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The Impact of Environmental Factors on the Physiology of the Atlantic surfclam, *Spisula solidissima*

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Abstract of the Thesis

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The Atlantic surfclam supports a multi-million dollar fishery in New York. Between 2002 and 2008, surfclam abundance in New York State waters along the south shore of Long Island has decreased by 63% in biomass and 75% in the total number of clams. Population age structure has drastically shifted since 2002 indicating the lack of recruitment. Only 3% of the population was made up of clams that were 6 years old and younger in 2006, compared to 21% in 2002. In 2008, 15% of the population was composed of clams less than 6 years in age. It is hypothesized that increased temperatures in recent years has caused stress in these animals, negatively impacting their physiology leading to a reduction in population size.

Studies were conducted on surfclam energy balance, scope for growth and immune function to investigate the impact of temperature on the physiology of these

animals. Results suggest energy reserves are used differently during warm and cooler years, which may impact survival and reproductive success. Further studies on scope for growth indicate an increased metabolic demand at 23°C compared to 19°C. Results also demonstrated a reduction in filtration rate at 23°C compared to 19°C which could cause an energetic imbalance during the critical period following spawning. Furthermore, short term energy usage was greater at 23°C, and data from immune defense studies imply surfclams are immuno-compromised at this temperature. These results strongly suggest that stressful summer temperatures negatively influence surfclam physiology.

Table of Contents

List of Figures

List of Tables

Introduction

The Atlantic Surfclam, *Spisula solidissima*, occurs from the Gulf of Maine to Cape Hatteras, North Carolina (Wigley and Emery 1968, Fay et al. 1983). In New York state waters, the surfclam supports a multimillion dollar fishery. Between 2002 and 2008, surfclam abundance has decreased by 63% in biomass and 75% in the total number of clams in New York State waters off the south shore of Long Island in the Atlantic Ocean (Davidson et al. 2007). Coupled with the population decline, there has been a lack of recruitment (Davidson et al. 2007) and prevalence of abnormal gonadal development in adult clams (Allam, 2007). Between the 2006 and 2008 NYS DEC population surveys, surfclam population biomass dropped 2.7 million bushels. Population ages have drastically shifted since 2002. Only 3% of the population was made up of clams that were 6 years old and younger in 2006, compared to 21% in 2002 (Davidson et al 2007). In 2008, 15% of the population was composed of clams less than 6 years in age. Although this indicates some recruitment has occurred, the abundance of young clams in the population still remains low compared to 2002. Both the decline in population since 2002 and lack of young clams show that no substantial recruitment has occurred over the past few years.

The Atlantic surfclam historically goes through periods of low recruitment interspersed with years of strong recruitment (Chintala and Grassle, 2001). As in other

bivalve species, recruitment success in *Spisula solidissima* is determined by various factors including the timing of spawning, food availability, larval settlement, growth, and predation (Weinberg, 1999). Temperature has been shown to control the timing of spawning (Ropes, 1968, Sephton, 1987), larval settlement (Chintala and Grassle, 2001), growth (Weissberger and Grassle, 2003), and predator abundance (Chintala and Grassle, 2001). Predation can have the most devastating effect on surfclam recruitment by eliminating entire year classes (Mackenzie Jr. et al., 1985). However, predation varies from year and rarely causes mortality of all recruits (Ma et al., 2006).

Other large scale environmental factors such as climate change can also have an impact on bivalve recruitment success. Warmer than average seasonal temperatures have been shown to negatively impact population dynamics through recruitment, mortality and growth in *Macoma balthica* and other bivalve species (Beukema et al., 2009). Mild winter temperatures may enhance predation on bivalve recruits (Frietas et al., 2007). Such an event may occur if spawning is initiated earlier in the year, at a time when food supply is poor (Bos et al., 2006; Beukema et al., 2009), or environmental conditions are not appropriate to support fast growth of new bivalve recruits. It was hypothesized by Weissberger and Grassle (2003) that juvenile clams which grow faster are better off compared to clams that grow at a slower rate, because some of the faster-growing clams are able to outgrow their predators and survive to age one.

The seasonal progression of energy storage and utilization is closely related to environmental conditions and the reproductive cycle (Sastry, 1970; Ojea et al., 2004; Darriba et al., 2005). Typically, energy reserves are stored as glycogen, lipids and

proteins prior to the beginning of gametogenesis (Ojea et al., 2004; Darriba et al., 2005). This usually occurs when food supply is plentiful (Darriba et al., 2005). These reserves are ultimately used for the production of gametes during gametogenesis (Darriba et al., 2005). Seasonal temperature changes have been shown to be important in initiating the gametogenic process (Martinez et al., 2003). Once gametogenesis begins, the use of these energy reserves are reflected by changes in the biochemistry of different tissues in the organism.

Biochemical components of bivalves have been shown to change throughout the reproductive cycle (Giese, 1967). Three of the most important biochemical reserves are glycogen, lipids, and proteins. Glycogen is the main energy reserve in marine bivalves and is essential to the gametogenic cycle (Beninger and Lucas, 1984; Dridi et al., 2007). Glycogen is converted into lipids, proteins and vitellogenin as gametogenesis begins (Blaise et al., 1999; Dridi et al., 2007). Vitellogenin is an important energy reserve for larval clams before they begin feeding on their own (Blaise et al., 1999). In general, maximum glycogen values are reached prior to gametogenesis, and decline there after (Dridi et al., 2007). Lipid and protein values are highest when the animal is ripe and generally decrease after spawning occurs (Dridi et al., 2007). The use of energy reserves during the reproductive period is not the same in all bivalve species and is dependent on environmental conditions and the genetic makeup of the organism. Under stressful conditions, glycogen, lipids and proteins will be used for maintenance needs as necessary (Beninger and Lucas, 1984). Use of these energy reserves for homeostatic needs will result in a decrease of the amount allocated for reproduction.

It has been shown in some bivalve species that recruitment success is partly determined by the energy use during gonadal maturation (Philippart et al., 2003). Increasing temperatures during the pre-vitellogenic period has been shown to elevate the metabolism of the individual, stimulating the use of energy reserves for maintenance needs, otherwise used for vitellogenesis (Martinez et al., 2003). In the study conducted by Martinez et al. (2003), it was also noted that increased temperatures early in the gametogenic process can result in a shortening of the gonad maturation period and a loss of quantity and quality of the gametes. This could potentially lead to a loss of gamete quality in the surfclam during years with an intense warming period early in the gametogenic process, ultimately leading to poor recruitment success.

Typically, temperature is the most important exogenous factor controlling the metabolism of exothermic organisms (Mao et al., 2006). Therefore, global temperature rise may have a strong influence on the metabolic needs of bivalves and their ability to balance energy reserves. The capacity of a bivalve to balance energetic demands is important for growth, reproduction and mortality of the individual (Buxton et al., 1981; Beukema et al., 2009). Organisms have adapted to tolerate temperatures between an upper and lower threshold. Their ability to survive is controlled by the organism's capacity to grow and reproduce within these limits (Newell and Branch, 1980). Years with warmer than average temperatures may increase the metabolic needs and affect the ability of the animal to balance energy reserves (Philippart et al., 2003, Beukema et al., 2009). As global temperatures continue to increase and alter the metabolic needs of these organisms, the capability of the animals to deal with further stress will be compromised. This could lead to mortality, especially during the summer months when temperatures are

most likely to exceed optimal ranges and elevate maintenance needs (Jansen et al., 2007b).

Previous studies have suggested that surfclams are physiologically constrained by temperature inshore, in shallow water (Cerrato and Keith, 1992) and they become stressed when temperatures reach 23°C (Kim and Powell, 2004). In the study conducted by Kim and Powell (2004), surfclams were surveyed off the Delmarva Peninsula. The findings of the study concluded that few clams remained inshore in shallow water, which was further supported by Weinberg (2005). During this survey, surfclams with abnormal gonadal growth and digestive gland atrophies were found. Gonadal abnormalities were classified as clams with follicles filled with degenerating gametes and cellular debris, and occasionally immature eggs were found floating within the lumen (Kim and Powell, 2004). Most of the clams with abnormal gonads had a lower condition index compared to clams with healthy gonads (Kim and Powell, 2004). Kim and Powell hypothesized the abnormal gonads to be caused by increasing water temperature or a poor food source. An alternative hypothesis they gave was that temperature stressed the clams, reducing their ability to feed, escalating their respiratory rate, and increasing the energy needed for tissue maintenance. This may cause clams to break down gonadal tissue as a source of energy to off-set malnutrition.

Warmer water temperatures can reduce the scope for growth in bivalves by increasing the respiratory rate and reducing the filtration rate at temperatures outside of the optimal range (Ali, 1970; Brock and Kofoed, 1987; Kim and Powell, 2004; Han et al., 2008). Filtration rate has been shown to increase with temperature in many different

bivalve species up to a critical temperature, after which it rapidly declines (Ali, 1970; Brock and Kofoed, 1987; Han et al., 2008). It is hypothesized that peak summer water temperatures in coastal waters of New York have exceeded the critical temperature for surfclams in recent years, causing a reduction in filtration rate. At high temperatures, it may become a requirement to pump large amounts of water over the gills. It has been shown that some species will stop feeding during high temperature stress and bypass particle sorting in order to pump a sufficient amount of water over the gills to receive enough oxygen (Ali, 1970). The resulting alteration of energy balance during stressful periods may lead to the formation of gonadal abnormalities in surfclams, causing a decline in reproductive success. It is hypothesized that the current population decline can be attributed to unsuccessful spawning as a result of abnormal gonadal growth and unfavorable environmental conditions.

From 1991-2009 the maximal summer water temperature reached in New York State coastal waters has increased 1.52°C; the lowest maximum temperature was 24.2°C in 1992 and 1996, and the warmest was 27.1°C in 2009 (NOAA Buoy 44025). These temperatures are possibly underestimates of inshore water temperatures, as this buoy is located 33 nautical miles south of Islip, New York. It is also important to point out that these temperatures are all surface water temperatures and that bottom temperatures are undoubtedly lower. However, as surface temperatures increase we can expect the temperature of the bottom water to also increase.

Temperature is an important factor regulating bivalve reproduction, energy metabolism, growth and immunity (Chen et al. 2007). Although temperature has not been

directly implicated as a cause of mortality in the Atlantic surfclam, it may play an important role by negatively affecting the immune status of the clam. Temperature changes have been shown to impact total hemocyte counts and phagocytic activity of hemocytes in many bivalve species, both seasonally and over the short term (Chen et al. 2007). In *Crassostrea gigas*, increased temperatures have been shown to increase hemocyte mortality, and reduce hemocyte locomotion and spreading (Gagnaire et al. 2006). Hemocytes are the main cellular defense in bivalves as they recognize, phagocytoze, and eliminate non-self particles by antimicrobial activities (Delaporte et al. 2006). A decline in hemocyte function caused by temperature stress may leave the surfclam more susceptible to opportunistic pathogens in the environment.

Reproduction can be more important than environmental factors in controlling the immune status of bivalves (Li et al. 2009b). The reproductive process is an energetically demanding process. Mass mortalities in Pacific oysters are often observed when the animals are ripe or just after spawning occurs (Li et al. 2007). Other species have also been shown to be negatively impacted by the gametogenic process. In a study conducted on the flat oyster *Ostrea edulis* it was shown that as gametogenesis proceeded, infection with *Bonamia ostreae* became more intense (Mirella da Silva et al. 2009). Although no direct cause and effect relationship was established, it is thought that gametogenesis is stressful to the oyster leaving them more susceptible to infection (Mirella da Silva et al. 2009). Interestingly, spawning has been shown to reduce phagocytosis, antimicrobial activity, adhesive capacity, and lysosomal stability of hemocytes in the Pacific oyster (Cho et al. 2005, Delaporte et al. 2006, Li et al. 2007). Similar processes may occur in the

Atlantic surfclam resulting in immune suppression after spawning. This may cause the Atlantic surfclam to be more susceptible to opportunistic pathogens at these times.

After spawning occurs, bivalves are suggested to be in a fragile state (Li et al. 2009b). Recovery from spawning depends on a proper food source and that there are no further stressors (Li et al. 2007). The period after bivalves spawn is a time when glycogen reserves are at their lowest levels. As mentioned above, glycogen is the main energy reserve in marine bivalves (Beninger and Lucas, 1984; Dridi et al., 2007). Glycogen levels are considered bio-indicators of environmental status and reflect a bivalve's capacity to sustain further stress (Patrick et al. 2006). Without sufficient energy reserves after spawning the animals will be more vulnerable to temperature stress or food deprivation, as they will not have the necessary resources to cope (Li et al. 2007). Spawning typically occurs at the time of year when water temperatures are warm and continuously warming. It is also known that the growth of many pathogens increases with temperature, making them more abundant at the time many bivalve species spawn (Li et al. 2007). It has been previously shown that pathogenic bacteria can invade the hemolymph of bivalves during stressful periods of the year (Garnier et al. 2007). Mortalities caused by bacteria can occur rapidly or over a period of several weeks (Garnier et al. 2007). The combined impact of temperature stress and spawning on the immune health of the clam could lead to bacterial infection. It is hypothesized that suboptimal summer temperatures cause immuno-suppression in the Atlantic surfclam enhancing the risk of infection by opportunistic pathogens.

Objectives

The objectives of this study were to investigate the potential link between increasing water temperatures and declining surfclam populations. First, historical surfclam population and temperature data were examined to explore trends in population changes and summer temperatures. Clams collected from the field were monitored monthly between March and September in 2008 and April and October in 2009, to assess the health of the current population. Studies were conducted on surfclam energetic balance, gonadal development, filtration rate, scope for growth, and immune function to determine if warmer temperatures are negatively impacting surfclam physiology.

Methods

Examination of historical population and temperature data

This part of the study looked at the percentage of seed clams (<100mm length) in the population and temperature data to examine any trends between the two. Temperature data was taken from NOAA's buoy 44025 from the years 1987-2009. Population data, including biomass and age structure have been collected since 1992 by the New York State Department of Environmental Conservation. The 1992-1996 surveys do not have the same parameters as the surveys from 1999-2008. The results may not be directly comparable. Statistics were only conducted on the surveys between 1999 and 2008. As well, in the 1992-1996 surveys, the dredge was not lined with as fine of a mesh as it was in surveys from 1999-2008. Therefore the seed estimates from 1992-1996 are likely low. The percentage of seed in the population combines various year classes that are below legal size, which gives an idea of potential recruitment to the fishery.

Health surveillance of the current population

Clams were collected March 27th, April 4th, April 30th, July 1st, August 4th, and September 22nd, in 2008. In 2009 clams were sampled on April 9th, May 8th, June 17th, July 14th, September 9th, and October 14th. From this point on these samples are referred to as March, April, May, July, August, September and April, May, June, July, September and October in 2008 and 2009 respectively. Samples were obtained from local fishermen

Bob Doxsee and Jim Reilly in 2008, and Mark Cummings in 2009. In 2008, clams were obtained from waters between Rockaway and Fire Island inlets from 1-3 miles offshore. In 2009, clams were acquired from an area west of Rockaway inlet between 1-3 miles offshore. During each sampling, the length, height and width of thirty clams was measured. They were then processed for histology, biochemistry and condition index (see below). In 2009, immune parameters were also measured. Clams were bled and differential hemocyte counts were made by flow cytometry (see below). Hemolymph was plated on Marine Agar (Becton Dickinson, #212185), TCBS (Becton Dickinson, #265020) and CHROMagar Vibrio (CHROMagar Microbiology, Paris, France) media to determine bacterial counts and diversity during the reproductive season.

The impact of temperature on gonadal development

A laboratory experiment began on March 24th 2008 and lasted until June 26th, 2008. The experiment consisted of two temperature treatments; one corresponding to a warm year and the other to a cooler year. Temperatures in the cool year ranged from 6.5-19°C, while those in the warm year ranged from 6.5-23°C. These temperatures were determined using data taken from NOAA's Buoy 44025 from 1992-2007. The warm temperatures were established by taking the top (warmest) 10% of the temperatures over the 15 year period, and the cool year the bottom (coolest) 10% of these temperatures. Unfortunately there are no records of bottom temperatures in this region, so they had to be estimated. Bottom temperatures were projected by matching the buoy's temperatures with a site in Long Island Sound (25 m depth), in which both surface and bottom temperatures have been recorded from 1992-2007. A positive correlation between surface

temperatures at the two sites allowed us to extrapolate the evolution of bottom water temperatures in Long Island Sound to the ocean.

Four tanks were used in this experiment, each containing 40 clams of 4-8 inches in length. Two tanks rested in a water bath simulating temperatures of a cool year and the others sat in a bath simulating temperatures of a warm year. Clams were collected from the site located at (40° 39.747 N, ¯73° 02.366 W). Approximately ten inches of sand was added to each tank, allowing the clams to burrow. Clams were fed a diet of live *Isochrysis galbana*, DT's Phytoplankton (Premium Reef Blend), and Reed Mariculture Shellfish Diet. The bulk of the diet consisted of Reed Mariculture Shellfish Diet, a highly concentrated shellfish diet (two billion cells/ml) and live *Isochrysis galbana*. DT's phytoplankton was added as a supplement to the main food sources. Live *Isochrysis* cultures were produced in the lab in six, 25 gallon cylinders. Temperature, salinity and *Isochrysis* concentrations were measured daily throughout the experiment. Seawater in experimental tanks was filtered using fluidized bed filters. Every other day the bottom of the tanks was siphoned clean and ten percent of the water was changed.

In order to determine the amount of algae needed to feed the clams, the average dry weight of a twenty clam sample was determined by extracting the meat from the shell and drying it in an oven at 60°C until there is no change in weight. Clams were fed 1.5% of their dry tissue weight per day (Chaparro, 1990; Utting and Millican, 1997). Salinity was measured using a refractometer, and was maintained at 31. *Isochrysis* concentrations were determined by taking a 1 ml sample of the culture, fixing it with 1 ml of 0.5% glutaraldehyde and counting the cells with a hemocytometer.

Due to unforeseen mortalities, clams were sampled at time zero, May 30th, June 2nd, and the experiment ended on June 26th. Temperatures on May 30th and June 2nd were 9.2°C in the cold treatment and 14.2°C in the warm treatment. During the final sampling the temperature was 17.7°C in the warm treatment and no clams remained in the cold treatment. Clams were processed for histology, biochemistry, and condition index (see below).

Once completed, the prevalence of gonadal abnormalities was determined using the formula: (number of positive clams/number analyzed) x 100.

Filtration rate and scope for growth

In October of 2008 surfclams were collected from the field, divided into two batches and acclimated for one week to 19°C or 23°C. Filtration rate, ingestion rate, assimilation efficiency, irrigatory efficiency, oxygen consumption and ammonia excretion were measured in both treatments (see below). A day before the experiment, clams were not fed to ensure that feces produced during the experiment were a result of feeding that day. Clams were individually placed in sealed, 3.5 liter aquariums containing filtered SW and rested in temperature controlled water baths. Water inside the aquaria was stirred using a magnetic stirrer to ensure homogeneity. Before any measurements were taken, clams were allowed to acclimate to the aquaria for one hour. During this time the tanks remained aerated and unsealed. Once measurements were ready to be taken, algae ($3x10^5$ cells/ml) was added and the tanks were sealed (Brock and Kofoed, 1987). DT's Phytoplankton (Premium Reef Blend) was used for the filtration rate study, representing a more diverse food source, as opposed to a monoculture such as *Isochrisis*

galbana. A control chamber was made in which algae was added without any clams. The experiment was conducted using twelve different clams of similar size per treatment.

Immune Defense and Short Term Energy Balance

Approximately 200 clams were collected on 10/14/09 and held in a flow through system at Flax Pond Marine Laboratory for 6 days to ensure no injured clams were used in the experiment. Four tanks were used in this experiment, two tanks rested in a water bath simulating optimal summer temperatures (19°C) and the others sat in a bath simulating stressful summer temperatures (23°C). Twenty five clams were placed in each tank at temperatures similar to the field during this time of the year (18.5°C) and at a salinity of 31 (10/20/09). Temperatures remained constant for four days (10/24/09) and were then increased over a six day period (10/30/09) in the warm treatment tanks to 23°C. Temperature in the cold treatment remained at 19°C for the length of the experiment. Temperature, salinity, and oxygen concentrations were measured daily. On 11/5/09, all clams were bled and measurements were made to determine hemocyte counts, viability, phagocytic activity, and reactive oxygen species production (ROS). Ten clams were taken from each treatment for biochemical analysis and condition index. The remaining animals were injected with a 300 µl solution of sea water containing *Vibrio alginolyticus* at a concentration of $2x10^9$ cells/ml and returned to their respective tanks. Mortalities were noted and clams that died were removed from the tanks as soon as possible and processed for biochemistry. The experiment concluded on 11/11/09 and the remaining clams were processed for biochemistry and condition index. Before processing, the remaining live animals were bled and bacterial counts were made by

spreading 100 µl of hemolymph on Marine agar plates and allowing the plates to incubate in the dark at room temperature for 96 hours.

Histology and Condition Index

Tissues sampled for histology include the visceral mass, gills, and the mantle. Tissue samples were placed in cassettes and fixed in formalin for up to a week before being processed. Tissues were embedded in paraffin wax, sectioned (5 µm thickness), mounted on slides and stained with Hematoxylin and Eosin.

The gametogenic stages were characterized according to a modified version of that used in Kim and Powell, 2004 (Appendix A). Pictures of the gametogenic stages can be viewed in appendix C and D for male and female gonads respectively. The intensity of abnormality conditions were ranked using the scale described in Kim and Powell (2004) (Appendix B) and can be viewed in appendix E. The intensity values were calculated for each month by averaging the values of each abnormal clam in that sample.

Condition index was measured using the formula CI= ((dry meat weight / Wet shell weight) x 100) modified from Lucas and Beninger (1985), which is a measure of the overall metabolic condition of the clam (Lucas and Beninger, 1985; Zarnoch and Schreibman, 2008).

Biochemical Analyses

In order to determine how temperature affects the use of important energy reserves in surfclams, biochemical measurements were made on gonadal tissue in clams collected from the field and experimental tanks in 2008. In 2009 mantle, adductor

muscle, and gill tissues were also processed in addition to the gonad from the field and lab experiments. Biochemical compounds that were measured include glycogen, lipids, vitellogenin and proteins (see below). Field samples were obtained monthly from local fishermen. After collection, samples were processed in the lab later that day and tissue samples were stored at -80°C until analyzed.

Glycogen

Glycogen was measured indirectly through an enzymatic reaction which releases glucose trapped in the glycogen molecules (Murat and Serfaty, 1974). Free glucose is measured through a phenol-sulfuric acid reaction (Dubois et al., 1956). Twenty milligrams of tissue was homogenized in 1 ml of ice cold citrate buffer (0.1 mol/liter, pH 4.2) in a 5 ml tube. To this, one additional milliliter of buffer is added and the homogenate is mixed and split between two 5 ml tubes. One tube is used to measure free glucose in the tissue and the other is used to measure glycogen. One additional milliliter of buffer is added to the tube used for measuring free glucose. To the other tube, 1 ml of buffer and amyloglucosidase (2 mg/ml) is added. This tube is then placed in a water bath for at least 2 hours between 25-30°C and the free glucose was then measured. The free glucose value is subtracted from the trapped glucose to yield the final glucose value. The amount of glycogen trapped in the tissue is proportional to the net glucose value. The volume of buffer and weight of tissue used are such as to obtain a range for total glucose values of 0.2-5 mg/ml of buffer.

Glucose measurements were made by adding 50 µl of 80% phenol (by weight) to each tube containing 2 ml of homogenate. Two milliliters of concentrated sulfuric acid

(95-98%) was added rapidly and directed at the center of the liquid surface to obtain good mixing. Tubes were allowed to stand 10 minutes and were shaken and placed for 10-20 minutes in a 25-30°C water bath. Readings were then taken on a spectrophotometer at a wavelength of 490 nm. Blanks are made by substituting buffer for the sugar solution. A separate blank containing buffer and amyloglucosidase was made for the part of the assay in which trapped glucose was measured. A standard curve is made with known concentrations of glucose.

Lipids

Lipids were measured gravimetrically using a modified version of Folch et al. (1957), obtained from Chester B. Zarnoch (Baruch College, 2008). Fifty milligrams of tissue was placed in a 15 ml tube and was homogenized in 5 ml of 2:1 chloroform-methanol. Homogenates were mixed for 1 hour. After samples were mixed, 1 ml of distilled water was added to each tube and they were centrifuged at 1600 g for 5 minutes to create a biphasic solution. A 2 ml sample of the lower phase (chloroform and lipids) was taken with a glass pipette and placed in an aluminum tray and allowed to evaporate under the fume hood. Trays were then placed in an oven at 110°C for 30 minutes, cooled and weighed. A blank was created by mixing 5 ml of 2:1 (v/v) chloroform-methanol and 1 ml of distilled water. The solution was allowed to sit for one hour, after which the volume of chloroform (lower layer) was measured by reading the volume on the 15 ml tube. This yields the proportion of chloroform in the original system. Total lipids were calculated by subtracting the weight of the tray from the weight of the tray plus lipids and

multiplying by the proportion of chloroform in the original system and dividing that product by the weight of the tissue used in the sample.

Protein

Protein was measured using the Pierce BCA protein assay reagent kit (Pierce, Rockford, IL) according to manufactures recommendations. One hundred milligrams of tissue was homogenized in 500 µl of phosphate buffer (0.1 M, pH 8). Samples were centrifuged at 12,000 g for 20 minutes at 4°C. After centrifugation 10 µl of the supernatant was added to a microcentrifuge tube containing 240 µl of phosphate buffer. A standard curve was made using bovine serum albumin.

Vitellogenin

Vitellogenin was measured by an alkaline-labile phosphorus (ALP) technique modified from Blaise et al. (1999), obtained from Francois Gagné (Environment Canada, 2008). Only gonad tissue was analyzed. Two hundred milligrams of gonad tissue was homogenized in 1 ml of buffer (125 mM NaCl, 25 mM Tris-HCl, 5 mM EDTA and 1 mM dithiothreitol, pH 8) with a Teflon pestle. The buffer was stored at 4°C and kept on ice during use. The homogenate was centrifuged at 12,000 g for 20 minutes at 4°C. An 800 µl volume of the supernatant was then placed in a 2 ml microcentrifuge tube and 430 µl of cold acetone was added. Tubes were then mixed and allowed to stand for 5 minutes, after which the sample was centrifuged at 10,000 g for 5 minutes. The supernatant was discarded and 500 µL of 1 M NaOH was added to the pellet and placed in a heat block at 60^{o}C for 30 minutes. Approximately every 5 to 10 minutes the samples were vortexed to

help dissolve the pellet. From this, 160 µl was withdrawn to measure total PO_4. This was measured by adding 160 µL sample + 1900 µl H_2O + 40 µL 100% Trichloroacetic acid (TCA) + 200 µL of 1% ammonium molybdate in 0.9 M H_2SO_4 + 200 µL of 1% ascorbic acid in a 5 ml tube. For samples and blanks, H_2O was added first followed by TCA and ammonium molybdate, then the sample was added and lastly ascorbic acid. Tubes were mixed for 10 minutes and allowed to stand for 10 additional minutes after mixing. Readings were then taken on a spectrophotometer at 20 and 40 minutes after the ascorbic acid was added. Measurements were first recorded at 815 nm (this indicates the color intensity of the reduced phosphomolybdate blue complex linked to the phosphate groups of Vitellogenin). Afterwards, readings at 444 nm were taken (this corrects for sample turbidity which may cause some interference with the blue color reading taken at 815 nm). Values were corrected using the following equation: Corrected value A815 = [1.023 x mean A815 value] – [0.09 x mean A444 value] (Stanton, 1968). A blank was made by substituting NaOH for the sample. A standard curve was made with known concentrations of phosphate. Data is expressed as µg ALP/ mg protein/ per gram of tissue.

Scope for Growth Analyses

Filtration Rate

At thirty minutes and one hour, 1 ml of seawater was sampled, and 1 ml of 0.5% glutaraldehyde was added to fix the cells. The concentration of algae cells was determined using a flow cytometer (Becton Dickinson FACScalibur). Filtration rate is expressed using the formula : Filtration rate =V/t * ln(Co/Ct), where Co is the

concentration of algae at time 0, Ct is the concentration at time t, t is the time in hours, and V is the volume of seawater in the chamber (Coughlan, 1969; Shumway et al., 1985). Four assumptions were made in order to calculate filtration rate. It was assumed that pumping rate was constant, the reduction in particles was not due to gravity, particle retention was 100% and the suspension remained homogeneous (Coughlan, 1969).

Ingestion Rate

Ingestion rate is expressed as the product of filtration rate and the energy content of the experimental diet (cal/hour) (Han et al., 2008).

Assimilation Efficiency

Assimilation efficiency was measured using the methods of Conover (1966). Glass fiber filters were combusted in a muffle furnace for 4 hours at 450°C and cooled prior to use. Food samples were filtered onto tared glass-fiber filters and washed with a 6% solution of ammonium formate and then with distilled water. Feces were collected from the experimental tanks with a pipette and underwent the same treatment as the food. Samples were then dried at 90˚C for 24 hours and combusted in a muffle furnace for 4 hours at 450°C, allowed to cool and were then weighed. Assimilation efficiency was then calculated using the formula U'= [(F'-E')/ (1-E') (F')] where F' is the ash free dry weight: dry weight ratio (fraction of organic matter) in the ingested food, and E' is the same ratio in a representative sample of feces. This method assumes that only the organic component of the food is significantly affected by digestion.

Oxygen Consumption

Oxygen consumption was measured every thirty minutes using a YSI 85 (YSI Incorporated, Yellow Springs, OH) and is expressed as (mg O_2 /g dw/ hr) (Brock and Kofoed, 1987). Oxygen consumption was transformed into energy using the conversion 1 mg O_2= 3.38cal (Elliott and Davison, 1975).

Ammonia Excretion

Ammonia concentrations (ug NH_4/g dw/hr) were measured at time zero and at the end of the experiment (60 minutes) using the methods of Solorzano (1969). Fifty milliliters of the experimental tank was pippetted out at time zero and after one hour. To this, 2 ml of a phenol solution, 2 ml of a 0.5% nitroprusside solution and 5 ml of an oxidizing solution were added, mixing after each addition. The phenol solution consisted of 10 grams of phenol in 100 ml of 95% ethyl alcohol. The oxidizing solution was made by adding 100 ml of an alkaline solution (100 grams of sodium citrate and 5 grams of NaOH in 500 ml of distilled water) to 25 ml of bleach on the day it was used. The color was allowed to develop at room temperature for one hour and was measured at 640 nm in a spectrophotometer. Blanks were made using distilled water. A standard solution was made by dissolving 0.956 grams of NH_4Cl in 1 L of water to give a stock solution containing 250 µg NH_3-N per ml. A standard curve was made from this by dilution. Ammonia excretion is converted into energy using the conversion 1mg NH_4=5.94cal (Elliott and Davison, 1975; Han et al., 2008).

Irrigatory Efficiency

Irrigatory efficiency is expressed as the volume of water cleared per unit oxygen uptake (ml H_2O/mg O_2/hr) modified from Brock and Kofoed (1987). Low filtration rates and high respiratory rates cause irrigatory efficiency to be low (Brock and Kofoed, 1987). Irrigatory efficiency is understood to be inversely related to the minimal maintenance food concentration; increasing the ratio means the food concentration needed for zero growth will decrease (Brock and Kofoed, 1987).

Scope for Growth

Scope for growth provides an estimate of energy status and is a useful approximation of how environmental stress affects the performance of the clam (Han et al., 2008). Scope for growth was measured using the formula: P= C-F-R-U, where P is the scope for growth, C is the energy of the food consumed, F is the amount of energy lost in the feces, R is the heat produced by oxygen consumption and U is the excretion product (ammonia). The caloric value of the feces and food were determined using the formula, cal/mg dw = (-.555 + .113 (%C) + .054 (C:N)) (Platt and Irwin, 1973). Carbon and nitrogen values were determined by processing the samples in a Carbon/Nitrogen Analyzer (CE Instruments, Flash EA 1112 Series).

Immunological Analyses

Bacteriology

Prior to bleeding, surfclams were washed under tap water to remove sediment and foreign materials. The area in which the needle was inserted was washed with 100% ethanol. Clams were bled by puncturing the membrane next to the umbo with a 21g1

needle attached to a 1 ml syringe. Hemolymph was transferred to a sterile micro-centrifuge tube and aliquots (100 µl) were spread on three different bacterial medias; Marine Agar (non-selective), CHROMagar Vibrio (for vibrio detection), and lastly thiosulfate citrate bile salts sucrose media (TCBS, also for vibrio detection). Plates were incubated in the dark at room temperature for 96 hours. Bacterial counts were made after 48 and 96 hours and the total number of colonies were estimated and the color was recorded. After 96 hours, pictures were taken of each plate containing bacteria.

Differential Hemocyte Counts

Differential hemocyte counts were made on both field and experimental samples. Hemolymph of field samples were preserved by taking 100 µl of hemolymph and adding 100 µl of 0.5% glutaraldehyde and 200 µl of filtered sea water. The samples were stored at 4°C. Prior to analysis by flow cytometry, 10 µl of SYBR Green x100 was added to each tube before incubation in the dark for 30 minutes. SYBR green dye was added to stain the DNA of the hemocytes in order to differentiate actual cells from debris. The same procedure was conducted on experimental samples, except 100 µl of hemolymph was added to 300 µl of filtered sea water. No glutaraldehyde was added because the samples were analyzed soon after they were taken from the animals. Granulocytes are characterized by high FSC and high SSC, agranulocytes have low FSC and SSC (Allam et al., 2002, Delaporte et al. 2006). Total hemocyte counts are expressed as the number of cells per milliliter.

Phagocytosis

Phagocytic activity of the hemocytes was measured as described by Delaporte et al. (2006). Briefly, 100 µl of hemolymph was added to 300 µl of filtered sterile sea water (FSSW) and10 µl of green fluorescent 2.2 µm beads (330,000 beads per/µl) in a 2 ml microcentrifuge tube. The samples were incubated in the dark at room temperature for 60 minutes and gently mixed, before being analyzed by flow cytometry. The phagocytic activity was estimated as the percentage of hemocytes that had engulfed three beads or more.

Reactive Oxygen Species (ROS)

Reactive oxygen species activity was measured following the protocol described in Moss and Allam (2006). Briefly, 200 µl of hemolymph was mixed with 200 µl of FSSW in a 1.5 ml microcentrifuge tube. One hundred micro liters of this mixture was plated in triplicate in a black 96 well plate. Twenty µl of Dichlorofluorescein-diacetate (10 mM) was added to each well. Readings were then taken on a plate reader at 485 nm excitation and 535 nm emission to determine native ROS production. Ten microliters of zymosan was added in two of the wells and plates were incubated in the dark at room temperature. Readings were taken five and thirty minutes after zymosan was added. Zymosan was prepared at a concentration of 20 mg/ml in seawater. It was boiled for 30 min at 100°C, washed twice and resuspend in seawater and held on ice prior to use.

Viability

Viability of the hemocytes was measured by adding 100 µl of hemolymph and 300 µl of filtered sterile sea water in a 5 ml flow cytometry tube. To this, 10 µl of Calcein

AM (10x final concentration) dye was added to label live cells and 10 µl of Ethidium homodimer (10x final concentration) dye was added to detect dead cells. The sample was incubated in the dark at room temperature for 30 minutes and measured by flow cytometry. Live and dead cells were counted based on their green (FL1) and red (FL3) fluorescence respectively.

Statistical Methods

Data were analyzed using SigmaStat (Version 3.10, Copyright 2004 Systat Software, Inc.). One-way analysis of variance (ANOVA), two-way ANOVA, student t-test's, Holm-Sidak tests, and Tukey tests were used as appropriate to determine the significance of the results from the different data sets. Results were considered significant at a p-value < 0.05. Results of each statistical test can be viewed in appendix K.

Results

Examination of historical population and temperature data

The percent seed clams (<100 mm length) was highest in the 1996 survey accounting for 45.1% of the population. The lowest percentage of seed occurred in 2006, making up just 1.6% of the population. Between 1999 and 2005 the percent seed significantly declined from 40.32% to 1.87% respectively ($p<0.001$) (Figure 1). Since 2005 seed levels have remained low compared to historical numbers. Since 1987 the average summer temperatures in New York state waters have increased approximately 0.0385°C per year. During the last decade, summer temperatures have increased at a rate of 0.1578°C per year (Figure 2).

Health surveillance of the current population

Histology/Condition Index

In 2008, 166 total clams were processed for histological analysis. Of the 166, 62 were male and 104 were female. The average length of the clams in 2008 was 121.64 ±0.94 mm. Histological data is summarized in table 1 and displayed graphically in figure 3 for 2008. Spawning was first noted in the July sample. Despite progressive ripening of the gonads over the season, a small percentage of clams in July (20%), August (23%) and September (10%) remained in developing stages, never forming mature gametes.

Approximately 31% of all clams were infected with nematode or cestode parasites in 2008; pictures of infection can be seen in appendix E. Abnormal gonad conditions were noted in every sample except for the last. The greatest percentage of abnormal clams occurred in late March corresponding to 46% of all clams in the sample and 63% of the females. Abnormal conditions were most intense in July (2.17 ±1.17).

In 2009, 179 clams were processed for histology. Of the 179, 65 were male and 114 were female. The average length of the animals in 2009 was 123.00 ±0.98 mm. Histological data is summarized in table 2, and displayed graphically in figure 4 for 2009. The first signs of spawning were detected in June. In July, 38% of the clams remained in developing stages. In October, 63% of the clams were in early developing stages following the summer spawn. About 15% of all clams in 2009 were infected with nematode or cestode parasites. Two clams in 2009 displayed signs of hemic neoplasia, which are thought to be the first ever documented cases in *Spisula solidissima* (Appendix E). The highest percentage of abnormal clams was noted during May, equaling 27% of the total sample and 28% of the females. The intensity of abnormal conditions was highest in both June and July (2.00 ±1, 1.1).

Condition index dropped significantly in 2008 from May through July ($p<0.001$) and continued to decline into September (Figure 5A). In 2009 condition index dropped significantly between April and May ($p<0.001$), May through June ($p<0.001$), and significantly increased from September into October (Figure 5B). Between years, condition index was significantly higher in 2008 in May ($p=0.001$) and July ($p<0.001$)

(Figure 5C). No significant differences in condition were observed between normal and abnormal clams in both 2008 and 2009 (Appendix C).

Gonad Biochemistry (2008)

Monthly Variability

In 2008 only gonad tissue was processed for biochemical analysis. The biochemical analysis is summarized in Table 3. Glycogen values increased significantly from late March to July ($p<0.001$) (Figure 6A). After July, glycogen remained stable into September. Between March and September, significant declines in carbohydrates were observed ($p<0.001$) (Figure 6B). Gonad lipids increased significantly from late March through May ($p<0.001$). Following the increase, a subsequent decline in lipid content was seen between May and July ($p<0.001$). Lipids dropped again from August to September ($p<0.001$) (Figure 6C). Proteins dropped dramatically between March and May ($p<0.001$) (Figure 6D). No significant differences were found in vitellogenin values between months (Figure 6E).

Males/Females

Differences were noted between male and female gonads over the 6 month period (Figure 7 A-E). In males, the data indicates a trend of increasing glycogen concentrations over time. Within females, glycogen greatly increased from March through July ($p=0.001$). Between sexes, glycogen was significantly higher in July ($p=0.024$). Total carbohydrates in males remained stable over time. In females, total carbohydrates significantly declined from March through September ($p<0.001$). In both sexes, lipids

significantly increased between March and May ($p<0.001$). Within females, lipids declined significantly between May and July ($p<0.001$), and August and September ($p<0.001$). Lipids were significantly higher in females from March through May. In males, protein levels decreased from March into May ($p<0.001$), after which they increased from July through September ($p=0.004$). A decline in protein values was observed between April and May ($p=0.001$) and May to July ($p<0.001$) in females. Proteins in female gonads were statistically greater during April ($p=0.022$) and May ($p<0.001$). A significant increase of vitellogenin occurred in males between March and May ($p=0.002$). Vitellogenin values declined in males from May to September ($p<0.001$). Considerable differences between male and females were observed in May ($p<0.001$) and July ($p=0.023$).

Normal/Abnormal

Abnormal clams displayed differences in biochemistry over the course of the spawning season when compared to normal clams that did not show abnormal gonadal growth (Figure 8A-E). Glycogen increased from March into August in clams that did not display abnormal conditions ($p=0.002$). Comparatively, in abnormal individuals glycogen remained stable over time. Carbohydrates did not change in either normal or abnormal individuals during the sampling period. Lipids in normal and abnormal clams increased from March through May ($p \leq 0.006$) and subsequently declined between May and July ($p<0.001$, $p=0.003$). Lipids in abnormal individuals were significantly higher in both March and April ($p=0.006$, $p=0.002$). Protein levels declined in normal and abnormal clams from April through July ($p<0.001$). Protein levels were significantly higher in

abnormal clams during July (p=0.030). Although no significant differences were observed in vitellogenin levels, the trends in the data suggest that this metabolite remained lower in abnormal clams throughout the sampling season when compared to normal clams.

Adductor Muscle Biochemistry (2009)

Monthly Variability

In 2009, biochemical components of the adductor muscle, gill, gonad and mantle tissues were measured, and overall values are displayed in table 4, 5, 6 and 7 respectively. Adductor muscle glycogen increased and decreased from month to month (Figure 9A). Glycogen declined between May-June and July-September ($p<0.001$) and increased from June to July and September to October ($p<0.001$). Adductor muscle total carbohydrates increased significantly from April to May ($p<0.001$), declined substantially from June through July ($p<0.001$) and remained low into October (Figure 9B). Lipids in the adductor muscle significantly increased from April to May ($p=<0.001$). Declines in lipids were statistically significant between July and September ($p=0.001$) (Figure 9C). Protein in the adductor muscle dropped between April and May ($p<0.001$) and remained stable through July when it increased to levels as high as April ($p<0.001$) (Figure 9D).

Male/Female

Biochemistry of the adductor muscle followed the same trends in both male and female gonads over the course of the season (Appendix D). Significant differences in protein between sexes were observed in September ($p=0.014$).

Normal/Abnormal

Trends between normal and abnormal clams were the same for all metabolites in the adductor muscle, except for glycogen (Appendix E). In normal clams, glycogen sharply dropped between May and June ($p<0.001$) and increased into July ($p<0.001$). Glycogen in abnormal individuals remained low until June after which it increased into July ($p=0.004$).

Gill Biochemistry (2009)

Monthly Variability

Glycogen in the gill remained stable between April and September and significantly increased from September to October ($p<0.001$) (Figure 10A). Gill carbohydrates declined from April through September ($p<0.001$), and increased from September to October ($p=0.006$) (Figure 10B). Lipids in the gill remained fairly constant from April to July and increased between July and October ($p<0.001$) (Figure 10C). Protein values in the gill remained constant over the course of the year except for a sharp decline between May and June ($p=0.001$). Protein levels rebounded the following month ($p=0.002$) (Figure 10D).

Male/Female

No significant differences were observed between male and female metabolites over the season (Appendix F).

Normal/Abnormal

Patterns of energy use in the gill differed between normal and abnormal individuals (Appendix G). Glycogen in normal clams declined into May ($p=0.003$) and remained stable thereafter. In abnormal clams, no significant changes were observed over the course of the year. Glycogen was significantly higher in normal clams in April ($p=0.025$). No significant differences were observed in gill carbohydrates, lipids or protein. However, protein levels in abnormal individuals remained higher than normal clams during each sampling date (Appendix G).

Gonad Biochemistry (2009)

Monthly Variability

Gonad glycogen significantly increased between June and October ($p<0.001$) (Figure 11A). Carbohydrates in the gonad decreased from April through September, significantly declining between April-July ($p<0.001$) and July-September ($p<0.001$) (Figure 11B). Similar to carbohydrates, lipids in the gonad continuously decreased from April to September; significant declines were seen between April and June ($p=<0.001$). Lipids recovered to levels similar to May between September and October ($p<0.001$) (Figure 11C). Protein in the gonad significantly decreased from April through May ($p=<0.001$) and remained stable into September (Figure 11D). Protein values re-established themselves between September and October ($p<0.001$) to levels comparable to April. Although differences in vitellogenin levels were not significant, the data implies that this metabolite increased in May and declined into October (Figure 11E).

Males/Females

Upon comparison of males to females, no statistically significant differences are found in gonad glycogen (Figure 12A). In males, carbohydrates significantly declined between May and July ($p=0.002$). Carbohydrates in females declined between April-May ($p=0.001$), May-July ($p=0.009$), and July-September ($p<0.001$) (Figure 12B). Between September and October, carbohydrates increased in females ($p=0.004$). Total carbohydrates were significantly higher in males during May ($p=0.008$) and September ($p=0.005$). In females, lipids dropped from May to June ($p<0.001$) and again between June and September ($p<0.001$). In October, lipid levels increased to values equivalent of June ($p<0.001$). Lipids were significantly higher in females compared to males during April and May ($p=0.001$, $p<0.001$) (Figure 12C). Protein began higher in females ($p=0.016$) and declined between April and May ($p=0.001$) (Figure 12D). Between September and October, proteins significantly increased in females ($p<0.001$). Proteins remained stable in males over the course of the year and increased between September and October ($p<0.001$). Within males, vitellogenin significantly decreased between June and October ($p=0.003$). Vitellogenin was significantly higher in males than females in June ($p=0.031$). Vitellogenin was stable in females between May and October (Figure 12E).

Normal/Abnormal

Glycogen in the gonad of normal clams remained stable through July (Figure 13A). In abnormal clams, glycogen levels were low until they increased between June and July ($p<0.001$), finishing significantly higher than normal clams ($p=0.001$). Carbohydrates significantly declined in normal clams between April and July ($p<0.001$),

and no significant changes were observed in abnormal clams (Figure 13B). The difference in carbohydrate levels between the two groups was statistically significant in June ($p=0.022$). Lipids followed similar trends in normal and abnormal clams, as both significantly declined between April and July ($p\leq0.006$) (Figure 13C). In normal individuals, proteins declined between April and May ($p=0.001$). Proteins were higher in abnormal clams ($p=0.043$) in April and did not change thereafter (Figure 13D). Although no significant differences were found in vitellogenin values between the two groups, differences are still observed graphically (Figure 13E). Vitellogenin in normal clams remained higher than abnormal clams, except for July.

Mantle Biochemistry (2009)

Monthly Variability

Mantle glycogen declined between April and June ($p=0.004$) and leveled off thereafter (Figure 14A). Carbohydrates increased from April to May ($p<0.001$) and significantly dropped between June-July ($p<0.001$) and July-October ($p=0.004$) (Figure 14B). Lipid values remained constant until June when they increased in July ($p=0.002$) and again in September ($p<0.001$) (Figure 14C). Mantle protein remained fairly stable between April and July. After July, an increase was seen in September ($p<0.001$), followed by a drop in October ($p<0.001$) (Figure 14D).

Male/Female

No differences were found between sexes in any metabolite in the mantle (Appendix H).

Normal/Abnormal

Differences in metabolite use in the mantle were discovered upon investigation of normal and abnormal clams (Appendix I). In abnormal individuals, glycogen significantly decreased from April to July ($p=0.001$). Significant differences were found between groups in April ($p=0.039$) and May ($p=0.020$). No significant differences in any other energy reserve were found in mantle tissues between groups. However, in normal calms, carbohydrates increased between April and May ($p=0.001$) and decreased between June and July ($p<0.001$). As well, lipids in normal clams increased between June and July ($p=0.008$).

Gonad Biochemistry 2008 vs. 2009

Metabolite levels in the gonad showed striking differences between 2008 and 2009. During all sampling dates, significant differences were found between one or more of the biochemicals between years (Table 8, Figure 15A-E). In all comparisons, levels are significantly higher in 2008 compared to 2009 for glycogen, carbohydrates, lipids (except for the September sample) and protein. Vitellogenin was the only metabolite that consistently remained higher in the 2009 samples over the entire season.

Immune Parameters

Total hemocyte counts declined from April to June after which they increased in July ($p=0.003$) and declined thereafter (Table 9, Figure 16A). The percent granulocytes in the hemocyte population increased from April to May ($p<0.001$), May to June ($p<0.001$),

and July to September ($p<0.001$) (Figure 16B). Percent granulocytes declined from June to July ($p=0.006$) and September to October ($p<0.001$).

Bacteriology:

The largest number of clams containing bacteria counts greater than 401 per 100 µl of hemolymph occurred in September (Figure 17A). Vibrio species were also the highest during September (Figure 17B, C). The lowest bacterial counts were observed in April.

The impact of temperature on gonadal development:

Histology/Condition Index

The average length of the clams in the cold treatment was 127.06 ±2.71 mm, and 122.28 ±2.32 mm in the warm treatment. As the experiment progressed, surfclam gonads ripened. During the one sampling date (5/30/08) in which both clams from the cold and warm treatments were sampled, a greater percentage of clams from the warm treatment (64.29%) were in secondary developmental stages and ripe stages compared to the cold treatment (58.33%) (Figure 18). Fifty seven percent of female clams in the cold treatment were considered to be abnormal in comparison to 86% in the warm treatment (Appendix J). Furthermore, the intensity of hemocyte infiltration in the gonad was more intense in the warm treatment (2.33 ±0.52) than the cold (1.80 ±0.84). Condition index was not significantly different between treatments (Figure 19).

Biochemistry

Clams sampled on 5/30/08 from the cold and warm treatments were processed to determine the biochemical composition of their gonad tissues. It is interesting to note that glycogen, lipids and vitellogenin were all higher from clams in the cold treatment, based on the trends in the graphs (Figure 20A,C, E). However, lipids were the only metabolite that was significantly higher in the cold treatment ($p=0.044$). In contrast, total carbohydrates and proteins were higher in clams from the warm treatment as observed in figure 20B and 20D. Due to unexplained mortalities no further samples from the cold treatment were obtained.

When comparing males and females between treatments, significant differences are found between lipids in males ($p=0.006$) (Figure 21C).

Lipids from normal clams in the cold treatment were significantly higher ($p=0.011$) than normal individuals in the warm treatment (Figure 22C). As well, lipids were significantly higher in abnormal clams in the warm treatment compared to normal clams in the warm treatment ($p=0.030$). Protein levels in gonad tissue from clams in the warm treatment were significantly higher among abnormal clams as compared to their normal counterparts ($p=0.001$) (Figure 22D).

Filtration rate and scope for growth

The average length of the clams in the cold treatment was 128.53 ±3.65 mm and 130.02 ±3.37 mm in the warm treatment. The 8th clam in the 19°C treatment was excluded from statistical analyses because it remained closed during the experiment. All physiological measurements conducted during this experiment showed that clams

exposed to 23°C were either stressed or had an increased metabolic demand compared to animals in the 19°C treatment (Figure 23A-H). Average ammonia excretion values were .15 μg NH_4-N/g dw/h in clams from the cold treatment and .25 μg NH_4-N/g dw/h from the warm treatment. Assimilation efficiency was significantly higher at 19°C ($p=0.015$). An approximately 50% reduction in filtration rate was observed at 23°C in contrast to 19°C ($p=0.034$). The amount of water cleared per unit of oxygen uptake, also known as irrigatory efficiency was significantly higher ($p=0.014$) in clams from the colder treatment. The amount of oxygen consumed per gram dry weight was higher at 23°C however, it was not statistically significant. Condition index of clams at 23°C was lower than those held at 19° ($p=0.029$). Ingestion rate was higher in clams from the 19°C treatment, although it was not significantly different. Lastly, scope for growth was significantly higher at 19°C compared to 23°C ($p=0.042$).

Immune Defense and Short Term Energy Balance

Biochemistry/Condition Index

The average length of the clams in the cold treatment was 121.40 ±2.28 mm and 117.46 ±2.41 mm in the warm treatment. Most metabolites measured during this experiment were lower in clams from the warm treatment compared to the cold treatment. One week after acclimation to their respective temperatures, patterns in the data suggest that glycogen and total carbohydrates in the adductor muscle were higher from clams held at 19°C (Figure 24A, B). Proteins in the adductor muscle were significantly higher in clams from the cold treatment ($p=0.047$) (Figure 24D) and there was no difference between lipids in the adductor muscle (Figure 24C). In the gill, lipids ($p=0.003$) were

greater in clams sampled from the cold tanks (Figure 25C). Although not statistically significant, glycogen and proteins in the gill were also higher in the cold treatment (Figure 25A, D). In contrast, total carbohydrates were higher in the gills from clams held at 23°C based on the trends in the graph (Figure 25B). Gonad glycogen and protein were both significantly higher in the cold treatment ($p=0.014$, $p=0.044$) (Figure 26A, D).On the other hand, there were no significant differences between total carbohydrates, lipids and vitellogenin (Figure 26B, C, E). In the mantle tissue, glycogen and lipids were significantly higher in animals from the 19°C treatment ($p=0.025$, $p=0.001$) (Figure 27A, C). Total carbohydrates and protein were also higher in the mantle tissue from the cold treatment although differences were not statistically different (Figure 27B, D).

Immune Parameters

Total hemocyte counts were higher in clams sampled from the 23°C tanks ($p <0.001$) (Figure 28A). There was no significant difference in the percentage of dead hemocytes between treatments. In contrast, the percent granulocytes in the 19°C tanks was significantly higher ($p<0.001$), than those in the warm tanks (Figure 28B).

Reactive oxygen species (ROS) values were significantly higher from clams in the cold treatment prior to stimulation ($p=0.037$). Thirty minutes post stimulation there was no difference between treatments (Figure 29).

There was no difference in the proportion of phagocytic hemocytes between treatments (Figure 30A). The number of beads engulfed by individual hemocytes (intensity) was higher in the cold treatment, yet not statistically significant (Figure 30B).

The phagocytic index (phagocytic hemocytes x fluorescence intensity) was higher in the cold treatment as compared to the warm treatment ($p=0.048$) (Figure 30C).

Following bacterial challenge, 81% of the population in the warm treatment perished. Seventy two percent of the clams in the warm treatment died within the first two days post injection (Figure 31). Thirty five percent of the population died in the cold treatment following injection. No clams died within the first two days following injection in the cold treatment.

Bacterial counts from clams remaining at the end of the experiment were different between treatments. The two remaining clams from the warm treatment contained bacteria counts greater than 350 per 100 µl of hemolymph. Only three (23%) of the clams from the cold treatment had bacteria numbers within this range. One type of bacteria was found in the two clams remaining in the warm treatment and a few of the clams in the cold treatment, including one with high bacterial counts. A variety of bacterial species were present in the remaining clams from the cold treatment.

Discussion

Examination of historical population and temperature data

The Atlantic surfclam population in New York state waters is at the lowest level since 2002 and the population consists mainly of older individuals indicating that little to no recruitment has occurred in recent years (Davidson et al., 2007). The percent seed in the surfclam population has drastically declined since 1999 meaning that few clams will be recruiting to the fishery in the coming years compared to historical levels. Between 1987 and 2009 the average summer temperature has increased about 0.0385°C per year. Within the last decade, summer temperatures have increased at a rate of 0.1578°C per year, suggesting that the current lack of seed may be partly due to the rise in summer temperatures during this time. During the summer, temperatures could exceed the optimal range for the Atlantic surfclam, which may cause stress in larvae, juveniles and adults. This may impact survival and recruitment success.

Surfclams, as other bivalves undergo years of poor recruitment and periods of exceptional recruitment success (Chintala and Grassle, 2001). A suite of factors could potentially impact recruitment in the surfclam. Previous studies on the surfclam and other bivalve species have shown temperature to play an important role in population dynamics (Chintala and Grassle, 2001; Beukema et al., 2009). Temperature can influence recruitment in a variety of ways including, growth of larvae, mortality, and predator

abundance. In the study by Chinatala and Grassle (2001), cool summers were associated with high larval settlement and a greater probability successful recruitment would occur. However, the study conducted by Chintala and Grassle (2001) took place in waters off New Jersey. Cooler water temperatures in the summer off New Jersey are associated with upwelling events that are typically followed by downwelling, bringing larvae from the surface to the benthos. This does not occur in New York waters due to the geographic position of Long Island and prevailing currents. Nevertheless, it is possible that cool temperatures have a similar impact in which more larvae are able to successfully settle out of the water column, increasing the chance that successful recruitment will occur. A study conducted by Snelgrove et al. (1998) found no significant differences in surfclam larval settling between 19.2-24.3°C. In recent years however, surface water temperatures have exceed 24°C for periods of time (Figure 32A).

An alternative explanation for the decline in seed clams may be partially attributed to changes in predator abundance. Many studies on surfclam recruitment dynamics have shown predation to be an important factor in recruitment success; even eliminating entire year classes (Mackenzie Jr. et al., 1985). It is generally accepted that years in which temperatures are above average result in higher predation, due to increased abundance and activity of predators (Freitas et al., 2007, Beukema et al., 2009). One of the largest sets of surfclams in New Jersey that was ever documented occurred following a hypoxic event (Garlo, 1982). Hypoxic conditions caused predators to perish or move out of the area. For the most part, surfclams were able to survive the low oxygen conditions and recruitment success was extremely high, which was thought to be a direct

result of reduced predation (Garlo, 1982). In other studies, it has been shown that juvenile abundance is significantly impacted by predation in the short term (Franz, 1976, 1977).

In general, smaller bivalves have a higher physiological demand compared to larger ones, due to faster growth rates (Bougrier et al., 1995). In oysters, fast growth during the first years of life combined with gonad development and spawning may stress young oysters making them more susceptible to mortality over the summer (Cotter et al., 2010). Above average summer temperatures may cause an energetic imbalance in juvenile surfclams, especially when they begin gametogenesis within the first and second years of life (Chintala and Grassle, 1995). This may cause stress in young surfclams, impeding their ability to survive.

On the other hand, warm water temperatures may stress adult clams, causing an energetic imbalance in which animals resorb gonad material as a source of energy (Kim and Powell, 2004). This could potentially impact the overall number or quality of the gametes spawned. Likewise, the impact of stress on the adult clam could alter the ability of the larvae to survive. Temperatures of 22°C are optimal for surfclam larval development (Loosanoff and Davis, 1963), while those above 24°C negatively impact fertilization (Clotteau and Dube, 1993). Temperatures in surface waters have exceeded 24°C for periods of time during the summer in recent years (Figure 32A). It is not known however how this has impacted development of the larvae or fertilization success. The combined impact of stress on the adult and impact this has on larvae could be detrimental to recruitment success. Also, continued stress throughout the summer may weaken any secondary spawning event that would take place in the fall. It is also plausible that stress over the summer could impact the capacity of the animal to build up energy stores used

for over-wintering and gametogenesis the following year. This is partly supported by biochemistry data from 2008 and 2009 (see below).

Lastly, as temperatures continue to warm, the type and amount of phytoplankton available to all life stages of the surfclam may be altered. Recent studies have shown global reductions in phytoplankton productivity and concentrations over the last decade (Behrenfeld et al., 2006) and century (Boyce et al., 2010) as temperatures have continuously warmed. As well, increased sea surface temperatures have been shown to strengthen stratification in the upper ocean, limiting nutrient inputs from deeper waters which can alter the phytoplankton composition (Goffart et al., 2002, Behrenfeld et al., 2006). Additionally, warmer temperatures have been shown to influence the timing and strength of the spring phytoplankton bloom in temperate waters (Lassen et al., 2010). A poor food source during the spring may significantly impact growth and reproductive processes in adult clams. As well, a poor food source during the summer may impact larval growth and survival and possibly the success of recruitment. Thus, potential changes in phytoplankton biomass and composition may partly account for the poor recruitment and decline in small surfclams over the last decade.

Health Surveillance of the current population

Histology/Condition Index

Surfclams generally follow a biannual cycle of spawning in New York, with a major mid-year spawn between June-August, and a minor spawn in the early fall (Ropes, 1968). In colder years, only one spawning event may be observed and it may be delayed (Ropes, 1968). A previous study on the reproductive stages of the surfclam by Jones et al. (1981) showed that animals were ripe by June and spawning took place between June and

July. In the same study, animals began to grow new gametes between September and November (Jones et al., 1981). In the current study, animals were sampled in 2008 and 2009 which were warm and cooler years respectively (Figure 32A, Table 10). Although both years were above average compared to the the twenty year mean, 2008 was warmer than 2009. This appears to have had implications on the reproductive cycle each year.

In 2008, spawning was spread out between July and September. Clams that partially spawned were first seen in July, accounting for 53% of the clams in the sample. The highest percentage (60%) of completely spent individuals was observed in September.

In 2009, clams began to spawn in June and a major spawn likely occurred between July and September. Many clams in October were observed with developing gonads. This may be a result of lower temperatures at this point of the year in 2009 compared to 2008.

Condition index also confirmed the histological results. In 2008, condition continuously dropped from May through September, indicating spawning was taking place (Figure 5A). In 2009 condition index decreased into September and increased in October, typical of energy replenishment following spawning (Figure 5B).

Between mid-March through April, waters begin to warm. In 2008, temperatures were higher in late March and increased at a faster rate through April compared to 2009 (Figure 32B). The beginning of gametogenesis is set by a temperature threshold, while the rate at which it proceeds depends on food and temperature (Delaporte et al., 2006).

The rate at which the water warmed in 2008 may have increased metabolic rates at a time when filtration rates or food sources were low, causing an imbalance in energy use and gains. This may have caused the animals to breakdown gonadal tissue as a source of energy as indicated by the high percentage (46%) of abnormal individuals in March of 2008. It was first hypothesized by Kim and Powell in 2004 that these abnormal conditions were a result of high temperature stress in the summer months. Based on the current results it appears that these conditions may be triggered by the rate temperature increases during late March into April. Abnormal conditions continue and become more intense as the temperature increases. This is likely due to increased metabolic needs of these individuals as the water continues to warm.

Gonad Biochemistry (2008)

Monthly Variability

In 2008, only gonad tissue was measured for biochemical analyses. The use of energy reserves varies among and within species (Ojea et al., 2004). Bivalves that use energy reserves to fuel reproduction follow a conservative reproductive pattern, as opposed to using food directly as a means to support gametogenesis (Darriba et al., 2005). It appears that the Atlantic surfclam follows a conservative reproductive pattern. Glycogen in the gonad was low between March and May implying that it may have been used to form lipids and proteins in the gonad or for shell and somatic growth. Various studies have shown glycogen to be the primary source of energy that is used in the reproductive process in many bivalves (Riley, 1976; Li et al., 2000; Li et al., 2006; Patrick et al., 2006). The lowest glycogen levels during spring corresponded to the

highest lipid values in the gonad. In the Pacific oyster, *Crassostrea gigas*, lipids and glycogen were shown to be inversely related. The maximal lipid values correspond to peak ripeness prior to the beginning of spawning (Dridi et al., 2007) (Figure, 6C). Carbohydrates other than glycogen were not used until after spawning took place suggesting that these resources were used for growth or to maintain homeostasis following spawning. Protein values have also been shown to be high when the animal is ripe (Li et al., 2000; Li et al, 2006). In 2008, protein declined during May at a time in which the highest percentage of ripe individuals was found. Either protein in the gonad was used to power the end of gametogenesis, or it was used for other metabolic needs at a time when glycogen values were low. Alternatively, protein concentrations may fluctuate as the result of dynamic changes in other compounds.

Due to the fact that vitellogenin is normalized to protein, the two were inversely related. Vitellogenin levels remained low over the course of the season, indicating that vitellogenesis was interrupted in some way. Sharp increases in temperature prior to vitellogenesis have been shown to stimulate the use of energy reserves otherwise used for this process, which may reduce the quality and quantity of gametes and shorten the maturation period (Martinez et al., 2003). The rate at which temperatures increased in 2008 in late winter may have caused a similar situation to occur as explained by Martinez et al (2003).

Males/Females

When comparing males to females, there are notable differences in the use and storage of metabolites in the gonad. Glycogen was low in both sexes early on, yet it

tended to be higher in females than males. This may be a result of its incorporation into the eggs of females or lack of use. Lipids were higher in females during the spring due the large portion of lipids in the eggs. Due to the complexity of the egg and need for structural support, protein values were higher in the female gonad during April and May when the most ripe animals were observed. Trends in the data indicate that vitellogenin remained higher in males during the season except for July. This is interesting because this protein is only found in background levels in males and is stored in the female egg as an energy source for the larvae. The results here may be due to a disruption of this process as described previously. It is clear that vitellogenin was lower in females early in the season and there was a delay in when the peak value is seen. This may have severe impacts on larval survival. During 2008, values peaked in July after initial spawning had taken place, meaning that it was not found in high levels in mature eggs that were initially spawned.

Normal/Abnormal

The pattern of the annual reproductive cycle may be thought of as a genetically controlled response to the environment (Sastry, 1970). If this is the case, normal and abnormal clams may have genetic differences with respect to the energy used in the reproductive cycle. Glycogen did not change in the gonads of clams displaying abnormal conditions. In 2008, trends displayed in the data imply that lipids were higher in abnormal clams for the entire season. These differences were significantly higher in March and April (<0.006). Although this may suggest these animals are in better reproductive condition than normal clams, it may just be the opposite. Higher lipid values

in March and April may indicate that these animals are putting more energy into reproduction than normal individuals (Delaporte et al., 2007). This may cause energy reserves in other tissues to become depleted, which may have caused these animals to break down their gonad tissue as a source of nutrition. Proteins of the gonad in 2008 follow similar trends to lipids and the explanation for this is the same. Vitellogenin values were lower in abnormal clams as suggested by the trends in the data. This may be due to the lack of energy available to synthesize this protein. It is possible that larval viability and survival may be compromised from these individuals.

Previous studies have shown differences in reproductive strategies of the Pacific oyster *Crassostrea gigas*, between oysters susceptible to a phenomenon known as summer mortality and those that are not. Oysters susceptible to summer mortality have been shown to put more resources into reproduction as seen by increased lipid contents in the gonad (Delaporte et al., 2007). The same trend is observed in this study when comparing abnormal to normal clams, indicating different reproductive strategies. It has been shown that oysters susceptible to these mortalities generally only partially spawn over the reproductive season (Delaporte et al., 2007). This can cause more stress on the animal which may inhibit any further spawning (Samain et al., 2007). Surfclams that display abnormal gonadal development may be more susceptible to mortality over the summer and during times of stress when compared to normal clams due to increased metabolic activity and energy use for the reproductive process. Lastly, a study conducted by Huvet et al. (2004) showed that genes involved in energy mobilization and immune function were expressed differently in susceptible oysters and resistant ones. Therefore,

there may be genetic differences between normal and abnormal clams which could be responsible for the observed differences.

Adductor Muscle Biochemistry (2009)

Monthly Variability

In 2009, biochemistry of the adductor muscle, gill, gonad, and mantle was measured. Looking at the energy balance between various tissues has shown to be more informative than looking at one particular tissue alone. Glycogen in the adductor muscle did not appear to play a large role in supporting the reproductive process. Sharp declines in adductor glycogen occurred in June and September at times in which glycogen was low in other tissues. The use of these reserves was likely for maintenance conditions. As glycogen was used in other tissues, it was selectively stored in the adductor muscle at various times of the year. Total carbohydrates in the adductor muscle significantly declined between June and July ($p<0.001$), indicating its use following spawning and during the summer months. Lipids in the adductor muscle were most likely used to support metabolic needs following the initial spawn as indicated by the decline in this metabolite between July and September. Protein declined significantly between April and May ($p<0.001$) implying its use to help fuel the end of the ripening process or for shell and somatic tissue growth.

Gill Biochemistry (2009)

Monthly Variability

Energy reserves in the gill did not appear to play a big role in either gametogenesis or maintenance needs. Glycogen remained constant over the course of the year and was stored in the gill in October, potentially for use later in the fall or during the winter. Changes in carbohydrate concentrations in the gill may reflect the abundance of food in the water. Highest values were observed early on in the season and during the last month of sampling, possibly matching the spring and fall phytoplankton blooms. Similar to glycogen, lipids did not significantly change until October. Protein values remained constant except for a significant drop in June. In other bivalves, drops of protein in the gill are associated with spawning (Li et al., 2009a), which may be the reason for the observed decline.

Gonad Biochemistry (2009)

Monthly Variability

Trends observed in the gonad in 2009 were similar to those seen in 2008. Glycogen was apparently used to aid in the production of lipids and protein early on in the season or for shell and somatic growth. Following its use early on, it was stored in the gonad during October. Following the same trend as 2008, in 2009 carbohydrates were possibly used in the gonad following spawning to help maintain energy balance and aid in the recovery process. Lipids were highest in April and May before spawning began and declined significantly between May and June ($p<0.001$) and continued to fall into September. Similar to 2008, a decline in protein values was observed as animals ripened suggesting that proteins were used to fuel the end of gametogenesis or for other metabolic needs at a time when glycogen values were low. Proteins may have also fluctuated due to

the use or storage of other compounds. Vitellogenin levels were highest in May and June corresponding to the months with the highest percentage of ripe individuals. Although not significantly different, the decline between May and July may be a result of the loss of this protein as it is released in the female egg during spawning. Histological results suggest that most clams had begun the next cycle of gametogenesis in October.

Males/Females

The only tissue showing biochemical differences between sexes in 2009 was the gonad. Carbohydrates were significantly lower in females than males during May and September, which may be due to the greater use of this energy source in females supporting the end of the reproductive process and the recovery after spawning. Lipids were higher in female gonads prior to spawning due to the incorporation of lipids in eggs. Protein was also higher in females due to the complexity of the eggs and need for protein as a structural support. Vitellogenin was significantly higher in males during June, and the trends in the data suggest that this was the case most of the year.

2008 vs. 2009

When comparing the biochemistry of the gonad between 2008 and 2009 substantial differences are seen. In 2007, temperatures were cooler than 2008 for much of the year (Figure 32A). Between July and September of 2007, temperatures were below or on the twenty year average for each month. This may have promoted energy storage following spawning in 2007. This was followed by a slightly above average winter between 2007 and 2008 in which the use of energy reserves may not have been

substantial (Table 10). Thus, glycogen, carbohydrates and proteins were higher in early 2008 when compared to 2009. This allowed clams in 2008 to put more energy into reproduction as attributed to the higher lipid and protein values in many months during the year. In 2008, from June on, temperatures were above average possibly causing energy reserves to be used for metabolic needs, leaving less available energy to be stored for over-wintering and use in 2009 for reproduction. Therefore, early in 2009, metabolite reserves in the gonad were lower than they were in 2008, meaning that less energy was available to put into gonadal growth. Thus, overall temperatures in the previous year may have an impact on energy use in the current year, which in turn may affect the amount of energy available for reproduction.

The most important difference between 2008 and 2009 was the lower vitellogenin in 2008 compared to 2009. One major difference in the years was the rate at which the water began to warm. In 2008, the water began at a higher temperature and increased at a faster rate between mid-March through April (Figure 32B). The vitellogenic process is typically one of the final processes to occur before individuals are ripe. Slow increases in temperature favor this process and improve the quality and quantity of the gametes (Martinez et al., 2003). It seems that in 2008 this process was disturbed as attributed to the lower vitellogenin levels. This may have impacted the survival of the larvae in 2008, especially when coupled with above average temperatures in July and August in 2008. In 2009, temperatures increased at a slower rate, supporting the vitellogenic process. Although less energy was presumably available for reproduction in 2009, the quality of the female gametes was potentially greater than that in 2008 as observed by the higher vitellogenin levels. This may have improved larval survival in 2009. Years in which the

water warms slowly may support the storage of vitellogenin in the eggs improving the chances of larval survival. This is important as temperatures in the summer have increased during the last decade (Figure, 2), potentially causing stress on larvae and adults, which may impede the success of recruitment.

Mantle Biochemistry

Monthly Variability

The mantle appeared to be an important tissue used for energy to support many physiological processes. Glycogen in the mantle declined significantly between April and June ($p=0.004$) suggesting its use to support the end of gametogenesis. Glycogen in the mantle has been shown to be strongly influenced by the reproductive process in other bivalves (Li et al., 2009a, b). It is also possible that the drop in glycogen may be due to use for shell and somatic tissue growth. Carbohydrates were used substantially after June, as indicated by the significant decline and low values into October ($p<0.001$). Low lipid values between April and June are an indication of their use for over-wintering, the beginning of gametogenesis, or for shell and somatic tissue growth. Lipid levels increased after June, implying that the mantle may be an important tissue in which lipids are stored for use in processes that occur during the fall and winter. Similar to lipids, protein was also low for about half of the sampling season suggesting the use of this metabolite over the winter or for the initiation of the gametogenic process.

Biochemistry of Normal/Abnormal Individuals

In many of the cases below, differences may not be statistically significant, yet the trends in the data are intriguing suggesting that there are differences between normal and abnormal individuals. In the adductor muscle of abnormal clams, glycogen remained low until June suggesting the enhanced use of these reserves for gametogenesis or growth. Although not significant, lipids were lower in abnormal individuals compared to normal clams during each month. Metabolite values in the gill support the idea that abnormal clams use more energy and have an increased metabolic demand compared to normal clams. From May through July, lipids were lower in the gills of abnormal clams. Protein was consistently higher in the gill of abnormal individuals, which is an indication of higher metabolic needs in other bivalves (Li et al., 2009a). In the gonad, glycogen values were lower in abnormal individuals until July, implying the increased use of glycogen for reproduction or other physiological processes. Lipids in the gonad were about the same in April and May and were higher in abnormal clams in June indicative of a greater energy use for the production of gametes (Delaporte et al., 2007). Gonad protein was higher in abnormal clams until July. Differences were significant in April ($p=0.043$), suggesting a larger portion of energy reserves in abnormal clams goes to gonadal development. Interestingly, vitellogenin was higher in normal clams during each month except for July, implying that eggs from abnormal individuals had less energy reserves for larvae. In the mantle, glycogen finished lower in abnormal clams and carbohydrate values remained lower in abnormal clams until July. The greater energetic demand for reproduction in abnormal clams may leave them in a vulnerable state as energy reserves will be lower than normal. This could compromise their ability to deal with further stress.

Summary

In summary, the different trends observed in each tissue shows that they play different roles in physiological processes. It appears that metabolites in the adductor muscle are important during stressful periods based on the time of year in which metabolite levels declined in this tissue. The gill only seems to be used as an energy source when necessary; otherwise metabolite levels are maintained over the season. The gill has been shown to exhibit the least dry weight loss in other studies as it helps to maintain the biochemical stability of the organism (Riley, 1976), supporting the current findings. Glycogen in the gonad and mantle appear to be important reserves in reproductive and growth processes. In most tissues, glycogen, lipids and proteins showed recovery in October of 2009. This implies environmental conditions were favorable, supporting the storage of reserves. It is likely that the ability of the animal to store energy during the fall will have implications on the strength of the reproductive process the following year and the capacity of the clam to survive the winter.

Immune Parameters

Some studies have shown hemocyte counts to be high at low temperatures and others have shown the opposite (Cho and Jeong, 2005). In the current study, hemocyte counts did not appear to be influenced by temperature but were impacted by spawning. In April and May, hemocyte counts were high, but agranulocytes (hemocytes characterized by the absence of granules in their cytoplasm) made up a large portion of the population. Agranulocytes do not undergo phagocytosis or release reactive oxygen species that are lethal to microbes. It is possible that clams do not spend extra energy on producing granulocytes at this time of the year because bacterial growth is slowest at the coldest

temperatures and the threat of infection is reduced. Alternatively, gametogenesis is in progress and the extra energy needed to make granulocytes is not available. In June, all hemocyte populations dropped, corresponding to the beginning of spawning. Based on low hemocyte counts alone, one may suspect surfclams to be immuno-compromised in October, although no conclusions can be made without further data. At this point in the year, energy reserves are being replenished and the animal may not have excess energy that can be incorporated into defenses to fight infection. As well, the normal gonad cleansing process requires hemocytes to infiltrate gonad tissues. This may leave less hemocytes in circulation available to fight invading pathogens.

Bacteriology

The greatest number of clams with high bacterial counts was observed in September, corresponding to the end of the spawn, warm temperatures, and low energetic reserves. The combination of spawning and warm temperatures may have weakened the ability of the animals to fight infection, allowing opportunistic pathogens to invade. As temperatures declined and animals recovered from spawning, bacteria counts declined. It is possible that spawning may cause surfclams to become immuno-deficient, allowing for pathogens to infiltrate. However, without any further data on other immune parameters no conclusions can be drawn.

The impact of temperature on gonadal development:

Histology/Condition Index

Results of the histology data from this study suggest that the maturation process was accelerated in the warm treatment when compared to the cold treatment. Warmer temperatures likely cause the animals to speed up the reproductive process in order to be coordinated with the environmental changes (Sastry, 1970; Delaporte et al., 2006). Increasing temperatures at a faster rate may increase metabolic needs, causing an imbalance in energy usage and gain. This is seen by the extremely high percentage (86%) of female clams displaying abnormal gonad conditions in the warm treatment. It is also apparent that the rate at which the water temperature increased in the cold treatment was large enough to create an energetic imbalance based on the high percentage of abnormal female clams in that treatment (57%). However, abnormal conditions were more intense in the warm treatment (Appendix J).

Biochemistry

Based on the findings in the study, it appears that the less mature animals in the cold treatment were able to produce gametes of higher quality, than animals in the warm treatment which produced gametes of lower quality. Although not all interactions are statistically significant, the data suggest that glycogen, lipids ($p=0.044$) and vitellogenin were higher in clams from the cold treatment, while protein and carbohydrates were lower. Lipid values in the female gonad have been shown to be indicators of oocyte quality and larval viability (Darriba et al., 2005). However, just lipid values alone may not tell the whole story unless vitellogenin values are also taken into consideration. Higher vitellogenin values in the gonad may improve the quality and viability of the egg and larvae. The data suggests that carbohydrates were used in clams from the cold

treatment to produce higher quality gametes. Animals in the warm treatment may have experienced an interruption of the vitellogenic process, diverting energy to other metabolic needs and less into reproduction. These findings support the results from the field data in 2008 and 2009 which were warm and cooler years respectively.

It is interesting to note that even though the interactions between treatments were not significant, the trends between normal and abnormal clams display similar patterns to those in the field in 2008 and 2009. Lipids were higher in abnormal clams from the warm treatment and lower in the cold treatment when compared to normal clams in their respective treatments. This matched the field data in 2008 and 2009 when temperatures were warmer and cooler respectively. Higher lipid levels in abnormal clams from the warm treatment indicate a greater use of energy for reproductive processes, following the findings from the field in 2008. Likewise, vitellogenin levels were highest in normal clams from the cold treatment and lowest in abnormal clams from the warm treatment, directly matching field results.

Filtration rate and scope for growth

Scope for growth has been used in numerous studies as an index of energy balance (Buxton et al., 1981). Increased metabolic demands and reduced filtration rates at temperatures outside the optimal range of the species will lead to a negative energy balance. In the current study, filtration rates were significantly lower at 23°C, compared to 19°C ($p=0.034$). A reduction in filtration rate limited the amount of food that was able to be ingested and subsequently the energy available to use for growth and reproduction. Concomitantly, surfclams excreted more ammonia and consumed greater amounts of

oxygen at 23°C, indicating a greater energetic demand. Although not significant, ingestion rates were lower in clams from the warm treatment, meaning their ability to capture food to meet the necessary energy requirements was altered. Likewise, scope for growth was significantly lower in clams from the 23°C treatment ($p=0.042$). Results of this experiment support the hypothesis stated by Kim and Powell (2004) that stressful summer temperatures may increase the metabolic demands and reduce the filtration rate of the surfclam. It is possible that this may cause abnormal conditions to intensify in the summer months as energy balance is negatively impacted.

Bivalves have been shown to alter their energy balance in various ways to avoid using more energy than is gained. A couple of ways this can be accomplished is by increasing the assimilation efficiency of the food ingested or by adjusting the irrigatory efficiency (Newell et al., 1977; Newell and Branch, 1980). Neither of these strategies was utilized by the animals exposed to higher temperatures during the current study. The ensuing net energy loss resulted in a greater amount of energy used during summer conditions. This could have an impact on the energy used towards a fall spawn, or may impact the ability of the clam to endure an extended period of stress. Lower body weights, as seen by a reduced condition indices at 23°C ($p=0.029$), may impact over-wintering success and the reproductive period the following year. Reduced condition towards the southern end of the Atlantic surfclams range due to temperature stress has been shown to increase the susceptibility of these animals to further warming and impact their survival (Marzec et al., 2010). In summary, 23°C is a stressful temperature to surfclams and can negatively impact the physiology of these animals, which may impact

their survival and reproduction. This may partly explain current population declines and lack of recruitment success.

Immune Defense and Short Term Energy Balance

Biochemistry

Seasonal cycles of energy storage and utilization are closely related to reproductive activity (Ojea et al., 2004). Following spawning, energy reserves may be depleted and the ability of the animal to deal with further stress could be compromised. The experiment conducted in this study occurred in October at a time of the year after spawning has occurred.

In general, patterns in the data suggest that many metabolites showed a greater decline in the warm treatment implying a negative energy balance. It is also apparent that animals were able to selectively use energy reserves from different tissues. For instance, glycogen, carbohydrates and proteins were used in the adductor muscle of clams held at 23°C, yet lipids were not used. Lipids, protein and glycogen in the gill all declined in animals from the warm treatment. The use of reserves in the gill may suggest that these energy stores are not necessary to over-wintering or that energy in other tissues were not great enough to support metabolic needs. Similar to the adductor muscle, the gonad of clams in the warm treatment displayed declines in glycogen and proteins without any changes in lipids. Differing from all other tissues, the mantle displayed losses in all metabolites measured. This again supports the idea that metabolites in the mantle are important reserves used by surfclams when energy is needed during stressful periods.

Glycogen and other carbohydrates are important energy sources during short term stress. Lipids and proteins are generally used for longer term stress, such as over-wintering. Excess use of proteins and lipids after spawning could negatively impact the survival of the animal over the next winter. Energy reserves typically used for long term stress may have been used by clams in the current study, as they may have had less short term energy stores at this time of the year. The breakdown of more complex metabolites and switch from anabolism to catabolism is likely energetically demanding. This could impact the survival of these individuals over the short term. The lack of lipid use in the adductor muscle and gonad may indicate that these pools of energy are important for use over the winter and were selectively untouched. Lastly, energy available to be allocated to other processes including immunity may no longer be available during unfavorable environmental conditions.

Immune Parameters

The immune status of an organism will impact its ability to fight off pathogens. This study assessed the ability of surfclams to fight a pathogenic bacteria species (*Vibrio alginolyticus*) during temperature stress. Hemocytes are the main defense in bivalves and are involved in the capture (phagocytosis) and killing of microbes through a sudden release of reactive oxygen species (Buggé et al., 2007). Animals should remain in a healthy immune status as long as they are not undergoing any stress and excess energy is available to support immune functions.

Hemocyte counts alone do not reflect the immune status of a bivalve. Total hemocyte counts were higher in animals from the warm treatment. However, the percent

granulocytes that made up the overall population were significantly higher ($p<0.001$) in the cold treatment, which may be a better indicator of immune status as these are the main blood cells involved in the killing of microbes. The percentage of dead cells between treatments was not significantly different. Prior to stimulation, ROS values were significantly higher in clams from the cold treatment. Following stimulation, ROS was higher in the warm treatment, but no significant differences were seen. Reactive oxygen species production may not be as important as phagocytosis. If the host cannot capture an invading pathogen, it will be much harder for the organism to destroy it. The proportion of phagocytic hemocytes and the intensity of phagocytosis which gives an index of the number of beads captured (Allam et al., 2002) were not significantly different between treatments. However, the phagocytic index was significantly higher in clams held at 19°C ($p=0.048$). Thus, clams subjected to the optimal temperature were able to more effectively capture invading organisms.

Post bacterial challenge, 81% of the clams in the warm treatment died in comparison to only 35% in the cold treatment. This strongly suggests animals in the warm treatment were immuno-compromised. This is further supported by the bacterial counts in remaining live individuals. The two remaining live clams in the warm treatment contained bacteria numbers exceeding 350 per 100 µl of hemolymph, suggesting that these animals did not clear the infection and likely would have died. Comparatively, only three (23%) of the thirteen remaining clams in the cold treatment had bacterial counts equally as high, showing that most of these animals were able to successfully clear the infection. Based on these results, surfclams are likely to be at higher risk of infections by opportunistic microorganisms and mortality during temperature stress. Although no

mortality event has been reported in New York state waters, it remains likely that some sort of mortality is occurring based on the magnitude of the population declines. It is possible that these declines could be partly due to bacterial infection. Even though bacterial infections have not been seen during histological analysis, field samplings are relatively scarce. In many cases, bacterial infections are often acute and can occur rapidly (within days) and the only thing that will remain is an empty shell (Malham et al. 2009).

Future Directions

Temperatures have risen 2-3°C in coastal waters over the past century (Weinberg, 2002). The thermal sensitivity of a cold water species declines at the warm extent of their range (Jansen et al., 2007a) and the range limits of large bodied species have shown to be less stable than that of smaller bodied animals (Roy et al., 2001). It has been demonstrated that surfclams are stressed at the southern end of their range as documented in the numerous studies by Kim and Powell (2004), Weinberg (2002, 2005), and Marzec et al. (2010). More recently, studies (Allam, 2007; Davidson et al., 2007) have shown a similar stress sensitivity of surfclams in New York, suggesting that the inshore and southern range of this large bodied bivalve may have shifted farther offshore and northwards respectively, as an apparent result of increasing ocean temperatures. While most studies have verified the impact of temperature stress on the adults, few studies have examined any potential stress temperature has on the larvae or more importantly how stress on the adult may impact the survivorship of the larvae.

In other adult bivalve species, stress has shown to impact reproductive investment and the performance of larvae and juveniles. Bivalve eggs are costly due to the large

amount of lipids that are stored in the egg (Honkoop et al., 1999). Reproductive investment in the adult can be determined by the number, size and energy content of the eggs (Honkoop et al., 1999). However, there is a trade off that bivalves must make between the number of eggs produced (fecundity) and the amount of energy put into each egg (Allen et al., 2006). The amount of energy put into each egg has been shown to impact the development and survival of the larvae and juvenile performance (Honkoop et al., 1999, Allen et al., 2006).

Under stress, the amount of energy invested in each egg may be diverted elsewhere for other metabolic needs. Stress can cause fewer eggs to be spawned and less energy reserves to be stored in the egg (Bayne et al., 1978, Bertram and Strathmann, 1998). Larvae are dependent on nutrient reserves in the egg before they begin feeding (Honkoop et al., 1999). The more energy reserves they have, the faster the larvae develop and metamorphose (Bertram and Strathmann, 1998, Honkoop et al., 1999). This improves the chances of survival as the larvae settle out of the water column and are no longer available to planktonic predators. Faster development may also improve juvenile performance as fewer energy reserves will have been used during metamorphosis and more can be used for growth or dealing with further stress (Allen et al., 2006). The ability of surfclams to grow quickly has been shown to improve their chances of survival as they outgrow many predatory species by age one (Cerrato and Keith, 1992).

Contrary to this, when metamorphosis is delayed by low energy reserves, a poor food source, or environmental conditions, the time spent in the plankton increases and chances of survival declines (Pechenik et al., 1998, Bos et al., 2007). Delaying

metamorphosis has been shown to lead to a smaller juvenile size, leading to poor juvenile performance and increased predation due to the smaller size making them available to more predators (Pechenik et al., 1998). All of these factors taken into consideration can have a significant impact on recruitment.

Surfclam larvae remain in the water column about 35 days at 14°C and 19 days at 22°C (Loosanoff and Davis, 1963, Weissberger and Grassle, 2003). Larvae have been shown to settle between May and December depending on when the larvae were fertilized (Weissberger and Grassle, 2003). The optimal temperature for larval growth was shown to be 22°C (Loosanoff and Davis, 1963, Marzec et al., 2010) and temperatures of 24°C and greater negatively impact fertilization (Clotteau and Dube, 1993, Marzec et al., 2010). Surfclam larvae have also been shown to survive without food for up to 15 days (Walker et al., 1998). All of these properties change from year to year depending on environmental factors. As well, stress on the adults may cause these factors to change, which may impact negatively impact larval survival.

The next logical step in surfclam research would be to examine the possible relationship between stress in the adults and the impact this has on larval survival. I hypothesize that during colder years, surfclams are able to store and utilize energy more efficiently than warmer years, translating to improved larval survival. During cool years it is thought that more energy is able to be invested into the eggs, thus increasing the quality of the egg and chances of larval survival. Given the current temperature trends in summer and stressful conditions larvae are likely to encounter, the more energy reserves the larvae have, the greater the chances of survival.

Temperature may also impact the quantity or quality of food in the environment. In a previous study conducted by Kim and Powell (2004), surfclams with digestive gland atrophies were observed. This suggests that food concentrations were low or of poor quality during the time the study took place. No studies however, have examined the type, quantity or quality of phytoplankton in the surface and benthos over the course of the year in relation to larval concentrations in the environment and how this impacts recruitment in the Atlantic surfclam. It is possible that the quality and concentration of food available to all life stages of the surfclam will have a significant impact on recruitment success in a given year. I hypothesize that during a warm year, phytoplankton quality and/or quantity is altered, negatively impacting recruitment success either directly as a poor larval food source or indirectly through the nutrition of the adult.

Overall, it is clear that temperature plays an important role in modulating the physiology, population and recruitment dynamics of the Atlantic surfclam, *Spisula solidissima*. As the world's oceans continue to warm, it can be expected that increasing temperatures will continue to negatively impact this species suggesting a dim future for the species in shallow coastal waters of New York State.

Table 1: Histological data from 2008 field samples

Month	Gametogenic Stage	% of Total	Abnormal Females	Abnormal Males	Total % Abnormal	%Abnormal Females	Intensity Average
March	D1	36	12	1	46	63	1.30
	D2	54					
	R	11					
April	D1	6	4	0	22	36	1.25
	D2	72					
	R	22					
May	D1	7	4	0	13	20	1.00
	D2	50					
	R	43					
July	D2	20	5	1	20	24	2.17
	R	7					
	S1	53					
	S2	7					
	S3	10					
	S4	3					
August	D2	23	3	1	13	14	1.50
	S1	27					
	S2	20					
	S3	13					
	S4	17					
September	D1	10	0	0	0	0	0.00
	S1	3					
	S2	13					
	S3	13					
	S4	60					

Table 2: Histological data from 2009 field samples

Month	Gametogenic Stage	% of Total	Abnormal Females	Abnormal Males	Total % Abnormal	%Abnormal Females	Intensity Average
April	D1	37	4	2	20	19	1.16
	D2	47					
	R	17					
May	D1	3	5	3	27	28	1.25
	D2	73					
	R	23					
June	D1	17	6	1	23	29	2.00
	D2	47					
	R	30					
	S1	3					
	S3	3					
July	D2	38	3	3	21	16	2.00
	R	28					
	S1	31					
	S2	3					
August	R	3	0	0	0	0	0.00
	S1	23					
	S2	20					
	S3	23					
	S4	30					
September	D1	63	0	0	0	0	0.00
	S1	7					
	S2	13					
	S3	7					
	S4	10					

Table 3: Biochemical composition of gonad samples in 2008

Gonad												
Month	March	St Error	April	St Error	May	St Error	July	St Error	August	St Error	September	St Error
Glycogen (mg per gram tissue)	3.801	0.821	4.311	0.588	4.674	0.703	7.125	0.776	6.492	0.583	6.565	0.705
Total Carbohydrates (mg per gram tissue)	21.715	1.715	21.201	0.745	16.978	0.678	15.435	2.178	17.755	1.412	10.561	2.867
Lipids (mg per gram tissue)	8.486	0.612	11.631	0.776	12.974	0.474	8.167	0.326	9.283	0.383	5.820	0.595
Proteins (mg per gram tissue)	43.655	2.615	50.799	3.164	29.347	3.560	19.262	2.395	22.721	1.600	31.361	5.184
Vitellogenin (µg ALP/ mg Protein (per gram tissue))	0.261	0.032	0.137	0.018	0.345	0.085	0.339	0.082	0.332	0.052	0.559	0.062

Table 4: Biochemical composition of adductor muscle samples in 2009

Adductor Muscle												
Month	April	St Error	May	St Error	June	St Error	July	St Error	September	St Error	October	St Error
Glycogen (mg per gram tissue)	7.936	1.257	11.120	2.229	1.788	0.378	15.107	1.236	6.138	1.665	17.139	1.003
Total Carbohydrates (mg per gram tissue)	23.089	1.407	33.216	2.308	31.661	2.099	10.373	1.038	9.257	0.576	8.793	0.542
Lipids (mg per gram tissue)	4.432	0.278	7.308	0.795	6.206	0.379	5.836	0.278	3.934	0.182	4.651	0.243
Proteins (mg per gram tissue)	42.302	2.628	25.341	2.094	25.619	2.191	30.158	1.257	52.144	4.189	42.234	5.749

Table 5: Biochemical composition of gill samples in 2009

Gill												
Month	April	St Error	May	St Error	June	St Error	July	St Error	September	St Error	October	St Error
Glycogen (mg per gram tissue)	1.649	0.327	0.763	0.152	1.327	0.252	0.982	0.168	1.395	0.169	4.394	0.424
Total Carbohydrates (mg per gram tissue)	9.439	0.496	8.593	0.249	8.199	0.314	7.480	0.335	5.699	0.257	7.020	0.289
Lipids (mg per gram tissue)	6.147	0.404	7.330	0.555	7.202	0.442	5.693	0.204	6.549	0.529	8.133	0.408
Proteins (mg per gram tissue)	14.439	1.450	16.061	2.163	7.336	1.683	15.196	1.201	15.194	1.724	13.985	1.857

Table 6: Biochemical composition of gonad samples in 2009

Gonad												
Month	April	St Error	May	St Error	June	St Error	July	St Error	September	St Error	October	St Error
Glycogen (mg per gram tissue)	2.619	0.480	2.014	0.505	1.185	0.210	2.214	0.820	1.843	0.805	4.380	0.765
Total Carbohydrates (mg per gram tissue)	15.671	0.554	14.140	0.481	13.682	1.040	10.642	0.485	7.208	0.530	9.133	0.444
Lipids (mg per gram tissue)	12.244	0.641	11.137	0.593	8.512	0.572	6.783	0.354	5.978	0.479	9.493	0.280
Proteins (mg per gram tissue)	27.582	2.252	16.824	2.659	16.800	1.755	19.912	1.754	14.774	1.424	31.944	1.443
Vitellogenin (µg ALP/ mg Protein (per gram tissue))	0.489	0.039	1.079	0.265	1.030	0.235	0.842	0.133	0.816	0.150	0.414	0.025

Table 7: Biochemical composition of mantle samples in 2009

Mantle												
Month	April	St Error	May	St Error	June	St Error	July	St Error	September	St Error	October	St Error
Glycogen (mg per gram tissue)	12.678	2.059	10.078	1.776	6.444	1.290	7.152	1.379	4.882	0.865	4.239	0.880
Total Carbohydrates (mg per gram tissue)	19.935	1.536	29.258	1.886	27.666	2.104	9.688	1.115	7.228	0.568	3.884	0.249
Lipids (mg per gram tissue)	4.477	0.417	4.471	0.214	4.299	0.339	5.745	0.252	8.795	0.368	8.383	0.362
Proteins (mg per gram tissue)	9.625	1.443	16.564	1.677	10.473	1.961	11.537	1.414	28.518	1.602	16.463	1.992

Table 8: Comparison of the biochemical composition of the gonad in 2008 and 2009 (ND= No data)

Month	March					April				
	2008	St Error	2009	St Error	p-value	2008	St Error	2009	St Error	p-value
Temperature	6.1		5.5			6.1		7.2		
Glycogen (mg per gram tissue)	3.801	0.821	ND			4.311	0.588	2.619	0.480	0.032
Total Carbohydrates (mg per gram tissue)	21.715	1.715	ND			21.201	0.745	15.671	0.554	<.001
Lipids (mg per gram tissue)	8.486	0.612	ND			11.631	0.776	12.244	0.641	0.550
Proteins (mg per gram tissue)	43.655	2.615	ND			50.799	3.164	27.582	2.252	<.001
Vitellogenin (µg ALP/per mg Protein (per gram tissue))	0.261	0.032	ND			0.137	0.018	0.489	0.039	<.001

Month	May					June				
	2008	St Error	2009	St Error	p-value	2008	St Error	2009	St Error	p-value
Temperature	9.4		10.3			19.8		17.1		
Glycogen (mg per gram tissue)	4.674	0.703	2.014	0.505	0.008	ND		1.185	0.210	
Total Carbohydrates (mg per gram tissue)	16.978	0.678	14.140	0.481	0.001	ND		13.682	1.040	
Lipids (mg per gram tissue)	12.974	0.474	11.137	0.593	0.019	ND		8.512	0.572	
Proteins (mg per gram tissue)	29.347	3.560	16.824	2.659	0.008	ND		16.800	1.755	
Vitellogenin (µg ALP/per mg Protein (per gram tissue))	0.345	0.085	1.079	0.265	0.007	ND		1.030	0.235	

Month	July					August				
	2008	St Error	2009	St Error	p-value	2008	St Error	2009	St Error	p-value
Temperature	22.1		21.7			23.4		23.2		
Glycogen (mg per gram tissue)	7.125	0.776	2.214	0.820	0.002	6.492	0.583	ND		
Total Carbohydrates (mg per gram tissue)	15.435	2.178	10.614	0.485	0.036	17.755	1.412	ND		
Lipids (mg per gram tissue)	8.167	0.326	6.783	0.354	0.006	9.283	0.383	ND		
Proteins (mg per gram tissue)	19.262	2.395	19.912	1.754	0.826	22.721	1.600	ND		
Vitellogenin (µg ALP/per mg Protein (per gram tissue))	0.339	0.082	0.842	0.133	0.005	0.332	0.052	ND		

Month	September					October				
	2008	St Error	2009	St Error	p-value	2008	St Error	2009	St Error	p-value
Temperature	19.9		21.0			18.2		17.4		
Glycogen (mg per gram tissue)	6.565	0.705	1.843	0.805	<.001	ND		4.380	0.765	
Total Carbohydrates (mg per gram tissue)	10.561	2.867	7.208	0.530	0.076	ND		9.133	0.444	
Lipids (mg per gram tissue)	5.820	0.595	5.978	0.479	0.866	ND		9.493	0.280	
Proteins (mg per gram tissue)	31.361	5.184	14.774	1.424	0.002	ND		31.944	1.443	
Vitellogenin (µg ALP/per mg Protein (per gram tissue))	0.296	0.062	0.816	0.150	0.004	ND		0.414	0.025	

Table 9: Differential hemocyte counts from the field in 2009

Month	Temperature °C	Total Hemocytes (per ml)	St Error	Total Granulocytes (per ml)	St Error	Total Agranulocytes (per ml)	St Error	Percent Granulocytes	St Error
April	7.2	7.88E+06	7.57E+05	1.44E+06	1.39E+05	6.44E+06	6.31E+05	18.86	1.02
May	10.3	7.83E+06	9.38E+05	2.59E+06	3.96E+05	5.24E+06	6.83E+05	34.72	2.56
June	17.1	1.92E+06	2.14E+05	1.53E+06	2.08E+05	3.97E+05	3.29E+04	75.40	2.13
July	21.7	5.86E+06	2.15E+06	2.94E+06	5.98E+05	3.12E+06	1.59E+06	64.84	4.40
September	21.0	3.05E+06	2.56E+05	2.38E+06	2.19E+05	6.65E+05	5.39E+04	76.75	1.43
October	17.4	1.20E+06	1.15E+05	7.73E+05	9.33E+04	4.30E+05	3.19E+04	62.39	1.70

Table 10: Average seasonal sea surface temperatures (°C) in 2008, 2009 and the last twenty years

Year	Winter	Spring	Summer	Fall
2008	7.2	8.3	21.3	17.3
2009	7.1	7.9	20.5	16.9
20 Year Mean	6.6	7.6	20.2	16.6

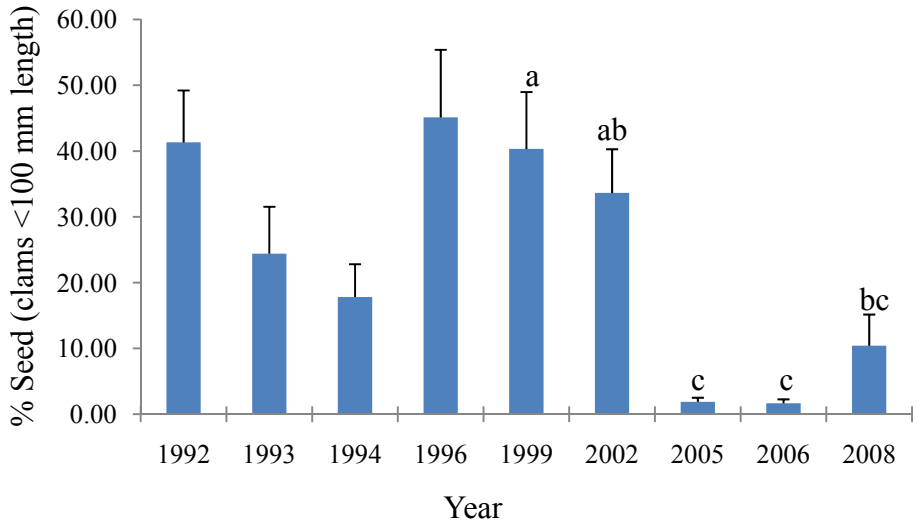


Figure 1: The percent seed (<100 mm length) in New York State Department of Environmental Conservation surfclam population assessments dating back to 1992. * The 1992-1996 surveys do not have the same parameters as the surveys from 1999-2008. The results may not be directly comparable. Statistics were only conducted on the surveys between 1999 and 2008. Samples not sharing a common letter are significantly different $p<0.05$.

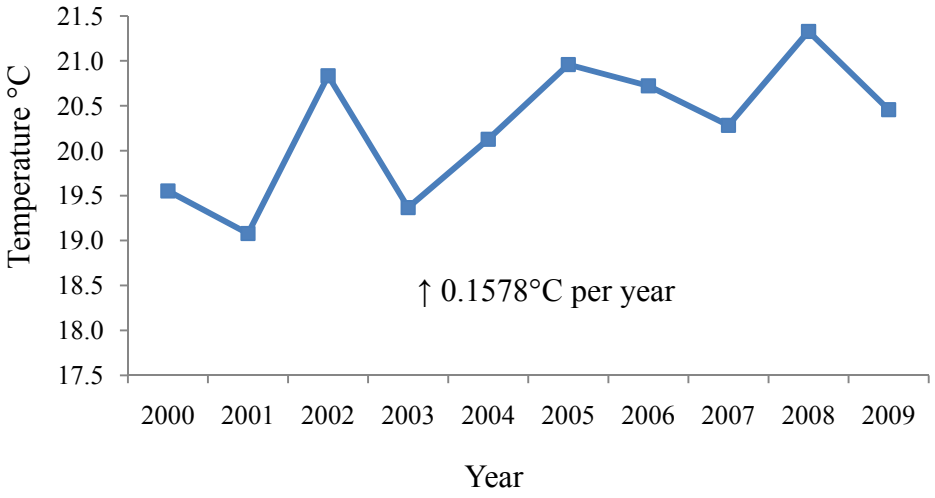


Figure 2: Average summer temperatures between 2000 and 2009.

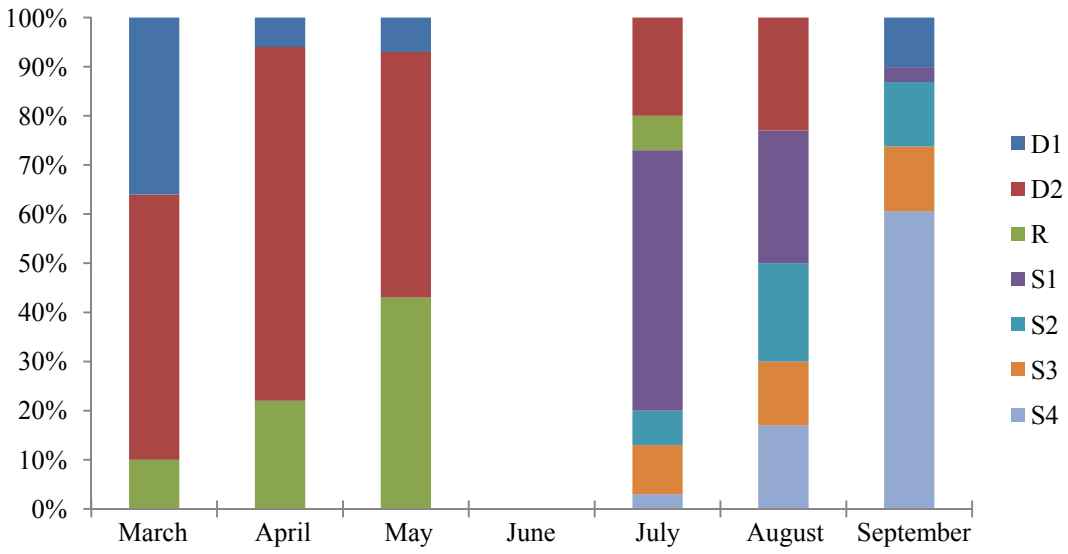


Figure 3: Gametogenic stages of clams collected in 2008. (D1=Developing Stage I, D2=Developing Stage II, R=Ripe, S1=Spawning Stage I, S2=Spawning Stage II, S3=Spawning Stage III, S4=Spawning Stage IV).

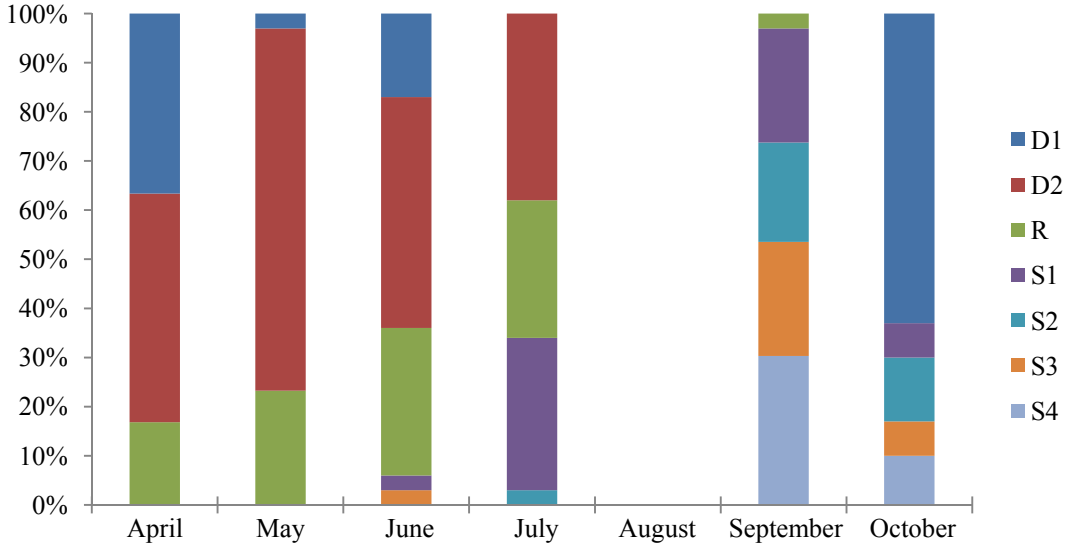


Figure 4: Gametogenic stages of clams collected in 2009. (D1=Developing Stage I, D2=Developing Stage II, R=Ripe, S1=Spawning Stage I, S2=Spawning Stage II, S3=Spawning Stage III, S4=Spawning Stage IV).

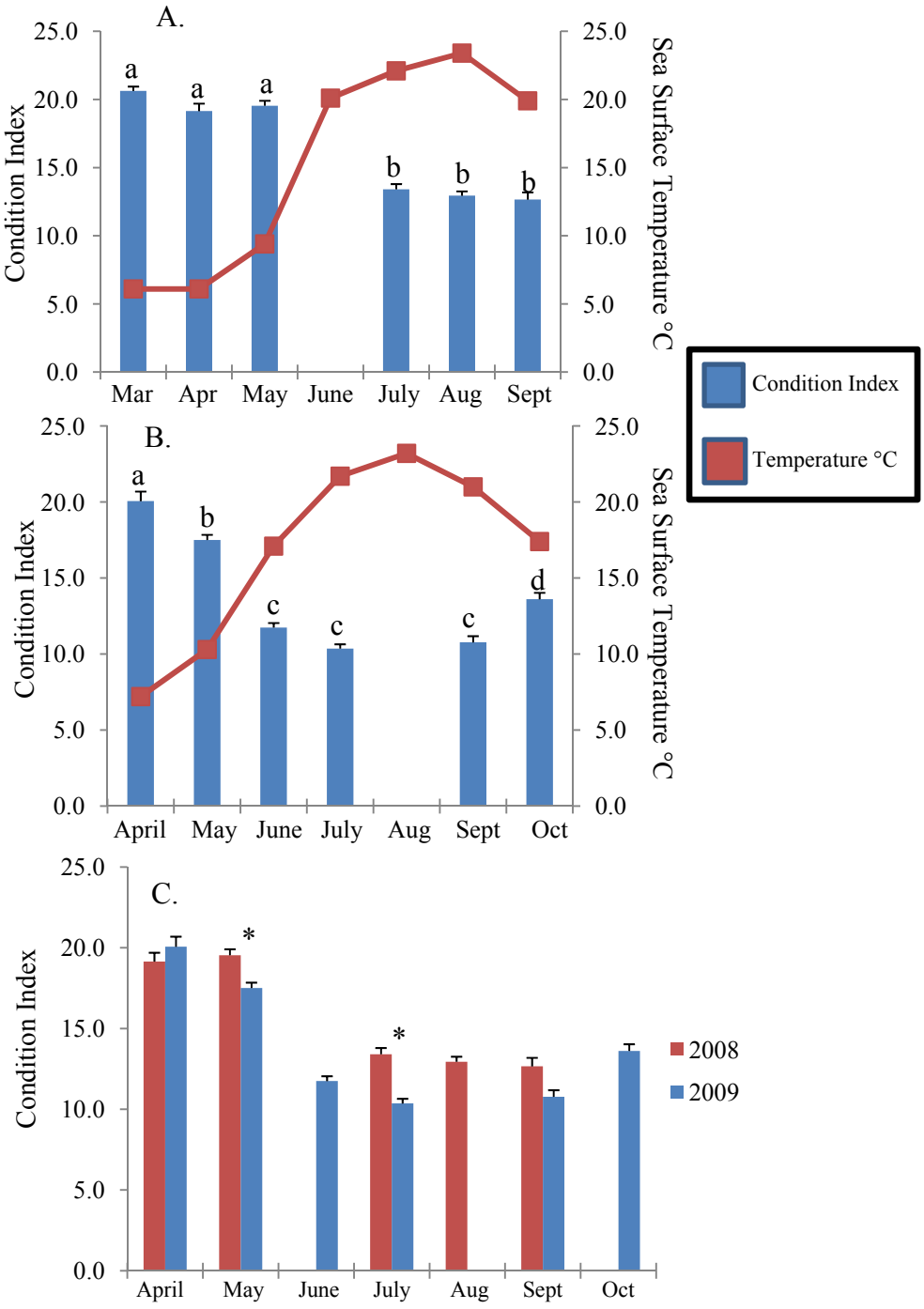


Figure 5: A-C, Condition index and sea surface temperature in 2008 (A), 2009 (B) and a comparison of condition index in 2008 vs. 2009 (C). An asterisk (*) indicates significance within a month between years. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

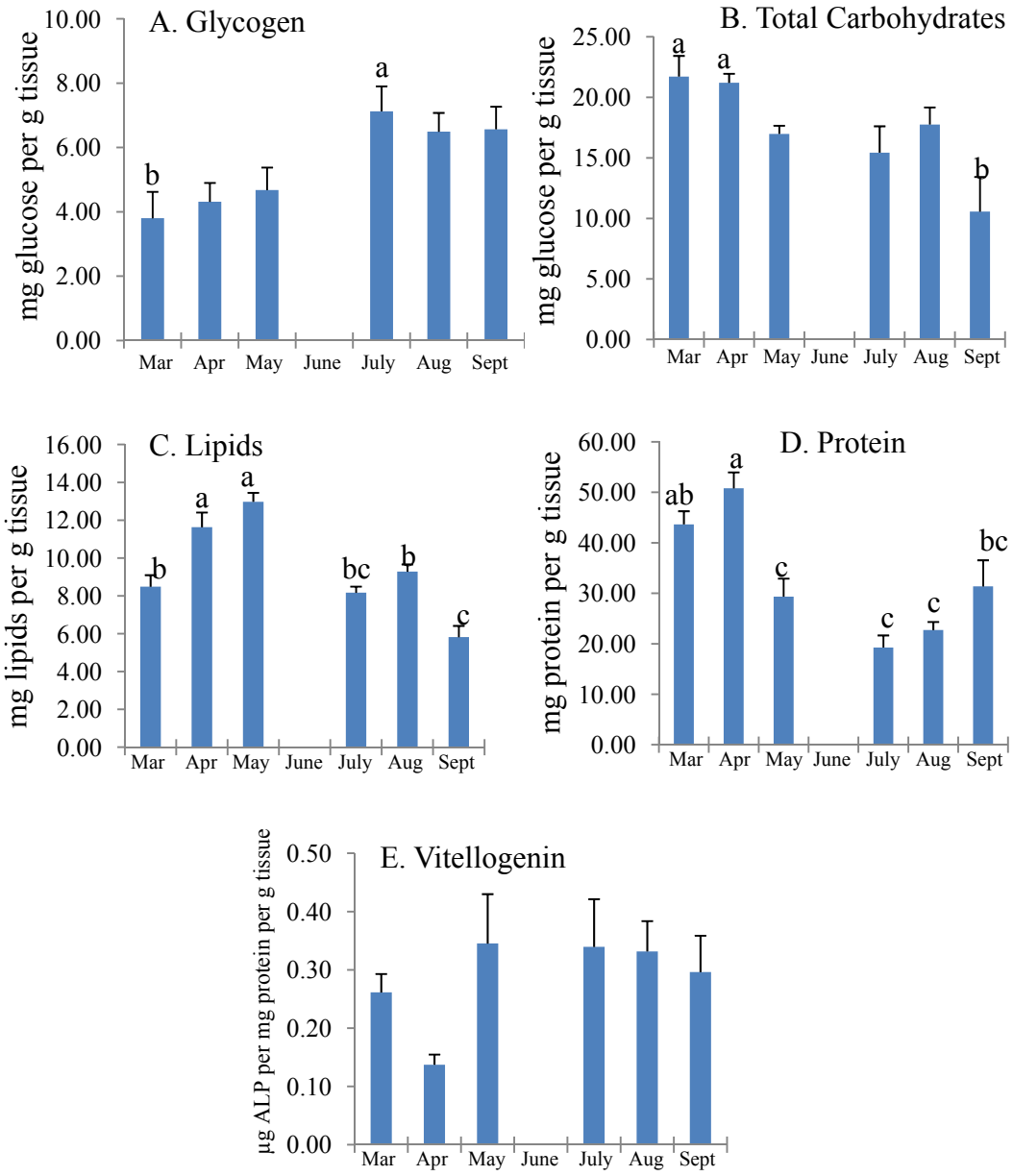


Figure 6: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of surfclam gonads in 2008. No samples were collected in June. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

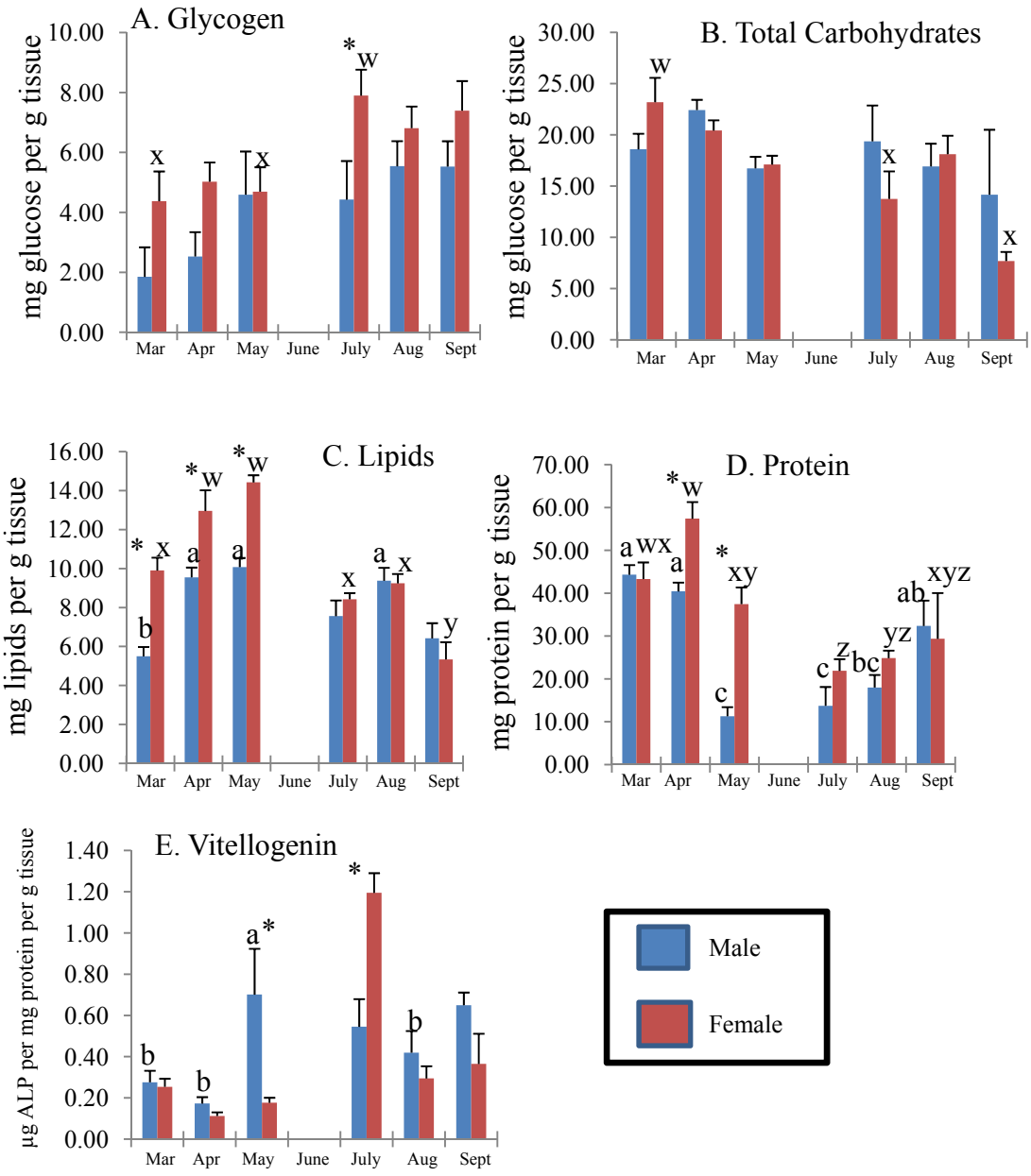


Figure 7: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of male and female surfclam gonads in 2008. No samples were collected in June. An asterisk (*) indicates significance between sexes within a month. Letters indicate significance between males (a,b,c,d) and females (w, x,y,z) between months. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

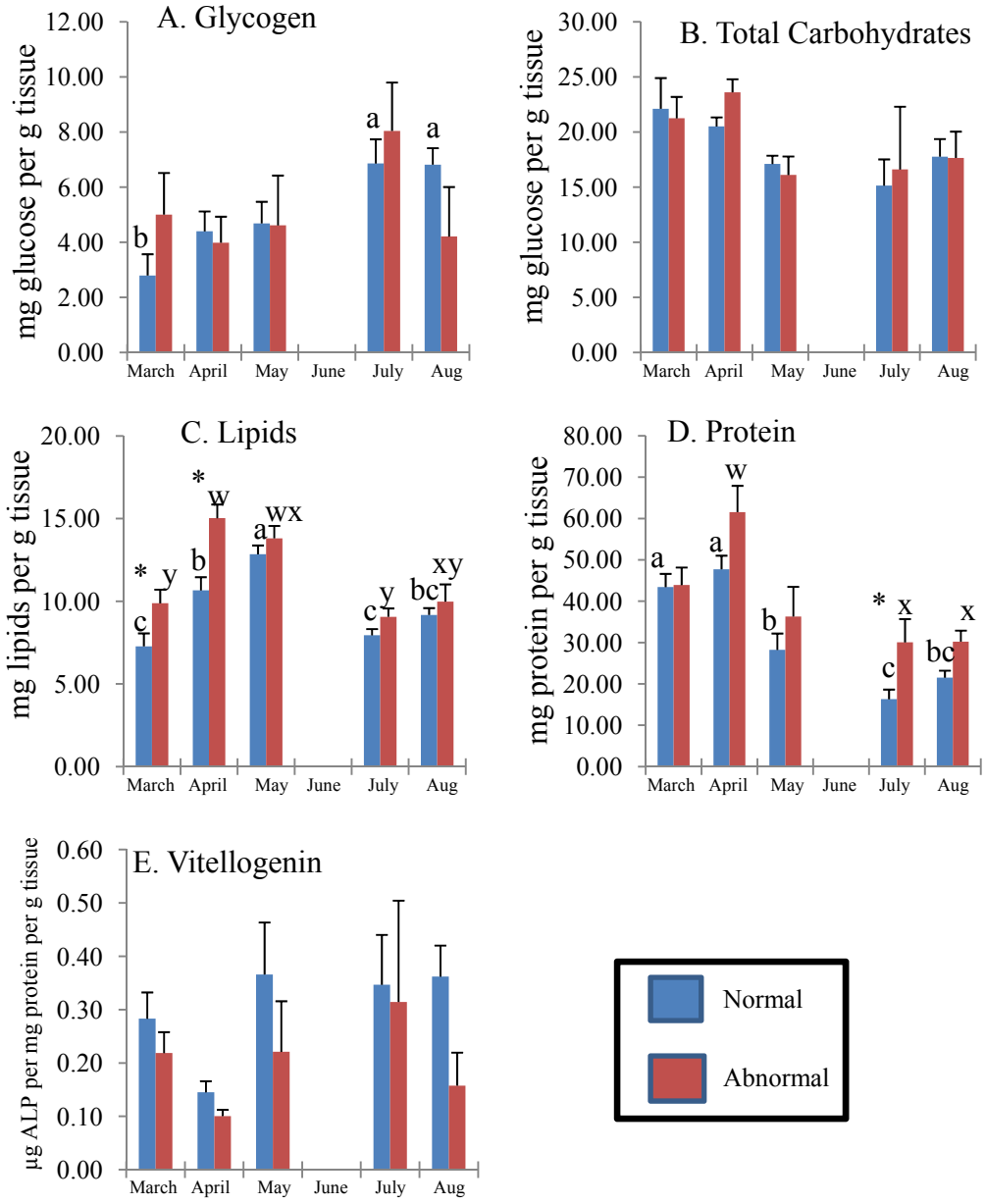


Figure 8: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of normal and abnormal surfclam gonads in 2008. No samples were collected in June. September is not included because no abnormal clams were observed during this month. An asterisk (*) indicates significance between groups within a month. Letters indicate significance between normal (a,b,c,d) and abnormal individuals (w, x,y,z) between months. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

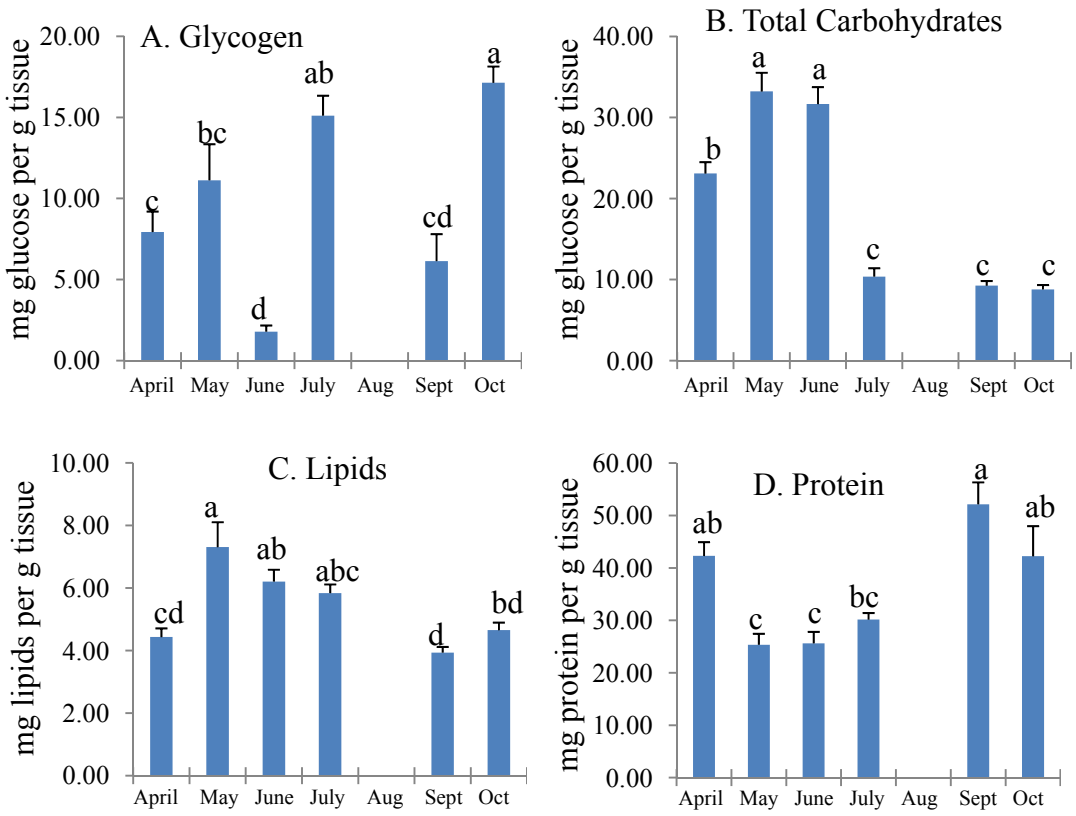


Figure 9: A-D, Glycogen (A), Total Carbohydrate (B), Lipid (C) and Protein (D) levels of surfclam adductor muscles in 2009. No samples were collected in August. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

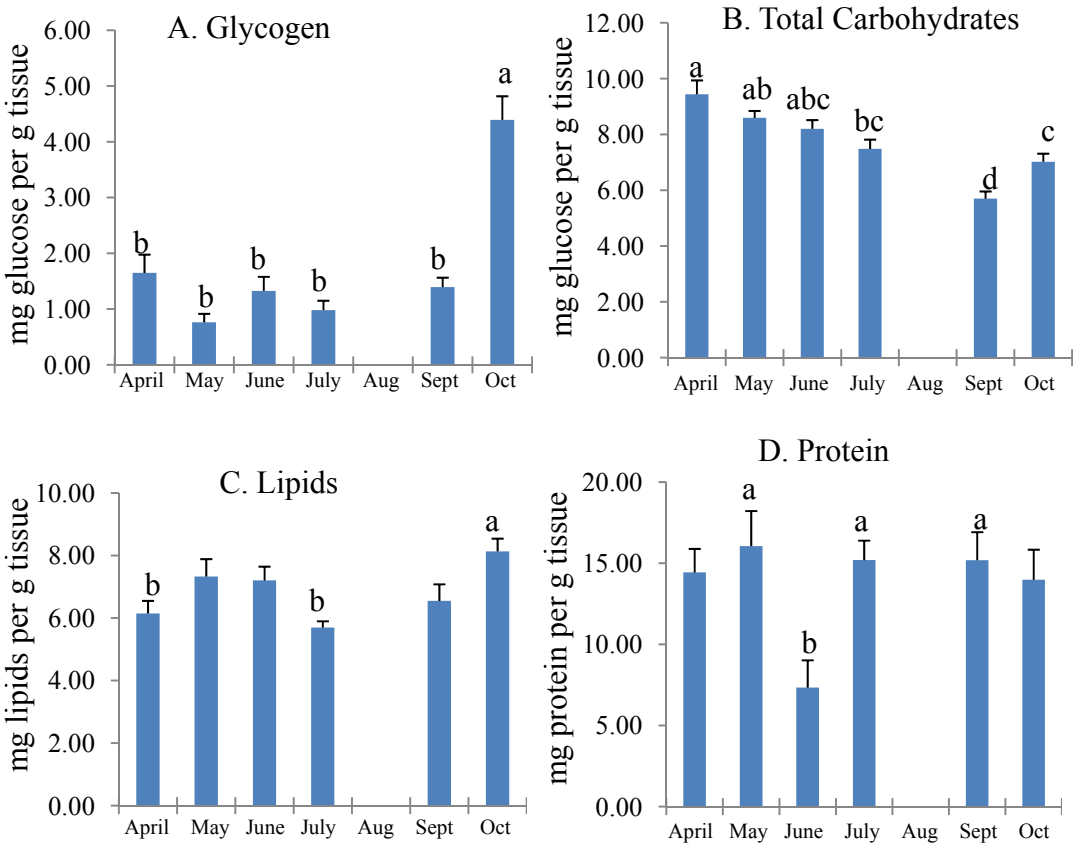


Figure 10: A-D, Glycogen (A), Total Carbohydrate (B), Lipid (C) and Protein (D) levels of surfclam gills in 2009. No samples were collected in August. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

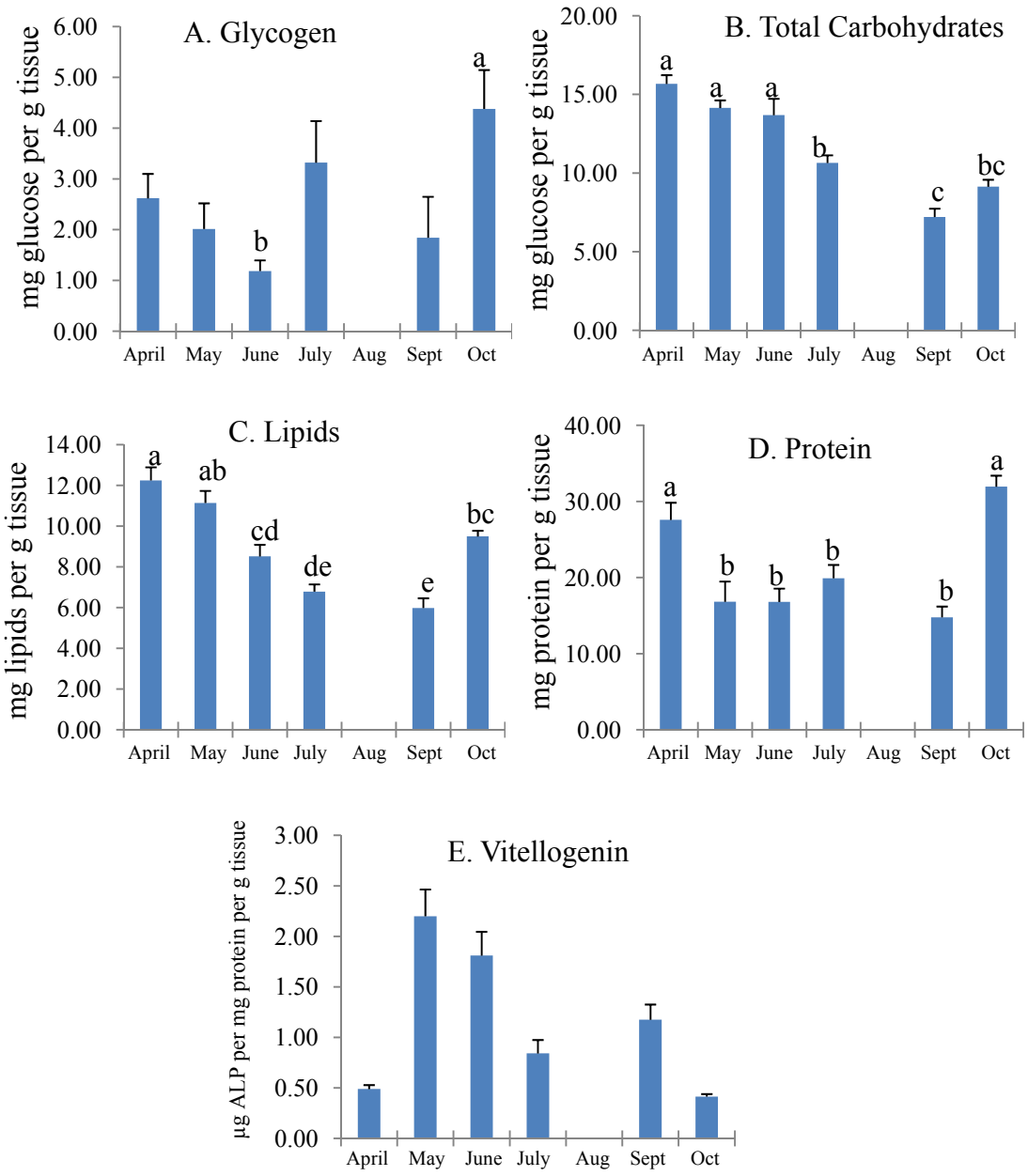


Figure 11: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of surfclam gonads in 2009. No samples were collected in August. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

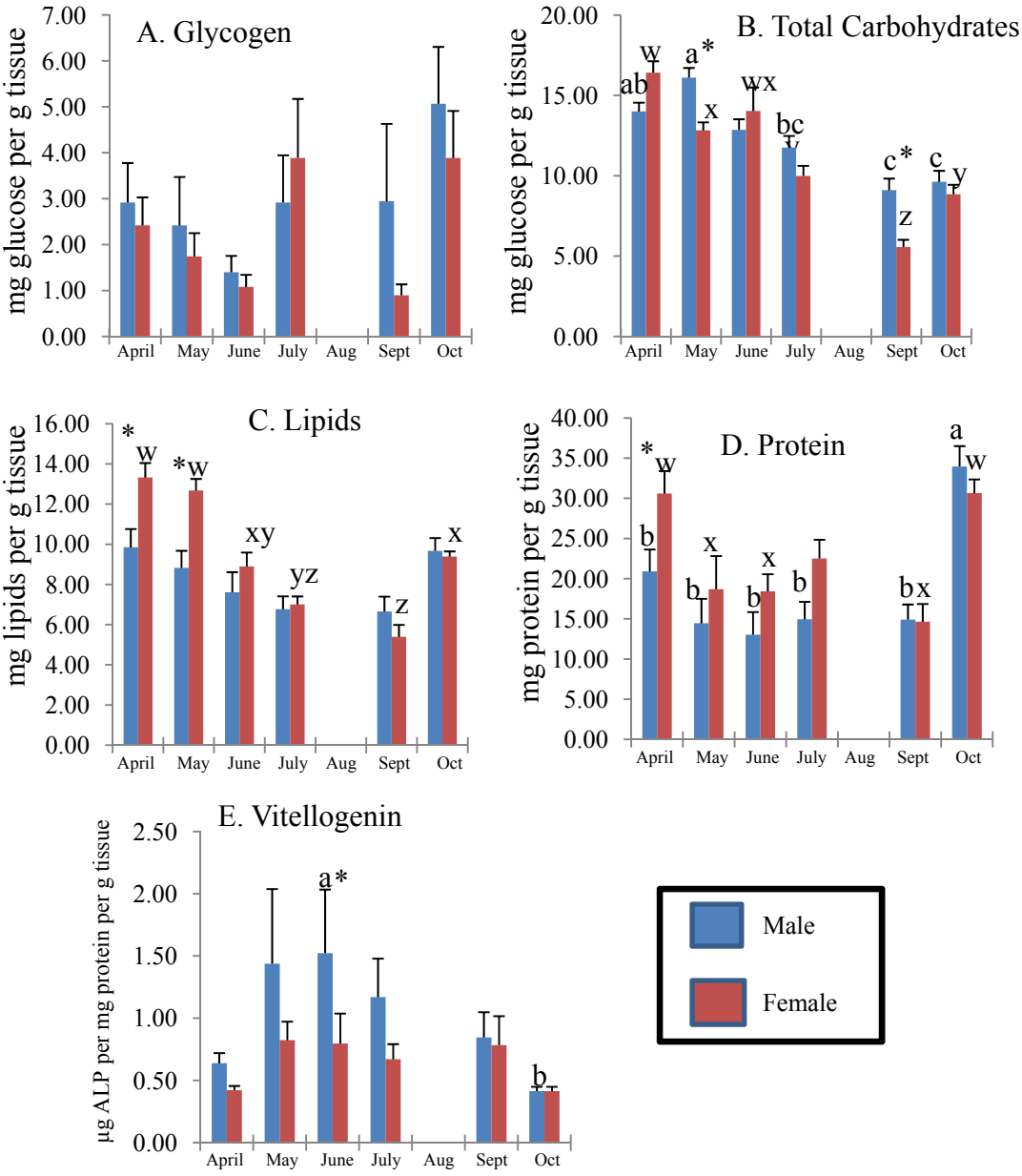


Figure 12: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of male and female surfclam gonads in 2009. No samples were collected in August. An asterisk (*) indicates significance between sexes within a month. Letters indicate significance between males (a,b,c,d) and females (w,x,y,z) between months. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

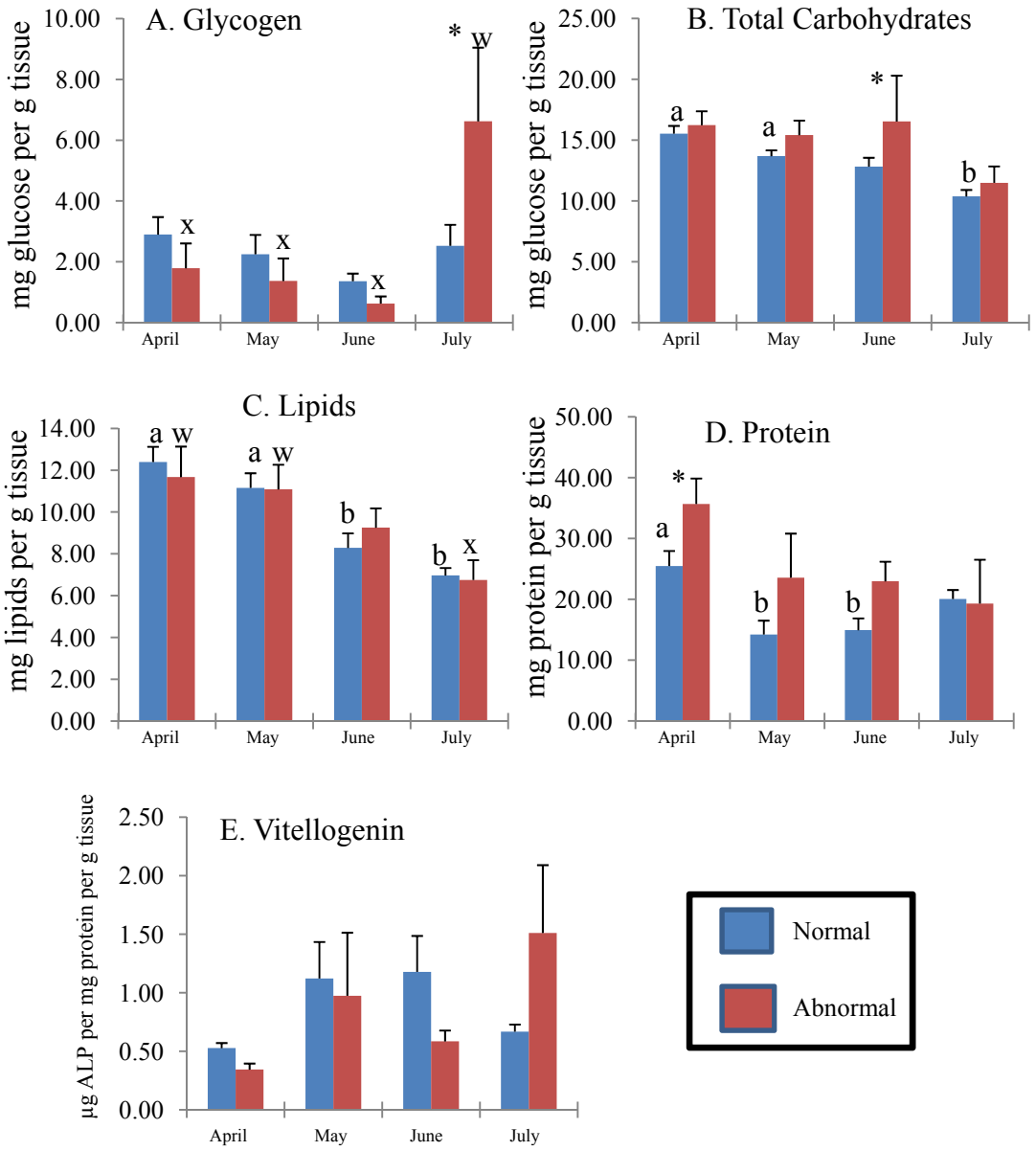


Figure 13: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of normal and abnormal surfclam gonads in 2009. No samples were collected in August and no abnormal clams were observed in September or October. An asterisk (*) indicates significance between groups within a month. Letters indicate significance between normal (a,b,c,d) and abnormal individuals (w, x,y,z) between months. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

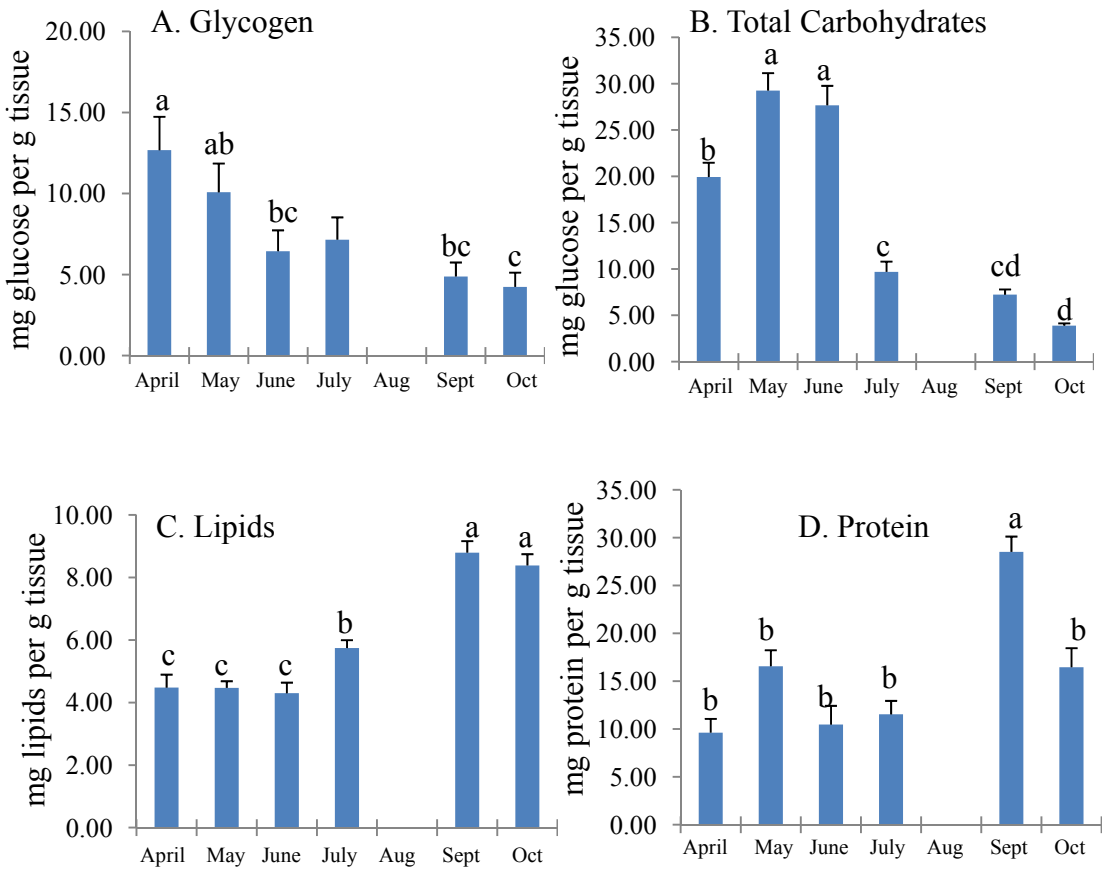


Figure 14: A-D, Glycogen (A), Total Carbohydrate (B), Lipid (C) and Protein (D) levels of the surfclam mantle in 2009. No samples were collected in August. Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

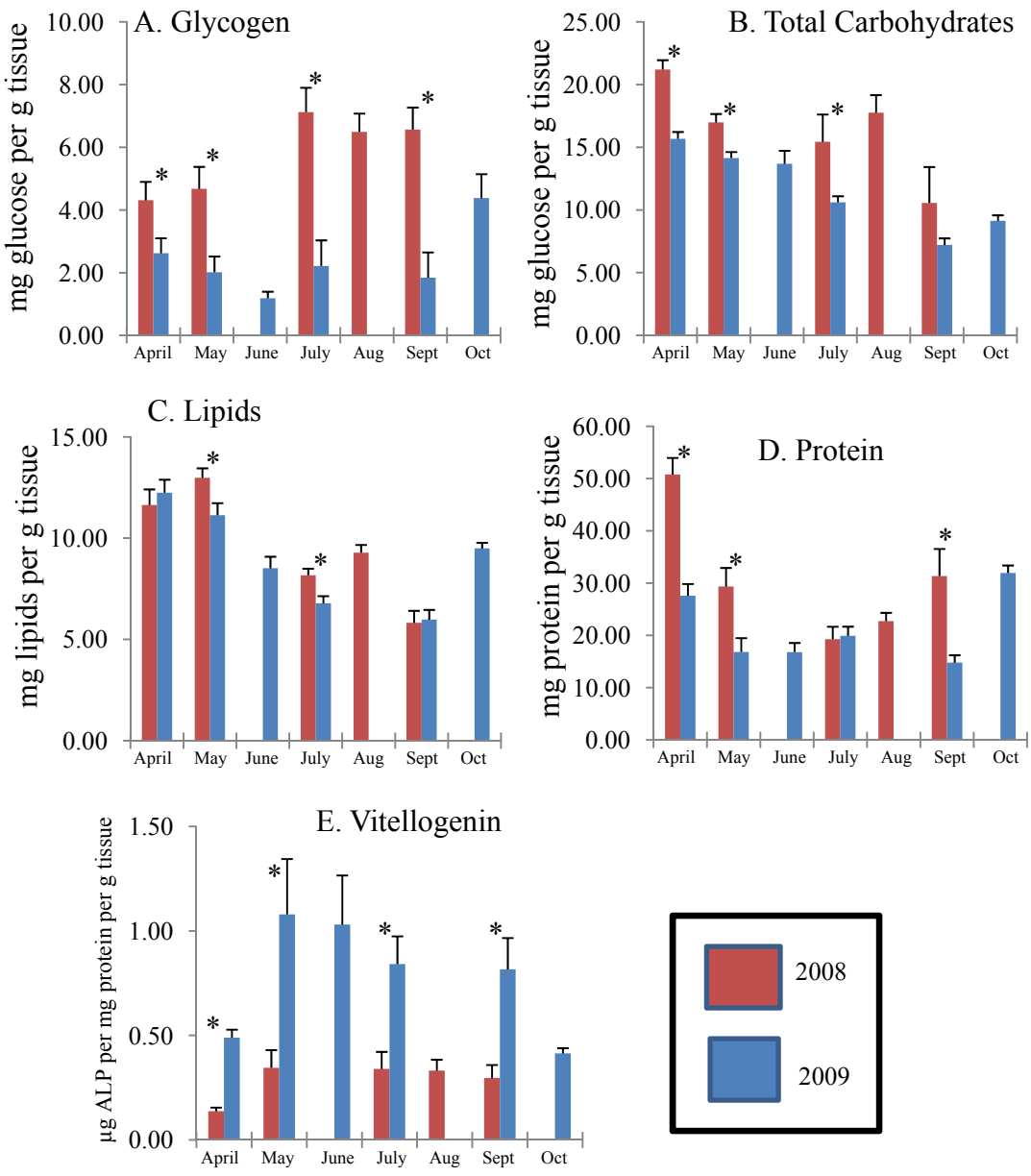


Figure 15: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of surfclam gonads in 2008 and 2009. Each value represents the mean + SE. An asterisk (*) indicates significance between years within a month.

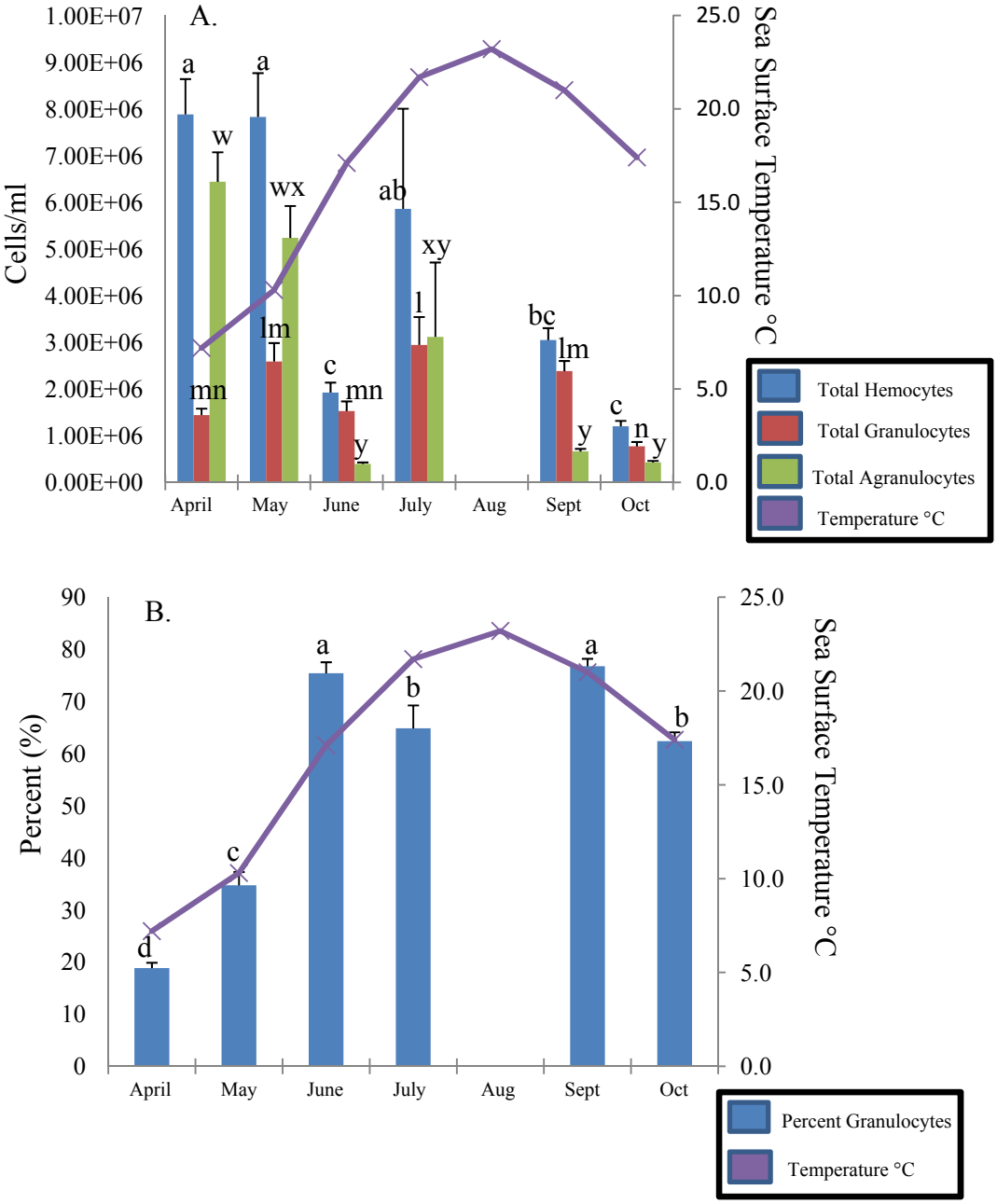


Figure 16: A-B, Differential hemocyte counts (A) and the percent granulocytes (B) from field samples in 2009. Letters indicate significance between Total hemocytes (a,b,c,d), Total Granulocytes (l,m,n,o),Total Agranulocytes (w, x,y,z), and Percent Granulocytes (a,b,c,d). Each value represents the mean + SE. Samples not sharing a common letter are significantly different $p<0.05$.

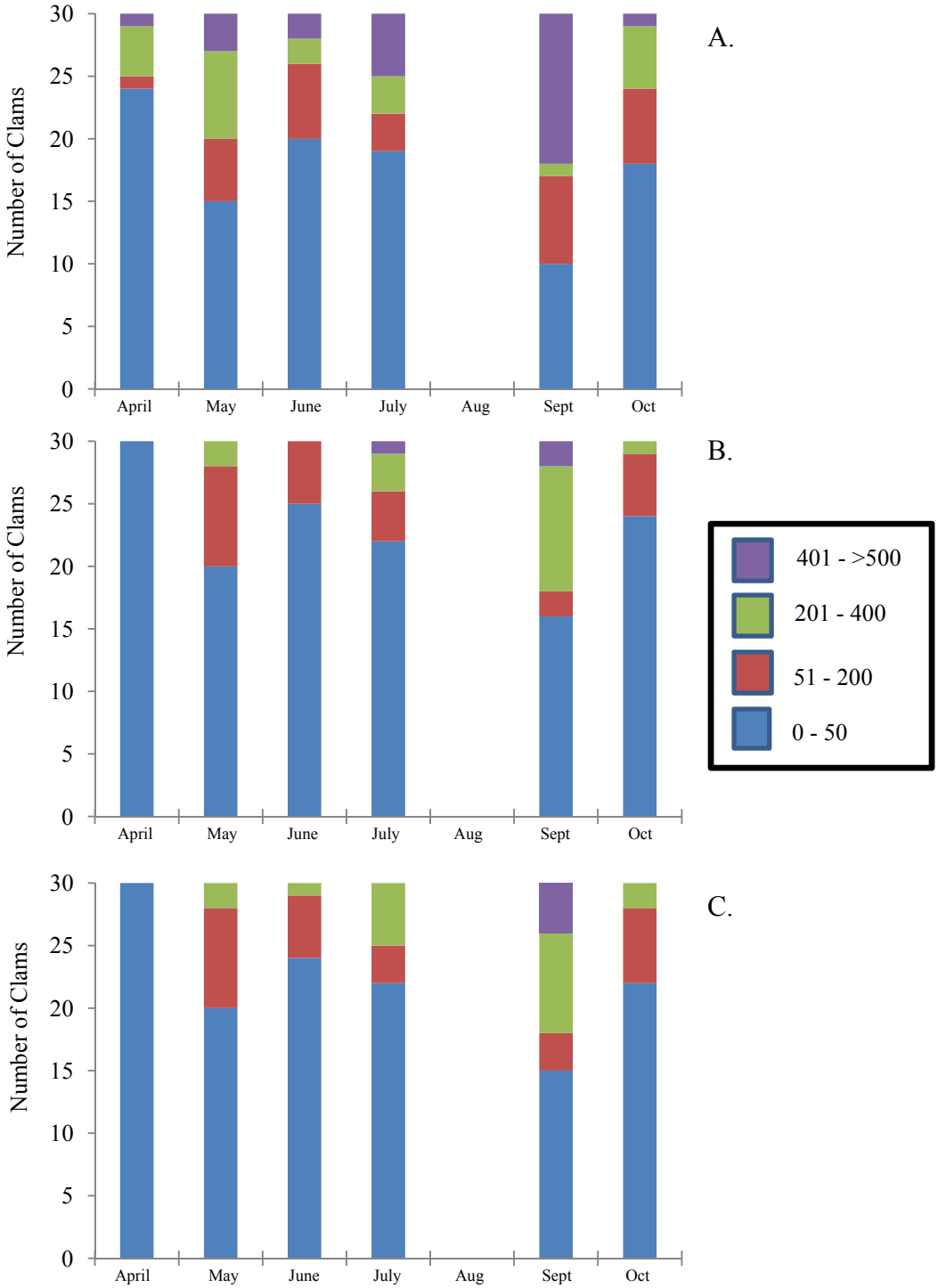


Figure 17: Bacteria counts (per 100µl hemolymph) from the field on Marine agar (A.), TCBS (B.), and CHROMagar (C.) medias.

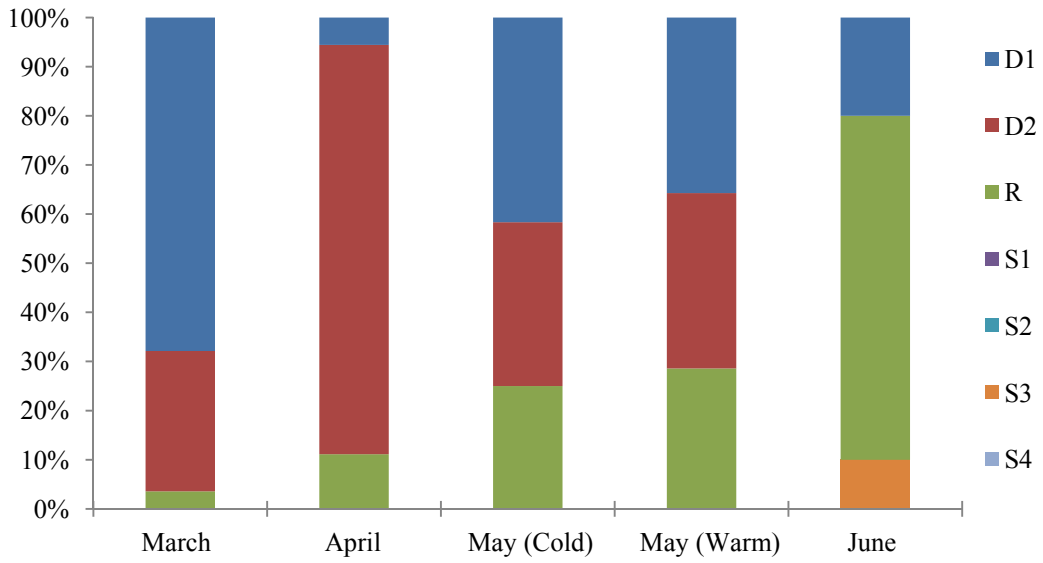


Figure 18: Gametogenic stages of clams from the impact of temperature on gonadal development study. (D1=Developing Stage I, D2=Developing Stage II, R=Ripe, S1=Spawning Stage I, S2=Spawning Stage II, S3=Spawning Stage III, S4=Spawning Stage IV).

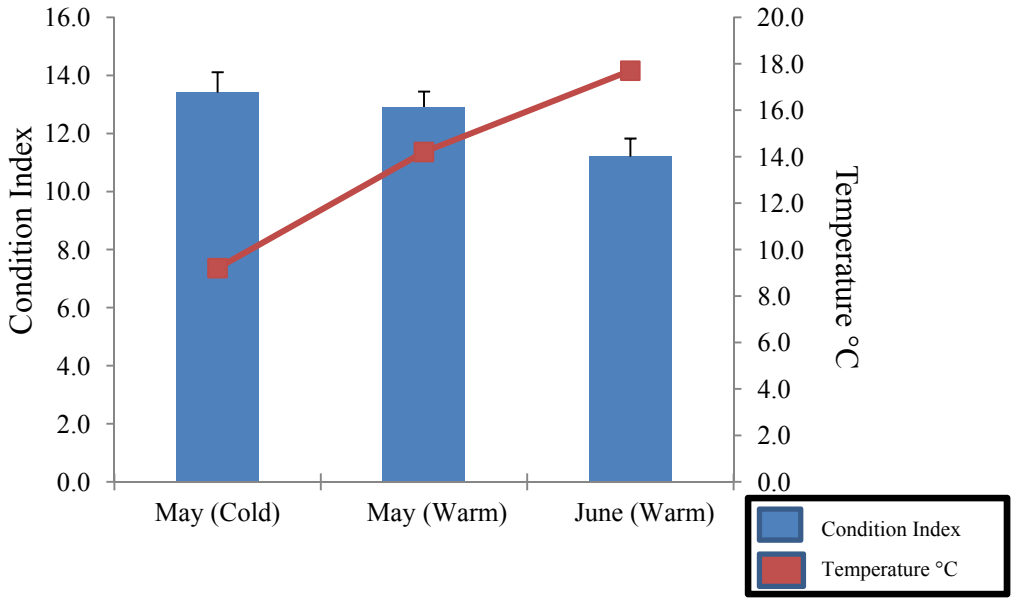


Figure 19: Condition index of clams in the cold and warm treatments from the impact of temperature on gonadal development study. Each value represents the mean + SE.

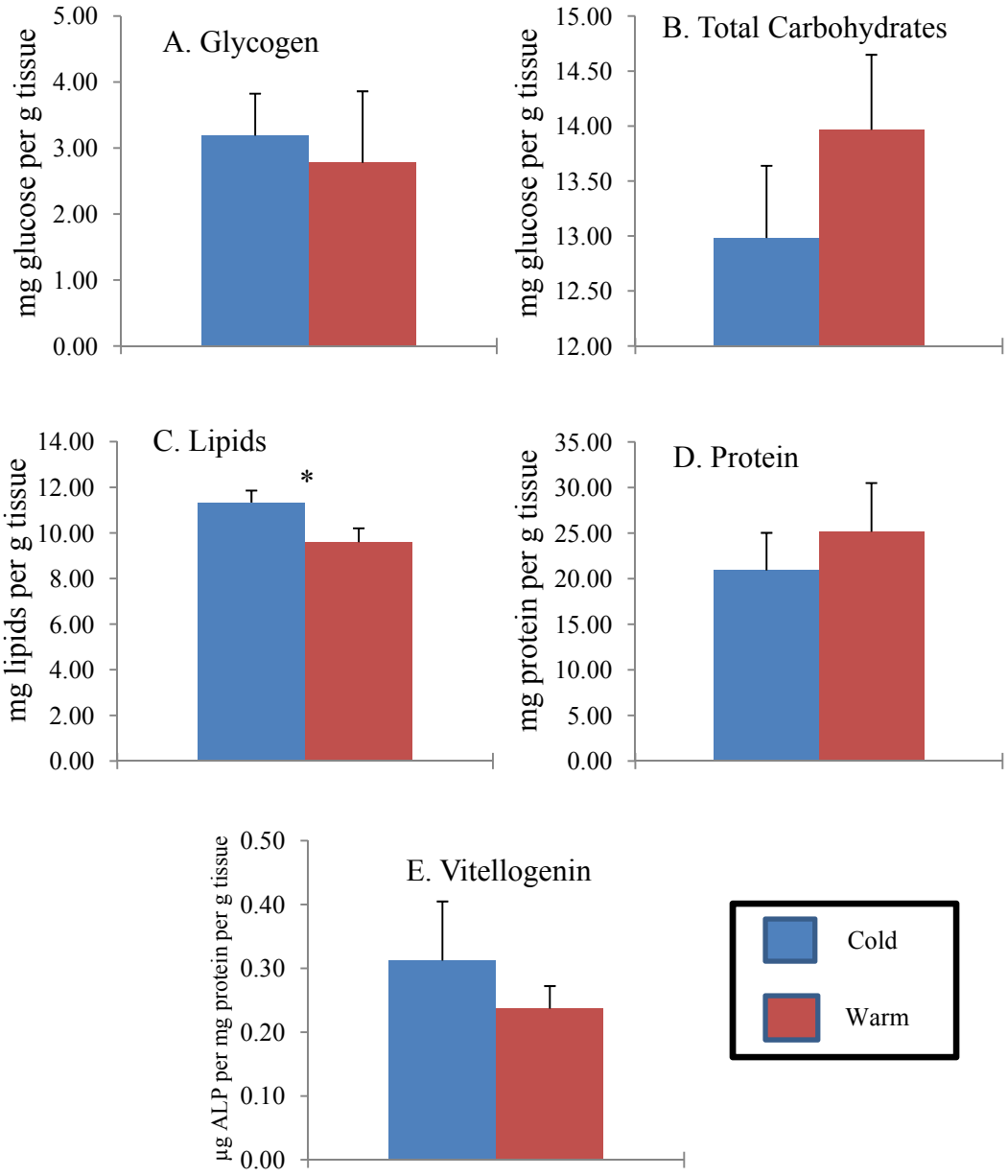


Figure 20: A-E, , Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of surfclam gonads from the impact of temperature on gonadal development experiment. The first biochemical measurements taken from the experiment occurred in May. Due to unexplained mortalities no further data is displayed. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments within a month.

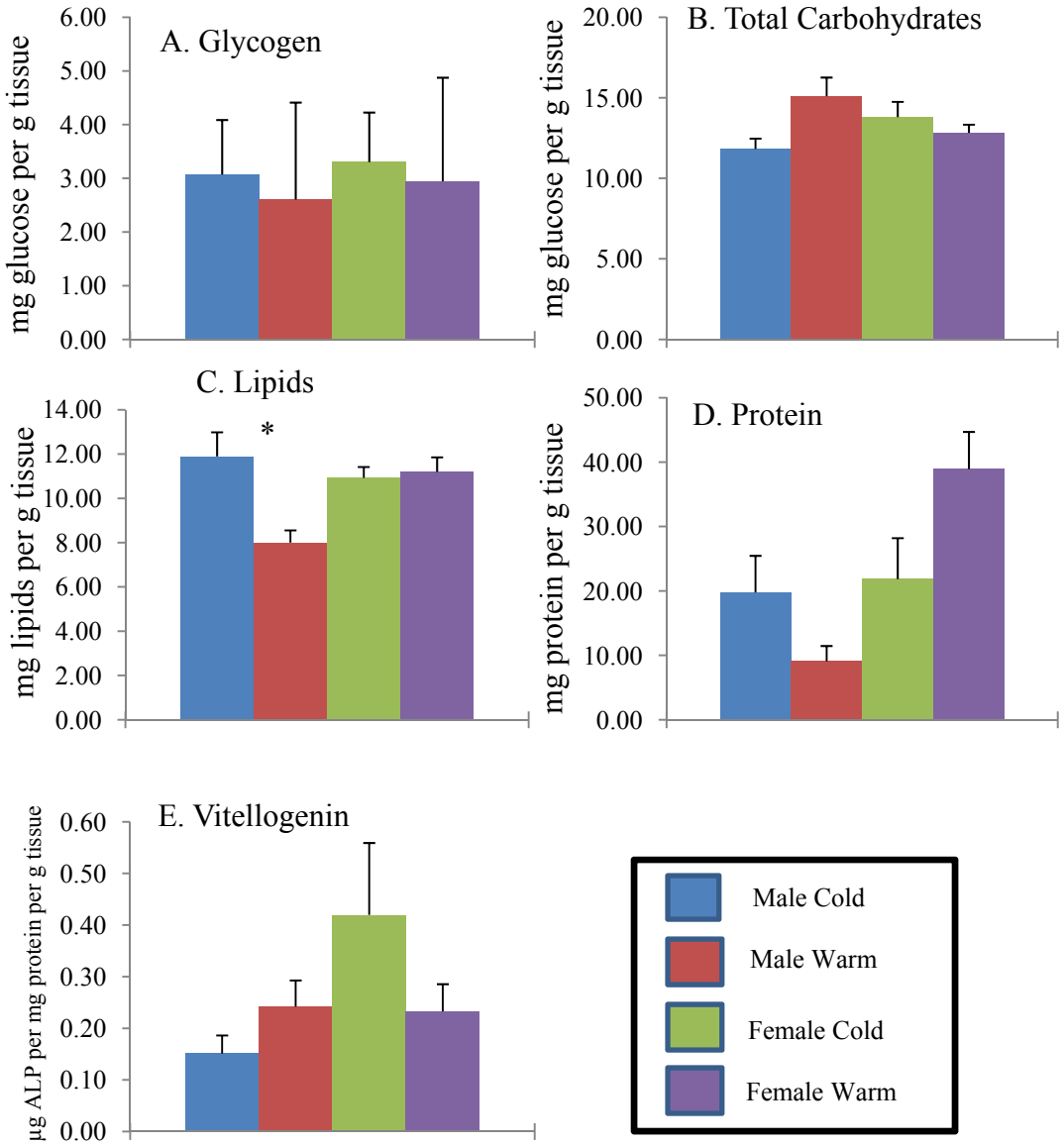


Figure 21: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of male and female surfclam gonads from the impact of temperature on gonadal growth experiment. The first biochemical measurements taken from the experiment occurred in May. Due to unexplained mortalities no further data is displayed. Each value represents the mean + SE. An asterisk (*) indicates significance within a sex between treatments.

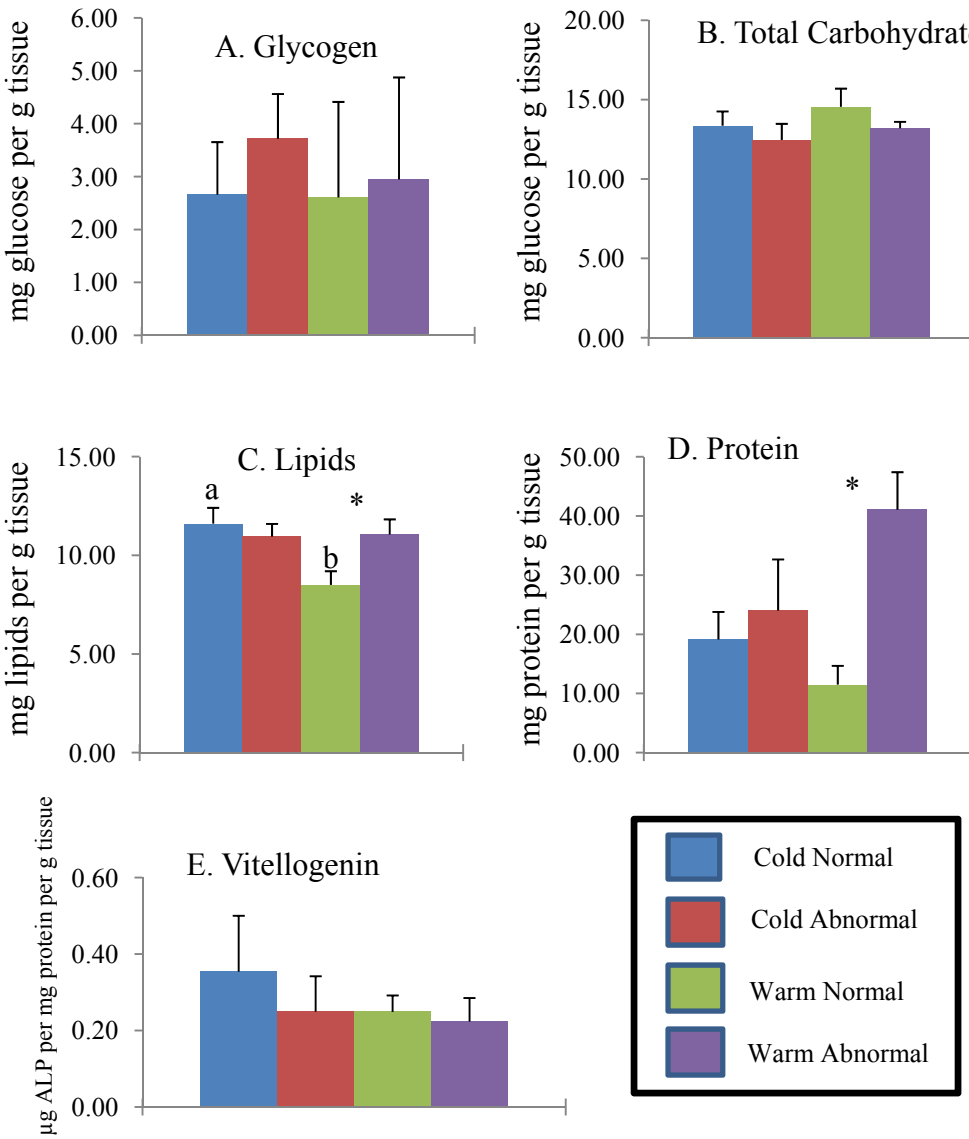


Figure 22: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of surfclam gonads in normal and abnormal clams from the impact of temperature on gonadal development experiment. The first biochemical measurements taken from the experiment occurred in May. Due to unexplained mortalities no further data is displayed. Each value represents the mean + SE. An asterisk (*) indicates significance within a treatment between groups. Letters indicate significance between normal individuals between treatments (a,b). Samples not sharing a common letter are significantly different $p<0.05$.

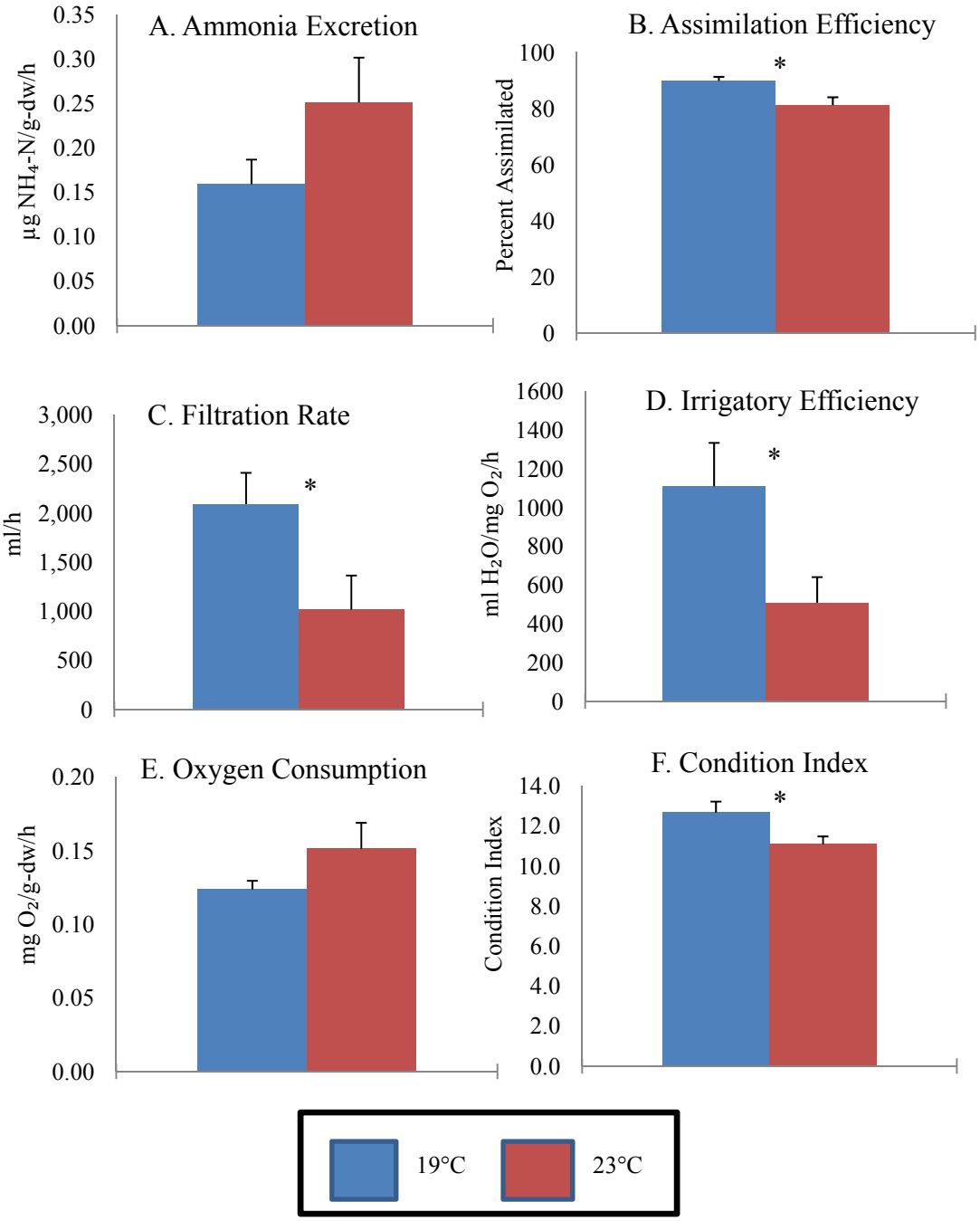


Figure 23: A-H, Physiological measurements made during the scope for growth study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments. The figure is continued on the next page.

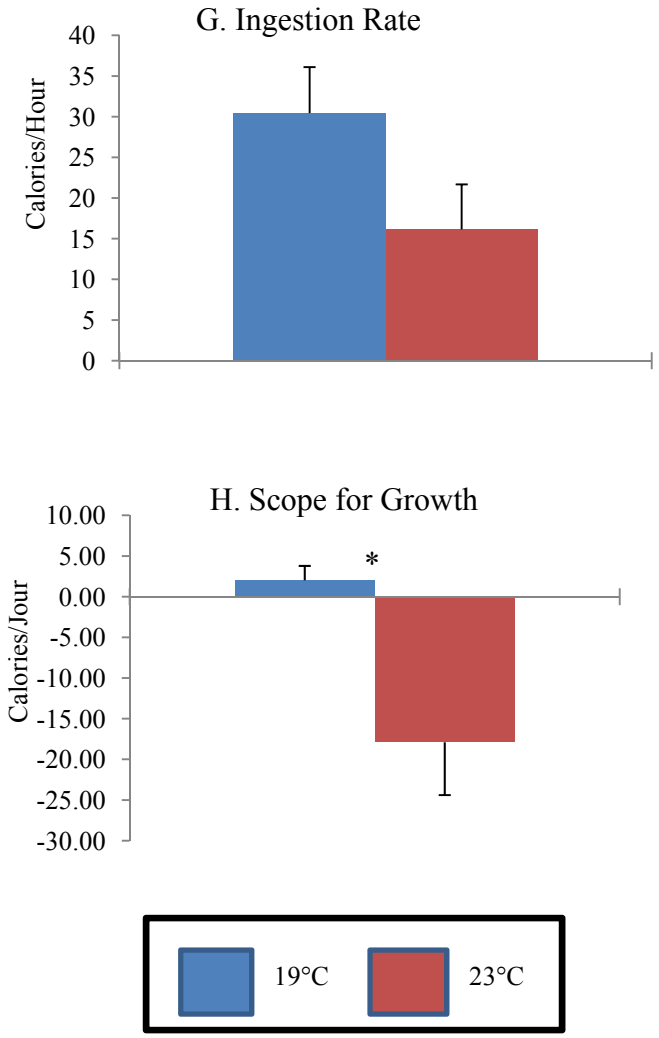


Figure 23 (continued): A-H, Physiological measurements made during the scope for growth study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

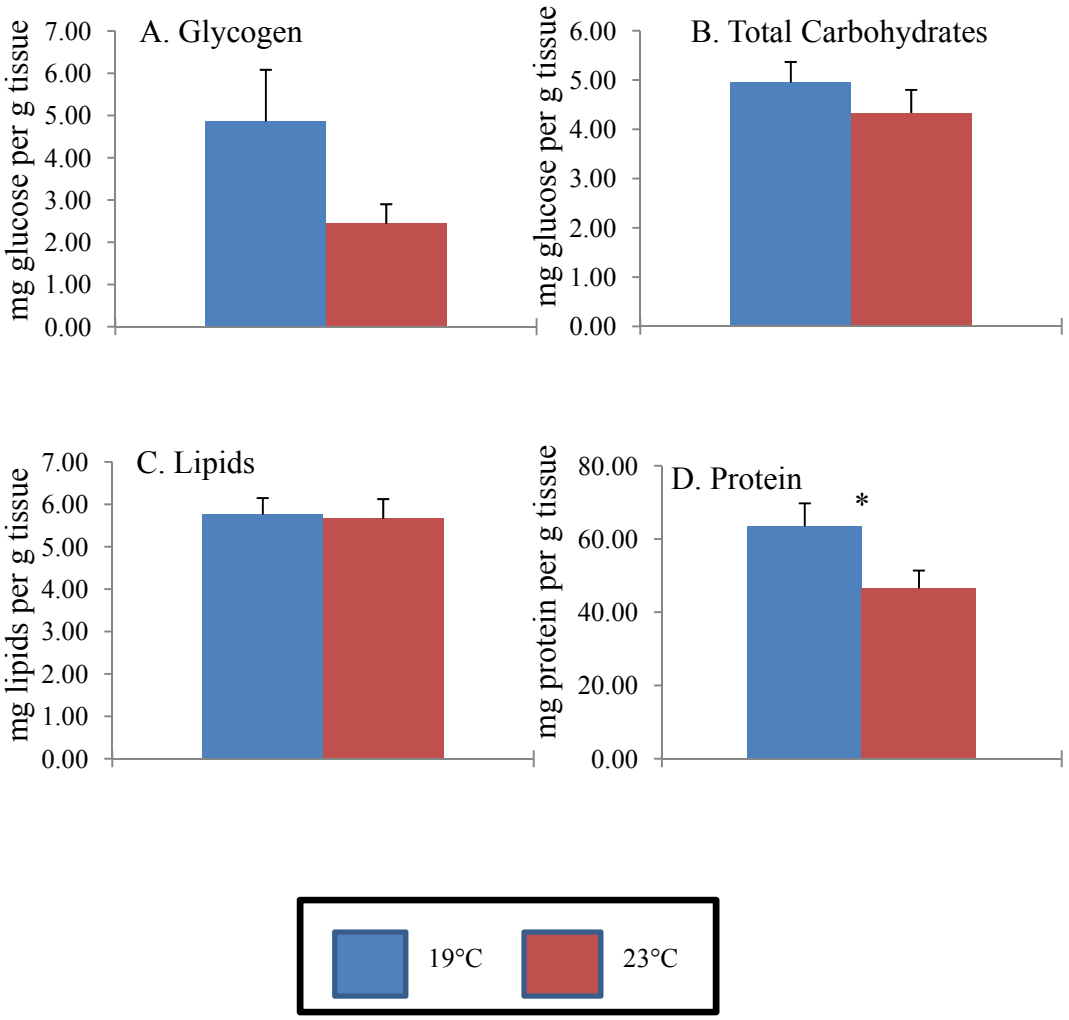


Figure 24: A-D, Glycogen (A), Total Carbohydrate (B), Lipid (C), and Protein (D) levels of surfclam adductor muscles from the short term energy balance study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

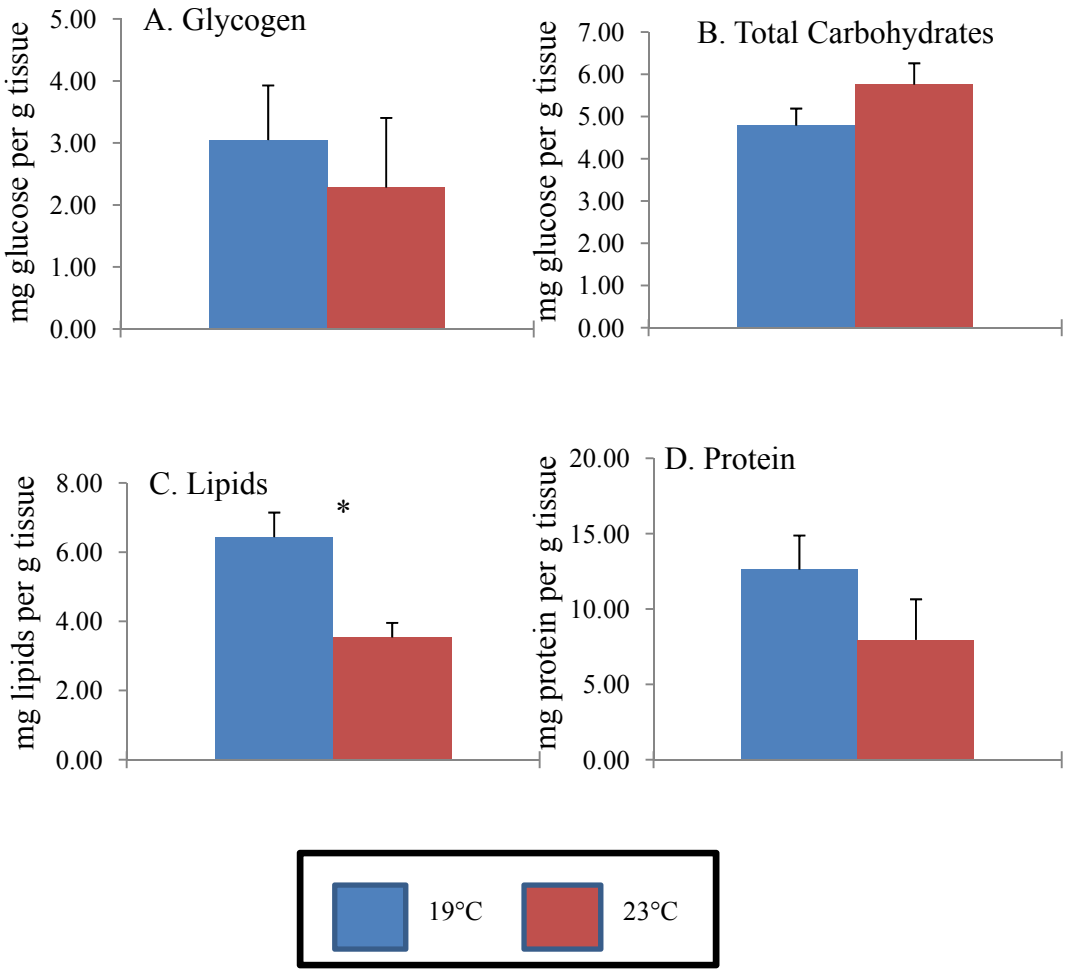


Figure 25: A-D, Glycogen (A), Total Carbohydrate (B), Lipid (C), and Protein (D) levels of surfclam gills from the short term energy balance study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

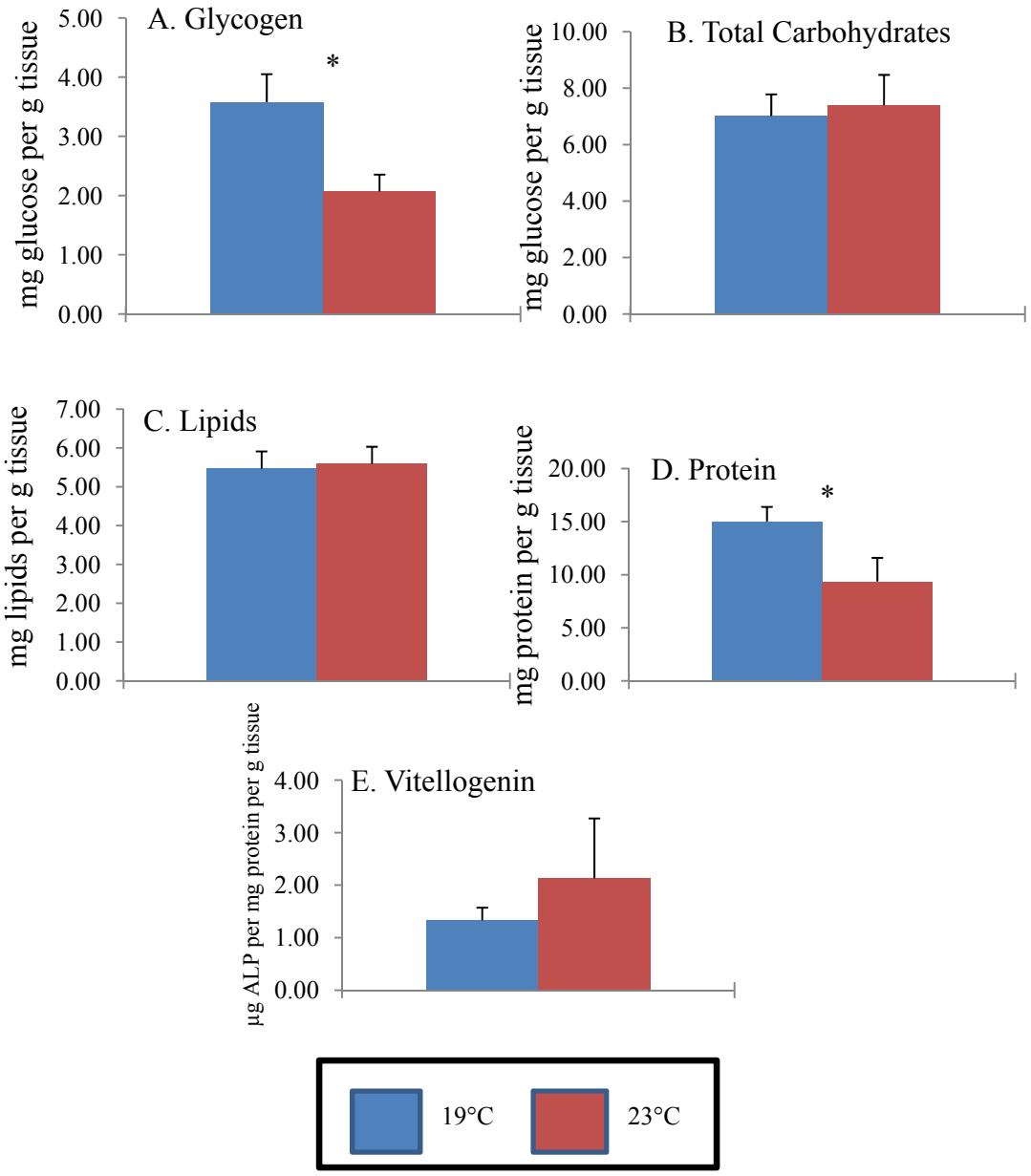


Figure 26: A-E, Glycogen (A), Total Carbohydrate (B), Lipid (C), Protein (D) and Vitellogenin (E) levels of surfclam gonads from the short term energy balance study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

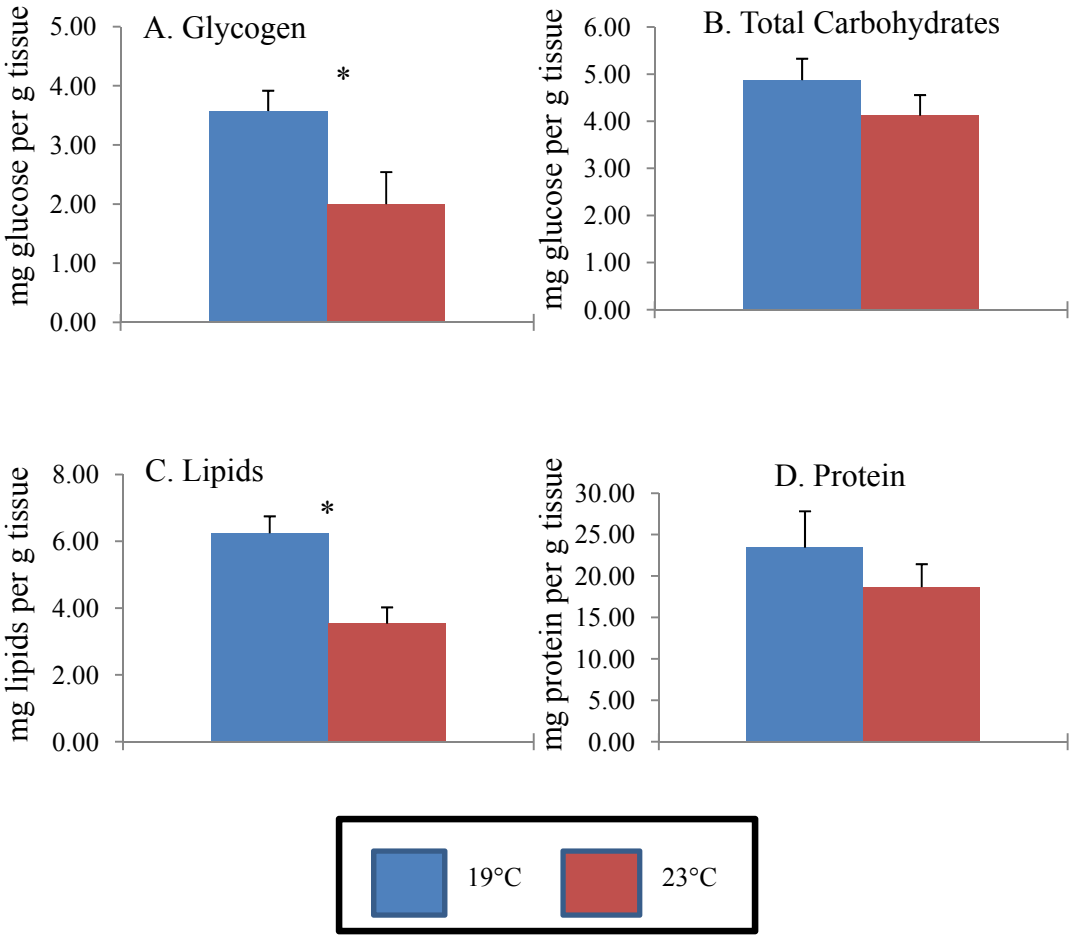


Figure 27: A-D, Glycogen (A), Total Carbohydrate (B), Lipid (C), and Protein (D) levels of the surfclam mantle from the short term energy balance study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

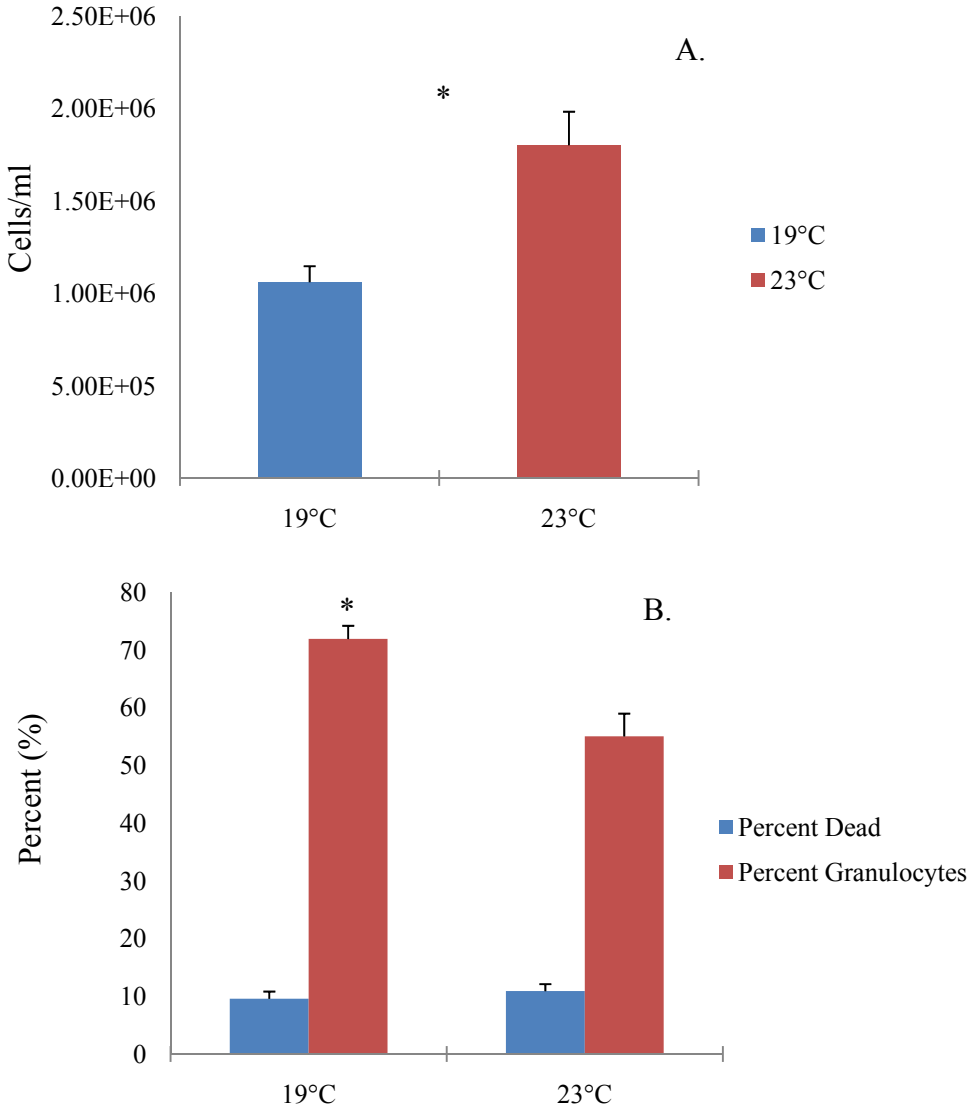


Figure 28: A-B, Total hemocyte counts (A) and the percent dead and granulocytes (B) from the immune defense study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

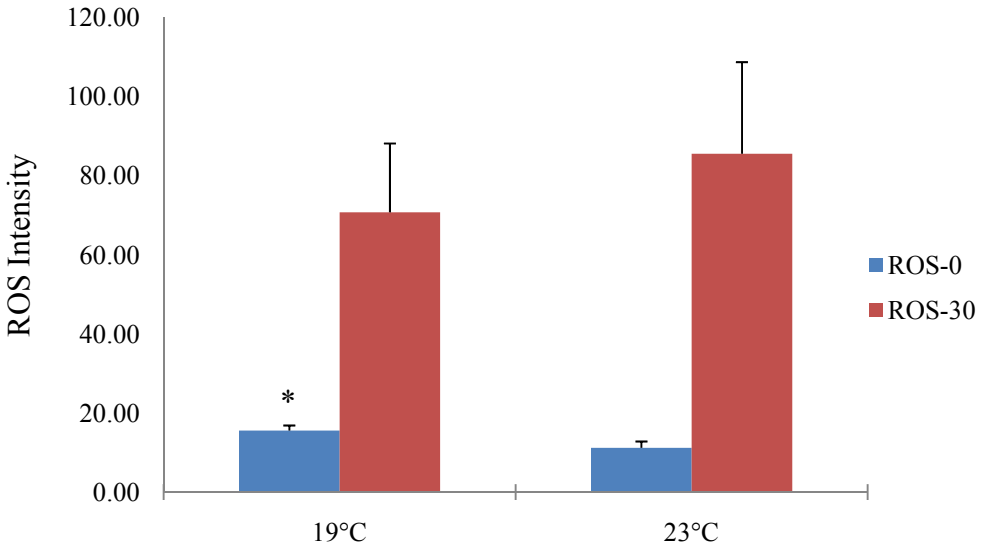


Figure 29: Production of reactive oxygen species in un-stimulated (ROS-0) and zymosan-activated (ROS-30) hemocytes from surfclams submitted to the cold or warm treatment of the immune defense study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

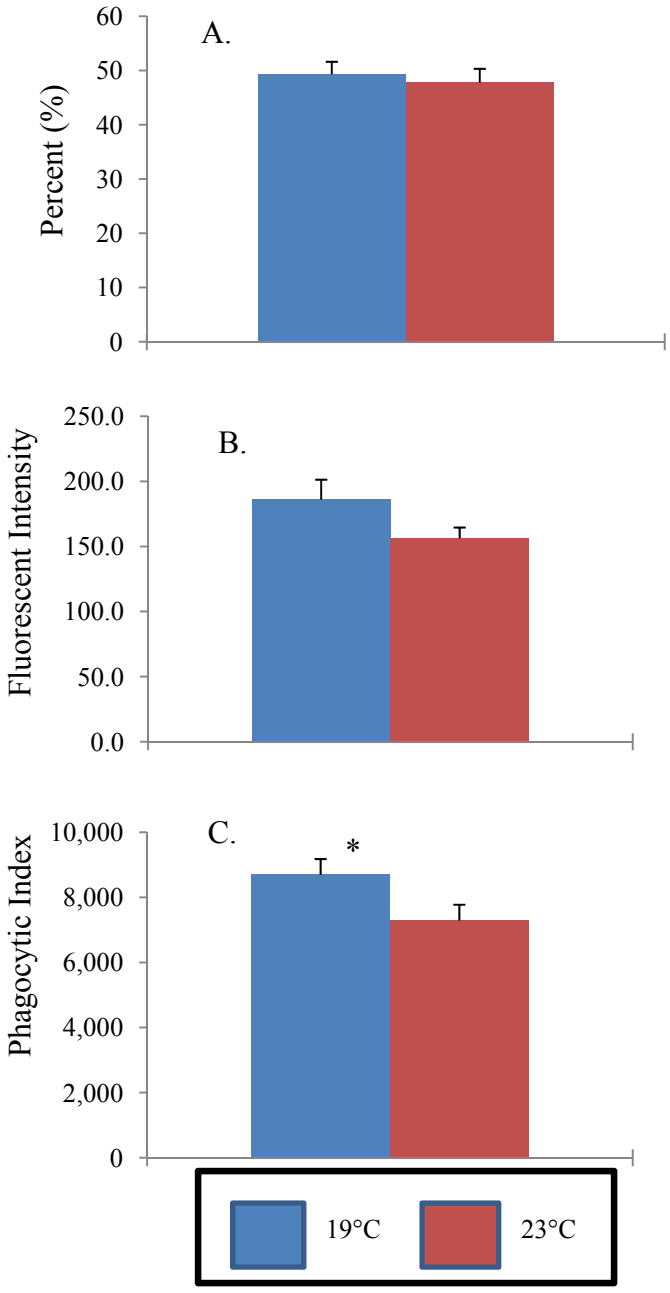


Figure 30: A-C, The proportion of phagocytic hemocytes (A), the fluorescent intensity of the phagocytic hemocytes (B) and the phagocytic index (C) of clams from the immune defense study. Each value represents the mean + SE. An asterisk (*) indicates significance between treatments.

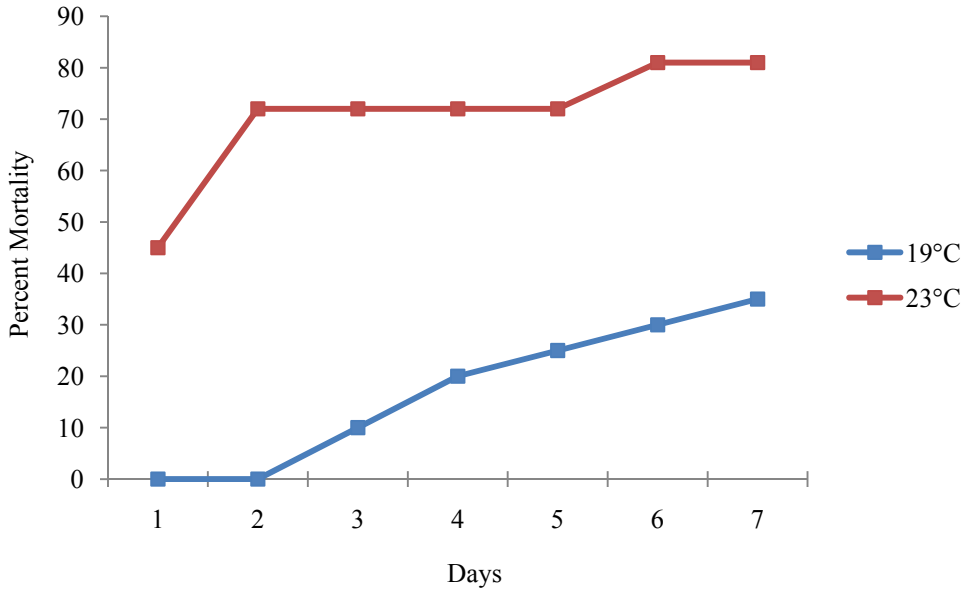
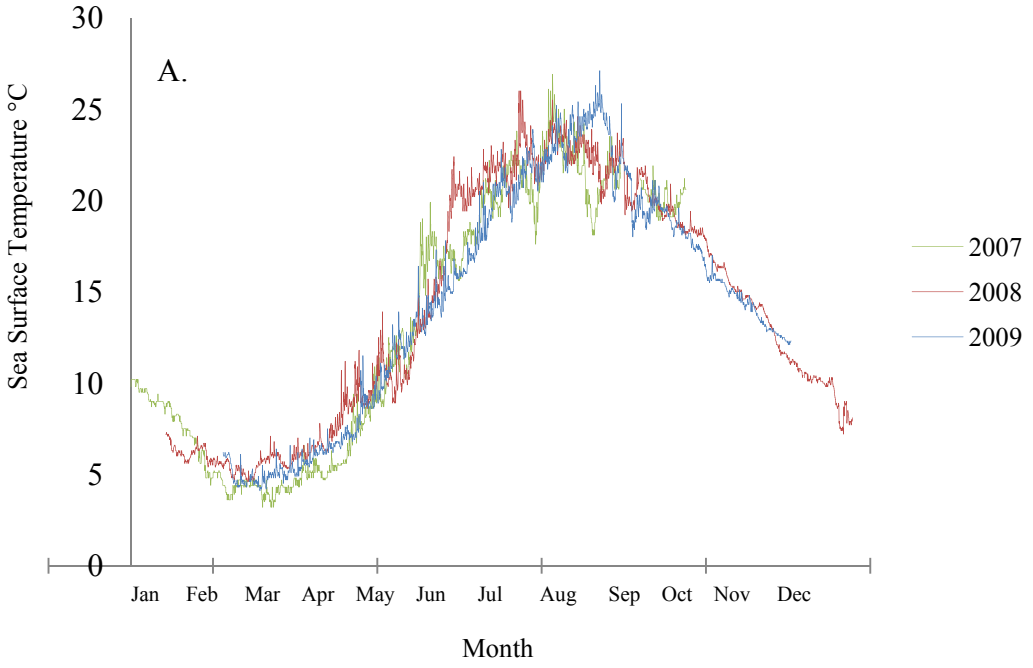


Figure 31: Percent mortality post challenge from the immune defense study.



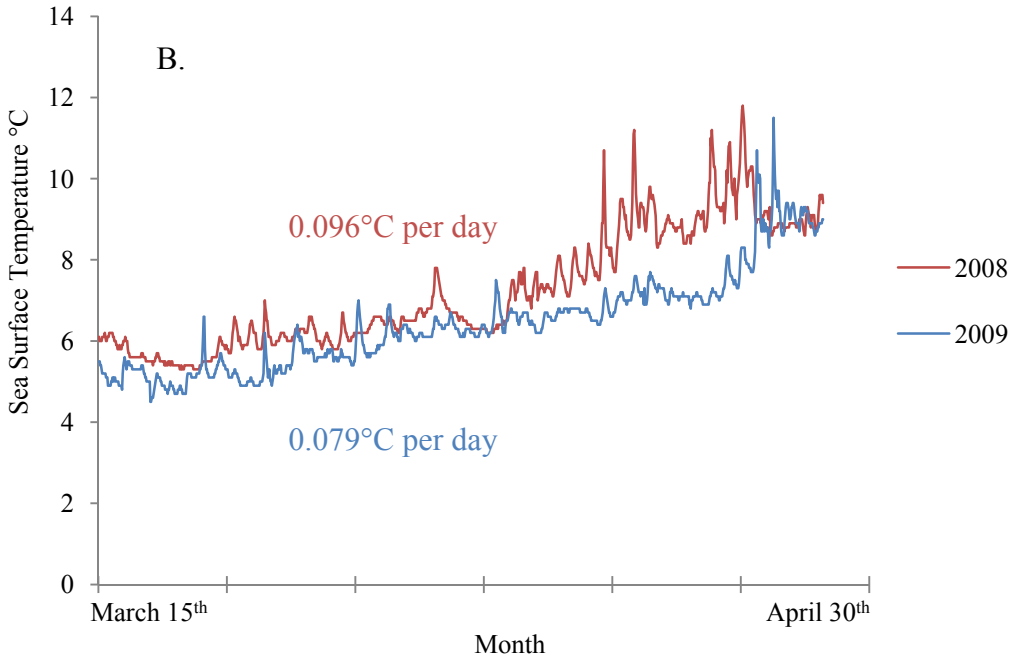


Figure 32: A-B, Sea surface temperature in 2007, 2008 and 2009 for the entire year (A) and late winter through early spring (B).

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Appendix A: A summary of gonad conditions detected in processed animals is presented below. Clams are ranked as belonging to one of the following 10 gonadal stages (modified from Kim and Powell, 2004):

I: Inactive (no gonadal activity, resting)
G1: Gametogenic I stage; gametogenesis has begun; no ripe gametes visible
G2: Gametogenic II stage; first ripe gametes appeared; gonad developed to about one-third of its final size.
D1: Developing I stage; Gonad increased in mass to about half the full ripe condition; each follicle contains about equal proportions of ripe and developing gametes.
D2: Developing II stage; Gametogenesis still progressing; follicles mainly contain ripe gametes.
R: Ripe stage; Gonad fully ripe, early stages of gametogenesis rare; follicles distended with ripe gametes; ova compacted into polygonal configurations; sperm with visible tails.
S1: Spawning stage I; Active emission of gametes has begun; gamete density reduced.
S2: Spawning stage II; Gonad about half empty.
S3: Spawning stage III; Gonadal area reduced; follicles about one-third full of ripe gametes.
S4: Spawning stage IV; Only residual gametes remain; some may be undergoing cytolysis.

Appendix B: Semi-quantitative scale used for the evaluation of abnormal gonadal development (Kim and Powell, 2004).

Score	Description
0	Normal Gonad.
1	Less than half the follicles are affected.
2	About half the follicles are affected.
3	More than half the follicles are affected.
4	All follicles are affected.

Appendix C: Condition index of normal and abnormal clams in 2008 and 2009.

	2008				2009			
	Normal	St Error	Abnormal	St Error	Normal	St Error	Abnormal	St Error
March	20.48	0.48	20.79	0.44	ND	ND	ND	ND
April	19.07	0.59	19.44	1.52	19.68	0.71	21.60	1.24
May	19.64	0.41	18.91	0.54	17.54	0.36	17.43	0.83
June	ND	ND	ND	ND	11.81	0.37	11.53	0.44
July	13.16	0.47	14.40	0.23	10.40	0.32	10.21	0.71
August	12.74	0.28	14.24	1.48	ND	ND	ND	ND

Appendix D: Glycogen, Total Carbohydrate, Lipid and Protein levels of male and female surfclam adductor muscles in 2009.

Adductor Muscle								
	Glycogen				Total Carbohydrates			
	Male	St Error	Female	St Error	Male	St Error	Female	St Error
April	8.29	2.74	7.75	1.35	19.81	2.66	24.49	1.60
May	9.68	2.72	12.27	3.46	30.86	4.04	34.78	2.78
June	1.64	0.34	1.85	0.54	28.67	3.48	32.94	2.60
July	12.01	2.46	15.95	1.29	10.38	2.22	10.51	1.19
August	ND	ND	ND	ND	ND	ND	ND	ND
September	5.57	2.08	6.42	2.34	9.66	0.94	8.90	0.72
October	18.75	1.92	16.21	1.12	9.43	0.94	8.42	0.67
	Lipid				Protein			
	Male	St Error	Female	St Error	Male	St Error	Female	St Error
April	4.42	0.49	4.44	0.35	43.34	5.44	41.86	3.03
May	6.33	1.01	7.96	1.14	26.63	3.08	24.48	2.87
June	5.98	0.74	6.30	0.45	24.03	2.10	26.30	3.02
July	5.62	0.52	5.98	0.35	27.66	1.79	31.87	1.63
August	ND	ND	ND	ND	ND	ND	ND	ND
September	4.24	0.29	3.67	0.22	43.22	1.77	59.95	7.25
October	5.06	0.24	4.41	0.35	39.45	9.65	43.85	7.33

Appendix E: Glycogen, Total Carbohydrate, Lipid and Protein levels of normal and abnormal surfclam adductor muscles in 2009.

Adductor Muscle								
	Glycogen				Total Carbohydrates			
	Normal	St Error	Abnormal	St Error	Normal	St Error	Abnormal	St Error
April	8.09	1.51	7.31	2.11	23.26	1.63	22.42	2.89
May	12.39	2.77	6.67	1.28	31.11	2.73	39.00	3.85
June	1.45	0.27	3.04	1.47	32.77	2.46	28.01	3.90
July	14.46	1.26	15.74	3.79	10.49	1.13	10.39	3.04
	Lipid				Protein			
	Normal	St Error	Abnormal	St Error	Normal	St Error	Abnormal	St Error
April	4.47	0.31	4.27	0.66	43.01	2.81	39.47	7.25
May	7.60	1.01	6.52	1.16	25.70	1.95	24.34	6.06
June	6.41	0.48	5.55	0.34	25.19	2.75	27.03	2.76
July	5.97	0.31	5.42	0.76	30.07	1.24	31.77	4.18

Appendix F: Glycogen, Total Carbohydrate, Lipid and Protein levels of male and female surfclam gills in 2009.

Gill								
	Glycogen				Total Carbohydrates			
	Male	St Error	Female	St Error	Male	St Error	Female	St Error
April	1.00	0.46	1.81	0.39	9.28	0.94	9.51	0.60
May	0.76	0.17	0.77	0.27	8.16	0.41	8.88	0.31
June	1.10	0.23	1.41	0.33	8.79	0.72	7.95	0.33
July	0.88	0.29	1.07	0.23	7.70	0.36	7.39	0.50
August	ND	ND	ND	ND	ND	ND	ND	ND
September	1.43	0.27	1.36	0.21	5.71	0.38	5.69	0.36
October	4.03	0.47	4.60	0.62	7.63	0.49	6.67	0.34
	Lipid				Protein			
	Male	St Error	Female	St Error	Male	St Error	Female	St Error
April	5.69	0.37	6.34	0.55	13.71	1.95	14.77	1.94
May	7.63	0.89	7.13	0.73	14.07	2.58	17.63	3.31
June	7.01	0.54	7.28	0.59	5.05	1.80	8.48	2.34
July	5.65	0.19	5.70	0.31	17.23	1.91	14.23	1.59
August	ND	ND	ND	ND	ND	ND	ND	ND
September	6.55	0.46	6.54	0.93	15.02	1.89	15.35	2.90
October	7.80	0.46	8.33	0.59	16.11	3.59	12.86	2.15

Appendix G: Glycogen, Total Carbohydrate, Lipid and Protein levels of normal and abnormal surfclam gills in 2009.

Gill								
	Glycogen				Total Carbohydrates			
	Normal	St Error	Abnormal	St Error	Normal	St Error	Abnormal	St Error
April	2.00	0.40	0.68	0.10	9.11	0.55	10.75	1.10
May	0.58	0.16	1.01	0.27	8.54	0.27	8.73	0.60
June	1.44	0.30	0.80	0.12	8.16	0.36	8.31	0.68
July	0.93	0.16	1.60	0.70	7.34	0.35	8.08	1.06
	Lipid				Protein			
	Normal	St Error	Abnormal	St Error	Normal	St Error	Abnormal	St Error
April	6.08	0.49	6.41	0.55	13.05	1.48	19.74	3.55
May	7.53	0.66	6.78	1.06	14.20	2.11	20.85	5.81
June	7.55	0.52	6.05	0.74	6.74	1.58	8.88	4.81
July	5.73	0.26	5.51	0.28	14.55	1.38	18.01	2.81

Appendix H: Glycogen, Total Carbohydrate, Lipid and Protein levels of male and female surfclam mantles in 2009.

Mantle								
	Glycogen				Total Carbohydrates			
	Male	St Error	Female	St Error	Male	St Error	Female	St Error
April	10.98	5.12	13.21	2.25	22.05	2.20	19.03	1.98
May	9.13	2.42	10.63	2.50	32.39	3.30	27.17	2.19
June	4.56	2.65	7.17	1.49	31.51	4.94	26.02	2.13
July	5.68	1.27	8.35	2.14	9.12	1.03	10.14	1.68
August	ND	ND	ND	ND	ND	ND	ND	ND
September	4.78	1.09	4.97	1.35	7.14	0.65	7.31	0.92
October	4.39	1.49	4.15	1.12	3.72	0.50	3.98	0.27
	Lipid				Protein			
	Male	St Error	Female	St Error	Male	St Error	Female	St Error
April	4.12	0.92	4.64	0.45	6.92	2.52	10.64	1.72
May	4.79	0.41	4.25	0.23	17.46	2.97	15.97	2.03
June	3.92	0.49	4.46	0.44	8.82	1.98	11.30	2.80
July	5.76	0.41	5.66	0.33	11.68	2.41	10.85	1.86
August	ND	ND	ND	ND	ND	ND	ND	ND
September	9.37	0.51	8.30	0.51	28.37	2.39	28.63	2.22
October	8.86	0.52	8.11	0.48	18.80	3.74	15.11	2.31

Appendix I: Glycogen, Total Carbohydrate, Lipid and Protein levels of normal and abnormal surfclam mantles in 2009.

Mantle								
	Glycogen				Total Carbohydrates			
	Normal	St Error	Abnormal	St Error	Normal	St Error	Abnormal	St Error
April	11.08	2.24	19.05	4.39	20.70	1.67	16.89	3.80
May	6.54	1.65	14.95	2.84	30.19	2.31	26.70	3.17
June	5.73	1.53	8.94	2.10	28.69	2.50	24.31	3.78
July	8.44	1.60	1.91	0.36	8.87	1.22	13.33	2.73
	Lipid				Protein			
	Normal	St Error	Abnormal	St Error	Normal	St Error	Abnormal	St Error
April	4.33	0.45	5.03	1.10	10.14	1.65	7.31	2.91
May	4.62	0.27	4.06	0.26	16.64	1.77	16.37	4.21
June	4.28	0.38	4.35	0.78	11.80	2.13	2.51	1.24
July	5.67	0.32	5.79	0.23	10.34	1.44	13.43	3.83

Appendix J: Histological data from the impact of temperature on gonadal development study.

Date	Gametogenic Stage	% of Total	Abnormal Females	Abnormal Males	Total % Abnormal	%Abnormal Females	Intensity Average
March	D1	68	6	1	25	32	1.67
	D2	29					
	R	4					
April	D1	6	4	0	22	36	1.00
	D2	83					
	R	11					
May (Cold)	D1	42	4	1	42	57	1.80
	D2	33					
	R	25					
May (Warm)	D1	36	6	0	43	86	2.33
	D2	36					
	R	29					
June (Warm)	D1	20	1	0	10	5	4.00
	R	70					
	S3	10					

Appendix K: Statistical tests and results

Field Data				
	Test	**D.F.**	**F**	**p-value**
Percent Seed	One-way ANOVA	4	6.81	<0.001
Condition Index 2008	One-way ANOVA	5	84.38	<0.001
Condition Index 2009	One-way ANOVA	5	91.89	<0.001
Condition Index 2008 vs. 2009	One-way ANOVA	9	66.05	<0.001
Gonad Glycogen 2008	One-way ANOVA	5	3.64	0.004
Gonad Total Carbohydrates 2008	One-way ANOVA	5	3.90	0.002
Gonad Lipids 2008	One-way ANOVA	5	19.62	<0.001
Gonad Proteins 2008	One-way ANOVA	5	12.96	<0.001
Gonad Vitellogenin 2008	One-way ANOVA	5	1.27	0.280
Gonad Glycogen Male vs. Female 2008	Two-way ANOVA			
	Sex	1	7.13	0.009
	Month	5	2.65	0.026
	Sex vs. Month	5	0.48	0.785
Gonad Total Carbohydrates Male vs. Female 2008	Two-way ANOVA			
	Sex	1	0.72	0.397
	Month	5	2.84	0.018
	Sex vs. Month	5	1.31	0.261
Gonad Lipids Male vs. Female 2008	Two-way ANOVA			
	Sex	1	23.25	<0.001
	Month	5	22.18	<0.001
	Sex vs. Month	5	5.95	<0.001
Gonad Proteins Male vs. Female 2008	Two-way ANOVA			
	Sex	1	11.78	<0.001
	Month	5	14.59	<0.001
	Sex vs. Month	5	3.01	0.013
Gonad Vitellogenin Male vs. Female 2008	Two-way ANOVA			
	Sex	1	9.01	0.003
	Month	5	2.61	0.027
	Sex vs. Month	5	3.40	0.006
Gonad Glycogen Normal vs. Abnormal 2008	Two-way ANOVA			
	Normal/Abnormal	1	0.01	0.943
	Month	4	3.11	0.018
	Normal/Abnormal vs. Month	4	0.99	0.416
Gonad Total Carbohydrates Normal vs. Abnormal 2008	Two-way ANOVA			
	Normal/Abnormal	1	0.07	0.779
	Month	4	2.22	0.070
	Normal/Abnormal vs. Month	4	0.17	0.953
Gonad Lipids Normal vs. Abnormal 2008	Two-way ANOVA			
	Normal/Abnormal	1	12.79	<0.001
	Month	4	14.63	<0.001
	Normal/Abnormal vs. Month	4	1.33	0.261
Gonad Proteins Normal vs. Abnormal 2008	Two-way ANOVA			
	Normal/Abnormal	1	8.55	0.004
	Month	4	14.22	<0.001
	Normal/Abnormal vs. Month	4	0.85	0.499
Gonad Vitellogenin Normal vs. Abnormal 2008	Two-way ANOVA			
	Normal/Abnormal	1	1.74	0.190
	Month	4	0.71	0.587
	Normal/Abnormal vs. Month	4	0.20	0.941

	Test	D.F.	F	p-value
Adductor Muscle Glycogen 2009	One-way ANOVA	5	19.39	<0.001
Adductor Muscle Total Carbohydrates 2009	One-way ANOVA	5	58.28	<0.001
Adductor Muscle Lipids 2009	One-way ANOVA	5	9.53	<0.001
Adductor Muscle Proteins 2009	One-way ANOVA	5	10.44	<0.001
Adductor Muscle Glycogen Male vs. Female 2009	Two-way ANOVA			
	Sex	1	0.41	0.525
	Month	5	17.03	<0.001
	Sex vs. Month	5	0.84	0.526
Adductor Muscle Total Carbohydrates Male vs. Female 2009	Two-way ANOVA			
	Sex	1	2.11	0.148
	Month	5	49.79	<0.001
	Sex vs. Month	5	0.73	0.605
Adductor Muscle Lipids Male vs. Female 2009	Two-way ANOVA			
	Sex	1	0.27	0.603
	Month	5	7.90	<0.001
	Sex vs. Month	5	0.95	0.449
Adductor Muscle Proteins Male vs. Female 2009	Two-way ANOVA			
	Sex	1	1.91	0.169
	Month	5	9.59	<0.001
	Sex vs. Month	5	0.99	0.424
Adductor Muscle Glycogen Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.27	0.604
	Month	3	9.89	<0.001
	Normal/Abnormal vs. Month	3	0.91	0.441
Adductor Muscle Total Carbohydrates Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.07	0.799
	Month	3	24.75	<0.001
	Normal/Abnormal vs. Month	3	1.67	0.179
Adductor Muscle Lipids Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	1.30	0.257
	Month	3	3.57	0.016
	Normal/Abnormal vs. Month	3	0.11	0.956
Adductor Muscle Proteins Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	1.18	0.281
	Month	3	4.40	0.006
	Normal/Abnormal vs. Month	3	1.28	0.288

	Test	D.F.	F	p-value
Gill Glycogen 2009	One-way ANOVA	5	23.96	<0.001
Gill Total Carbohydrates 2009	One-way ANOVA	5	15.40	<0.001
Gill Lipids 2009	One-way ANOVA	5	4.08	0.002
Gill Proteins 2009	One-way ANOVA	5	2.70	0.023
Gill Glycogen Male vs. Female 2009	Two-way ANOVA			
	Sex	1	1.13	0.289
	Month	5	20.82	<0.001
	Sex vs. Month	5	0.23	0.949
Gill Total Carbohydrates Male vs. Female 2009	Two-way ANOVA			
	Sex	1	0.47	0.496
	Month	5	13.67	<0.001
	Sex vs. Month	5	0.84	0.523
Gill Lipids Male vs. Female 2009	Two-way ANOVA			
	Sex	1	0.19	0.667
	Month	5	3.61	0.004
	Sex vs. Month	5	0.20	0.961
Gill Proteins Male vs. Female 2009	Two-way ANOVA			
	Sex	1	0.06	0.809
	Month	5	2.76	0.021
	Sex vs. Month	5	0.67	0.646
Gill Glycogen Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.51	0.479
	Month	3	0.74	0.530
	Normal/Abnormal vs. Month	3	2.97	0.038
Gill Total Carbohydrates Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	2.43	0.122
	Month	3	4.40	0.006
	Normal/Abnormal vs. Month	3	0.62	0.601
Gill Lipids Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	1.11	0.294
	Month	3	1.73	0.165
	Normal/Abnormal vs. Month	3	0.58	0.629
Gill Proteins Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	6.08	0.015
	Month	3	4.78	0.004
	Normal/Abnormal vs. Month	3	0.35	0.792

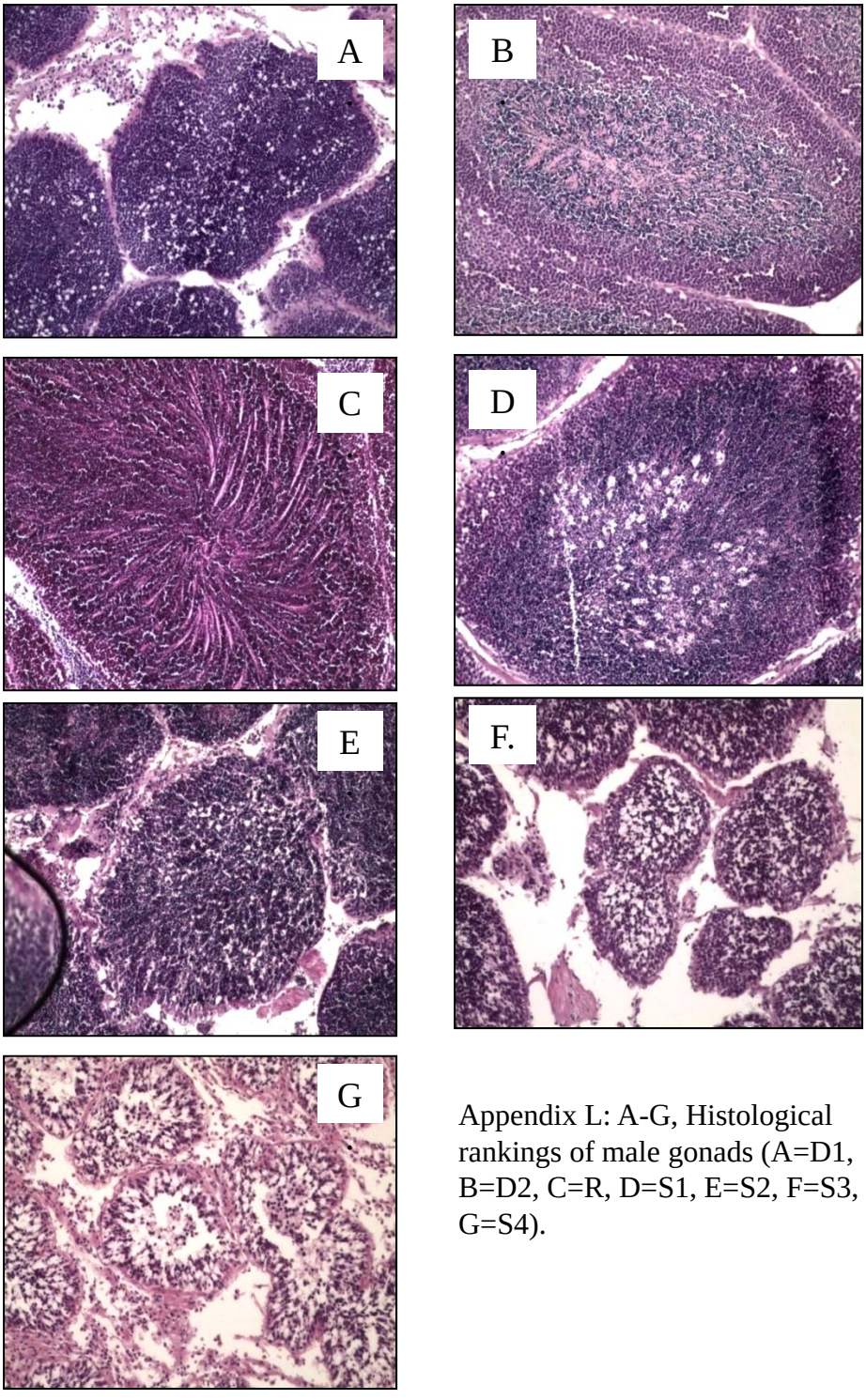
	Test	D.F.	F	p-value
Gonad Glycogen 2009	One-way ANOVA	5	3.85	0.003
Gonad Total Carbohydrates 2009	One-way ANOVA	5	26.75	<0.001
Gonad Lipids 2009	One-way ANOVA	5	23.36	<0.001
Gonad Proteins 2009	One-way ANOVA	5	12.85	<0.001
Gonad Vitellogenin 2009	One-way ANOVA	5	2.93	0.015
Gonad Glycogen Male vs. Female 2009	Two-way ANOVA			
	Sex	1	1.29	0.260
	Month	5	3.51	0.006
	Sex vs. Month	5	0.50	0.773
Gonad Total Carbohydrates Male vs. Female 2009	Two-way ANOVA			
	Sex	1	3.48	0.064
	Month	5	24.86	<0.001
	Sex vs. Month	5	3.55	0.005
Gonad Lipids Male vs. Female 2009	Two-way ANOVA			
	Sex	1	9.24	0.003
	Month	5	20.05	<0.001
	Sex vs. Month	5	4.60	<0.001
Gonad Proteins Male vs. Female 2009	Two-way ANOVA			
	Sex	1	5.86	0.017
	Month	5	12.75	<0.001
	Sex vs. Month	5	1.56	0.174
Gonad Vitellogenin Male vs. Female 2009	Two-way ANOVA			
	Sex	1	6.77	0.010
	Month	5	3.47	0.005
	Sex vs. Month	5	0.83	0.534
Gonad Glycogen Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.35	0.558
	Month	3	6.73	<0.001
	Normal/Abnormal vs. Month	3	4.36	0.007
Gonad Total Carbohydrates Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	4.97	0.028
	Month	3	6.46	<0.001
	Normal/Abnormal vs. Month	3	0.68	0.569
Gonad Lipids Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.00	0.990
	Month	3	11.74	<0.001
	Normal/Abnormal vs. Month	3	0.28	0.838
Gonad Proteins Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	7.56	0.007
	Month	3	5.34	0.002
	Normal/Abnormal vs. Month	3	1.04	0.379
Gonad Vitellogenin Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.01	0.924
	Month	3	1.91	0.133
	Normal/Abnormal vs. Month	3	2.03	0.114

	Test	D.F.	F	p-value
Mantle Glycogen 2009	One-way ANOVA	5	5.67	<0.001
Mantle Total Carbohydrates 2009	One-way ANOVA	5	59.18	<0.001
Mantle Lipids 2009	One-way ANOVA	5	38.43	<0.001
Mantle Proteins 2009	One-way ANOVA	5	16.42	<0.001
Mantle Glycogen Male vs. Female 2009	Two-way ANOVA			
	Sex	1	1.39	0.240
	Month	5	4.02	0.002
	Sex vs. Month	5	0.19	0.966
Mantle Total Carbohydrates Male vs. Female 2009	Two-way ANOVA			
	Sex	1	2.85	0.093
	Month	5	58.86	<0.001
	Sex vs. Month	5	0.97	0.439
Mantle Lipids Male vs. Female 2009	Two-way ANOVA			
	Sex	1	0.66	0.417
	Month	5	37.71	<0.001
	Sex vs. Month	5	0.92	0.471
Mantle Proteins Male vs. Female 2009	Two-way ANOVA			
	Sex	1	0.00	0.974
	Month	5	14.66	<0.001
	Sex vs. Month	5	0.38	0.865
Mantle Glycogen Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	2.18	0.144
	Month	3	5.04	0.003
	Normal/Abnormal vs. Month	3	3.11	0.031
Mantle Total Carbohydrates Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.76	0.385
	Month	3	14.69	<0.001
	Normal/Abnormal vs. Month	3	0.98	0.405
Mantle Lipids Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	0.05	0.831
	Month	3	2.93	0.037
	Normal/Abnormal vs. Month	3	0.47	0.703
Mantle Proteins Normal vs. Abnormal 2009	Two-way ANOVA			
	Normal/Abnormal	1	1.18	0.281
	Month	3	4.40	0.006
	Normal/Abnormal vs. Month	3	1.28	0.288

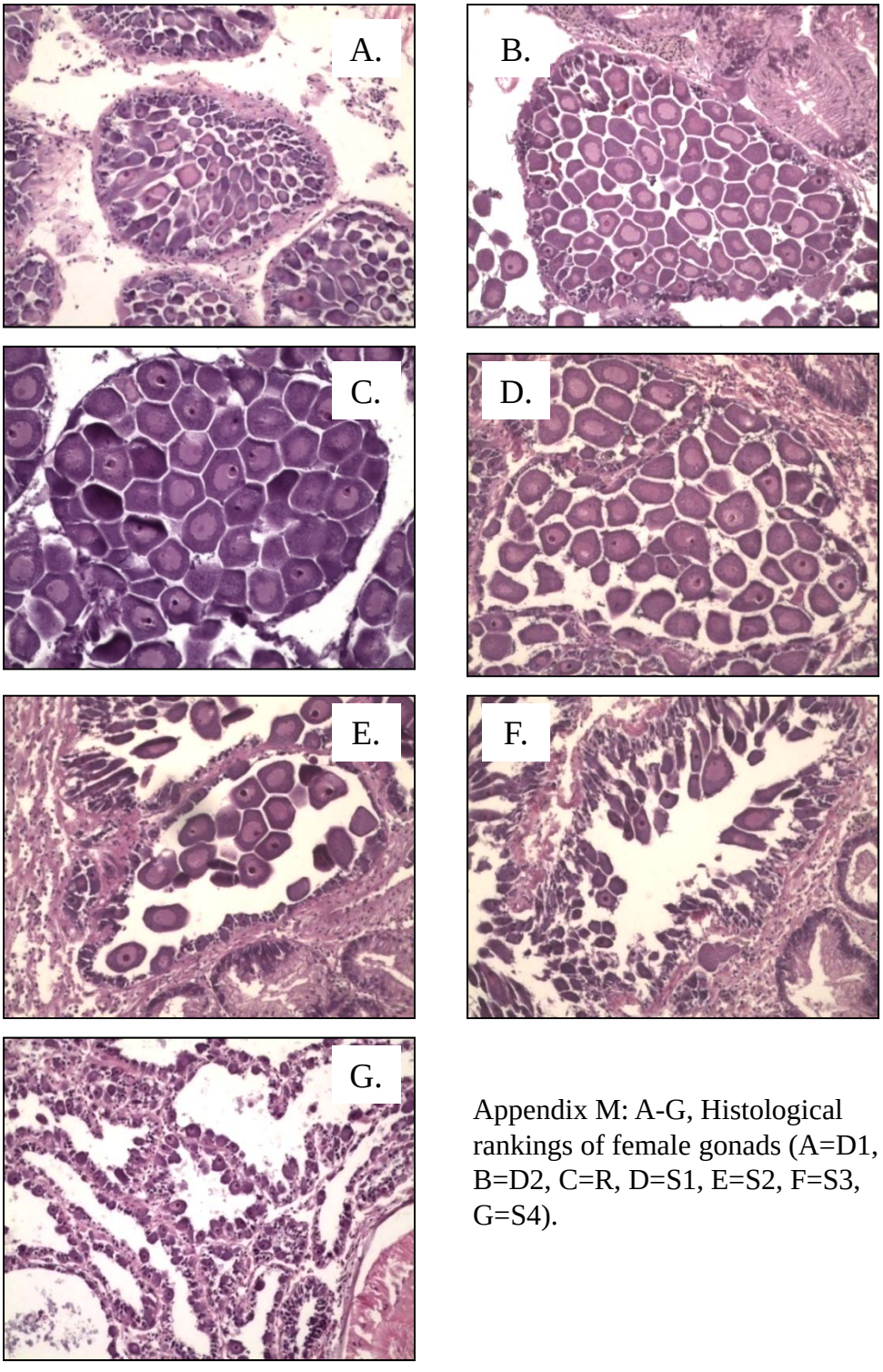
	Test	D.F.	F	p-value
Gonad Glycogen 2008 vs. 2009	One-way ANOVA			
	April	1	5.02	0.032
	May	1	7.84	0.008
	July	1	10.71	0.002
	September	1	17.34	<0.001
Gonad Total Carbohydrates 2008 vs. 2009	One-way ANOVA			
	April	1	36.48	<0.001
	May	1	11.66	0.001
	July	1	4.61	0.036
	September	1	3.33	0.076
Gonad Lipids 2008 vs. 2009	One-way ANOVA			
	April	1	0.36	0.550
	May	1	5.83	0.019
	July	1	8.26	0.006
	September	1	0.03	0.866
Gonad Proteins 2008 vs. 2009	One-way ANOVA			
	April	1	37.52	<0.001
	May	1	7.53	0.008
	July	1	0.05	0.826
	September	1	10.20	0.002
Gonad Vitellogenin 2008 vs. 2009	One-way ANOVA			
	April	1	44.98	<0.001
	May	1	7.89	0.007
	July	1	8.68	0.005
	September	1	9.55	0.004
Total Hemocytes Field 2009	One-way ANOVA	5	9.01	<0.001
Total Granulocytes Field 2009	One-way ANOVA	5	6.60	<0.001
Total Agranulocytes Field 2009	One-way ANOVA	5	12.95	<0.001
Percent Granulocytes Field 2009	One-way ANOVA	5	93.60	<0.001

The Impact of Temperature on Gonadal Development				
	Test	**D.F.**	**F**	**p-value**
Gonad Glycogen	Students t-test			0.734
Gonad Total Carbohydrates	Students t-test			0.313
Gonad Lipids	Students t-test			0.044
Gonad Proteins	Students t-test			0.548
Gonad Vitellogenin	Students t-test			0.413
Gonad Glycogen Males	Students t-test			0.360
Gonad Total Carbohydrates Males	Students t-test			0.601
Gonad Lipids Males	Students t-test			0.006
Gonad Proteins Males	Students t-test			0.185
Gonad Vitellogenin Males	Students t-test			0.224
Gonad Glycogen Females	Students t-test			0.336
Gonad Total Carbohydrates Females	Students t-test			0.558
Gonad Lipids Females	Students t-test			0.755
Gonad Proteins Females	Students t-test			0.071
Gonad Vitellogenin Females	Students t-test			0.251
Normal Cold vs. Abnormal Cold Glycogen	Students t-test			0.450
Normal Cold vs. Abnormal Cold Total Carbohydrates	Students t-test			0.528
Normal Cold vs. Abnormal Cold Lipids	Students t-test			0.565
Normal Cold vs. Abnormal Cold Proteins	Students t-test			0.594
Normal Cold vs. Abnormal Cold Vitellogenin	Students t-test			0.609
Normal Warm vs. Abnormal Warm Glycogen	Students t-test			0.909
Normal Warm vs. Abnormal Warm Total Carbohydrates	Students t-test			0.350
Normal Warm vs. Abnormal Warm Lipids	Students t-test			0.030
Normal Warm vs. Abnormal Warm Proteins	Students t-test			0.001
Normal Warm vs. Abnormal Warm Vitellogenin	Students t-test			0.740
Normal Cold vs. Normal Warm Glycogen	Students t-test			0.979
Normal Cold vs. Normal Warm Total Carbohydrates	Students t-test			0.440
Normal Cold vs. Normal Warm Lipids	Students t-test			0.011
Normal Cold vs. Normal Warm Proteins	Students t-test			0.201
Normal Cold vs. Normal Warm Total Vitellogenin	Students t-test			0.473
Abnormal Cold vs. Abnormal Warm Glycogen	Students t-test			0.681
Abnormal Cold vs. Abnormal Warm Total Carbohydrates	Students t-test			0.485
Abnormal Cold vs. Abnormal Warm Lipids	Students t-test			0.920
Abnormal Cold vs. Abnormal Warm Proteins	Students t-test			0.142
Abnormal Cold vs. Abnormal Warm Vitellogenin	Students t-test			0.813

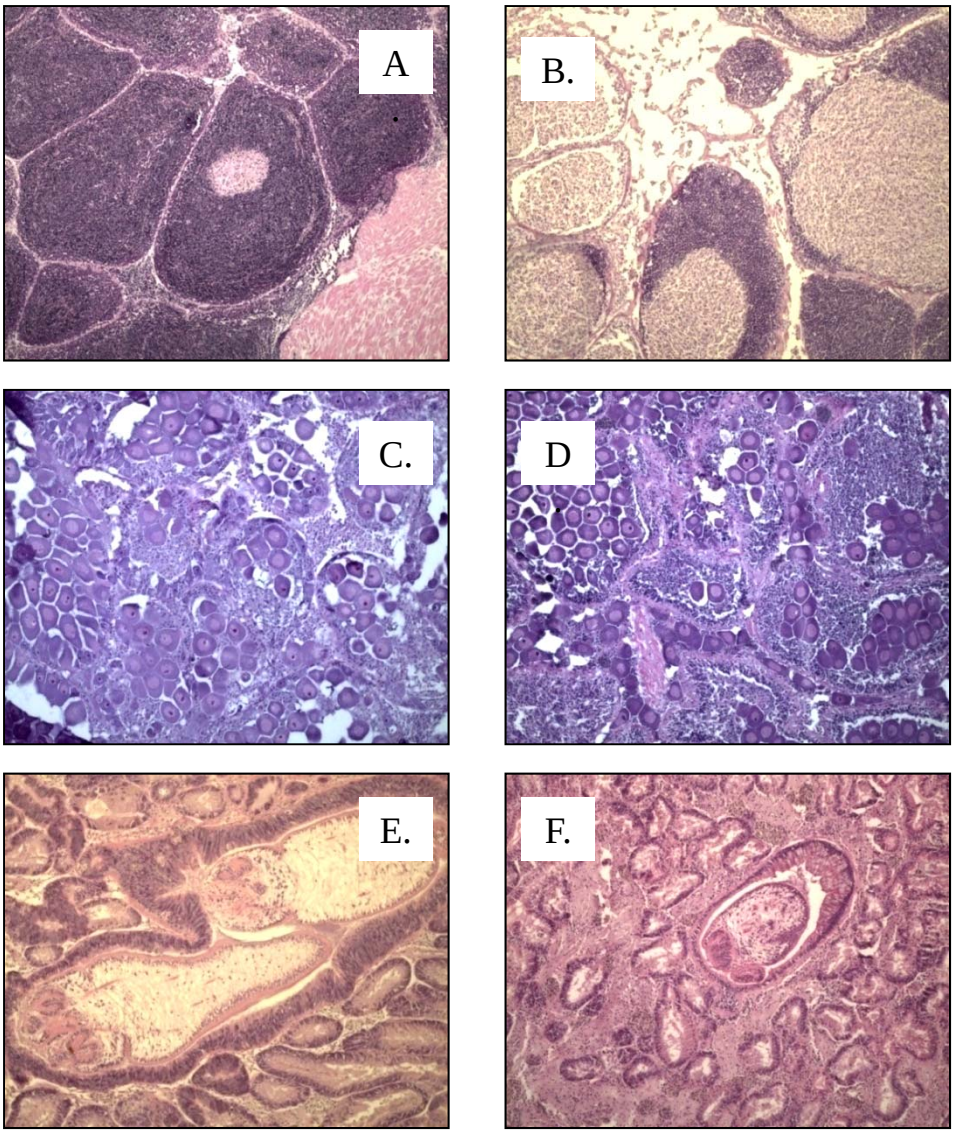
Filtration Rate and Scope for Growth				
	Test	**D.F.**	**F**	**p-value**
Ammonia Excretion	Students t-test			0.135
Assimilation Efficiency	Students t-test			0.015
Filtration Rate	Students t-test			0.034
Irrigatory Efficiency	Students t-test			0.040
Oxygen Consumption	Students t-test			0.168
Condition Index	Students t-test			0.029
Ingestion Rate	Students t-test			0.077
Scope for Growth	Students t-test			0.042
Immune Defense and Short Term Energy Balance				
	Test	**D.F.**	**F**	**p-value**
Adductor Muscle Glycogen	Students t-test			0.079
Adductor Muscle Total Carbohydrates	Students t-test			0.332
Adductor Muscle Lipids	Students t-test			0.876
Adductor Muscle Proteins	Students t-test			0.047
Gill Glycogen	Students t-test			0.598
Gill Total Carbohydrates	Students t-test			0.150
Gill Lipids	Students t-test			0.003
Gill Proteins	Students t-test			0.202
Gonad Glycogen	Students t-test			0.014
Gonad Total Carbohydrates	Students t-test			0.778
Gonad Lipids	Students t-test			0.851
Gonad Proteins	Students t-test			0.044
Gonad Vitellogenin	Students t-test			0.183
Mantle Glycogen	Students t-test			0.025
Mantle Total Carbohydrates	Students t-test			0.245
Mantle Lipids	Students t-test			0.001
Mantle Proteins	Students t-test			0.370
Total Hemocytes	Students t-test			<0.001
Percent Dead	Students t-test			0.458
Percent Granulocytes	Students t-test			<0.001
Reactive Oxygen Species un-stimulated (ROS-0)	Students t-test			0.037
Reactive Oxygen Species zymosan activated (ROS-30)	Students t-test			0.605
Percent Phagocytic Hemocytes	Students t-test			0.652
Fluorescent Intensity of Phagocytic Hemocytes	Students t-test			0.121
Phagocytic Index	Students t-test			0.048



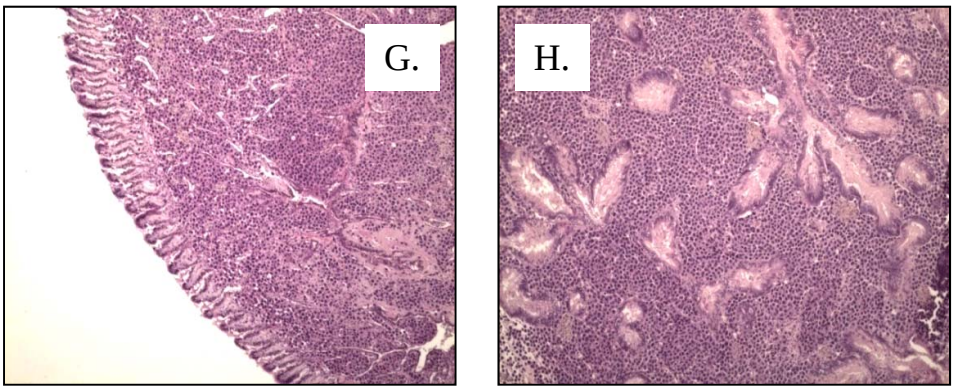
Appendix L: A-G, Histological rankings of male gonads (A=D1, B=D2, C=R, D=S1, E=S2, F=S3, G=S4).



Appendix M: A-G, Histological rankings of female gonads (A=D1, B=D2, C=R, D=S1, E=S2, F=S3, G=S4).



Appendix N: A-H, Histological snapshots of abnormal gonad conditions, parasites and hemic neoplasia. (A-B=abnormal male gonad; C-D=abnormal female gonad; E-F= cestode and nematode parasites; G-H= hemic neoplasia in the gill and digestive gland). The figure is continued on the next page.



Appendix N (continued): A-H, Histological snapshots of abnormal gonad conditions, parasites and neoplasia. (A-B=abnormal male gonad; C-D=abnormal female gonad; E-F= cestode and nematode parasites; G-H= neoplasia in the gill and digestive gland).