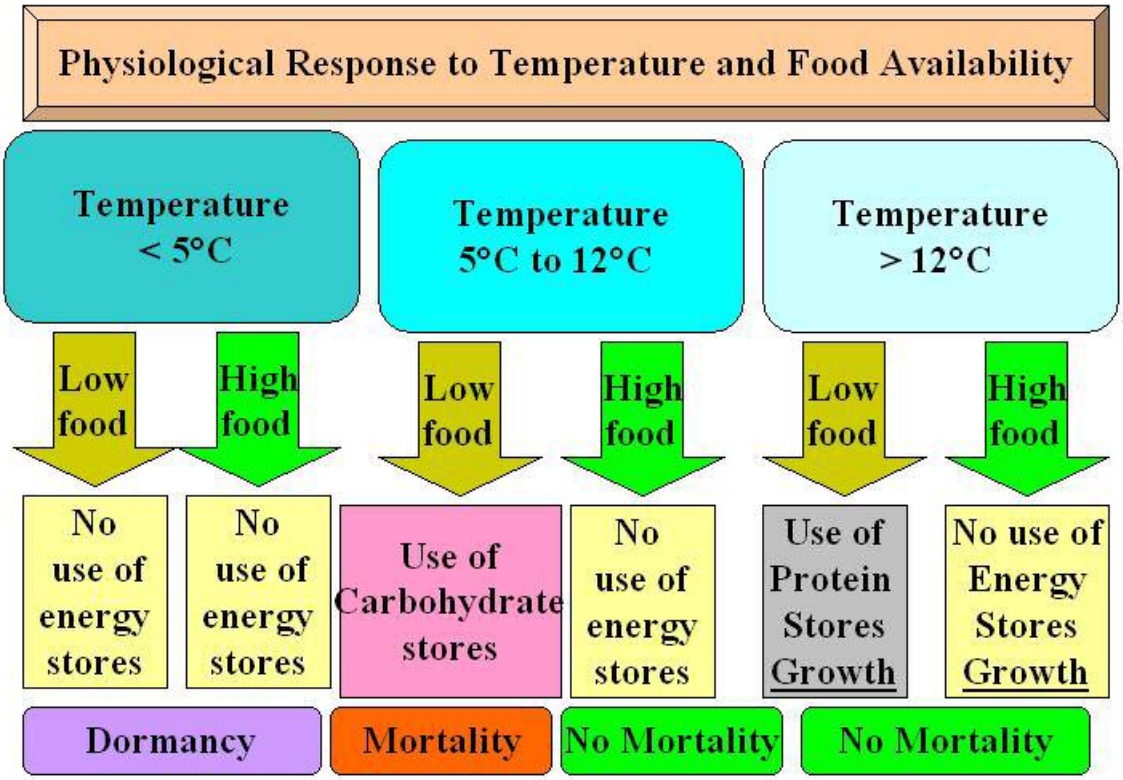

Fig. 24. A schematic describing the physiological response of hard clams to the interactions of temperature and food availability as it pertains to over-winter mortality.

Laboratory experiment

The purpose of the laboratory experiments was to investigate the significance of clam condition prior to the winter and to study its impact on over-winter mortality. The experiments were also designed to identify the interaction of clam condition prior to winter to the availability of high and low food levels when water temperature increases (from 4º to 14ºC) and how it impacts on mortality. Additionally, growth and biochemical parameters were measured to corroborate findings from the field experiments. Lastly, metabolic rates were studied under varying temperatures to test our hypotheses of temperature-related clam physiological activity.

The results of the laboratory experiment should be interpreted with caution since mortality was observed throughout the experimental period. This is unlike the field studies, where significant mortality did not occur until the spring. Similar results were obtained in laboratory experiments on *Donax vittatus* where heavy mortalities were observed throughout the study (Ansell and Sivadas, 1973). The similarity of response is interesting since hard clams are also similar in metabolic function and temperature response strategy. This is apparently something many aquaculturists have experienced when they have attempted to over-winter clams in a hatchery or laboratory setting .

Prior to the start of the laboratory experiment, several laboratory pilot trials were conducted (2003-2005) to assess variables such as sediment type, temperature variations, and food rations in the experimental design. In these pilot trials, as well, mortalities ranging from 40% to 80% were observed. It appears as though there is an inherent problem associated with keeping juvenile hard clams in the laboratory for extended periods of time (more than four weeks). This mortality is the result of an unknown

mechanism not related to over-winter mortality. Therefore, it was concluded that a certain proportion of the mortality experienced in the laboratory experiment would be considered "background mortality". An obvious solution to over-winter mortality would be to keep clams in a controlled environment through the winter, but mortality still occurs. The reason for this mortality is unknown. We believe that our results warrant consideration because they do provide insight into the mechanisms of over-winter mortality and also provide important data on the physiological condition of clams undergoing mortality of an unknown cause associated with being in a laboratory setting. This information may be useful in future studies on understanding the mechanisms of this mortality.

The results of the laboratory experiment do not show any significant difference in mortality between the high food and low food treatments in both low condition groups. The high condition group with the low food treatment did not differ from either of the low condition groups. The high condition group that received the high food treatment differed significantly from the low condition receiving low food treatment, but didn't differ significantly from the other groups. Our results suggest that juvenile hard clams with a high condition index before winter that are exposed to high food as water temperatures increase, will have the greatest survival and that the low condition clams with the low food treatment as temperatures rise will have the lowest survival. Hard clams going into the winter with a low condition index but are exposed to high food in the spring will experience similar mortality to clams with a high condition index before winter but are exposed to low food as water temperatures climb. These results highlight the fact that high food availability as water temperature increases is the most important

factor in determining over-winter mortality. However, caution should be taken in the interpretation of these results due to the potential interaction of the background mortality. The results dictate the need for repetition of these experiments in both laboratory and field. It would also be especially interesting to determine the cause of the laboratory-associated mortality so that aquaculturists might have another tool to overcome the field over-winter mortality.

The biochemical changes observed at the start of the experiment compared to the samples taken after four weeks at 4ºC were interesting. In the field studies, no changes were observed when temperatures are below 5ºC. However, in this laboratory experiment a significant decrease in protein content was observed in both the high condition and low condition groups. As discussed above (see: Biochemical analysis), protein content only decreased in field studies under conditions when water temperatures were warmer than 12ºC and food availability was low, causing the clams to use protein to support growth. Therefore, the use of protein content as an energy substrate in the laboratory is different from the field observations. Curiously, Ansell and Sivadas (1973) showed that protein was the main energy substrate used by *Donax* in their studies. Perhaps, the unexplained laboratory-related mortalities noted in both our studies are somehow related to the depletion of protein content.

When water temperatures were increased from 4ºC to 14ºC and the food treatments were applied, protein content did not decrease in any of the treatment group except for the low condition clams with low food availability. This was also the only treatment where a statistically significant difference in mortality was observed when compared to the high condition and high food treatment clams. Carbohydrate content

decreased in all treatments except for the high condition and high food treatment. In all cases where carbohydrate content decreased, it fell below the 10% tissue weight threshold. However, mortality was also high in the treatment where carbohydrate content did not decrease, so that the levels of carbohydrates cannot be used to explain mortality. In addition, the low condition index and high food treatment had a carbohydrate content of 56µg $mgDW^{-1}$ which is lower than would be expected since the high food should be enough to support metabolic demands. One explanation for this could be that the decrease in temperature from 14ºC to 4ºC may have resulted in a high metabolic demand and since the low condition clams started the experiment with only 116µg $mgDW^{-1}$of carbohydrates, a value close to the 10% tissue weight threshold the clams were challenged and succumbed prior to the food treatments. As in the field experiments, lipids followed the same trends as protein.

The results of the studies on metabolic rates and the histological analysis of digestive tubules support the hypotheses that were put forth describing clam metabolic activity in relation to temperature. **Oxygen consumption rates** observed at 14ºC before the start of the trial were comparable to unpublished data by V.M. Bricelj as discussed in Grizzle et al. (2001) on juvenile hard clams of similar shell length. It is interesting to note that the high condition clams were more active (consumed more oxygen) than the low condition clams. When the temperature was decreased to 4ºC oxygen consumption rates were dropped to approximately 1% of the measured values observed at 14ºC. This is no doubt indicative that the clams are entering winter dormancy and a concomitant metabolic depression. When the water temperature was increased, oxygen consumption rates increased. The low condition clams resumed consumption rates similar to the initial

measurements, while the high condition clams were not consuming as much oxygen as measured initially. This may be due to the reduction in condition experienced by the high condition clams through the experimental period. However, the overall pattern of this metabolic rate follows the anticipated trend.

The rate of **ammonium excretion** decreased as the water temperature was decreased from 14ºC to 4ºC, however, it did not drop below 5% of the initial measured value. When the water temperature was returned to 14ºC, ammonium excretion rates increased significantly. The lack of a significant decrease at 4ºC and the overwhelmingly high increase at 14ºC indicates that protein utilization was occurring in clams at both temperatures. As mentioned earlier, all treatments experienced a decrease in protein content during the 4ºC treatment. This may be related to the mortality that was occurring throughout the experiment. In *M. edulis*, high ammonia excretion rates are common when the mussels are stressed due to starvation or low salinity (Bayne et al. 1976). The clams in the laboratory experiment were not held in a low salinity (27 ppt), but were not feeding at 4ºC, as indicated by the histology of the digestive tubules. There hasn't been much work done on exploring excretion rates in juvenile hard clams under varying environmental and physiological conditions therefore making comparisons difficult. Our study should help fill this void.

The high rate of ammonium excretion impacted the results of the atomic ratio of oxygen consumed to nitrogen excretion. In almost all cases, the **O: N** ratio was below 7, indicative of protein catabolism. At 4ºC, the low O: N may have been a result of near zero oxygen consumption. However, excretion rate was not reduced to a level as low as the rate of oxygen consumption at this temperature. The persistent catabolism of proteins

as indicated by a low O: N, was also observed in *Donax* by Ansell and Sivadas (1973). This may provide another piece of information linked to the mortality experienced throughout the laboratory experiment.

The results of the **immunocompetence** assays suggest that hard clams can maintain their defense mechanisms even under periods of physiological stress. This is indicated by a frequency of 40% or greater of phagocytosed yeast cells. These values were similar to phagocytotic activity in *Ostrea edulis*, which ranged from 37% to 64% (Witt, 2003). There was a difference between high condition clams and low condition clams at 14ºC, suggesting that immunocompetence is related to physiological activity. The maintenance of this immunological function is important to note, since *Vibrio* infection have been suggested as a possible mechanism for over-winter mortality (Kraeuter and Castagna, 1984). The neutral red assay indicated that the hemocytes were not being challenged under any of the conditions tested and that the integrity of the lysosomal membrane is maintained with changes in temperature or physiological condition. A retention time of 120 minutes is double of what was found in *Ostrea edulis* (Witt, 2003) and similar to values measured in *Mytilus edulis* (Harding et al., 2004).

The **histological analysis** of digestive tubules showed a clear relationship between temperature and intracellular absorption and gut condition. The preponderance of type 1 and type 2 tubules at 14ºC are indicative of tubules that are digesting and absorbing food particles. The type 3 tubules, which are most prevalent at 4ºC, indicate that tubules are disintegrating and absorption and digestion of food particles is markedly decreased. The relative number and ratio of the four tubule types at the various temperature regimes demonstrate that when a continuous supply of food is available to

the clams, all the tubules will be similar in structure, that is the same type. On the other hand, when exposed to a variable supply of food, all four types are observed which may reflect a period of preparedness for digestion (Robinson et al., 1981).

SUMMARY

After conducting four years of field studies and an intense laboratory trial, it is evident that over-winter mortality is the result of a complex of interactions of temperature dynamics and food availability and that is closely linked to the physiological condition of the organism. Over-winter mortality is greatest when water temperature increases to greater than 5ºC and there is little food available to support an increase in metabolic rate. This 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. This is probably more likely due to lower food values observed at DHB or an interaction of food availability and sediment type rather than to sediment type alone. The quantity of carbohydrates in clams prior to winter may be important in determining their ability to survive. Although more work should be done on this, it seems 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.

Our observations suggest that it would be advisable for aquaculturists and natural resource mangers to determine seasonal dynamics of food availability at field sites before planting hard clam seed. In addition, to determining food availability, flow and sediment type should be analyzed. 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 over-winter 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 over-winter

mortality because in other estuaries, where primary productivity is limited by nutrients, over-winter 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, NY often experiences blooms of picoplankton (< 1 to 4µm) 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 5º and 12ºC, the juvenile clams would have to use energy reserves to support metabolism and this could lead to mortality as demonstrated in this study. This 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). Another interesting example of how phytoplankton community structure may influence hard clams is in Narragansett Bay, where winter-spring blooms of diatoms historically occurred every year up until the 1970's when the bloom was reduced in intensity, duration or did not occur at all (Oviatt, 1994). This is apparently due to changes in nutrient loadings and increased water temperatures (Oviatt et al., 2002). The impact this will have on wild hard clam populations and the aquaculture industry has yet to be determined.

Our data suggest that the physiological condition of the clams be determined prior to planting to ensure high carbohydrate content. Conditioning trials during this research have shown that a one-week feeding of a 3% algal dry weight per clam wet weight at 17ºC will increase carbohydrate content from 155µg $mgDW^{-1}$ to 190µg $mgDW^{-1}$, this is an increase of 18%. Such an increase could potentially make a significant difference in

the survival of hard clams during metabolically stressful periods. This short conditioning period, however, needs to be tested in the field and must also be shown to be an economically viable option for industry before it can be implemented. Therefore, a comprehensive study simultaneously measuring food quality and quantity along with physiological condition of clams through the over-winter period should be conducted across several different estuaries in order to gain a broader scope of understanding how these ecological and physiological processes impact the survival of juvenile aquacultured hard clams.

APPENDIX

Table 1.
Clam condition index, shell length, and tissue dry weight at Dead Horse Bay during the winter of 2001-2002.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/20/2001	1.33 ± 0.12	-	9.87 ± 0.19	-	13.48 ± 1.40	-
12/4/2001	1.33 ± 0.07	NS	10.09 ± 0.22	NS	13.74 ±1.00	NS
12/18/2001	1.11 ± 0.04	NS	9.74 ± 0.19	NS	10.92 ± 0.62	NS
1/2/2002	1.44 ± 0.10	NS	11.05 ± 0.25	NS	16.44 ± 1.49	NS
1/15/2002	1.50 ± 0.09	NS	10.52 ± 0.27	NS	16.25 ± 1.33	NS
1/29/2002	1.39 ± 0.06	NS	10.24 ± 0.19	NS	14.48 ± 0.89	NS
2/12/2002	1.53 ± 0.09	NS	10.56 ± 0.25	NS	16.61 ± 1.41	NS
2/24/2002	1.42 ± 0.07	NS	10.16 ± 0.25	NS	14.82 ± 1.08	NS
3/11/2002	1.64 ± 0.08	NS	10.56 ± 0.21	NS	17.68 ± 1.24	NS
3/26/2002	1.86 ± 0.11	NS	10.99 ± 0.28	NS	21.12 ± 1.74	NS
4/8/2002	2.04 ± 0.08	P < 0.0001	11.27 ± 0.19	NS	23.23 ± 1.25	NS
4/23/2002	2.18 ± 0.15	P < 0.0001	12.50 ± 0.43	P < 0.0001	28.90 ± 3.00	P < 0.0001
5/6/2002	2.22 ± 0.12	P < 0.0001	11.94 ±0.26	P < 0.0001	27.27 ± 0.42	P < 0.0001
5/21/2002	3.17 ± 0.12	P < 0.0001	14.78 ±0.30	P < 0.0001	47.86 ± 2.61	P < 0.0001
6/4/2002	4.36 ± 0.16	P < 0.0001	16.77 ± 0.29	P < 0.0001	74.11 ± 4.03	P < 0.0001
6/17/2002	5.46 ± 0.27	P < 0.0001	19.00 ± 0.53	P < 0.0001	107.10 ± 7.02	P < 0.0001

Table 2.
Clam condition index, shell length, and tissue dry weight at Floyd Bennett Field during the winter of 2001-2002.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/23/2001	1.30 ± 0.26	-	10.00 ± 0.22	-	13.32 ± 1.09	-
12/7/2001	1.40 ± 0.28	NS	10.06 ± 0.24	NS	14.45 ± 1.37	NS
12/21/2001	1.51 ± 0.30	NS	9.91 ± 0.18	NS	15.10 ± 0.60	NS
1/4/2002	1.26 ± 0.25	NS	9.84 ± 0.18	NS	12.64 ± 0.99	NS
1/18/2002	1.40 ± 0.29	NS	10.13 ± 0.23	NS	14.56 ± 1.23	NS
1/31/2002	1.50 ± 0.31	NS	10.12 ± 0.23	NS	15.60 ± 1.20	NS
2/14/2002	1.38 ± 0.28	NS	9.83 ± 0.20	NS	13.82 ± 1.08	NS
3/1/2002	1.30 ± 0.27	NS	9.77 ± 0.16	NS	12.83 ± 0.74	NS
3/14/2002	1.48 ± 0.29	NS	10.15 ± 0.19	NS	15.24 ± 0.88	NS
3/28/2002	1.56 ± 0.32	NS	10.24 ± 0.32	NS	16.73 ± 1.87	NS
4/11/2002	1.53 ± 0.28	NS	10.10 ± 0.22	NS	16.00 ± 1.34	NS
4/25/2005	2.19 ± 0.41	$P < 0.0001$	10.89 ±0.29	$P < 0.0001$	26.80 ± 1.99	$P < 0.0001$
5/9/2005	2.67 ± 0.67	$P < 0.0001$	12.75 ± 0.39	$P < 0.0001$	34.89 ± 3.18	$P < 0.0001$

Table 3.
Clam condition index, shell length, and dry weight at Dead Horse Bay during the winter of 2002-2003.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/3/2002	1.01 ± 0.04	-	9.76 ± 0.16	-	10.08 ± 0.55	-
11/19/2002	1.04 ± 0.21	NS	9.63 ± 0.19	NS	10.15 ± 0.48	NS
12/2/2002	1.02 ± 0.20	NS	9.76 ± 0.18	NS	10.14 ± 0.60	NS
12/17/2002	1.04 ± 0.04	NS	10.11 ± 0.22	NS	10.74 ± 0.67	NS
1/3/2003	1.15 ± 0.09	NS	10.03 ± 0.22	NS	11.61 ± 0.91	NS
1/17/2003	0.79 ± 0.04	NS	9.50 ± 0.20	NS	7.72 ± 0.57	NS
1/29/2003	0.89 ± 0.04	NS	9.59 ± 0.21	NS	8.68 ± 0.59	NS
2/13/2003	0.86 ± 0.05	NS	9.40 ± 0.26	NS	8.37 ± 0.71	NS
2/27/2003	0.96 ± 0.04	NS	9.64 ± 0.18	NS	9.40 ± 0.60	NS
3/13/2003	0.89 ± 0.04	NS	9.40 ± 0.20	NS	8.45 ± 0.52	NS
3/25/2003	0.88 ± 0.19	NS	9.43 ± 0.24	NS	8.42 ± 0.28	NS
4/23/2003	0.87 ± 0.05	NS	9.74 ± 0.19	NS	8.59 ± 0.07	NS
5/8/2003	0.92 ± 0.05	NS	9.47 ± 0.23	NS	8.89 ± 0.65	NS
5/22/2003	1.16 ± 0.07	NS	9.94 ± 0.26	NS	11.87 ± 0.95	NS
6/5/2003	1.16 ± 0.05	NS	10.24 ± 0.21	NS	12.13 ± 0.76	NS

Table 4.
Clam condition index, shell length, and tissue dry weight at Floyd Bennett Field during the winter of 2002-2003.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/3/2002	1.01 ± 0.04	-	9.76 ± 0.16	-	10.08 ± 0.55	-
11/19/2002	1.06 ± 0.07	NS	9.27 ± 0.20	P < 0.0001	10.00 ± 0.80	NS
12/2/2002	1.10 ± 0.03	NS	9.50 ± 0.12	NS	10.52 ± 0.43	NS
12/17/2002	1.08 ± 0.04	NS	9.74 ± 0.18	NS	10.69 ± 0.59	NS
1/3/2003	1.07 ±0.06	NS	9.48 ± 0.20	NS	10.44 ± 0.79	NS
1/17/2003	0.88 ± 0.04	NS	9.86 ± 0.17	NS	8.77 ± 0.49	NS
1/29/2003	0.92 ±0.07	NS	9.37± 0.24	NS	8.96 ± 0.95	NS
2/13/2003	1.01 ± 0.04	NS	9.55 ± 0.18	NS	9.76 ± 0.51	NS
2/27/2003	1.08 ± 0.03	NS	9.77 ± 0.16	NS	10.64 ± 0.44	NS
3/13/2003	1.04 ± 0.04	NS	9.66 ± 0.21	NS	10.20 ± 0.57	NS
3/25/2003	1.03 ± 0.06	NS	9.71 ± 0.19	NS	9.96 ± 0.55	NS
4/10/2003	0.91 ± 0.04	NS	9.23 ± 0.18	NS	8.56 ± 0.52	NS
4/23/2003	1.03 ± 0.06	NS	9.65 ± 0.24	NS	10.22 ± 0.94	NS
5/8/2003	1.37 ± 0.07	P < 0.0001	10.17 ± 0.23	NS	14.25 ± 1.03	P = 0.007
5/22/2003	1.76 ± 0.07	P < 0.0001	11.57 ± 0.27	P < 0.0001	20.67 ± 1.31	P < 0.0001
6/5/2003	1.86 ± 0.09	P < 0.0001	11.88 ± 0.31	P < 0.0001	22.72 ± 1.70	P < 0.0001

Table 5.
Clam condition index, shell length, and tissue dry weight planted in the sand sediment at Dead Horse Bay during the winter of 2003-2004.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/21/2003	0.42 ± 0.02	-	6.66 ± 0.09	-	2.85 ± 0.13	-
12/10/2003	0.32 ± 0.02	NS	6.12 ± 0.22	NS	2.04 ± 0.16	NS
1/7/2004	0.37 ± 0.03	NS	6.48 ± 0.22	NS	2.51 ± 0.28	NS
2/6/2004	0.37 ± 0.02	NS	6.86 ± 0.19	NS	2.64 ± 0.22	NS
3/8/2004	0.34 ± 0.03	NS	6.77 ± 0.21	NS	2.43 ± 0.18	NS
4/3/2004	0.33 ± 0.02	NS	6.32 ± 0.15	NS	2.18 ± 0.22	NS
5/1/2004	0.36 ± 0.01	NS	6.32 ±0.30	NS	2.30 ± 0.10	NS
5/19/2004	0.82 ± 0.15	P < 0.0001	7.48 ± 0.54	NS	6.50 ± 1.61	P = 0.009
6/3/2004	1.48 ± 0.08	P < 0.0001	9.41 ± 0.26	P < 0.0001	14.41± 1.14	P < 0.0001

Table 6.
Clam condition index, shell length, and tissue dry weight planted in mud sediment at Dead Horse Bay during the winter of 2003-2004.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/21/2003	0.42 ±0.02	-	6.66 ± 0.09	-	2.85 ± 0.13	-
12/10/2003	0.31 ± 0.03	NS	7.21 ± 0.20	NS	1.90 ± 0.26	NS
1/7/2004	0.37 ± 0.04	NS	6.62 ± 0.29	NS	2.55 ± 0.35	NS
2/6/2004	0.41 ± 0.02	NS	6.94 ± 0.30	NS	2.93 ± 0.25	NS
3/8/2004	0.35 ± 0.02	NS	6.91 ± 0.28	NS	2.44 ± 0.33	NS
4/3/2004	0.43 ± 0.02	NS	6.68 ± 0.33	NS	2.91 ± 0.26	NS
5/1/2004	0.57 ± 0.11	NS	7.57 ± 0.54	NS	4.43 ± 1.08	NS
5/19/2004	0.97 ± 0.27	P < 0.0001	7.75 ± 0.78	NS	7.65 ± 3.75	P < 0.0001
6/3/2004	1.29 ± 0.16	P < 0.0001	8.45 ± 0.35	NS	10.90 ± 2.41	P < 0.0001

Table 7.
Clam condition index, shell length, and tissue dry weight planted in sand at Floyd Bennett Field during the winter of 2003-2004.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/21/2003	0.42 ± 0.02	-	6.66 ± 0.09	-	2.85 ± 0.13	-
12/9/2003	0.37 ± 0.03	NS	6.39 ± 0.20	NS	2.47 ± 0.24	NS
1/6/2004	0.35 ± 0.02	NS	6.47 ± 0.16	NS	2.33 ± 0.20	NS
2/4/2004	0.37 ± 0.02	NS	6.50 ± 0.20	NS	2.50 ± 0.25	NS
3/5/2004	0.40 ± 0.02	NS	6.77 ± 0.19	NS	2.76 ± 0.21	NS
4/3/2004	0.44 ± 0.03	NS	7.17 ± 0.28	NS	3.30 ± 0.33	NS
4/29/2004	0.66 ± 0.06	$P < 0.009$	6.99 ± 0.24	NS	4.70 ± 0.53	NS
5/17/2004	0.97 ± 0.09	$P < 0.0001$	7.79 ± 0.32	$P = 0.01$	7.81 ± 0.94	$P < 0.0001$
6/1/2004	2.17 ± 0.19	$P < 0.0001$	11.22 ±0.45	$P < 0.0001$	25.34 ± 3.41	$P < 0.0001$

Table8.
Clam condition index, shell length, and tissue dry weight planted in mud (DHB) at Floyd Bennett Field.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/21/2003	0.42 ± 0.02	-	6.66 ± 0.09	-	2.85 ± 0.13	-
12/9/2003	0.29 ± 0.03	NS	6.14 ± 0.19	NS	1.80 ± 0.13	NS
1/6/2004	0.35 ± 0.03	NS	6.68 ± 0.24	NS	2.50 ± 0.31	NS
2/4/2004	0.38 ± 0.02	NS	6.94 ± 0.19	NS	2.71 ± 0.23	NS
3/5/2004	0.37 ± 0.03	NS	6.60 ± 0.27	NS	2.58 ± 0.31	NS
4/3/2004	0.38 ± 0.03	NS	6.52 ± 0.21	NS	2.46 ± 0.28	NS
4/29/2004	0.49 ± 0.04	NS	6.64 ± 0.19	NS	3.36 ± 0.35	NS
5/17/2004	1.14 ± 0.07	$P < 0.0001$	8.01 ± 0.36	$P < 0.0001$	9.31 ± 1.39	$P < 0.0001$
6/1/2004	1.83 ± 0.09	$P < 0.0001$	10.68 ± 0.29	$P < 0.0001$	20.14 ± 1.52	$P < 0.0001$

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Table 9.
Clam condition index, shell length, and tissue dry weight at Dead Horse Bay during the winter of 2004-2005.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/13/2004	1.22 ± 0.04	-	9.79 ± 0.14	-	12.16 ± 0.59	-
11/23/2004	1.19 ± 0.06	NS	9.45 ± 0.15	NS	11.44 ± 0.72	NS
12/9/2004	1.17 ± 0.04	NS	9.45 ± 0.18	NS	11.19 ± 0.63	NS
12/22/2004	1.20 ± 0.05	NS	9.72 ± 0.19	NS	11.87 ± 0.68	NS
1/10/2005	1.14 ± 0.03	NS	9.29 ± 0.11	NS	10.62 ± 0.40	NS
1/27/2005	1.21 ± 0.04	NS	9.60 ± 0.18	NS	11.77 ± 0.61	NS
2/7/2005	1.22 ± 0.06	NS	9.86 ± 0.22	NS	12.26 ± 0.84	NS
2/21/2005	1.28 ± 0.05	NS	10.08 ± 0.21	NS	13.03 ± 0.74	NS
3/8/2005	1.21 ± 0.05	NS	9.55 ± 0.19	NS	11.73 ± 0.76	NS
3/24/2005	1.13 ± 0.07	NS	9.30 ± 0.20	NS	10.43 ± 0.71	NS
4/8/2005	1.14 ± 0.05	NS	9.74 ± 0.22	NS	11.35 ± 0.74	NS
4/26/2005	1.22 ± 0.05	NS	9.47 ± 0.17	NS	11.68 ± 0.61	NS
5/10/2005	1.49 ± 0.06	NS	10.39 ± 0.20	NS	15.79 ± 0.95	NS
5/24/2005	1.86 ± 0.08	P <0.0001	11.44 ± 0.25	P <0.0001	21.71 ± 0.14	P <0.0001
6/6/2005	1.65 ± 0.07	P <0.0001	11.56 ± 0.26	P <0.0001	19.51 ± 1.27	P <0.0001
6/22/2005	3.43 ± 0.15	P <0.0001	13.92 ± 0.29	P <0.0001	48.72 ± 3.01	P <0.0001

Table 10.
Clam condition index, shell length, and tissue dry weight at Floyd Bennett Field during the winter of 2004-2005.

Sample Date	Condition Index ±SEM	Significance	Shell Length (mm) ±SEM	Significance	Tissue Dry Weight (mg) ±SEM	Significance
11/12/2004	1.22 ± 0.04	-	9.79 ± 0.14	-	12.16 ± 0.59	-
11/23/2004	1.26 ± 0.06	NS	9.84 ± 0.25	NS	12.74 ± 0.96	NS
12/9/2004	1.20 ± 0.04	NS	9.71 ± 0.17	NS	11.82 ± 0.58	NS
12/22/2004	1.18 ± 0.05	NS	9.66 ± 0.17	NS	11.56 ± 0.66	NS
1/10/2005	1.16 ± 0.04	NS	9.68 ± 0.16	NS	11.33 ± 0.56	NS
1/27/2005	1.16 ± 0.03	NS	9.51 ± 0.16	NS	11.18 ± 0.51	NS
2/7/2005	1.18 ± 0.05	NS	9.60 ± 0.20	NS	11.59 ± 0.75	NS
2/21/2005	1.25 ± 0.07	NS	9.87 ± 0.23	NS	12.66 ± 0.97	NS
3/8/2005	1.17 ± 0.04	NS	9.50 ± 0.19	NS	11.26 ± 0.59	NS
3/24/2005	1.19 ± 0.06	NS	9.81 ± 0.23	NS	11.96 ± 0.91	NS
4/8/2005	1.06 ± 0.05	NS	9.39 ± 0.18	NS	10.09 ± 0.62	NS
4/26/2005	1.39 ± 0.06	NS	10.33 ± 0.21	NS	14.62 ± 0.87	NS
5/10/2005	1.30 ± 0.05	NS	9.85 ± 0.21	NS	13.11 ± 0.84	NS
5/24/2005	1.86 ± 0.09	P < 0.0001	11.21 ± 0.30	P < 0.0001	21.47 ± 1.51	P < 0.0001
6/6/2005	1.73 ± 0.08	P < 0.0001	11.55 ± 0.25	P < 0.0001	20.40 ± 1.41	P < 0.0001
6/21/2005	3.68 ± 0.16	P < 0.0001	15.28 ± 0.33	P < 0.0001	57.38 ± 3.63	P < 0.0001

Table 11.
Condition index, shell length, and tissue dry weight of high condition clams during the laboratory experiment.

Sample	Condition Index ±sem	Significance	Shell Length (mm) ±sem	Significance	Tissue Dry Weight (mg) ±sem	Significance
14ºC	1.70 ± 0.09	-	11.72 ± 0.30	-	20.52 ± 1.51	-
4ºC	1.29 ± 0.08	P = 0.006	11.02 ± 0.36	NS	14.84 ± 1.40	NS
14ºC High food	1.37 ± 0.18	NS	11.24 ± 0.61	NS	16.00 ± 3.12	NS
14ºC Low food	1.28 ± 0.13	NS	12.50 ± 0.01	NS	16.15 ± 1.15	NS

Table 12.
Condition index, shell length, and tissue dry weight of low condition clams during the laboratory experiment.

Sample	Condition Index ±sem	Significance	Shell Length (mm) ±sem	Significance	Tissue Dry Weight (mg) ±sem	Significance
14ºC	1.30 ± 0.08	-	10.70 ± 0.30	-	14.49 ± 1.33	-
4ºC	1.34 ± 0.07	NS	10.99 ± 0.34	NS	15.06 ± 1.25	NS
14ºC High food	1.07 ± 0.17	NS	11.16 ± 1.17	NS	12.66 ± 3.09	NS
14ºC Low food	0.67	NC*	10.20	NC*	6.80	NC*

*NC = not calculated

Table 13.
Growth rates of shell and tissue dry weight during the final four weeks (May-June) of all experimental periods (2002-2005).

Year and Site	Growth in shell length (mm week^{-1})	Growth in tissue dry weight (mg week^{-1})	Temperature
2002 - DHB	1.1	14.8	17º-22ºC
2003- FBF	0.43	2.1	14º-15ºC
2004 - DHB - Sand	0.77	3.0	16º- 20ºC
2004 - DHB - Mud	0.22	1.61	16º- 20ºC
2004 - FBF- Sand	1.1	5.16	15º- 20ºC
2004 – FBF - Mud	1.01	4.2	15º- 20ºC
2005- DHB	0.63	6.8	15º- 21ºC
2005 - FBF	1.0	9.0	15º- 21ºC

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