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

Jesse Hornstein, Emmanuelle Pales Espinosa, Robert M. Cerrato, Kamazima M.M. Lwiza, Bassem Allam*

School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11794-5000, USA

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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 clamming 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; Clotteau 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),

* Corresponding author.
E-mail address: Bassem.Allam@stonybrook.edu (B. Allam).

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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, phagocytoze, 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 clamming 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 ± 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, 3.5 l 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 = $V/t * \ln(Co/Ct)$, where V is the volume of seawater in the chamber, t is the time in hours, Co is the concentration of algae at time 0 and Ct 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 preweighed 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 O_2/hr). Oxygen consumption was transformed into energy (for calculation of scope for growth) using the conversion 1 mg O_2 = 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 (μg NH_4-N/h). Ammonia excretion was converted into energy using the conversion 1 mg NH_4 = 5.94 cal (Elliott and Davison, 1975; Han et al., 2008).