



The influence of temperature stress on the physiology of the Atlantic surfclam, *Spisula solidissima*



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ABSTRACT

Atlantic surfclam populations have significantly declined in state and federal waters from the south shore of Long Island, New York to the Delmarva Peninsula since the early 2000s. Previous studies have demonstrated that surfclams in this geographic range show signs of physiological stress, suggested to be a result of increasing ocean temperatures. In this study, we examined the effect of 2 temperature regimes (19 °C and 23 °C) on surfclam physiology. These temperatures were chosen because they represent maximal (23 °C) and minimal (19 °C) temperatures prevailing in New York clamping areas during summer. Results demonstrated enhanced energy metabolism and significant reductions in filtration rate, scope for growth, and immune functions in clams exposed to the warmer temperature treatment. Although net energy gains remained positive in both treatments under our experimental conditions, the findings suggest that temperature stress is involved in the recent observations of surfclams in poor condition. The impact of elevated temperatures on phytoplankton quantity/quality and other environmental variables in combination with the direct impact on surfclam filtration and metabolic rates could lead to a negative energy balance. While some uncertainties remain about population-scale impacts of overall warming trends, we fear that future increases in temperature may lead to the collapse of the Atlantic surfclam between New York and Virginia, especially within inshore regions.

1. Introduction

The Atlantic surfclam, *Spisula solidissima*, is an important commercially harvested marine bivalve occurring from the Gulf of Maine to Cape Hatteras, North Carolina from the shallow sub-tidal zone to approximately 50 m depth (Wigley and Emery, 1968; Merrill and Ropes, 1969; Ropes, 1980; Fay et al., 1983). Surfclam populations have drastically declined in federal and state waters in areas to the south of New York since the early 2000s (Northeast Fishery Science Center (NEFSC), 2003; Normant, 2005; Weinberg, 2005; Weinberg et al., 2005; MAFMC, 2008). More recently, state surveys in New York demonstrated population declines of 72% between 2002 and 2012 (Dahl and Hornstein, 2010; O'Dwyer and Hornstein, 2013). Thermal stress has been implicated as the main factor for the declines in the mid-Atlantic region of the United States (Kim and Powell, 2004; Weinberg, 2005; Northeast Fishery Science Center (NEFSC), 2017; Marzec et al., 2010; Narváez et al., 2014; Munroe et al., 2016) and is also thought to be in part responsible for the declining population in New York state waters (Davidson et al., 2007; Dahl and Hornstein, 2010; O'Dwyer and Hornstein, 2013).

Temperatures have risen 2–3 °C along the coast of North America over the past century (Drinkwater, 1996; Levitus et al., 2000; Weinberg, 2002) and water temperatures are projected to increase over 2 °C in the next 50–100 years in the mid-Atlantic (Frumhoff et al., 2007; Munroe et al., 2016). Previous studies have suggested that Atlantic surfclams are physiologically constrained by temperature inshore, in shallow water (Cerrato and Keith, 1992) and they become stressed when temperatures exceed 20 °C (Weinberg, 2005; Marzec et al., 2010). Stress in surfclams above 20 °C is demonstrated by a reduction in burrowing ability (Savage, 1976), termination of growth at 23.9 °C (Saila and Pratt, 1973; Goldberg and Walker, 1990; Walker and Heffernan, 1994; Spruck et al., 1995; O'Beirn et al., 1997), diminished fertilization success at 24 °C and mortality between 27 and 30 °C (Saila and Pratt, 1973; Goldberg and Walker, 1990; Cloteau and Dube, 1993; Walker and Heffernan, 1994; Spruck et al., 1995; O'Beirn et al., 1997). Studies have also indicated the sensitivity of surfclams to thermal stress at the southern end of their range as documented by abnormal gonadal development (Kim and Powell, 2004), bathymetric shifts in the population distribution (Weinberg, 2005), low condition indices inshore (Marzec et al., 2010), poor reproductive success (Narváez et al., 2014),

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and a decline in maximum shell size from 1982 to 2016 (Munroe et al., 2016). Studies in New York state waters have presented surfclams with abnormal gonadal development, signifying signs of physiological stress in New York (Allam, 2007; Dahl and Hornstein, 2010; O'Dwyer and Hornstein, 2013).

Temperature is known to be an important factor influencing the energy use for basal metabolic needs which can be used for growth, reproduction, immunity and other physiological processes in marine bivalves (Bayne and Newell, 1983; MacDonald and Thompson, 1986; Bayne and Hawkins, 1990; Chen et al., 2007). Temperatures outside the optimal range of a bivalve can reduce the scope for growth by increasing the respiratory rate and reducing the filtration rate (Ali, 1970; Brock and Kofoed, 1987; 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). A reduction in surfclam filtration rates under temperature stress may reduce the ability of the animal to obtain food, lowering energetic gains or resulting in a negative scope for growth (net negative energy gain) (Munroe et al., 2013; Narváez et al., 2014).

Temperature is also an important factor regulating bivalve immune defenses (Abele et al., 2002; Hégaret et al., 2003a, 2003b; Liu et al., 2004; Paillard et al., 2004; Gagnaire et al., 2006; Chen et al., 2007; Monari et al., 2007). Temperature changes have been shown to impact total hemocyte counts, phagocytic activity and other functions of hemocytes in many bivalve species, both seasonally and over the short term (Fisher et al., 1987, 1989; Auffret and Oubella, 1994; Oubella, 1996; Paillard et al., 2004). For example, in the oyster *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, phagocytose, and eliminate non-self particles by antimicrobial activities (Delaporte et al., 2006). The effect of temperature on Atlantic surfclam immunity is unknown and warrants investigation since an adverse effect of increasing summer temperatures on immunity may leave the surfclam more susceptible to opportunistic pathogens in the environment.

In this study, we used field temperature data to determine two temperature regimes representing the maximal (23 °C) and minimal (19 °C) summer temperatures measured in New York clamping areas between 1992 and 2007. We then studied the effect of these 2 temperature regimes on filtration rate, scope for growth, energy metabolism, and immunity in the Atlantic surfclam. Findings are discussed in light of observations of surfclams in poor physiological condition in recent surveys in the Delmarva region and along the south shore of Long Island.

2. Materials and methods

2.1. Filtration rate and scope for growth

2.1.1. General experimental design

In October of 2008, surfclams (129.28 ± 11.93 mm in length, mean \pm SD) were collected from the field by a hydraulic dredge, divided into two batches and gradually acclimated for one week to 19 °C or 23 °C (salinity of 31). These temperatures were determined using data taken from NOAA's Buoy 44025 from 1992 to 2007. The cool (19 °C) and warm (23 °C) temperatures were established by respectively taking the average of the 10% coolest or the 10% warmest of the temperatures over the 16 year period. 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 ($n = 12$ /treatment) were individually placed in 24 (12 replicates per treatment) sealed, 3.51 aquariums containing filtered seawater and

rested in temperature controlled water baths. Water inside the aquaria was mixed using a magnetic stirrer to ensure homogeneity. Before any measurements were taken, clams were allowed to acclimate to the aquaria for 1 h. During this time the tanks remained aerated and unsealed. Once measurements were ready to be taken, algae (4×10^4 cells ml $^{-1}$) (Goldberg, 1985) was added and the tanks were sealed. DT's Premium Reef Blend Phytoplankton mix (DT's Plankton Farm, Sycamore, IL) was used for the filtration rate study, representing a diverse food source, optimal for clam growth (Pales Espinosa and Allam, 2006). Control chambers with algae and without any clams were used to account for algae settling, if any, in each temperature treatment.

2.1.2. Filtration rate

At 30 min and 1 h, 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 (FACSCalibur, BD Biosciences, CA, USA). The 488 nm argon and the 635 red diode lasers were used for excitation. A minimum of 10^4 events were analyzed. Filtration rate is expressed using the formula: Filtration rate = $V/t \ln(C_0/C_t)$, where V is the volume of seawater in the chamber, t is the time in hours, C_0 is the concentration of algae at time 0 and C_t is the concentration at time t (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 (confirmed in control chambers that did not contain surfclams), particle retention was 100% and the suspension remained homogeneous (Coughlan, 1969).

2.1.3. Ingestion rate

Ingestion rate is expressed as the product of filtration rate and the energy content of the experimental diet (cal/h) which is calculated as described below (Han et al., 2008). Ingestion rate follows the same four assumptions previously described for filtration rate.

2.1.4. Assimilation efficiency

Assimilation efficiency was measured using the methods of Conover (1966). Glass fiber filters were combusted in a muffle furnace for 4 h at 450 °C and cooled prior to use. Food samples were filtered on pre-weighed 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 dried at 90 °C for 24 h and combusted in a muffle furnace for 4 h at 450 °C, allowed to cool and were then weighed. Assimilation efficiency was 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.

2.1.5. Oxygen consumption

Oxygen consumption was measured every 30 min using a YSI 85 (YSI Incorporated, Yellow Springs, OH) and is expressed as (mg O₂/hr). Oxygen consumption was transformed into energy (for calculation of scope for growth) using the conversion 1 mg O₂ = 3.38 cal (Elliott and Davison, 1975).

2.1.6. Ammonia excretion

Ammonia concentrations were measured at time zero and at the end of the experiment (60 min) using the phenol-hypochlorite method described in Solorzano (1969). Ammonia excretion is expressed as (μ g NH₄N/h). Ammonia excretion was converted into energy using the conversion 1 mg NH₄ = 5.94 cal (Elliott and Davison, 1975; Han et al., 2008).

2.1.7. Irrigatory efficiency

Irrigatory efficiency is expressed as the volume of water cleared per unit oxygen uptake (ml H₂O/mg O₂/h) (Brock and Kofoed, 1987). Low filtration rates and high respiratory rates cause irrigatory efficiency to be low (Brock and Kofoed, 1987). Irrigatory efficiency is considered 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).

2.1.8. 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: $P = (C * A) - (R + U)$, where P is the scope for growth (cal/hr), C is the ingestion rate, A is the assimilation efficiency, R is the cost of oxygen consumption and U is the excretion cost (ammonia) (Goldberg, 1985; Widdows and Johnson, 1988; Sobral and Widdows, 1997; Navarro et al., 2000; Widdows et al., 2002; Mubiana and Blust, 2007). The caloric value of the food was determined using the formula, cal/mg dw = $(-0.555 + 0.113 (\%C) + 0.054 (\text{C:N}))$ (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).

2.2. Energy metabolism and immune defense

2.2.1. General experimental design

Surfclams (119.68 ± 12.29 mm in length, mean ± SD) were collected in October of 2009 and held in flow-through seawater tables under an initial acclimation for 6 days. Four tanks were used in this experiment. Fifteen clams were placed in each tank and all tanks were initially held at a temperature of 19 °C coinciding with the field temperature at that time of the year (18.5 °C) and at a salinity of 31. Clams in the two tanks used for the warm treatment were gradually (over a one week period) brought up to 23 °C. Temperature in the cold treatment remained at 19 °C for the length of the experiment. Each day, clams were fed DT's Premium Reef Blend Phytoplankton mix (DT's Plankton Farm, Sycamore, IL) and temperature, salinity, and oxygen concentrations were measured. Six days after incubation at stabilized temperatures, clams were bled (400 µl hemolymph/clam) and measurements were made to determine hemocyte counts, viability, phagocytic activity, and reactive oxygen species production (ROS) as described below. In addition, 10 clams were taken from each treatment for biochemical analysis of adductor muscle and mantle tissues as described below. These tissues were chosen because they have been shown to be important areas where bivalves store energy for physiological processes (Barber and Blake, 1983; Berthelin et al., 2000; Ojea et al., 2004; Darriba et al., 2005; Dridi et al., 2007). Remaining clams were injected with 300 µl of sea water containing the opportunistic marine pathogen *Vibrio alginolyticus* at a concentration of $2 \times 10^9 \text{ cells ml}^{-1}$ and returned to their respective tanks (see "Bacteriology" section below). Mortalities were noted and clams that died were immediately removed from the tanks. The experiment concluded one week post injection and remaining live animals were bled and bacterial counts were determined as described below.

2.2.2. Biochemistry

Biochemicals measured included lipids, protein, glycogen and total carbohydrates. Glycogen was measured following enzymatic digestion of the gonad homogenate with amyloglucosidase, releasing glucose trapped in the glycogen molecules (Murat and Serfaty, 1974). Free glucose was then measured through a phenol-sulfuric acid reaction according to Dubois et al. (1956), using glucose as a standard. Total free carbohydrates were determined by taking a subsample of tissue homogenate prior to digestion with amyloglucosidase. Lipids were measured gravimetrically according to Folch et al. (1957). Protein was

measured using the Pierce BCA protein assay reagent kit (Pierce, Rockford, IL) according to manufacturer's recommendations. Bovine serum albumin was used as the standard for protein measurements.

2.2.3. Hemocyte counts

To determine hemocyte counts, 100 µl of hemolymph was added to 300 µl of filtered sterile sea water (FSSW) and 10 µl of a SYBR Green solution (25 µg ml⁻¹ final concentration) was added to each tube before incubation in the dark for 30 min. SYBR green dye was added to stain the DNA of the hemocytes in order to differentiate actual cells from debris. Counts were made post incubation using flow cytometry. Total hemocyte counts are expressed as the number of cells per milliliter using flow cytometry flow rate and time data. Light scatter parameters of each hemocyte were then used to differentiate between granulocytes and agranulocytes (Allam et al., 2002; Delaporte et al., 2006).

2.2.4. 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 FSSW and 10 µl of green fluorescent 2.2 µm beads ($3.3 \times 10^5 \text{ beads } \mu\text{l}^{-1}$) in a 2 ml microcentrifuge tube. The samples were incubated in the dark at room temperature for 60 min under gentle mixing, before being analyzed by flow cytometry. The phagocytic activity was estimated as the percentage of hemocytes that had engulfed three beads or more based on fluorescence intensity.

2.2.5. 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 µl of this mixture was plated in triplicate in a black 96 well plate. Twenty microliters of Dichlorofluorescein-diacetate (10 mM) was added to each well. Production of ROS was initiated by adding 10 µl of zymosan A suspension (20 mg ml⁻¹ in FSSW, Sigma) in two wells and fluorescence was measured after 30 min of incubation in the dark at room temperature using a plate reader (Wallac 1420, Perkin Elmer) at 485 nm excitation and 535 nm emission. Signals in wells activated with zymosan A were corrected by subtracting the values obtained from the third replicate (unstimulated). ROS activity was expressed as mean fluorescence in arbitrary units (AU) per 10^4 hemocytes.

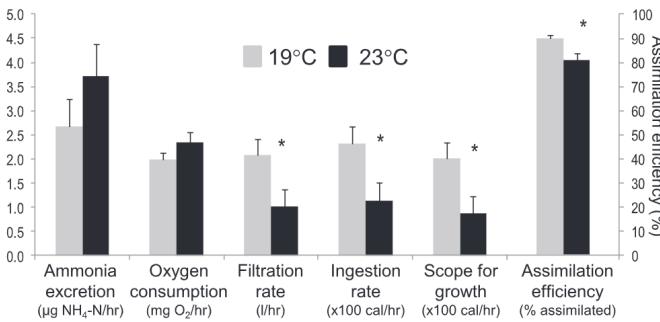
2.2.6. Hemocyte viability

Viability of the hemocytes was measured by adding 100 µl of hemolymph and 300 µl of FSSW in a 5 ml flow cytometry tube. To this, 10 µl of Calcein AM (25 µg ml⁻¹ final concentration) dye was added to label live cells and 10 µl of Ethidium homodimer (25 µg ml⁻¹ concentration) dye was added to detect dead cells. The sample was incubated in the dark at room temperature for 30 min and measured by flow cytometry. Live and dead cells were identified and counted based on their green (FL1) and red (FL3) fluorescence respectively.

2.2.7. Bacteriology

Bacteria used in the challenge experiment were grown on Marine agar (Difco™) plates for 24 h at room temperature. They were recovered from the plates and suspended in sterile artificial seawater (salinity of 30), washed with seawater by centrifugation (2000 g for 15 min) and re-suspended in seawater ($2 \times 10^9 \text{ cells ml}^{-1}$).

Bacterial counts were determined in hemolymph from surviving clams at the end of the experiment. Prior to bleeding, surfclams were washed under tap water and 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 21 g needle attached to a 1 ml syringe. Hemolymph was transferred to a sterile micro-centrifuge tube and aliquots (100 µl) were spread on Marine agar plates and incubated in the dark at room temperature for 96 h before bacterial colonies were counted.



2.3. Data analysis

Flow cytometry data was initially processed using Becton Dickinson's CellQuest Pro software to identify different cell subpopulations. A student's *t*-test was used to test for differences between the two treatment groups (19 and 23 °C) using SigmaStat (Systat Software, Inc., San Jose, California, USA). Principal component analysis was conducted on the biochemistry data to test for overall differences between treatments. LogRank survival curves were used to test for differences in mortality among clams injected with *V. alginolyticus* and incubated at 19 and 23 °C. Data were log10 or arcsin transformed when needed to meet assumptions before statistical testing, but results shown in figures are presented as non-transformed values. Results were considered significant at $p < 0.05$.

3. Results

3.1. Filtration rate and scope for growth

Temperature treatments impacted several physiological parameters of Atlantic surfclams (Fig. 1). Average ammonia excretion values were 2.49 $\mu\text{g NH}_4\text{-N}/\text{hr}$ in clams from the cold treatment and 3.72 $\mu\text{g NH}_4\text{-N}/\text{hr}$ from the warm treatment and the amount of oxygen consumed was also higher at 23 °C (2.35 $\text{mg O}_2/\text{h}$) compared to 19 °C (1.98 $\text{mg O}_2/\text{h}$), however differences were not statistically significant. A significant ($p = 0.034$) reduction in filtration rate (51%), and consequently ingestion rate, was observed at 23 °C in contrast to 19 °C. Ingested food was assimilated to a significantly higher degree at 19 °C ($p = 0.015$). Irrigatory efficiency was significantly higher ($p = 0.018$) in clams from the colder treatment (1110.58 $\text{ml H}_2\text{O}/\text{mg O}_2/\text{h}$) in contrast to the warm treatment (424.0 $\text{ml H}_2\text{O}/\text{mg O}_2/\text{h}$) (results not displayed). Scope for growth (energy available for growth, reproduction, immunity and other physiological processes) was significantly higher at 19 °C compared to 23 °C ($p = 0.025$).

3.2. Energy metabolism and immune defense

In all eight comparisons of tissue biochemical parameters, values were greater at 19 °C as compared to 23 °C. Glycogen and proteins in the adductor muscle were significantly lower in clams maintained at 23 °C (Fig. 2a). Additionally, glycogen and lipids were significantly lower in the mantle tissue from clams maintained at 23 °C as compared to those from the 19 °C group (Fig. 2b). Principal component analysis of all biochemistry data combined (extracted Component 1) showed significant differences between both treatments ($p = 0.002$) (Fig. 2c).

Total hemocyte counts were higher in clams maintained at 23 °C as compared to those held at 19 °C ($p < 0.001$) (Fig. 3). There was no significant difference in the percentage of dead hemocytes between treatments (8 to 10%, data not shown). Similarly, there was no

Fig. 1. Physiological parameters measured during the scope for growth study. Each value represents the mean + SE ($n = 12/\text{treatment}$). All parameters are plotted against the left Y-axis (units are displayed below the labels) excluding the assimilation efficiency (right Y-axis). An asterisk (*) indicates significance between treatments (Student's *t*-test, $p < 0.05$).

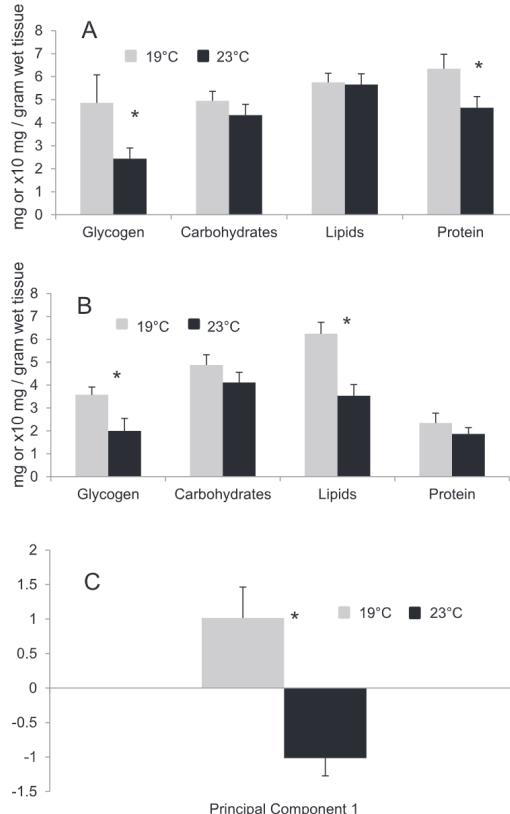


Fig. 2. Biochemical parameters measured in the adductor muscle (a) and mantle (b) in surfclams maintained at 19 or 23 °C. Each value represents the mean + SE ($n = 10/\text{treatment}$). Protein values in each graph are displayed as $\times 10$. Panel (c) shows the first principal component of a principal component analysis of all biochemical parameters in both tissues combined. An asterisk (*) indicates significance between groups ($p < 0.05$, Student's *t*-test).

difference in ROS production between treatments. In contrast, the percent granulocytes in clams from the 19 °C treatment were significantly higher ($p < 0.001$) than that measured in clams from the 23 °C tanks (Fig. 3).

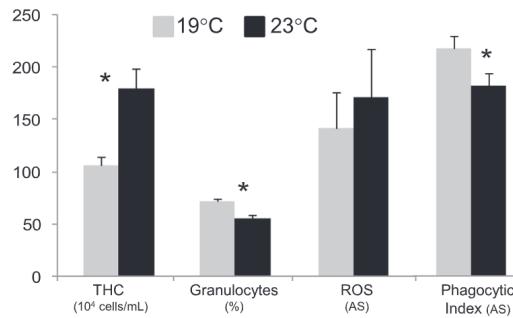


Fig. 3. Immune parameters in surfclams maintained at 19 or 23 °C. Each value represents the mean \pm SE ($n = 20$ and 11 clams for 19 °C and 23 °C treatments respectively). Units for each parameter are displayed below the labels (AS: arbitrary scale). An asterisk (*) indicates significance between treatments ($p < 0.05$, Student's *t*-test).

The proportion of phagocytic hemocytes as well as the number of beads engulfed by individual hemocytes (relative fluorescence intensity) was higher in clams maintained at 19 °C (50% and 200 relative fluorescence units, respectively) compared to those held at 23 °C (48% and 150 RFU, yet not statistically significant). The phagocytic index (% phagocytic hemocytes \times fluorescence intensity) was significantly higher in the 19 °C treatment compared to the 23 °C treatment ($p < 0.05$) (Fig. 3).

Mortality between groups was significantly higher in clams from the 23 °C treatment ($p < 0.001$). Following bacterial challenge, 81% of the clams maintained at 23 °C perished. Seventy two percent of these clams died within the first two days post injection (Fig. 4). Mortality in clams maintained at 19 °C and challenged with *V. alginolyticus* began on day 3 but was low overall (35%). Bacterial counts in hemolymph from clams remaining at the end of the experiment differed between treatments. The two remaining clams from the 23 °C treatment contained bacteria counts $> 3.5 \times 10^3$ cfu ml $^{-1}$, while only three (23%) of the clams from the 19 °C treatment had bacteria numbers within this range. In contrast, the other clams in the 19 °C treatment averaged 2.4×10^2 cfu ml $^{-1}$.

4. Discussion

The results of this study demonstrated enhanced metabolic demands, greater energy use and a poor immune status of clams held at 23 °C compared to those reared at 19 °C. Net energy gains (scope for

growth) were significantly higher in clams maintained at 19 °C, leaving more energy available for growth, reproduction and immunity. Use of adductor muscle protein and mantle glycogen and lipids was significantly higher in clams held at 23 °C. Although not all differences between metabolites were statistically significant, the general trends in the data show greater energy use in clams from the 23 °C treatment which is further supported by the principal component analysis of biochemistry data between groups. Additionally, the immune status of the clams in the 23 °C treatment was significantly reduced in phagocytic index of hemocytes, with significantly higher clam mortality following bacterial challenge.

Scope for growth has been used in numerous studies as an index of energy balance (Warren and Davis, 1967; Dame, 1972; Bayne et al., 1973; Bayne, 1976; Buxton et al., 1981). Increased metabolic demands and reduced filtration rates at temperatures outside the optimal range of the species diminish energetic gains. In a previous study, Kim and Powell (2004), surveyed surfclams off the Delmarva Peninsula and showed that few clams remained inshore in shallow water, which was further supported by Weinberg (2005). During the survey, surfclams with abnormal gonadal growth and atrophied digestive glands were observed, signifying physiological stress. The authors hypothesized the abnormal gonadal growth to be caused by increasing water temperatures which increased energy demand for tissue maintenance and reduced filtration rates leading to starvation and subsequent break down of gonadal tissue as a source of energy.

Results of the current study based on a series of laboratory experiments, strongly support the hypothesis of Kim and Powell (2004). Filtration rates were significantly lower at 23 °C, compared to 19 °C, suggesting that 23 °C is a suboptimal temperature. A reduction in filtration rate limited the amount of food that was ingested and energy available for other physiological processes. Concomitantly, surfclams excreted more ammonia and consumed greater amounts of oxygen at 23 °C, indicating an enhanced energetic demand. Additionally, irrigatory efficiency was significantly lower in clams from the warm temperature treatment demonstrating increased food requirements for maintenance needs. Furthermore, assimilation efficiency was negatively influenced by temperature as indicated by the significantly lower ability of clams to assimilate ingested food at 23 °C. Likewise, scope for growth was significantly lower in clams from the 23 °C treatment which may inhibit the ability of the surfclams to cope with additional stress.

Although scope for growth was significantly lower at 23 °C, net energy gains remained positive. A reduction in the energy available for growth is however likely to lead to low tissue weights, starvation mortality, and a reduction in overall size which has been described in the Delmarva region (Weinberg, 2002; Kim and Powell, 2004; Weinberg, 2005; Marzec et al., 2010; Narváez et al., 2014; Munroe et al., 2016). Additionally, indirect effects of increasing temperature not accounted for in the current study may also be at play in the field. For instance, high seawater temperature in the field may lead to modifications of the quantity and/or quality of phytoplankton resulting in a negative energy balance. Global reductions in phytoplankton concentrations have been documented over the last century (Boyce et al., 2010) and past decade (Behrenfeld et al., 2006). Furthermore, climate change has been shown to negatively influence phytoplankton species composition (shifting from large diatoms to smaller species/flagellates) and microalgal biomass found in the spring bloom and throughout the year (Lehman, 2000; Goffart et al., 2002; Lassen et al., 2010). A poor food source during the spring may interfere with reproductive conditioning and storage of energy for use following spawning and during stressful periods. Phytoplankton of low nutritional quality and/or quantity may not provide the resources necessary for large bodied bivalves such as surfclams to maintain all of their metabolic needs (Taylor, 1960; Powell et al., 1995; Kim and Powell, 2004; Munroe et al., 2013). It is not known how the energy of the food used in the current study compares to that measured during the summer in the natural environment. Energy availability for scope for growth may also be

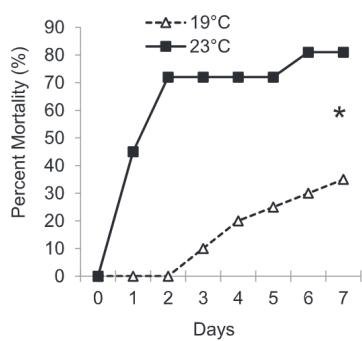


Fig. 4. Cumulative mortality in clams injected with *V. alginolyticus* and maintained at 19 or 23 °C. An asterisk (*) indicates a significant difference between treatments ($p < 0.05$, LogRank test).

reduced locally due to intraspecific competition in high density areas. As a matter of fact, growth rates have been shown to be poorer in areas of higher densities (Weinberg and Helser, 1996; Weinberg, 2002). In addition, density dependent growth has been demonstrated in surfclams from New York to the Delmarva region (Fogarty and Murawski, 1986; Cerrato and Keith, 1992; Weinberg, 1998; Weinberg, 2002). Thus, in areas in which competition for resources is high, reductions in phytoplankton quantity and quality and increasing temperatures are likely to significantly impact energy available for growth, reproduction and immunity.

Moreover, the sizes of the animals used in this study (129.28 ± 11.93 mm in length, mean \pm SD) do not represent the largest size classes found in surfclam populations in state and federal waters. For example, surfclams between 140 and 180 mm are commonly found in New York state waters (Dahl and Hornstein, 2010; O'Dwyer and Hornstein, 2013), and surfclams are known to grow to a maximal size of approximately 200 mm in length (Weinberg and Helser, 1996). Large marine bivalves require greater energetic demands to meet tissue maintenance needs as compared to smaller ones (Powell et al., 1995; Munroe et al., 2013; Narváez et al., 2014; Munroe et al., 2016), meaning that larger surfclams (> 140 mm) may have a negative scope for growth if maintained under the warm temperature used in this study (e.g. 23 °C).

Surfclams smaller in size than the ones used in this study may be able to maintain a positive scope for growth under temperatures similar or even warmer than those used in this study due to the lower energetic demands of smaller individuals (Cerrato and Keith, 1992; Munroe et al., 2016). Surfclams mature at a size of about 40 mm (Ropes, 1979) and, some within months after settlement at lengths < 5 mm (Chintala and Grasse, 1995; Chintala, 1997; Northeast Fishery Science Center (NEFSC), 2017). The additional energy cost associated with early sexual maturity, in addition to temperature, fishery, and other environmental stressors may limit the maximum size, growth rate, and lifespan of Atlantic surfclams (Cerrato and Keith, 1992; Munroe et al., 2016). This could have a significant impact on the commercial fishery and higher level trophic predators that rely on surfclams as a food source (Munroe et al., 2013, 2016). Overall, these intricate interactions make it difficult to predict how surfclams populations will respond to current and projected climate conditions.

Bivalves have been shown to alter their energy balance in various ways to avoid net negative gains. For example, they may increase the assimilation efficiency of the food ingested or adjust the irrigatory efficiency (Newell et al., 1977; Newell and Branch, 1980). Neither strategy was utilized by the animals exposed to higher temperatures during the current study, resulting in less energy gained under stressful summer conditions, potentially limiting the amount available for reproduction, immunity and other physiological processes. Limited energetic reserves could impact the ability of the clam to endure an extended high temperature period as well as spawning success in the fall. Additionally, it has been suggested that warmer temperatures can increase predation pressure on bivalve recruits (Freitas et al., 2007) and predation has been shown to have a significant influence on surfclam recruitment (Mackenzie Jr. et al., 1985). Spawning during the fall when temperatures begin decreasing may provide refuge from significant predation; reducing the strength of the fall spawn may have significant impacts on recruitment for a given year.

Continuous reductions in scope for growth will ultimately lead to starvation, use of internal energy sources and mortality, which is especially true for large bodied bivalves such as surfclams (Narváez et al., 2014; Munroe et al., 2016). Reduced metabolites in the adductor muscle and mantle tissues showed that the use of internal energy sources started within a one week period in clams maintained at 23 °C. These findings suggest that resources available to these animals for other physiological functions such as reproduction or immunity were limited, leaving the clams with both a strained energy budget and a compromised immune system. Such a scenario would have direct

consequences at organismal (slower growth rates, enhanced susceptibility to infections) and population (reduced fecundity) levels.

Previous studies have shown that short term but frequent exposure to temperatures outside of the thermal range of the clam *Macoma balthica* during summer led to its disappearance in the southern limit of its range in Spain (Jansen et al., 2007). For surfclams, large mortality events were documented in the Delmarva region by the National Marine Fisheries Service (NMFS) in 2003, and subsequently by Kim and Powell (2004) and Weinberg (2005). Although no mortality events have been documented in New York state waters, surfclam biomass declined 72% between 2002 and 2012 (Dahl and Hornstein, 2010; O'Dwyer and Hornstein, 2013), suggesting significant mortality well above natural and fishing levels. Overall, fewer clams remain inshore between New York and Virginia today in comparison to population sizes reported between 10 and 20 years ago (Kim and Powell, 2004; Normant, 2005; Weinberg, 2005; Dahl and Hornstein, 2010).

Temperature also plays an important role in directly regulating the immune defenses of marine bivalves (Paillard et al., 2004). The immune status of an organism will impact its ability to fight off pathogens. In this study, we assessed surfclams ability to fight the pathogenic bacteria *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 and other microbial processes (Buggé et al., 2007). Animals should be able to maintain an efficient immune status as long as they are not stressed and sufficient energy is available to support immune functions.

Our results suggest that clams maintained at 23 °C were immune-compromised. While total hemocyte counts were higher in animals from the 23 °C treatment, significantly higher percentages of granulocytes were measured in clams from the 19 °C treatment. Granulocytes in bivalves represent the hemocyte subpopulation involved in most defense processes including phagocytosis and the production and release of antimicrobial peptides (Mitta et al., 1999; Hégaret et al., 2003b; Perrigault et al., 2011). Results from the phagocytosis assay further supported a decrease in immune performance in clams maintained at 23 °C as shown by a significant reduction in the phagocytic index as compared to clams held at 19 °C ($p = 0.048$). These findings of lower immune performances in clams maintained at 23 °C were corroborated by higher mortality in this group (81%) following challenge with *V. alginolyticus* as compared to clams held at 19 °C. This is further supported by higher bacterial counts in remaining live individuals maintained at 23 °C in contrast to those held at 19 °C.

Although it is widely understood that concentrations of *Vibrio* species generally increase as water temperatures rise, both of the experimental temperatures are within the optimal range for a wide range of pathogenic vibrios, including *V. alginolyticus* (Kaneko and Colwell, 1973; Ayres and Barrow, 1978; Janda et al., 1988; Drake et al., 2007). Hence, we hypothesize that increased clam mortality and higher bacterial counts among survivors in clams maintained at 23 °C as compared to 19 °C were driven by the detrimental effect of the higher temperature on surfclam defense rather than a beneficial effect on the bacteria, although a combination of both processes cannot be ruled out. Overall, the reduced ability of surfclams to fight pathogens during temperature stress puts them at a higher risk of infection by opportunistic micro-organisms. Although bacterial infections have not been observed during histological analyses conducted on surfclams from the field, samplings are relatively scarce and prior investigations did not specifically target bacterial infections. In many cases, bacterial infections are often acute and resulting mortalities can occur rapidly (within days) and the only thing remaining is an empty shell (Malham et al., 2009).

In conclusion, ecologically-relevant high temperature had a significant negative impact on scope for growth, energy metabolism, and the immunity of adult Atlantic surfclams. Although, scope for growth was significantly lower at 23 °C as compared to 19 °C, net energy gains remained positive under our experimental conditions, suggesting that other factors in combination with temperature stress (e.g. food

availability) may contribute to the poor physiological condition of surfclams in recent surveys along the south shore of Long Island and the Delmarva Peninsula. A variety of factors influence scope for growth in marine bivalves, two of the most important being temperature and food. Increasing temperatures have been shown to negatively impact food resources for the Atlantic surfclam, which could lead to net negative energy gains, especially during the summer when temperatures are warmest. Rising temperatures in the future may further truncate the southern range of the surfclam (Weinberg, 2005) and limit the ability of the population to grow and reproduce successfully (Marzec et al., 2010) which may lead to the disappearance of the surfclam inshore between New York and Virginia. Nevertheless, an evaluation of the effect of temperature on the scope for growth and reproductive effort of different size classes (particularly smaller individuals) is needed for a better understanding of the potential impact of projected climate conditions on surfclam populations.

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