Behavioral and physiological effects of ocean acidification and warming on larvae of a continental shelf bivalve

Raymond Czaja Jr.[1], Robert Holmberg[2], Emmanuelle Pales Espinosa[1], Daniel Hennen[3], Robert Cerrato[1], Kamazima Lwiza[1], Jennifer O'Dwyer[4], Brian Beal[2,5], Kassandra Root[2], Hannah Zuklie[2], Bassem Allam[1*]

[1]School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11790-5000

[2]Downeast Institute, 39 Wildflower Lane, P.O. Box 83 Beals, ME 04611

[3]Northeast Fisheries Science Center, 166 Water Street Woods Hole, MA 02543-1026

[4]New York State Department of Environmental Conservation, East Setauket NY1173

[5]University of Maine at Machias, 116 O'Brien Avenue, Machias, ME 04654

*Corresponding author

Bassem Allam

School of Marine and Atmospheric Sciences

Stony Brook University

Stony Brook, NY 11790-5000

United States

E-mail: bassem.allam@stonybrook.edu

Phone: 1 (631) 632 8745

Abstract

The negative impacts of ocean warming and acidification on bivalve fisheries are well documented but few studies investigate parameters relevant to energy budgets and larval dispersal. This study used laboratory experiments to assess developmental, physiological and behavioral responses to projected climate change scenarios using larval Atlantic surfclams *Spisula solidissima solidissima,* found in northwest Atlantic Ocean continental shelf waters. Ocean warming increased feeding, scope for growth, and biomineralization, but decreased swimming speed and pelagic larval duration. Ocean acidification increased respiration but reduced immune performance and biomineralization. Growth increased under ocean warming only, but decreased under combined ocean warming and acidification. These results suggest that ocean warming increases metabolic activity and affects larval behavior, while ocean acidification negatively impacts development and physiology. Additionally, principal component analysis demonstrated that growth and biomineralization showed similar response profiles, but inverse response profiles to respiration and swimming speed, suggesting alterations in energy allocation under climate change.

Keywords: Ocean acidification, ocean warming, bivalve larvae, surfclam, behavior, energy budget

Highlights:

- Warming and acidification impacts on surfclam larvae were investigated
- Warming increased larvae feeding, scope for growth and biomineralization
- Warming decreased swimming speed and pelagic larval duration
- Acidification increased respiration but reduced immunity and biomineralization

1. Introduction

1.1 Background

Increasing carbon dioxide emissions are affecting physical and chemical properties of the ocean (Caldeira and Wickett, 2003). These effects include ocean warming, resulting from an enhanced greenhouse effect which causes more solar radiation to be absorbed by the ocean, and ocean acidification (OA), which occurs as atmospheric carbon dioxide is absorbed by the ocean, thereby shifting carbonate chemistry equilibria (e.g., decreased seawater pH and calcium carbonate mineral saturation state). The latest Intergovernmental Panel on Climate Change (IPCC) assessment report predicts average, coastal sea surface temperatures (SST) increases over 3°C and pH decreases over 0.4 by the end of the 21st century, under the "business-as-usual-path" Representative Concentration Pathway (RCP8.5) (Pörtner et al., 2019). Within shelf waters of the northeast United States, the Middle Atlantic Bight (MAB) not only hosts numerous commercially important shellfish species, but may be particularly sensitive to climate change. The MAB has warmed three times faster than the global average rate (Saba et al., 2016) and experiences relatively low pH and buffering capacity (Wanninkhof et al., 2015). Numerous studies have linked MAB oceanography, climate change and shellfish fisheries production. For example, the northward and deep-water shift of American lobster stock, including its collapse in southern New England, is believed to be driven by ocean warming(Pearce and Balcom, 2005; Wahle et al., 2015). Additionally, models that assume decreased sea scallop, *Placopecten magellanicus*, growth due to OA have predicted over a 50% decrease in production by 2050 under RCP8.5 (Cooley et al., 2015; Rheuban et al., 2018). However, there are gaps in knowledge regarding interactive climate change influences on diverse responses including those related to the energy budget, immune functioning and larval dispersal.

As ectothermic organisms that typically possess calcified shells, mollusks may be particularly susceptible to climate change phenomena, specifically, OA. In a meta-analysis of OA effects on marine organisms, Kroeker et al. (2010) found that mollusks exhibited the lowest survival rates compared to other taxa such as echinoderms and crustaceans, suggesting bivalves may be more sensitive to OA. As broadcast spawners, bivalves typically produce planktotrophic larvae that remain in the water column for multiple weeks before settlement (Loosanoff and Davis, 1963). Physiological tolerances as well as energy acquisition and expenditure during the larval stage may be different than that of the adult stage (Bayne, 1965; Peteiro et al., 2018); therefore, it is important to study responses to climate change at various life stages. Additionally, larval bivalves often possess shells with different mineralogy than adults (i.e., aragonite, as opposed to an aragonite-calcite mixture), which may affect how different life stages respond to OA (Fuller and Lutz, 1988; Weiss et al., 2002). While there have been significant contributions toward understanding climate change effects on adult bivalves, meta-analyses reveal fewer published studies on climate change effects on bivalve larvae (Clements and Darrow, 2018). Additionally, while there have been recent studies analyzing interactive ocean warming and OA effects on marine bivalves (Cole et al., 2016; Van Colen et al., 2018; Matoo et al., 2021; Bosch-Belmar et al., 2022), meta-analyses (Kelley and Lunden, 2017; Cattano et al., 2018; Leung et al., 2022) again have identified a need for additional studies examining effects of OA and ocean warming on bivalve larvae. A better understanding of interactive climate change effects on bivalve larvae allows scientists to more accurately predict shifts in recruitment, population size and structure, dispersal and distribution patterns.

Of particular interest is the Atlantic surfclam, *Spisula solidissima solidissima* (hereafter referred to as 'surfclam') that supports a $30 million dollar fishery in the northeast U.S.. Surf-

clams are distributed between Nova Scotia, Canada and Cape Hatteras, North Carolina and primarily live in continental shelf waters (Wigley and Emery, 1968); however, near the northern end of their distribution, they can be found in the lower intertidal in shallow bays. To the south, they are replaced by the southern subspecies, *Spisula solidissima similis*, which may be found in warmer, shallow waters (e.g., Long Island Sound) as far north as southern New England (Hare et al., 2010). Previous studies have shown that increased temperatures may lead to decreased survival for juveniles (Acquafredda et al., 2019), decreased adult scope for growth (Hornstein et al., 2018), shifts in distributions to cooler water (Timbs et al., 2019) and reduced fishing yields (Hennen et al., 2018) for surfclams. Although sensitive to moderate ocean warming (temperatures above 20°C) (Munroe et al., 2016; Hornstein et al., 2018; Acquafredda et al., 2019), adult surfclams may be resilient to moderate OA (pH of 7.51) but sensitive to severe OA (pH of 7.31) (Pousse et al., 2020). Additionally, Meseck et al. (2021) found that larval surfclam growth increased under a moderate OA scenario (pH 7.63), but decreased under a severe OA scenario (pH 7.47). However, no studies have examined interactive ocean warming and OA effects on surfclam larvae. It is known that 20-22°C represents the ideal temperature for surfclam larvae and recruit cultivation in an aquaculture setting (Loosanoff and Davis, 1963; Acquafredda et al., 2019), but it is not known how surfclam larvae will respond to discrete, forecasted, ocean warming-based temperature changes. Analyzing responses to combined ocean warming and OA scenarios is important, as previous studies have shown that ocean warming may exacerbate or mitigate OA effects on bivalve larvae, depending on factors such as metabolic trade-offs (Harney et al., 2016), thermal thresholds (i.e., extremes of the temperature treatments) (Ko et al., 2014), and local adaptation (Cole et al., 2016; Van Colen et al., 2018). Interestingly, Pousse et al. (2022) found that via dynamic energy budget model simulations, combined OA and ocean

warming may yield faster juvenile surfclam growth near the end of the century, highlighting the importance and complexity of assessing multiple stressors in tandem.

While quantifying growth and mortality to ocean warming and OA is important, other biological (e.g., physiological and behavioral) responses are needed to better understand how fisheries will respond to climate change. Examining physiological responses (e.g., feeding and respiration rates) under both ocean warming and OA scenarios may provide energy budget insights and a mechanistic explanation for changes in energy dependent processes (e.g., growth rates). For example, Gray et al. (2017) found that OA negatively impacted larval mussel, *Mytilus californianus*, feeding physiology, thereby delaying development and growth. Such physiological responses have been examined for adult, but not larval surfclams (Pousse et al., 2020). Less studied than physiological responses are behavioral responses (Espinel-Velasco et al., 2018; Wang and Wang, 2020). Behavioral responses such as a swimming speed, may not only factor into energy budgets and thereby be related to physiological responses, but may affect dispersal patterns via controlling water column position (Garland et al., 2002; North et al., 2008; Hubbard and Reidenbach, 2015). Therefore, it is important to understand how climate change may impact swimming behavior. To date, only one study has been published regarding OA effects on bivalve swimming behavior (Meyer-Kaiser et al., 2019), and three studies have been published regarding combined OA and ocean warming effects on gastropod larvae swimming behavior (Zhang et al., 2014; Fonseca et al., 2020; Kavousi et al., 2021). Also relevant to dispersal patterns is pelagic larval duration (PLD), or the amount of time spent in the water column before settlement (Levin, 2006). Shorter PLDs typically yield lower dispersal distances and different dispersal paths (Ospina-Alvarez et al., 2018; McGeady et al., 2022). While PLD is

often a function of growth rates, few studies have measured changes in bivalve larvae PLD in response to OA and ocean warming (Lawlor and Arellano, 2020).

The primary objective of this study was to assess the interactive effects of ocean warming and OA scenarios on a suite of understudied, fisheries-relevant responses for bivalve larvae using the Atlantic surfclam as a model species. The following three hypotheses were tested: 1) ocean warming and OA will affect physiological responses such as clearance and respiration rates, 2) ocean warming and OA will affect swimming behavior and PLD, 3) physiological and behavioral responses are linked, potentially due to energy allocation. Analyzing both physiological and behavioral responses in bivalve larvae to climate change may provide wholistic insights regarding fisheries responses to climate change.

2. Materials and Methods

2.1 Husbandry, Maintenance and Water Chemistry

The experimental trial took place in the Ocean and Coastal Acidification Laboratory at the Downeast Institute (DEI) in Beals, Maine, USA. The experimental system is designed around the Apex aquarium controller platform (Neptune Systems, Morgan Hill, CA), consisting of 30 11-L conical experimental tanks independently configurable to any combination of seawater pH and temperature treatments (precise to 0.01 pH units and 0.1 ºC, respectively). Seawater pH control operates in a feedback loop: temperature-compensated pH is monitored in real time using pH and temperature probes (Oakton EW-35805-67 and Neptune Systems PRBTMPJR, respectively); the aquarium controller activates a solenoid valve when pH rises above a programmed setpoint, dosing CO_2 gas through a diffuser (reducing pH); the aquarium controller deactivates the solenoid valve when the setpoint is reached (stabilizing pH). pH monitoring and

CO_2 mixing occurs independently in each tank, achieving true replication (Cornwall and Hurd, 2016). The Apex firmware was modified to allow for pH calibration on the total hydrogen ion scale (pH_T) using synthetic seawater buffers prepared at DEI, which are most appropriate for the seawater pH range and reduce measurement errors associated with differences in ionic strength and composition between buffers and sample (Paulsen and Dickson, 2020). Seawater temperature is adjusted in a similar feedback loop using custom-designed heat jackets that wrap around each tank.

A randomly-interspersed, fully factorial design was employed to test simultaneously three temperature and two pH treatments (N = 5; all sample sizes refer to replicate tanks, unless otherwise stated). Temperature treatments (17, 20 and 23°C) were chosen to represent past (1970), current (2020) and future (2100) mean summer whole-water column temperatures, respectively, in New York Bight inner-shelf waters (Alexander et al., 2018; Thorne et al., 2020). Two pH treatments (7.7 and 7.3) were chosen to represent current (2020) and future (2100) mean summer whole-water column pH, respectively, in the same habitat (Thorne et al., 2020; Wright-Fairbanks et al., 2020). Treatments were chosen with the New York Bight as a target region. The range of temperatures within the New York Bight also overlaps with northern and southern latitudes across the surfclam distribution. For example, 17°C represents a temperature that surfclam larvae may experience present day in Southern New England, and 23°C represents a temperature that surfclam larvae may experience present day in the southern MAB (Ropes, 1968; Mann, 1985; Weissberger and Grassle, 2003). Therefore, these temperature treatments have implications for spatial variability throughout the distribution of the surfclam. Additionally, Czaja Jr et al. (2023)found that in the New York Bight, summer temperature negatively affects

recruitment, suggesting a potential mechanism where larvae respond negatively to ocean warming.

Broodstock (100-150 mm) were collected during low tide from an intertidal flat on Deer Isle, Maine (44°16'40.5"N, 68°40'48.9"W) on 15 November 2020 and conditioned at DEI until spawning on 16 June 2021. Spawning and fertilization were conducted according to standard commercial hatchery procedures. Briefly, spawning was induced via heat shock (25°C) after adults were held at 12°C overnight. Sperm from six males were combined and added to eggs from one female. Therefore, maternal effects on different replicates should be nonexistent. After approximately 30 minutes of gamete incubation, during which gametes were gently mixed every five minutes to resuspend eggs, fertilized eggs were removed from remaining male gametes, as approximately 95% of the eggs showed the presence of polar bodies. Larvae were stocked in experimental tanks at densities of 10 larvae ml^{-1} and were at ambient conditions (21°C, pH of 7.8) for four hours before acclimation to treatment conditions. Acclimation occurred by adjusting temperature 1°C and the pH 0.1 units every three hours in each tank until treatment conditions were met. Holding tanks were stocked with filtered seawater (FSW) pumped into the lab from nearby Black Duck Cove and filtered to 1 μm. Larvae were fed ad libitum (algal concentrations were maintained at 20,000 cells ml^{-1} for the first 15 days and 50,000 cells ml^{-1} for the last 15 days) using a 1:1 mix of the haptophyte *Tisochyrsis lutea* (Tahitian strain) and the diatom *Chaetoceros muelleri* (Hawaiian strain). Tanks were cleaned, water replaced and larvae graded on a three-day cycle such that ten tanks were cleaned daily. Salinity was measured from the holding tank every 1-2 days. Experiments were terminated after 30 days.

Throughout the experimental trial, seawater pH_T and temperature measurements from each tank were logged once per minute using a custom VBA (Visual Basic for Applications)

macro that scraped real-time sensor data from the Apex. Seawater samples (50 ml) were collected weekly from each tank and preserved using mercuric chloride for later total alkalinity (TA) analysis. Upon conclusion of the trial, erroneous log data (i.e., sensor readouts during calibrations or water changes) were removed and pH_T and temperature were averaged by tank. Salinity was measured in the preserved seawater samples using a digital refractometer (Sper Scientific 300035, accurate/precise to 1 ppt). TA was measured using a spectrophotometrically-guided titration method accurate/precise near 1 µmol kg^{-1} SW (Yao and Byrne, 1998; Liu et al., 2015) adapted to the Cary 60 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA) using a custom ADL (Applications Development Language) script. Measurements were averaged by tank. TA method accuracy was verified using CO_2 in seawater certified reference material (CRM, batch #162) supplied by the Dickson lab (Scripps Institution of Oceanography, UC San Diego). Remaining seawater carbonate chemistry parameters (partial pressure of CO_2, pCO_2; dissolved inorganic carbon, DIC; saturation state of aragonite, Ω_{Ar}) were calculated for each tank from pH_T, temperature, salinity, and TA using CO2Sys v2.1 (Pierrot et al., 2006) (K_1, K_2 from Lueker et al. (2000); K_{HSO4} from Dickson (1990); B_T from Uppstrom (1974)).

2.2 Developmental Responses

Mortality was quantified by estimating total number of remaining live larvae (via ciliary movement, swimming and shell contents) on day 30 relative to the initial number of live larvae in each tank, and is presented as a percent by subtracting the percent remaining alive on day 30 from 100. On days 5, 11, 16, 23 and 30, larvae were preserved via glutaraldehyde (0.5%) fixation and stored at -20°C for growth rate and biomineralization analyses. Growth rate was measured (50 larvae per tank) by the increase in larvae length through time via image analysis (ImageJ) on microscope-captured photos. Biomineralization was measured (20 larvae per tank) via cross-

polarized light microscopy similar to Wessel et al. (2018) on larvae preserved on days 16, 23 and 30. Briefly, biomineralization was quantified by calculating the mean grey scale value (hereafter referred to as biomineralization index) for individual larva in ImageJ, such that a grey scale value of zero represents black shell material (no biomineralization) and a grey scale value of 255 represents white shell material (100% biomineralization). This approach quantifies biomineralization via observed birefringence and is based on the principle that more crystalline calcium carbonate yield more (i.e., brighter) birefringence (Weiss et al., 2002). Photos for growth rate and biomineralization analyses were measured on a Nikon Eclipse TE2000-S inverted compound microscope (100x magnification).

2.3 Physiological Responses

Respiration rate assays were conducted on larvae (day 20-24) after modifying the approach of Waldbusser et al. (2015). Approximately 75 larvae were placed in a 4.5 ml capped cuvette with the respective treatment seawater. Each cuvette contained a Pyroscience oxygen-sensor spot that used a fiber-optic cable to transmit real-time oxygen concentration measurements to a computer. Assays lasted three hours and were kept at room temperature (21°C). Three controls were conducted without larvae to estimate background oxygen loss. Background oxygen loss was subtracted to estimate the total loss in oxygen due to larvae respiration from time zero to time three hours. Oxygen loss per $hour^{-}$ was standardized to exact larvae counts per cuvette and to estimated larvae biovolume. Biovolume was estimated using the equation for the volume of a sphere where each radius is represented by half the larval length, width, and height.

Clearance rate assays were conducted on day 14 larvae via modifying the approach of Ginger et al. (2013). Approximately 200 larvae were placed in a 50 ml centrifuge tube with 30

ml of FSW and 50,000 cells ml^{-1} of *T. lutea*. Tubes were placed in a temperature-controlled water bath (for the appropriate temperature treatment) for six hours. At the start and end of the assay, 2 ml from each tube were fixed with 0.5% glutaraldehyde to estimate algae concentrations. Algae concentrations were estimated via FlowCam, a continuous imaging flow cytometer. Approximately 0.1 ml of sample were analyzed with auto-image mode, a 300 µm FlowCell, a 4x objective, a minimum cell diameter of 1 µm and a speed dial setting of 10 at fast mode. Clearance rate as particle loss $hour^{-1}$ was standardized to exact larvae counts per tube and to estimated larvae biovolumes. Scope for growth was then calculated as [(clearance rate x absorption efficiency) - (respiration rate)]. Assumptions and values used for scope for growth calculations were based on Gray et al. (2017). Briefly, clearance rate and respiration rate were standardized to biovolume, clearance rate was converted into µJoules h^{-1} assuming an energetic cell content of 0.61 µJoules algae $cell^{-1}$ (Sprung, 1983), absorption efficiency was assumed to be 0.38 (Sprung, 1983) and respiration rate was converted into µJoules h^{-1} assuming 1 nL of O_2 to be 20.1 µJoules of respired energy (Crisp, 1971). Scope for growth calculations were performed on clearance and respiration rate values that came from the same tank, with two exceptions. In these two exceptions, clearance rate and respiration rate were measured from closely matched individuals from the same treatment but from different tanks. This was necessary because time limitations prohibited a complete set of measurements from all tanks. Clearance rate was measured for Tank 10 (17°C & 7.3) and Tank 25 (17°C & 7.7), but respiration rate was not measured from these tanks. These were combined with respiration rate measurements from Tank 22 (17°C & 7.3) and Tank 14 (17°C & 7.7), respectively, where no clearance rate measurements were taken. It was felt that including these two exceptions would beneficially reduce the effects of unbalanced treatment replicates for the data analysis.

Immune performance assays began on day 13 after modifying the approach of Schwaner et al. (2020). Larvae were exposed to bacteria (*Vibrio* spp.) cocktails (sensu Schwaner et al., 2020) for five days in 16.8 ml 6-well microplates with no aeration but in temperature controlled water bath (for the appropriate temperature treatment). Each well contained 100 larvae and 12 mL of FSW. Wells were dosed with 10,000 colony forming units per ml (CFU ml^{-1}) at the beginning of the assay and then 100,000 CFU ml^{-1} halfway through the assay. For each treatment, three control wells without bacteria were used. Immune performance was assessed as a function of percent mortality on the final day on ~50 larvae per well via ciliary movement, swimming and shell contents (high mortality equating low immune performance).

2.4 Behavioral Responses

Swimming responses were measured on days 5, 11, 16 and 23 via microscope video (Accu-scope Excelis HD camera attached to a Nikon SMZ745T dissection microscope) recording analysis software in ImageJ via the wrMTrck plugin (see Gamain et al. 2020 for details regarding the image analysis technique used by wrMTrck). For each well in a 6 well plate, 15 ml of FSW was added with ~50 larvae. Preliminary analyses showed that swimming responses were unaffected by densities between 20 and 100 larvae ml^{-1}, whereas for densities of 100 larvae ml^{-1} or greater, the software yielded biased (higher) swimming speeds (likely due to double counting of larvae and increased larval collisions). Preliminary analyses also showed no significant differences in swimming responses when using video durations between 5 and 45 seconds. However, a video duration of 15 seconds was chosen as a conservative approach to minimize potential measurement error associated with shorter videos. When running wrMTrck, program settings were adjusted from Gamain et al. (2020) to improve analyses at the video resolution used (Table S1). Swimming speed was calculated as mm $second^{-1}$, and percent swimming was

calculated as the percent of larvae, from each tank, that swam at any time point for each 15-second video. Swimming speed was also analyzed when standardized to larval length, however, outcomes did not change. Therefore, raw swimming speeds were reported and analyzed. PLD was determined by the presence of a 'searching foot' (Rodriguez-Perez et al. 2019; Fig. S1), as this stage of development indicates the larva is nearing settlement and seeking substrate for attachment. Every day from day 21 to 30, approximately 50 larvae from each tank were analyzed for the presence of a searching foot. Settlement percent was calculated as the percent of larvae from each tank that displayed settlement behavior at any time point for a 15-second period. Analyzing settlement percent through time allows for quantifying PLD as the day on which 50% of the larvae were ready to settle. All behavioral metrics were measured within one hour of removing larvae from holding tanks at room temperature (21°C). For all assays on live larvae (i.e., physiological and behavioral responses), larvae were removed from tanks by gently pipetting (via 50 ml serological pipettes) the appropriate amount of tank water (i.e., if 50 larvae were needed, 500 ml of tank were pipetted) into an appropriately sized beaker. Larvae were then concentrated using a 40 μm sieve after which, larval counts were performed to determine if the needed number of larvae were obtained for the assay.

2.5 Statistics: Analyses of Variance and PLD

All statistical testing was performed in R version 4.0.2 (base packages for ANOVAs). Two-way ANOVAs with pH and temperature as fixed, categorical factors were used to compare mortality (N = 5), immune performance (N = 3), respiration rate (N = 3), clearance rate (unbalanced with N = 3-4) and scope for growth (N = 3) among treatments. Sample sizes for individual responses are lower than the total number of tanks as some responses contained replicates that yielded extreme outliers (values outside three times the interquartile range) and/or

replicates with too few larvae to yield a reliable signal. For growth rate, heterogeneity of regression slopes with time precluded application of a two-way factorial ANCOVA of the variables of interest (pH and temperature). Therefore, growth rate differences were analyzed via a two-way ANOVA on calculated, linear growth rates ($\frac{L30-L5}{25}$), where L30 is larval length on day 30, L5 is larval length on day 5 and N=4. A three-way ANOVA, including two-way and three-way interactions, with pH, temperature and time (continuous) was initially used to compare biomineralization (N = 4), swimming speed (N = 4-5) and percent swimming (N = 4-5) among treatments. A repeated measures design to remove temporal pseudoreplication was used by including tank as a random effect (i.e., because the same tanks were measured through time). When time interactions were present, a two-way ANOVA was used for individual time points. Three separate time points were used and a Bonferroni correction was applied, using an adjusted alpha (α') of 0.0167. For clearance rate, swimming speed and percent swimming ANOVAs, a Type II Sum of Squares was used because of the unbalanced experimental design (Underwood, 1997). Multiple comparisons were carried out using Tukey's test for balanced designs and a Dunnett-Tukey-Kramer (DTK package) test for unbalanced designs. For DTK multiple comparisons, the outcome was considered significant if confidence intervals of estimated differences did not contain zero. An extended Box-Cox analysis (Sokal and Rohlf, 1981) was used to identify the best power transformation of the data to meet normality and homogeneity of variance assumptions of the ANOVAs.

It was expected that settlement rate through time should yield a logistic function, with an asymptote of zero percent settlement at time zero (day 21) and an asymptote of 100 percent settlement at time final (day 30). However, data for multiple treatments would not converge to a logistic function because of high variance and because settlement rates were not high enough at

the end of the experiment (day 30) (i.e., asymptotes were not achieved). Therefore, because the data were unable to be appropriately modelled as an autoregressive logistic function, settlement percent through time was analyzed as a line plot time series. Settlement percent for each treatment was averaged across tanks and in two-day bins to minimize variability (e.g., settlement percent on day 21 was averaged with day 22). PLD was then quantified as the first day on which the median surpassed 50% settlement.

2.6 Statistics: Multivariate Analyses

Principal component analysis (PCA) was used via the R packages 'vegan' (Oksanen et al., 2013), 'ggbiplot' (Vu, 2011) , 'cluster' (Maechler et al., 2013) and 'factoextra' (Kassambara and Mundt, 2017) to examine potential relationships between responses related to energy use including growth rate, clearance rate, respiration rate, swimming speed, scope for growth and biomineralization. These responses (hereafter referred to as the energy budget profile) allowed for 16 tanks to be used in PCA, as not all tanks were used for every response metric. Swimming speed and biomineralization data on the oldest larvae available (day 23 and day 30, respectively) were used for PCA. All data were standardized and made unitless by subtracting by the mean and dividing by the standard deviation (for each individual response). Biplots were examined visually to assess relationships between different response metrics. K-means cluster analysis was used to identify groups of data points (and their associated treatments) which were most alike. To determine the appropriate numbers of clusters, the 'average silhouette method' was used via the 'fviz_nbclust' function.

3. Results

3.1 Water Chemistry

All water chemistry parameters remained reasonably stable (within pH treatments), with TA exhibiting the highest variance (Table 1, Fig. S2). Increasing pCO_2 successfully reduced pH, increased DIC and reduced Ω_{Ar} (Table 1, Fig. S2). The highest pCO_2 occurred in the 23°C & 7.3 treatment (Table 1, Fig. S2). The lowest Ω_{Ar} occurred in the 17°C & 7.3 treatment and the highest Ω_{Ar} occurred in the 23°C & 7.7 treatment (Table 1, Fig. S2).

3.2 Developmental Responses

Larvae did not experience any significant difference in mortality between treatment groups (Table 2, Fig. 1). Although mortality was highly variable and seemingly high, average larval mortality (82.5%) was similar to other studies on surfclam larvae (Hurley and Walker, 1997; Meseck et al., 2021). Larvae experienced significantly higher growth rates (i.e., increase in larval length through time) at 23°C & 7.7 than all other treatments, but did not experience differences in growth rates between any other treatment groups (Fig. 2). Larvae experienced no difference in biomineralization between treatment groups on days 16 and 23 (Fig. 3, Table S2). However, on day 30, larvae experienced significantly lower biomineralization at pH 7.3 than 7.7 and at 17°C than 20°C ($p = 0.006$) and 23°C ($p = 0.015$) (Fig. 3, Table S2).

3.3 Physiological Responses

Respiration rate was significantly impacted by pH only, but clearance rate and scope for growth were significantly impacted by temperature only (Table 2). Larvae experienced a significantly higher respiration rate at pH 7.3 than pH 7.7 (Table 2, Fig. 4). Larvae experienced a significantly higher clearance rate at 23°C than at 20°C ($p = 0.0472$) and 17°C ($p = 0.0051$) (Fig. 5). Larval scope for growth was significantly higher at 23°C than at 17°C ($p = 0.0143$, Fig. 6). Larvae at pH 7.3 experienced significantly higher mortality when challenged with *Vibrio* spp. (lower immune performance) than larvae at pH 7.7 (Table 2, Fig. 7). For immune performance

assays, larvae exposed to bacteria generally had higher mortality than controls (Fig. S3), with an average of 5.34% of observed mortality being due to bacteria exposure.

3.4 Behavioral Responses

The percent of larvae swimming was not significantly different between treatment groups, but did significantly increase after day 5 (Table 3, Fig. 8A). Larval swimming speed also increased through time (Table 3, Fig. 8B). Larvae also swam significantly slower at 23°C, than at 20°C and 17°C (Table 3, Fig. 8B). Larvae from the 23°C & 7.7 treatment experienced a PLD of just above 25.5 days (Fig. 9). Larvae from all other treatment did not achieve 50% settlement by day 29.5 and therefore have a PLD of longer than 29.5 days (Fig. 9).

3.5 Multivariate Statistics

The silhouette plot showed that the optimal number of clusters was two (Fig. S4). The first cluster contained all three data points from the 23°C & 7.7 treatment, where larvae experienced increased growth and biomineralization. All other data points (and treatments) were contained in the second cluster, where larvae experienced decreased growth and suppressed physiological responses (clearance and respiration rate). The two dimensions represented by the biplot explain ~66% of the variance (Fig. 10). Within this biplot, vector directions show that within the first two principal components, three pairs of responses were changing in the same direction. The first pair was biomineralization and growth rate, the second pair was swimming speed and respiration rate, and the third pair was clearance rate and scope for growth rate (Fig. 10). Vector directions also showed that within the first two principal components, biomineralization and growth rate were changing in the opposite direction as swimming speed and respiration rate (Fig. 10).

Discussion

4.1 General Larval Performance

Previous studies have demonstrated that ocean warming and/or OA may not significantly affect mortality rates of mollusk larvae (Gobler and Talmage, 2014; Fonseca et al., 2020), but may significantly affect other responses (i.e., non-lethal responses) including immune performance (Schwaner et al., 2020), clearance rate (Cole et al., 2016), respiration rate (Gray et al., 2017), biomineralization (Wessel et al., 2018), growth rate (Meseck et al., 2021) and behavior (Fonseca et al., 2020). For example, Gobler and Talmage (2014) found that for larval Eastern oysters, *Crassostrea virginica*, projected OA did not significantly affect mortality but did significantly affect biomineralization, and Fonseca et al. (2020) found that for larval Netted whelk, *Tritia reticula*, projected ocean warming and OA did not affect mortality but did significantly affect swimming behavior. Such variable responses may be due to experimental design decisions, as more severe OA scenarios and lower pH may more likely lead to higher mortality. For example, Gobler and Talmage (2014) found that a moderate OA scenario (pH of 7.68) did not yield increased mortality for larval oysters, but Barros et al. (2013) found that a severe OA scenario (pH of 7.37) did increase mortality for larval oysters. These variable responses also highlight the importance if considering a suite of responses. More specifically, considering a suite of responses is important as survival under environmental changes may come at the cost of immune functioning (Rauw, 2012), growth (Harrington et al., 2019) and normal behavior (Holt and Jørgensen, 2014). The present study supports this idea as ocean warming and OA did not affect surfclam larval mortality, but ocean warming affected growth rate, clearance rate, biomineralization and behavioral responses and OA affected growth rate, immune performance, respiration rate and biomineralization. Although it should be noted that while

ocean warming and OA did not directly lead to increased mortality, decreased fitness in the natural environment and delayed metamorphosis (i.e., longer PLD) caused by climate change stress may indirectly lead to increased mortality via mechanisms such as increased susceptibility to predators (Jackson and Strathmann, 1981; Sponaugle et al., 2006).

4.2 Physiological Responses and the Energy Budget

pH had no significant effect on growth at 17°C and 20°C, but at 23°C, a pH of 7.3 yielded lower larval growth than at 7.7 (Fig. 2). Additionally, at ambient pH levels, temperature increased larval growth. These results suggest that surfclam larvae respond positively to ocean warming at specific pH levels. It should be noted that while increased food availability may offset climate change stress, the algae concentrations used in the present study (20,000-50,000 cells/ml) fall within the range of other studies, including OA studies on surfclam larvae (Talmage and Gobler, 2011; Meseck et al., 2021). Therefore, there is little evidence to suggest food availability artifacts in the present study. The positive impacts of increased temperature contrasts previous findings in a general context, as a meta-analysis of climate change impacts on marine larvae found that calcifying larvae typically respond negatively to ocean warming (Przeslawski et al., 2015). This contrast is particularly noteworthy, as the present study used a relatively large temperature change of +6°C total, or +3°C of projected warming, and still found that such an OW scenario increased growth. Although, it should be noted that in an aquaculture setting, 20-22°C has been identified as the optimal temperature range for rearing larval surfclams (Loosanoff and Davis, 1963; Fay et al., 1983). Therefore, this temperature increase does not appear stressful for surfclam larvae. Nevertheless, the outcome of increased growth under projected warming, contrasts some previous findings, as Munroe et al. (2016) found increases in

current bottom water temperature of >1°C can lead to decreased adult surfclam growth. Furthermore, the present study found that temperature increased mean larval scope for growth, but Hornstein et al. (2018) found that adult surfclam scope for growth was lower at 23°C than 19°C, providing further contrast. However, other studies have found that adult bivalves exhibit stronger, more negative responses to ocean warming than juveniles or larvae (Pörtner and Farrell, 2008; Stevens and Gobler, 2018), potentially because larvae and juveniles have lower total metabolic rates. In further contrast to the present findings, sea scallops, *P. magellanicus*, which also occupy cool, MAB continental shelf waters, had lower larval growth at 19°C than lower temperatures (Culliney, 1974). While both surfclams and sea scallops occupy continental shelf waters, surfclams are found more inshore, exposing them (and presumably their larvae) to warmer waters than sea scallops and potentially yielding increased adaptive ocean warming responses. Indeed, other studies have found that larval bivalves in shallow and/or warmer habitats may be tolerant to ocean warming (Cole et al., 2016; Lawlor and Arellano, 2020). Providing further support for this hypothesis, Loosanoff and Davis (1963) found that 20-22°C is the optimal temperature range for culturing larval surfclams, compared to 10-15°C for larval ocean quahogs, *Arctica islandica*, which also occupy deeper MAB continental shelf waters. These results also suggest that the temperature-induced recruitment failure observed for surfclams in the New York Bight by Czaja Jr et al. (2023) is not likely due to harmful ocean warming effects on larval fitness. As another relevant comparison, Pousse et al. (2022) found that OA alone decreased simulated growth of juvenile surfclams near the end of the century, but combined OA and ocean warming increased simulated growth. The present study found potential opposite trends were ocean warming alone increased growth, but combined OA and ocean

warming decreased growth. This contrast highlights the importance of life stage specific responses to additive, if not synergistic stressors.

The interactive ocean warming and OA effects on larval surfclam larval development have been documented for other bivalve larvae including brooding flat oysters, *Ostrea angasi* (Cole et al., 2016), and northern bay scallops, *Argopecten irradians irradians* (Talmage and Gobler, 2011). Growth rate differences may have been observed between pH treatments only at 23°C because Ω_{Ar} was the highest (1.50) for the 23°C and 7.7 treatment, potentially yielding the most ideal conditions for development. Carbonate chemistry parameters and temperature interact via a negative relationship where warmer water retains a higher Ω_{Ar} (relative to cooler waters) under increased CO_2 due to decreased solubility (Millero, 2007). Such interactions have been observed in the field where ocean warming is slowing the OA-induced Ω_{Ar} decline in the Arctic (Yamamoto- Kawai et al., 2011) and in previous lab experiments where warmer water reduced dissolution processes for adult mollusks under high pCO_2 (Noisette et al., 2016). Furthermore, Gray et al. (2017), found that Ω_{Ar} may best predict bivalve larvae responses to OA, thereby potentially explaining why the 23°C and 7.7 pH treatment yielded the highest growth rate. While the underlying cellular mechanisms are outside of the scope this study, other OA-marine invertebrate studies have found that OA-induced hemolymph pH decreases can lead to disrupted ion regulation and altered enzyme activity, thereby decreasing growth (Pörtner et al., 2004).

Further potential explanations regarding growth rate responses to ocean warming and OA may involve physiological responses that affect the energy budget. For example, larvae experienced lower respiration rate under OA conditions (Fig. 4), suggesting larvae were coping with abiotic stress (potentially via energy conservation). Therefore, less energy was available for growth. Additionally, growth and clearance rate were highest at 23°C (Fig. 5), suggesting

potential metabolic depression at lower temperatures. PCA suggested that growth rate and respiration rate show opposite response profiles (Fig. 10). Therefore, it is possible that for the 23°C and pH 7.7 treatment, more energy was available to increase growth rate because less energy was used for maintenance, as represented by respiration. Counter to expectation, PCA did not show a link between growth rate and clearance rate, but larvae experienced higher clearance rates under ocean warming conditions. This difference in clearance rate may explain why larvae grew faster at 23°C than 17°C, but does not explain why larvae grew faster at 23°C and 7.7 than the 20°C treatments, as larval clearance rates did not significantly differ between 23°C and 20°C. However, the opposite responses profiles for growth rate and swimming speed leads to the possibility of an energy trade-off such that when more energy is allocated to growth, less energy is allocated to locomotion. Therefore, larvae may have grown faster at 23°C than 20°C because less energy is used for locomotion at 23°C and therefore more energy remains available for growth. Such a hypothesis has never been investigated in marine invertebrate larvae, but support for an energy trade-off between growth and locomotion when faced with environmental variability (e.g., temperature and food availability) has been found for marine fish (Billerbeck et al., 2001; Killen et al., 2014). PCA also suggested that growth rate may be linked with biomineralization (Fig. 10). This relationship aligns with previous studies that found when under the influence of ocean warming and/or OA, larval shell properties respond similarly to growth (Miller et al., 2009). This suggests that when more energy is allocated to linear growth, more energy may be simultaneously allocated to shell development (e.g., increasing shell thickness or different crystalline structure). This distinction is specified because increased linear growth does not always lead to increased shell development. For example Talmage and Gobler (2010) found that shell length increased in larval hard clams (*Mercenaria mercenaria*) grown at a pCO_2 of 750

as compared 1500 ppm, while shell thickness did not change. However, it should be noted that other studies have found a growth-calcification trade-off where reduced pH increases calcification costs, which then decreases shell length (Ramesh et al., 2017; Sanders et al., 2018). The potential lack of this trade-off for surfclam larvae suggests that they may be relatively tolerant to OA.

While many studies have documented negative impacts of climate change on adult bivalve immune performance (Wang et al., 2016; Nardi et al., 2018; Huang et al., 2022), surprisingly few studies have analyzed impacts of climate on larval bivalve immune performance. However, Schwaner et al. (2020) found that larval hard clams (*M. mercenaria*) experienced higher pathogen-induced mortality under OA, aligning with the results of the present study. Such higher mortality may be due to increased bacterial growth under OA conditions or decreased host immunity (e.g., hemocyte activity), as Schwaner et al. (2020) indeed found higher *Vibrio*. spp. concentrations under acidified conditions. Additionally, Elston et al. (2008) found that larval oyster and clam mortality events on the west coast of North America were linked to *Vibrio* spp. blooms and warmer waters, suggesting increased temperature may worsen pathogen-induced mortality. This contrast results of the present study where temperature had no significant impact on bacteria-induced mortality (Fig. 7). Furthermore, while adult bivalve immune responses may differ than those of larvae, Hornstein et al. (2018) showed that adult surfclam immune performance responds negatively to ocean warming. This further highlights that larval surfclams are more resilient to ocean warming than adult surfclams. While it is unknown if pathogens such as *Vibrio* spp. regularly leads to mortality of wild population surfclams, the results of the present study suggest that OA may exacerbate *Vibrio* spp. risks for surfclams, at least during the larval stage. Furthermore, while temperature did not affect

pathogen-induced mortality in the present study, warming coastal waters may support larger *Vibrio* spp. populations, providing an avenue by which ocean warming may increase surfclam risks to pathogens (Le Roux et al., 2016).

4.3 Behavioral Responses

A vast literature exists regarding the effects of ocean warming and OA on swimming behavior of larvae of fauna other than mollusks (e.g., fish, echinoderms and crustaceans) (Chan et al., 2015; Cominassi et al., 2019; Gravinese et al., 2020); however, few studies have focused on the combined effects of ocean warming and OA on larval mollusk swimming behavior. Four studies investigating OA impacts on mollusk swimming behavior revealed either decreased swimming speeds at projected pH levels (Zhang et al., 2014; Fonseca et al., 2020), or no change in swimming speed (Meyer-Kaiser et al., 2019; Kavousi et al., 2021), the latter aligning with the results of the present study. Additionally, studies on swimming behavior of non-mollusk marine invertebrates have found that echinoderm larvae do not change swimming behavior in response to OA; (Chan et al., 2015), aligning with the results of the present study.

The present study found that surfclam larvae decrease swimming speed in response to ocean warming (Fig. 8), contrasting previous studies that found that bivalve larvae typically increase swimming speed in response to ocean warming, due to increased metabolic activity, (Hidu and Haskin, 1978). While decreased swimming speed in response to ocean warming would be expected if upper thermal tolerance thresholds are passed, surfclam larval growth increased in response to ocean warming, suggesting thermal tolerance thresholds were not passed. Therefore, the observed decreased swimming speed in response to ocean warming may be considered unexpected. While decreasing swimming speed in response to ocean warming is unexpected, it is not unprecedented in marine larvae. For example, Cominassi et al. (2019) found

that for European sea bass larvae, swimming speed was lower at 20°C than 15°C even though growth increased at 20°C. Therefore, it is possible that different optimal thermal windows exist for growth and swimming behavioral responses. Additionally, Mann and Wolf (1983) reported presence or absence of swimming *A. islandica* larvae in an artificial stratified water column and found that later stage larvae did not swim in temperatures above 20°C. Therefore, inner continental shelf bivalves such as *S. solidissima* and *A. islandica* may produce larvae with constrained upper limit thermal sensitivities, as these bivalves experience lower temperatures relative to estuarine bivalves.

It is well documented that changes in bivalve larvae swimming behavior may lead to dispersal changes (North et al., 2008; Burgess et al., 2021). This occurs due to swimming behavior controlling vertical position, such that larvae higher in the water column in the surface mixed layer may experience greater advection than those at depth (Garland et al., 2002; North et al., 2008; Daigle et al., 2016). For example, Chen et al. (2021) used biophysical models to test the relative impact of diel swimming behavior against thermocline-seeking swimming behavior for sea scallop (*Placopecten magellanicus*) larvae in the MAB and found that such differences in swimming behavior can lead to ~10% changes in settlement success in different regions (e.g., Georges Bank vs. Southern New England). However, it is not known if decreases in swimming speed documented in the present study may lead to significant dispersal pattern changes. Additional biophysical modelling studies are needed to assess if swimming speed differences of less than 0.5 mm^{-s} changes will lead to negligible or large changes in dispersal patterns for surfclam larvae in the MAB.

Under ocean warming-only conditions, our results suggest a low PLD of ~26 days, however under combined ocean warming and OA conditions, as well as present conditions, our

results suggest a PLD greater than ~30 days (Fig. 9). These results align well with previous studies that document a PLD for surfclam larvae from 19-36 days (Loosanoff and Davis, 1963; Ropes, 1980). A longer PLD can be considered analogous to delayed metamorphosis, as both concepts highlight extended time in the water column before settlement, and therefore may affect dispersal patterns (Pechenik, 1990). Delayed metamorphosis for mollusk larvae under stressful conditions is well established in the literature (Bayne, 1965; Pechenik, 1984; Talmage and Gobler, 2009), but directly linking climate changed-induced longer PLD (i.e., delayed metamorphosis), to altered larval dispersal patterns is not as well documented. Under ocean warming conditions, a shorter PLD may compound the effects of decreased swimming speeds, as previous studies have also found that shorter PLDs can lead to shorter dispersal distances (Shanks et al., 2003; Phelps et al., 2015; Ospina-Alvarez et al., 2018). Of particular geographic relevance, Gilbert et al. (2010) found that for sea scallop (*P. magellanicus*) larvae in the MAB, changes in PLD of 5 days may lead to significant changes in dispersal patterns, more specifically, connectivity between subpopulations, by up to a factor of 10. Such dispersal changes corresponded with decreases in settlement of up to 81%. Therefore, ocean warming-induced decreases in PLD and swimming speed may decrease dispersal distances of surfclam larvae. Previous studies have examined climate change impacts on marine larvae dispersal (Andrello et al., 2015; Lacroix et al., 2018). For example, Figueiredo et al. (2022) found that ocean warming-induced (increase to 29°C from 27°C) changes in larval coral (*Acropora* spp.) survival and PLD led to an average 7% decrease in dispersal distance and a 20% decrease in larval retention. To the knowledge of these authors, this is the only study that has examined how climate change induced alterations in PLD will affect marine invertebrate larval dispersal. Such studies are needed for different phyla and in different systems.

4.4 Conclusions

These results suggest that OA may have moderate, negative effects on surfclam larvae physiology and development, as lone OA effects were only observed for immune performance and biomineralization. However, ocean warming may have stronger but positive effects on surfclam larvae physiology, development and behavior. More specifically, ocean warming may increase clearance rate and biomineralization, thereby positively affecting growth and development (assuming that the structural defense associated with a more mineralized shell outweighs any swimming behavior cost associated with a denser shell). Additionally, ocean warming may decrease PLD and swimming speed, potentially affecting dispersal patterns (see references in previous paragraphs, as well as O'Connor et al., 2007). Interactive ocean warming and OA effects were observed on larval growth where under ocean warming only, larval growth is higher, but under ocean warming and OA, larvae growth is lower. These results can be used by resource managers to make projections affecting the surfclam fishery. To make projections more accurate, however, additional studies are needed to investigate how food availability may impact surfclam larvae responses to ocean warming and OA. Food availability may not only be affected by climate change, but has the potential to interact with ocean warming and OA effects on bivalve larvae (Cole et al., 2016). Nevertheless, results from this study not only provide insight regarding climate change impacts on a declining and valuable continental shelf bivalve fishery, but may also encourage other scientists to conduct additional experimental climate change work that examines multiple fisheries-relevant responses. Such approaches provide holistic insights regarding climate change impact on bivalve fisheries and therefore may allow for more informed management decisions.

Tables:

Table 1: Mean seawater carbonate chemistry parameters (± SD) for each treatment. * denotes combined mean, pooled standard deviation (where parameters were first averaged by tank and then averaged again by treatment).

Treatment	Salinity (PSS)*	Temp (°C)*	pH*	TA (umol kg^{-1})*	DIC (umol kg^{-1})	pCO_2 (uatm)	Ω_{Ar}
17°C & 7.7	35.00 ± 0.00	17.05 ± 0.02	7.70 ± 0.01	2145.31 ± 33.76	2057.088± 34.90	910.78 ± 30.38	1.22 ± 0.02
17°C & 7.3	35.00 ± 0.00	17.07 ± 0.04	7.34± 0.01	2120.13 ± 5.70	2142.60 ± 5.78	2185.36 ± 42.65	0.56 ± 0.01
20°C & 7.7	34.80 ± 0.45	20.00 ± 0.02	7.71 ± 0.00	2161.98 ± 22.72	2060.04 ± 21.68	921.92 ± 12.31	1.38 ± 0.01
20°C & 7.3	34.40 ± 0.55	19.96 ± 0.09	7.33 ± 0.02	2154.84 ± 32.18	2175.48 ± 40.88	2382.84 ± 128.15	0.61 ± 0.03
23°C & 7.7	35.00 ± 0.00	22.99 ± 0.04	7.70 ± 0.01	2130.61 ± 40.85	2015.98 ± 42.32	928.10 ± 41.21	1.50 ± 0.04
23°C & 7.3	35.00 ± 0.00	22.96 ± 0.03	7.31 ± 0.02	2158.41 ± 35.17	2168.40 ± 32.80	2447.20 ± 89.23	0.67 ± 0.03

Table 2: Two-way ANOVA output and power exponent (per extended Box-Cox transformation procedure) for mortality, clearance rate, respiration rate, scope for growth, immune performance and growth rate (displayed as change in larval length through time). Bolded *p*-values denote a significant difference.

Variable	Value	Mortality	Clearance Rate	Respiration Rate	Scope for Growth	Immune Performance	Growth Rate
Temperature	df	2	2	2	2	2	2
	F	0.873	6.992	1.34	6.06	0.405	19.13
	p	0.431	**0.007**	0.230	**0.015**	0.676	**3.51e-05**
pH	df	1	1	1	1	1	1
	F	0.840	2.779	4.918	0.021	5.272	23.48
	p	0.368	0.114	**0.047**	0.888	**0.041**	**0.0001**
Temperature * pH	df	2	2	2	1	2	2
	F	0.184	0.642	0.868	1.327	0.376	19.11
	p	0.833	0.539	0.444	0.302	0.695	**3.53e-05**
Total	df	29	17	17	17	17	23
Residual	df	24	12	12	12	12	18
Power Transformation Exponent		n/a	0.40	0.23	n/a	0.22	n/a

Table 3: Three-way ANOVA output and power exponent (per extended Box-Cox transformation procedure) for biomineralization, swimming speed and percent swimming. Bolded *p*-values denote a statistically significant difference.

Variable	Value	Biomineralization	Swimming Speed	Percent Swimming
Temperature	df	2	2	2
	F	2.581	12.098	0.953
	p	0.098	**0.001**	0.403
pH	df	1	1	1
	F	13.971	0.002	0.759
	p	**0.001**	0.968	0.394
Time	df	1	1	1
	F	215.626	31.458	52.508
	p	**< 2e-16**	**4.844-07**	**6.377e-10**
Temperature * pH	df	2	2	2
	F	0.533	0.540	0.461
	p	0.594	0.591	0.637
Temperature * Time	df	2	2	2
	F	5.015	1.903	0.062
	p	**0.011**	0.158	0.940
pH * Time	df	1	1	1
	F	7.427	0.234	0.008
	p	**0.009**	0.630	0.932
Temperature * pH * Time	df	2	2	2
	F	1.774	0.743	0.404
	p	0.181	0.480	0.669
Total	df	71	95	95
Residual	df	60	77	77
Power Transformation Exponent		n/a	n/a	1.49

Figure Captions:

Fig. 1 Bar plot displaying percent mortality of larvae on day 30 (N = 5, ± SE). No significant differences were detected. pH of 7.7 can be considered the control.

Fig. 2: Bar plot displaying growth rate (N = 4, ± SE) in microns per day. Growth rate was calculated from larvae length measurements on days 5 and 30. Different letters (a, b) indicate significant differences within each pH treatment. pH 0f 7.7 can be considered the control.

Fig. 3: Line plot displaying mean biomineralization (N = 4, ± SE) on days 16, 23 and 30. Triangles and solid lines denote treatments of pH 7.7 (control), whereas circle and dashed lines denotes treatments of pH 7.3. Biomineralization is displayed as biomineralization index (i.e., grey scale value). Different letters (a, b, c and d) indicate significant differences on day 30 measurements, as no significant differences were detected for other days.

Fig. 4: Bar plot displaying respiration rate, standardized to individual larva biovolume, (N = 3, ± SE) on day 20-24 larvae. Respiration rate is displayed as picomoles (pmol) of oxygen consumed $hour^{-1}$. Different letters (a, b) indicate significant differences between pH treatments, as no significant temperature (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 5: Bar plot displaying clearance rate, standardized to individual larva biovolume, (unbalanced, N = 3-4, ± SE) on day 14 larvae. Clearance rate is displayed as number of algae cells consumed $hour^{-1}$. Different letters (a, b) above color shadings for temperature indicate significant differences, as no significant pH (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 6: Bar plot displaying scope for growth (N = 3, ± SE) as microjoules h^{-1} standardized to individual larvae (day 21). Different letters (a, b) above color shadings for temperature indicate significant differences, as no significant pH (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 7: Bar plot displaying immune performance (N = 3, ± SE) (represented as percent mortality in response to *Vibrio* spp. exposure), on day 18 larvae. Different letters (a, b) indicate significant differences between pH treatments, as no significant temperature (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 8: Line plot displaying mean (± SE) percent swimming (A) and swimming speed (B) (both unbalanced, N = 4-5) for each treatment on days 5, 11, 16 and 23. Triangles and solid lines denote treatments of pH 7.7 (control), whereas circle and dashed lines denotes treatments of pH 7.3. Different letters (a, b) above color shadings for temperature indicate significant differences, as no significant pH (or interaction) effect was detected. No significant differences were observed for percent swimming (A). Analyses for A are separate from B.

Fig. 9: Line plot displaying PLD (N = 6, ± SE) as the percent of larvae settled for each treatment from day 21.5 to 29.5 Triangles and solid lines denote treatments of pH 7.7 (control), whereas

circle and dashed lines denotes treatments of pH 7.3. The black dashed line denotes 50% settlement.

Fig. 10: Principal component biplot displaying relationships between energy budget profile response metrics including biomineralization, respiration rate, clearance rate, swimming speed, scope for growth and growth rate. Biplot also displays k-mean cluster analysis results where similar data points are grouped in one of four clusters (grey ovals). pH of 7.7 can be considered the control.

Figures:

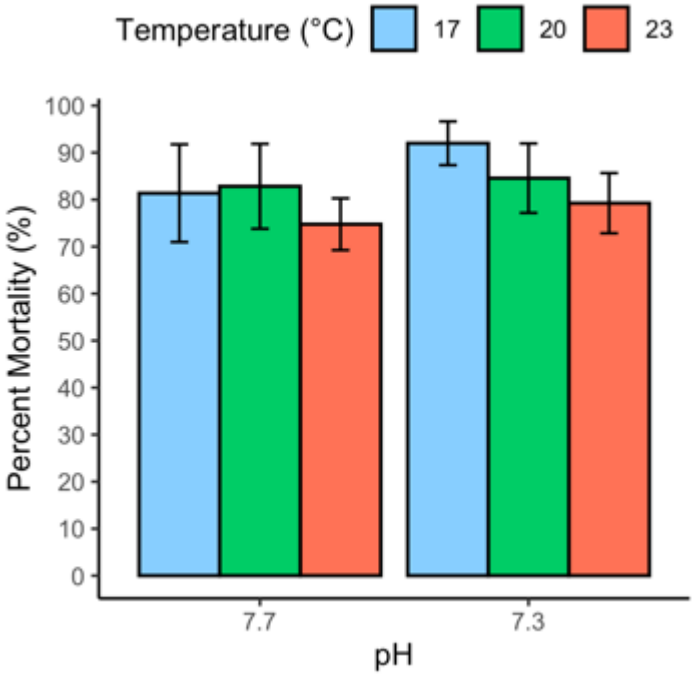

Fig. 1

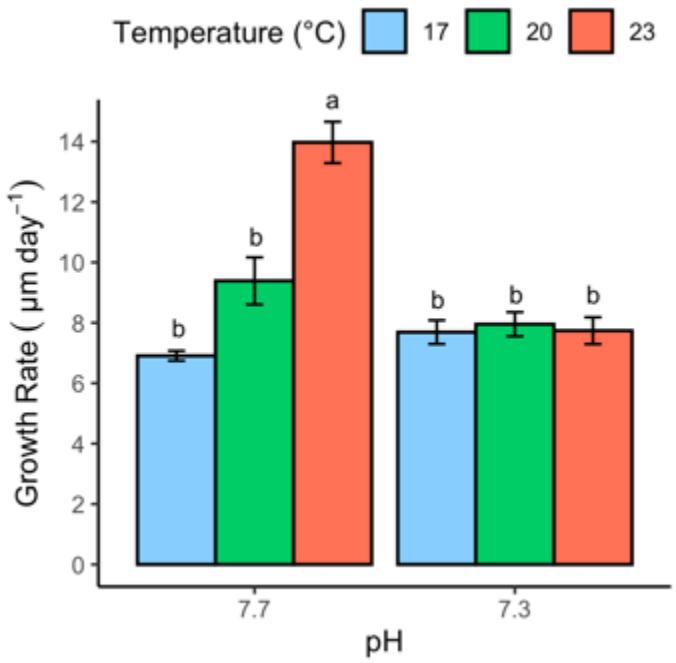
Temperature (°C)
17
20
23
Growth Rate (μm day⁻¹)
0
2
4
6
8
10
12
14
a
b
b
b
b
b
7.7
7.3
pH

Fig. 2

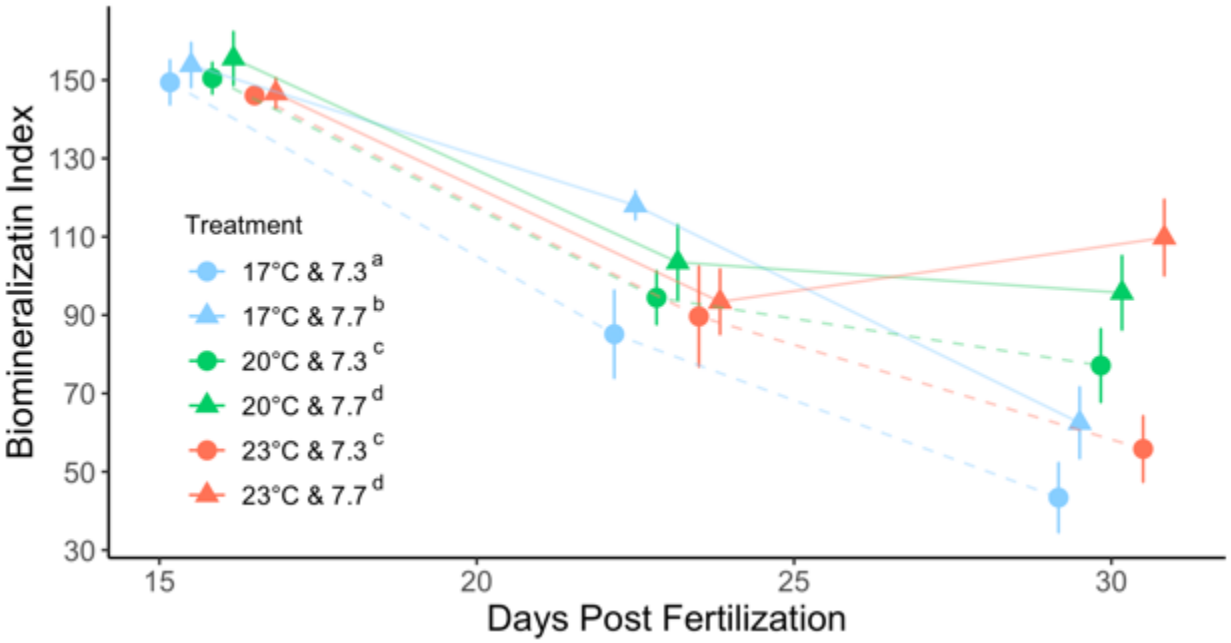
Biomineralizatin Index
30
50
70
90
110
130
150
15
20
25
30
Days Post Fertilization
Treatment
17°C & 7.3 a
17°C & 7.7 b
20°C & 7.3 c
20°C & 7.7 d
23°C & 7.3 c
23°C & 7.7 d

Fig. 3

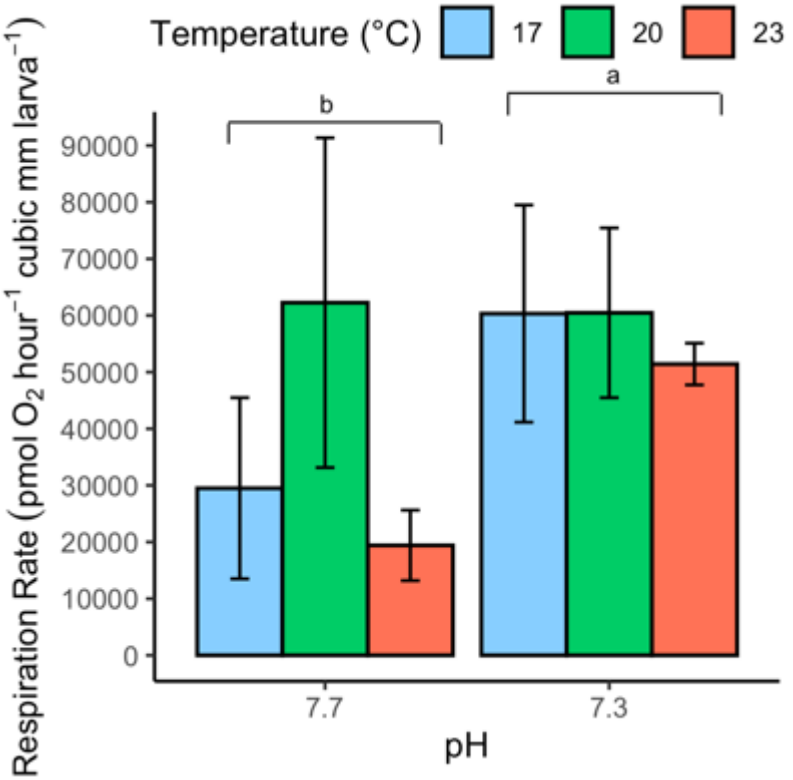
Temperature (°C)
17
20
23
b
a
Respiration Rate (pmol O_2 hour^{-1} cubic mm larva^{-1})
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
7.7
7.3
pH

Fig. 4

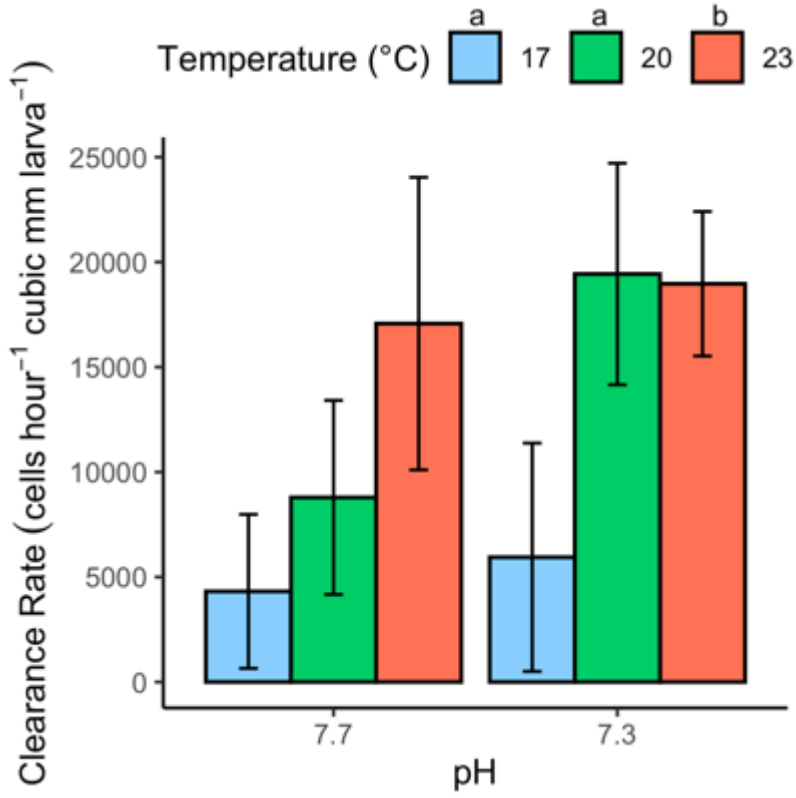
a
a
b
Temperature (°C)
17
20
23
Clearance Rate (cells hour^{-1} cubic mm larva^{-1})
25000
20000
15000
10000
5000
0
7.7
7.3
pH

Fig 5

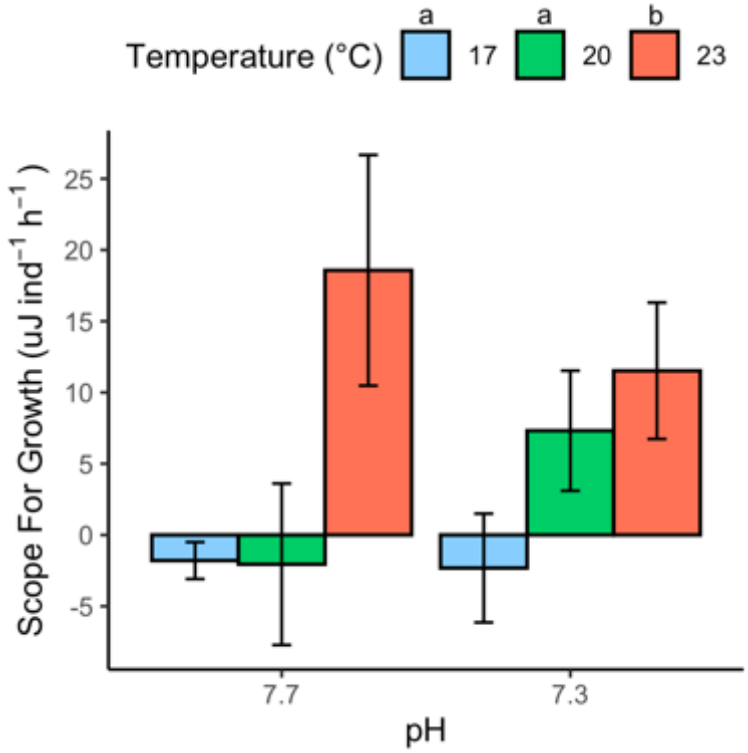
a
a
b
Temperature (°C)
17
20
23
Scope For Growth (uJ ind^{-1} h^{-1})
25
20
15
10
5
0
-5
7.7
7.3
pH

Fig. 6

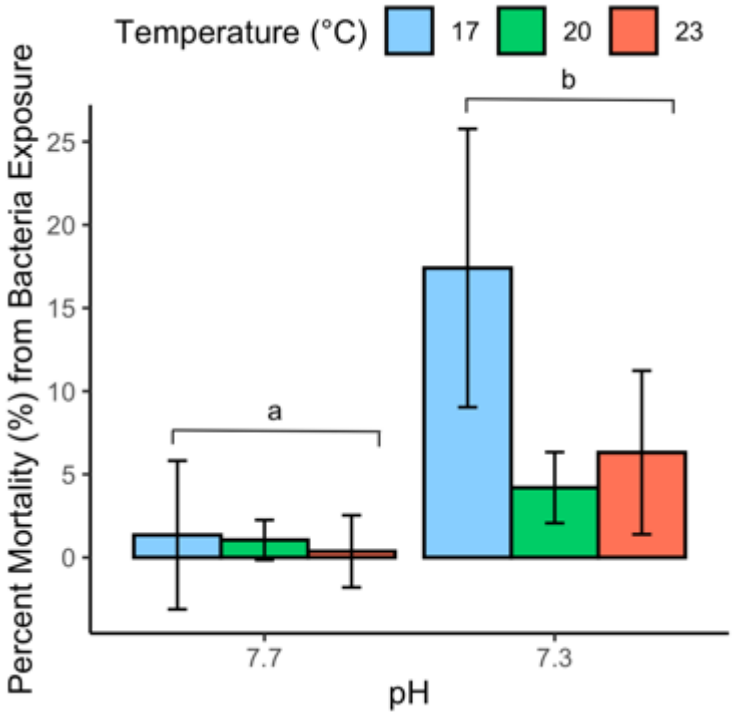
Temperature (°C)
17
20
23
b
a
Percent Mortality (%) from Bacteria Exposure
25
20
15
10
5
0
7.7
7.3
pH

Fig. 7

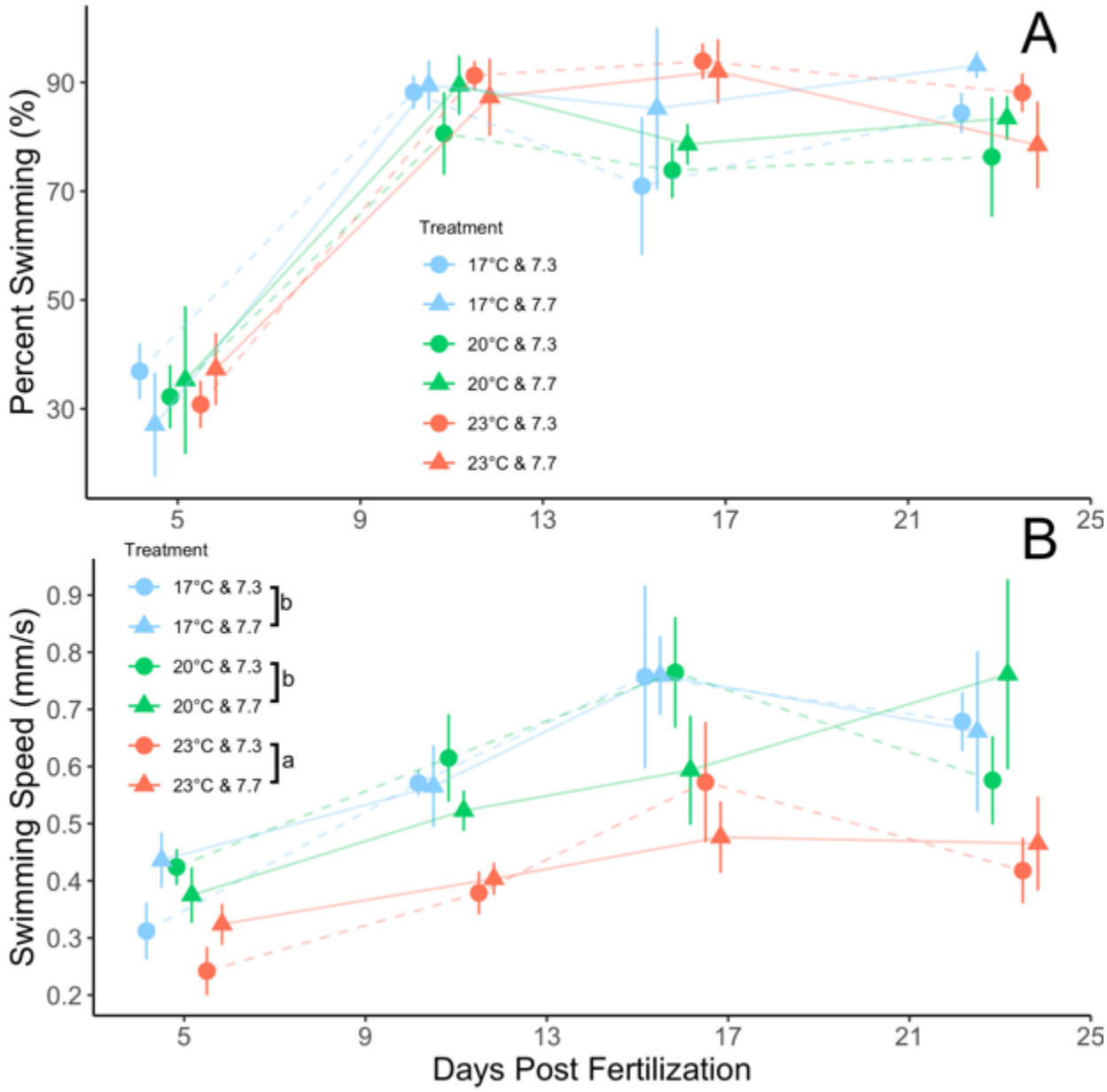
A
Percent Swimming (%)
90
70
50
30
Treatment
17°C & 7.3
17°C & 7.7
20°C & 7.3
20°C & 7.7
23°C & 7.3
23°C & 7.7
5
9
13
17
21
25
B
Swimming Speed (mm/s)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
b
b
a
Days Post Fertilization

Fig. 8

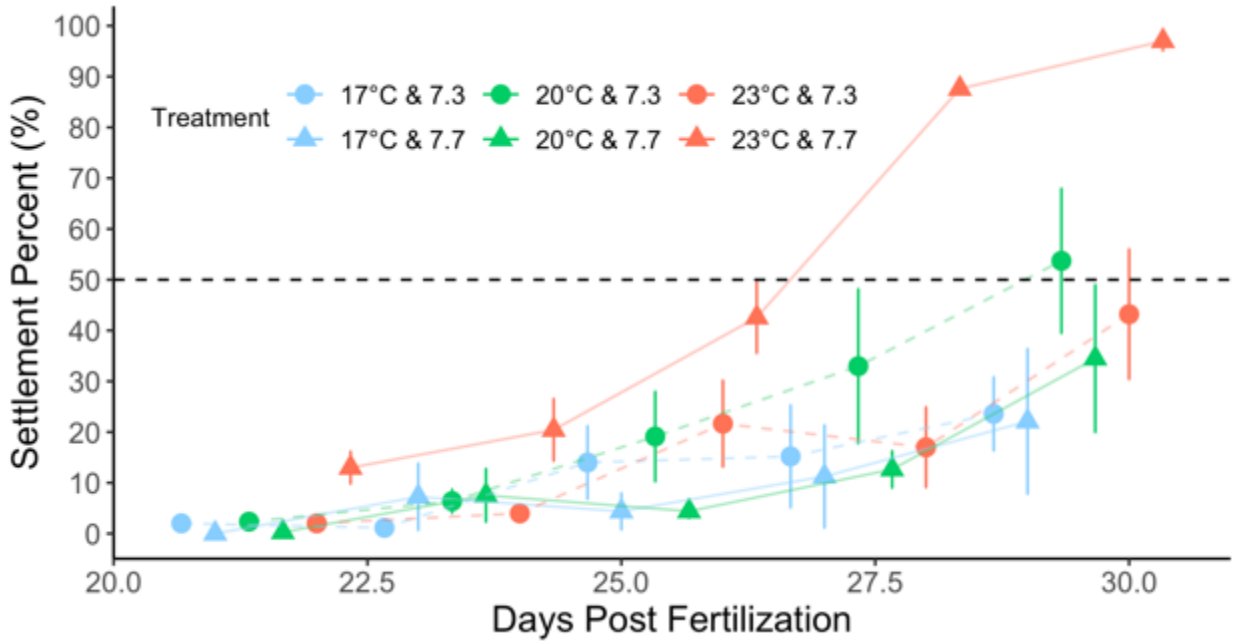
Settlement Percent (%)
Days Post Fertilization
Treatment
17°C & 7.3
20°C & 7.3
23°C & 7.3
17°C & 7.7
20°C & 7.7
23°C & 7.7
100
90
80
70
60
50
40
30
20
10
0
20.0
22.5
25.0
27.5
30.0

Fig. 9

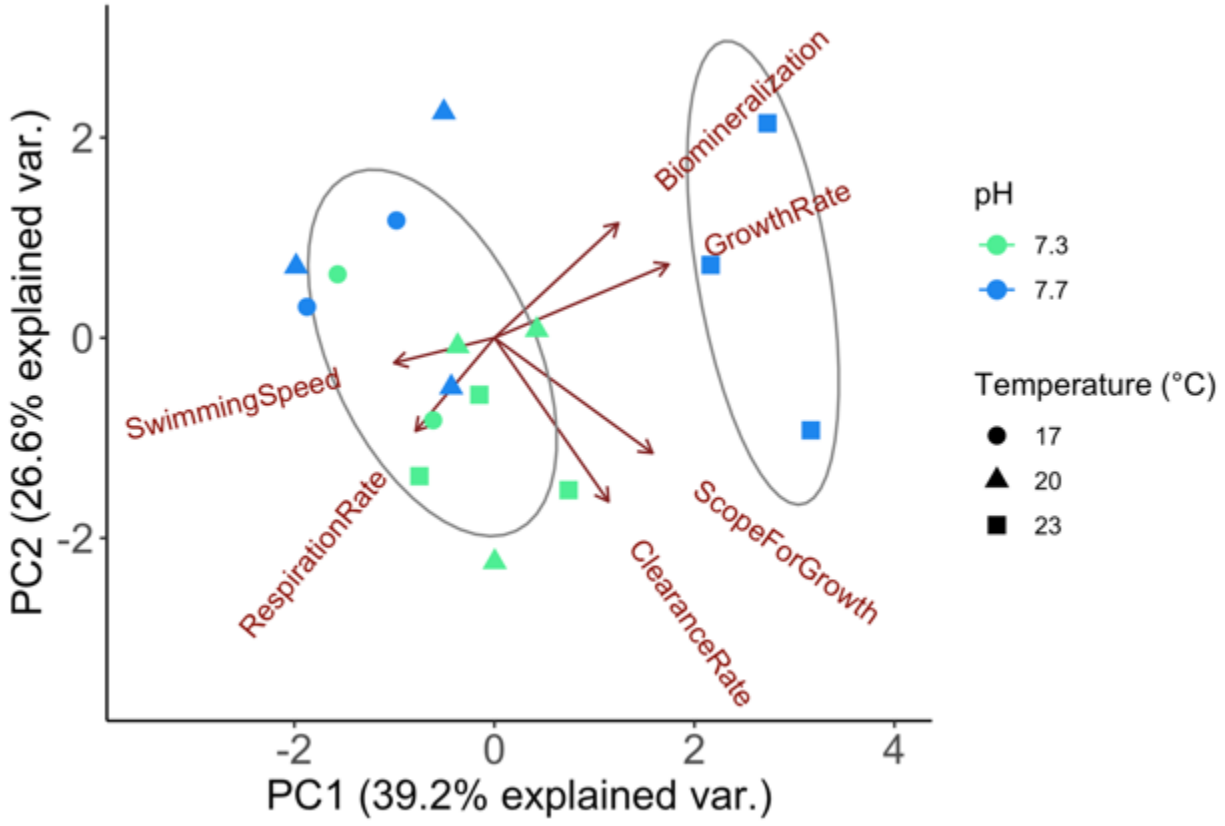
PC2 (26.6% explained var.)
PC1 (39.2% explained var.)
Biomineralization
GrowthRate
SwimmingSpeed
RespirationRate
ClearanceRate
ScopeForGrowth
pH
7.3
7.7
Temperature (°C)
17
20
23
2
0
-2
-2
0
2
4

Fig. 10

Supplementary Material

Table S1: Settings used in wrMTrck plugin for tracking larvae.

Program Setting	Value
Minimum size (pixel^2)	50
Maximum size (pixel^2)	10,000
Maximum velocity (pixels/frame)	300
Maximum area change (%)	99
Minimum track length	50
Threshold for turn	2.0
Size of bin for speed histogram (pixels/frame)	0.0
Show path length	Yes
Show labels	Yes
Show positions	Yes
Show paths	No
Show summary	Yes
Smoothing	Yes
Raw data (0: off, 3: AR + histogram)	0
Frame per second	2
Background subtraction (0: off)	25
Threshold method	0
Size of labelling font	16

Table S2: Two-way ANOVA output for multiple timepoints for biomineralization. Bolded *p*-values denote a statistically significant difference.

Variable	Value	Day 16	Day 23	Day 30
Temperature	df	2	2	2
	F	0.614	0.628	7.609
	p	0.552	0.545	**0.004**
pH	df	1	1	1
	F	2.597	4.019	18.392
	p	0.124	0.060	**0.0004**
Temperature * pH	df	2	2	2
	F	0.074	1.391	3.352
	p	0.929	0.274	0.058
Total	df	23	23	23
Residual	df	18	18	18

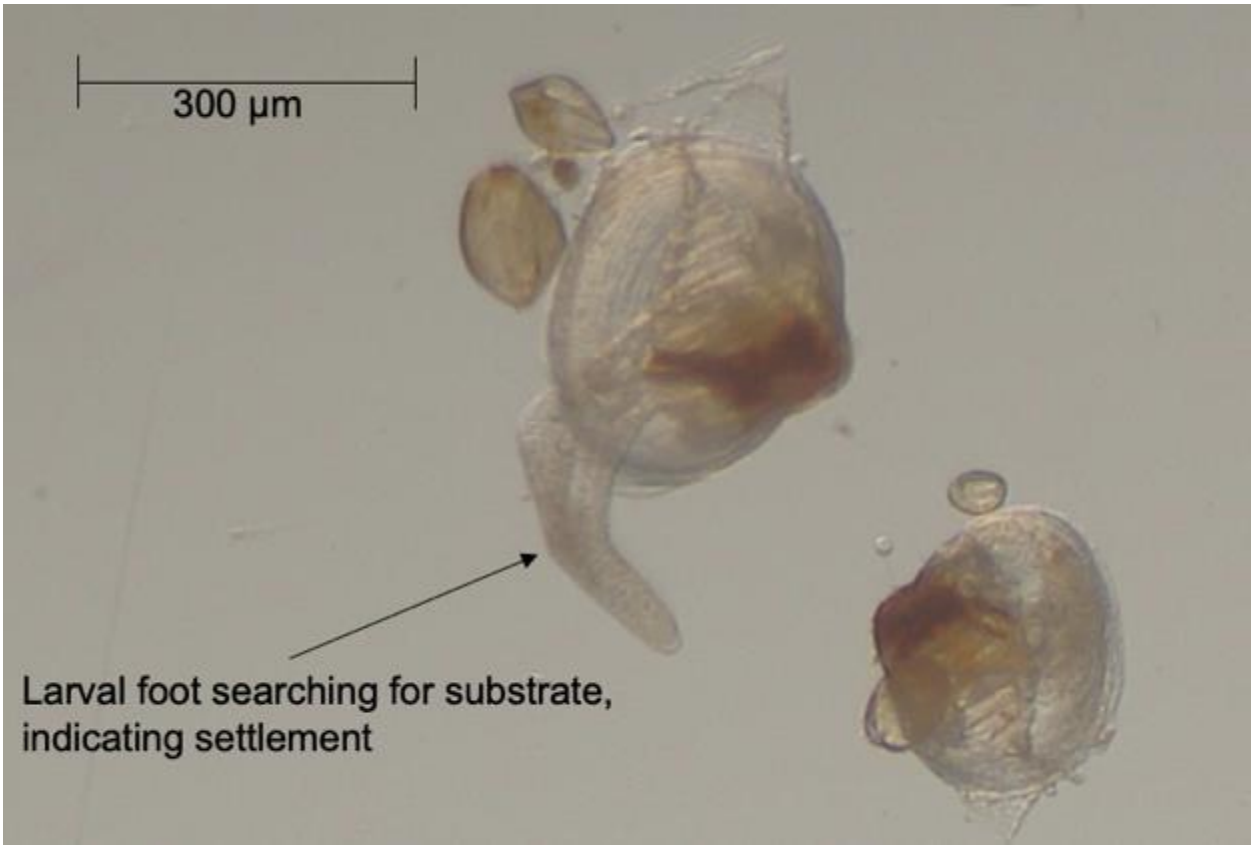

Figure S1: Two live, day 25 old larvae, one of which is displaying settlement behavior by using its foot to search for substrate to attach.

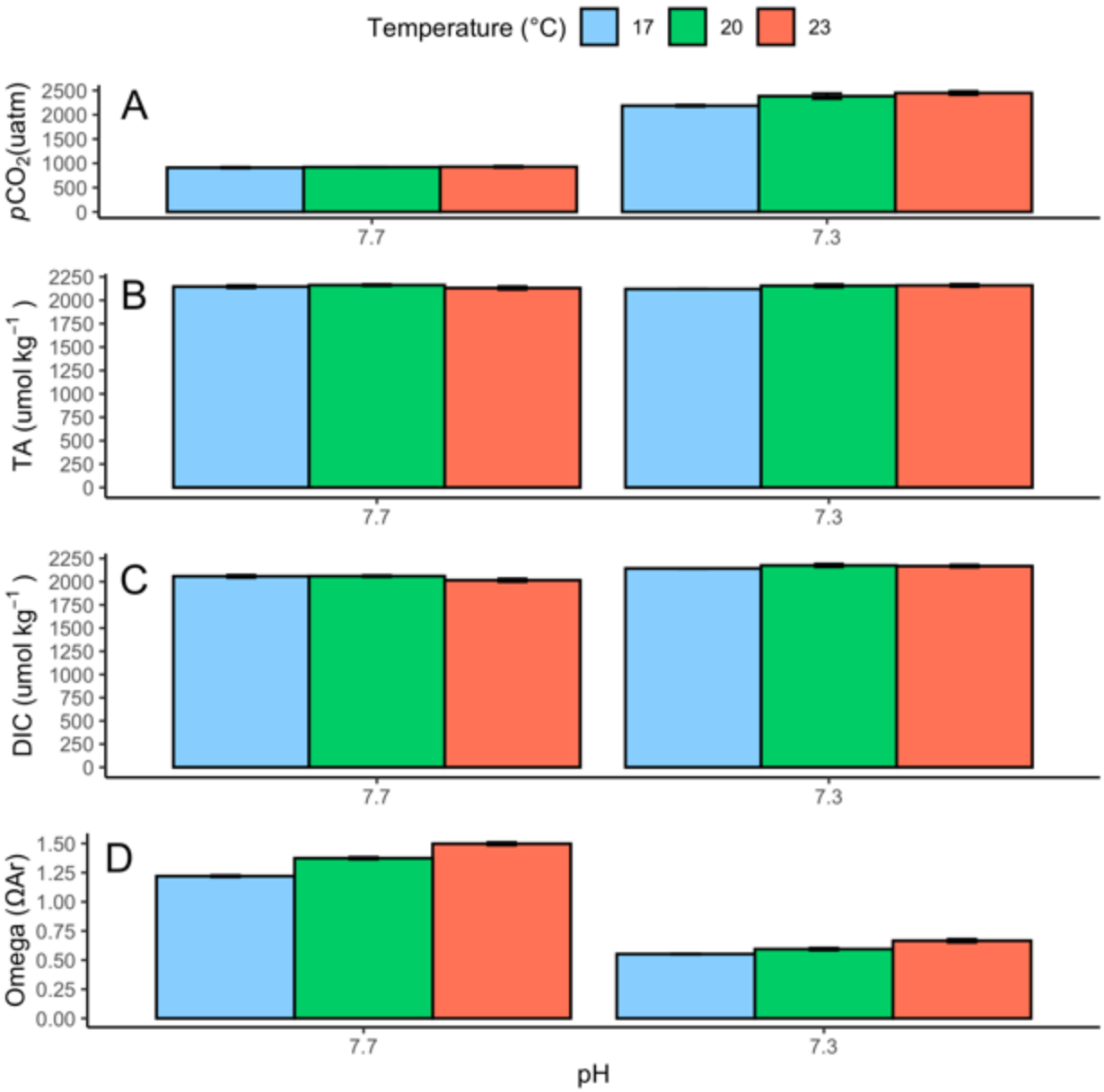

Figure S2: Bar plot displaying carbonate chemistry parameters including pCO_2 (A), TA (B), DIC (C) and Omega (D) for each tank from each treatment (N = 5, ± SE). Legend applies to A, B, C and D.

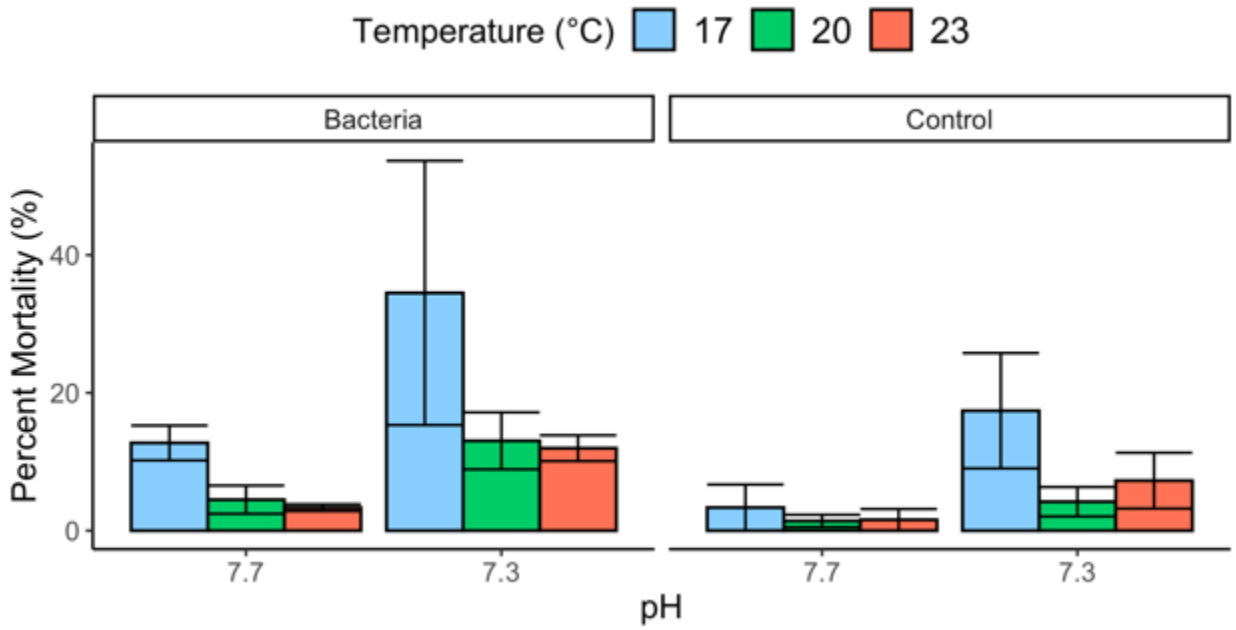

Figure S3: Bar plot displaying raw immune performance assay mortality values (N = 5, ± SE) for larvae in both wells with bacteria and wells without bacteria (i.e., controls).

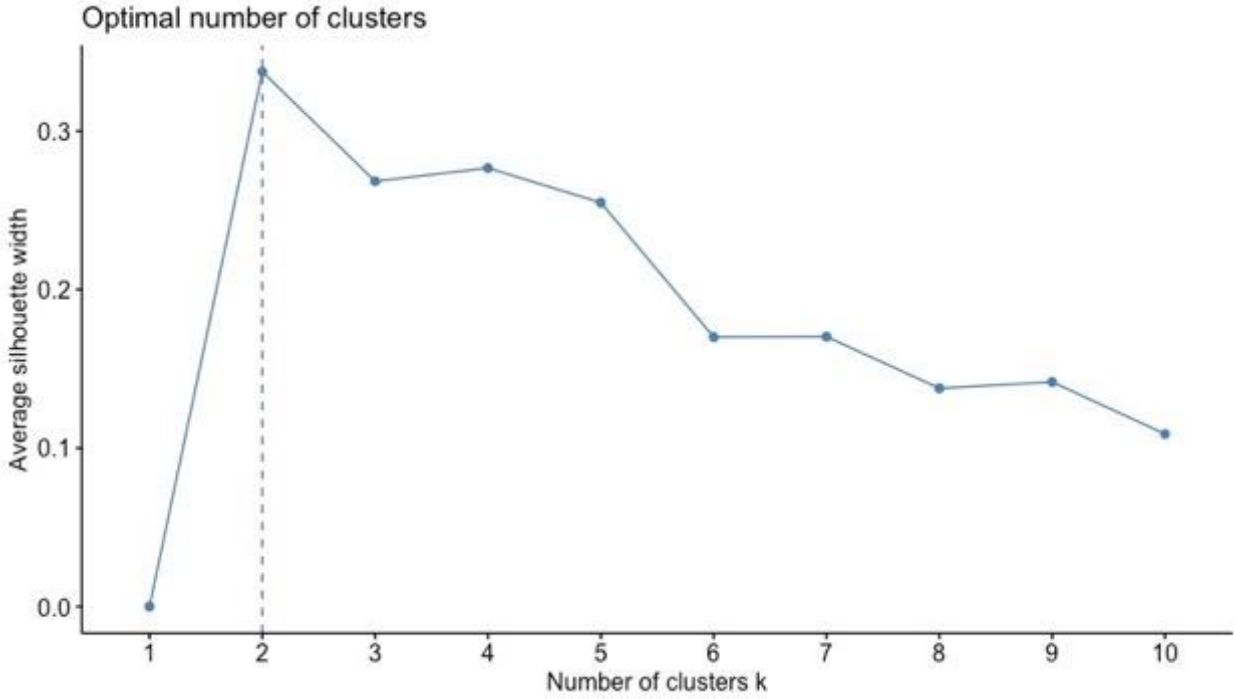

Figure S4: Average silhouette plot displaying the optimal number of clusters for k-means cluster analysis.

Author Statements

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Behavioral and physiological effects of ocean acidification and warming on larvae of a continental shelf bivalve

Raymond Czaja Jr.[1], Robert Holmberg[2], Emmanuelle Pales Espinosa[1], Daniel Hennen[3], Robert Cerrato[1], Kamazima Lwiza[1], Jennifer O'Dwyer[4], Brian Beal[2,5], Kassandra Root[2], Hannah Zuklie[2], Bassem Allam[1*]

[1]School of Marine and Atmospheric Sciences, Stony Brook University, Stony Brook, NY 11790-5000

[2]Downeast Institute, 39 Wildflower Lane, P.O. Box 83 Beals, ME 04611

[3]Northeast Fisheries Science Center, 166 Water Street Woods Hole, MA 02543-1026

[4]New York State Department of Environmental Conservation, East Setauket NY1173

[5]University of Maine at Machias, 116 O'Brien Avenue, Machias, ME 04654

*Corresponding author

Bassem Allam

School of Marine and Atmospheric Sciences

Stony Brook University

Stony Brook, NY 11790-5000

United States

E-mail: bassem.allam@stonybrook.edu

Phone: 1 (631) 632 8745

Abstract

The negative impacts of ocean warming and acidification on bivalve fisheries are well documented but few studies investigate parameters relevant to energy budgets and larval dispersal. This study used laboratory experiments to assess developmental, physiological and behavioral responses to projected climate change scenarios using larval Atlantic surfclams *Spisula solidissima solidissima,* found in northwest Atlantic Ocean continental shelf waters. Ocean warming increased feeding, scope for growth, and biomineralization, but decreased swimming speed and pelagic larval duration. Ocean acidification increased respiration but reduced immune performance and biomineralization. Growth increased under ocean warming only, but decreased under combined ocean warming and acidification. These results suggest that ocean warming increases metabolic activity and affects larval behavior, while ocean acidification negatively impacts development and physiology. Additionally, principal component analysis demonstrated that growth and biomineralization showed similar response profiles, but inverse response profiles to respiration and swimming speed, suggesting alterations in energy allocation under climate change.

Keywords: Ocean acidification, ocean warming, bivalve larvae, surfclam, behavior, energy budget

Highlights:

- Warming and acidification impacts on surfclam larvae were investigated
- Warming increased larvae feeding, scope for growth and biomineralization
- Warming decreased swimming speed and pelagic larval duration
- Acidification increased respiration but reduced immunity and biomineralization

1. Introduction

1.1 Background

Increasing carbon dioxide emissions are affecting physical and chemical properties of the ocean (Caldeira and Wickett, 2003). These effects include ocean warming, resulting from an enhanced greenhouse effect which causes more solar radiation to be absorbed by the ocean, and ocean acidification (OA), which occurs as atmospheric carbon dioxide is absorbed by the ocean, thereby shifting carbonate chemistry equilibria (e.g., decreased seawater pH and calcium carbonate mineral saturation state). The latest Intergovernmental Panel on Climate Change (IPCC) assessment report predicts average, coastal sea surface temperatures (SST) increases over 3°C and pH decreases over 0.4 by the end of the 21st century, under the "business-as-usual-path" Representative Concentration Pathway (RCP8.5) (Pörtner et al., 2019). Within shelf waters of the northeast United States, the Middle Atlantic Bight (MAB) not only hosts numerous commercially important shellfish species, but may be particularly sensitive to climate change. The MAB has warmed three times faster than the global average rate (Saba et al., 2016) and experiences relatively low pH and buffering capacity (Wanninkhof et al., 2015). Numerous studies have linked MAB oceanography, climate change and shellfish fisheries production. For example, the northward and deep-water shift of American lobster stock, including its collapse in southern New England, is believed to be driven by ocean warming(Pearce and Balcom, 2005; Wahle et al., 2015). Additionally, models that assume decreased sea scallop, *Placopecten magellanicus*, growth due to OA have predicted over a 50% decrease in production by 2050 under RCP8.5 (Cooley et al., 2015; Rheuban et al., 2018). However, there are gaps in knowledge regarding interactive climate change influences on diverse responses including those related to the energy budget, immune functioning and larval dispersal.

As ectothermic organisms that typically possess calcified shells, mollusks may be particularly susceptible to climate change phenomena, specifically, OA. In a meta-analysis of OA effects on marine organisms, Kroeker et al. (2010) found that mollusks exhibited the lowest survival rates compared to other taxa such as echinoderms and crustaceans, suggesting bivalves may be more sensitive to OA. As broadcast spawners, bivalves typically produce planktotrophic larvae that remain in the water column for multiple weeks before settlement (Loosanoff and Davis, 1963). Physiological tolerances as well as energy acquisition and expenditure during the larval stage may be different than that of the adult stage (Bayne, 1965; Peteiro et al., 2018); therefore, it is important to study responses to climate change at various life stages. Additionally, larval bivalves often possess shells with different mineralogy than adults (i.e., aragonite, as opposed to an aragonite-calcite mixture), which may affect how different life stages respond to OA (Fuller and Lutz, 1988; Weiss et al., 2002). While there have been significant contributions toward understanding climate change effects on adult bivalves, meta-analyses reveal fewer published studies on climate change effects on bivalve larvae (Clements and Darrow, 2018). Additionally, while there have been recent studies analyzing interactive ocean warming and OA effects on marine bivalves (Cole et al., 2016; Van Colen et al., 2018; Matoo et al., 2021; Bosch-Belmar et al., 2022), meta-analyses (Kelley and Lunden, 2017; Cattano et al., 2018; Leung et al., 2022) again have identified a need for additional studies examining effects of OA and ocean warming on bivalve larvae. A better understanding of interactive climate change effects on bivalve larvae allows scientists to more accurately predict shifts in recruitment, population size and structure, dispersal and distribution patterns.

Of particular interest is the Atlantic surfclam, *Spisula solidissima solidissima* (hereafter referred to as 'surfclam') that supports a $30 million dollar fishery in the northeast U.S.. Surf-

clams are distributed between Nova Scotia, Canada and Cape Hatteras, North Carolina and primarily live in continental shelf waters (Wigley and Emery, 1968); however, near the northern end of their distribution, they can be found in the lower intertidal in shallow bays. To the south, they are replaced by the southern subspecies, *Spisula solidissima similis*, which may be found in warmer, shallow waters (e.g., Long Island Sound) as far north as southern New England (Hare et al., 2010). Previous studies have shown that increased temperatures may lead to decreased survival for juveniles (Acquafredda et al., 2019), decreased adult scope for growth (Hornstein et al., 2018), shifts in distributions to cooler water (Timbs et al., 2019) and reduced fishing yields (Hennen et al., 2018) for surfclams. Although sensitive to moderate ocean warming (temperatures above 20°C) (Munroe et al., 2016; Hornstein et al., 2018; Acquafredda et al., 2019), adult surfclams may be resilient to moderate OA (pH of 7.51) but sensitive to severe OA (pH of 7.31) (Pousse et al., 2020). Additionally, Meseck et al. (2021) found that larval surfclam growth increased under a moderate OA scenario (pH 7.63), but decreased under a severe OA scenario (pH 7.47). However, no studies have examined interactive ocean warming and OA effects on surfclam larvae. It is known that 20-22°C represents the ideal temperature for surfclam larvae and recruit cultivation in an aquaculture setting (Loosanoff and Davis, 1963; Acquafredda et al., 2019), but it is not known how surfclam larvae will respond to discrete, forecasted, ocean warming-based temperature changes. Analyzing responses to combined ocean warming and OA scenarios is important, as previous studies have shown that ocean warming may exacerbate or mitigate OA effects on bivalve larvae, depending on factors such as metabolic trade-offs (Harney et al., 2016), thermal thresholds (i.e., extremes of the temperature treatments) (Ko et al., 2014), and local adaptation (Cole et al., 2016; Van Colen et al., 2018). Interestingly, Pousse et al. (2022) found that via dynamic energy budget model simulations, combined OA and ocean

warming may yield faster juvenile surfclam growth near the end of the century, highlighting the importance and complexity of assessing multiple stressors in tandem.

While quantifying growth and mortality to ocean warming and OA is important, other biological (e.g., physiological and behavioral) responses are needed to better understand how fisheries will respond to climate change. Examining physiological responses (e.g., feeding and respiration rates) under both ocean warming and OA scenarios may provide energy budget insights and a mechanistic explanation for changes in energy dependent processes (e.g., growth rates). For example, Gray et al. (2017) found that OA negatively impacted larval mussel, *Mytilus californianus*, feeding physiology, thereby delaying development and growth. Such physiological responses have been examined for adult, but not larval surfclams (Pousse et al., 2020). Less studied than physiological responses are behavioral responses (Espinel-Velasco et al., 2018; Wang and Wang, 2020). Behavioral responses such as a swimming speed, may not only factor into energy budgets and thereby be related to physiological responses, but may affect dispersal patterns via controlling water column position (Garland et al., 2002; North et al., 2008; Hubbard and Reidenbach, 2015). Therefore, it is important to understand how climate change may impact swimming behavior. To date, only one study has been published regarding OA effects on bivalve swimming behavior (Meyer-Kaiser et al., 2019), and three studies have been published regarding combined OA and ocean warming effects on gastropod larvae swimming behavior (Zhang et al., 2014; Fonseca et al., 2020; Kavousi et al., 2021). Also relevant to dispersal patterns is pelagic larval duration (PLD), or the amount of time spent in the water column before settlement (Levin, 2006). Shorter PLDs typically yield lower dispersal distances and different dispersal paths (Ospina-Alvarez et al., 2018; McGeady et al., 2022). While PLD is

often a function of growth rates, few studies have measured changes in bivalve larvae PLD in response to OA and ocean warming (Lawlor and Arellano, 2020).

The primary objective of this study was to assess the interactive effects of ocean warming and OA scenarios on a suite of understudied, fisheries-relevant responses for bivalve larvae using the Atlantic surfclam as a model species. warming and OA will affect physiological responses such as clearance and respiration rates, 2) ocean warming and OA will affect swimming behavior and PLD, 3) physiological and Analyzing both physiological and behavioral responses in bivalve larvae to climate change may provide wholistic insights regarding fisheries responses to climate change.

2. Materials and Methods

2.1 Husbandry, Maintenance and Water Chemistry

The experimental trial took place in the Ocean and Coastal Acidification Laboratory at the Downeast Institute (DEI) in Beals, Maine, USA. The experimental system is designed around the Apex aquarium controller platform (Neptune Systems, Morgan Hill, CA), consisting of 30 11-L conical experimental tanks independently configurable to any combination of seawater pH and temperature treatments (precise to 0.01 pH units and 0.1 ºC, respectively). Seawater pH control operates in a feedback loop: temperature-compensated pH is monitored in real time using pH and temperature probes (Oakton EW-35805-67 and Neptune Systems PRBTMPJR,); the aquarium controller activates a solenoid valve when pH rises above a programmed setpoint, dosing CO_2 gas through a diffuser (reducing pH); the aquarium controller deactivates the solenoid valve when the setpoint is reached (stabilizing pH). pH monitoring and

CO_2 mixing occurs independently in each tank, achieving true replication (Cornwall and Hurd, 2016). The Apex firmware was modified to allow for pH calibration on the total hydrogen ion scale (pH_T) using synthetic seawater buffers prepared at DEI, which are most appropriate for the seawater pH range and reduce measurement errors associated with differences in ionic strength and composition between buffers and sample (Paulsen and Dickson, 2020). Seawater temperature is adjusted in a similar feedback loop using custom-designed heat jackets that wrap around each tank.

A randomly-interspersed, fully factorial design was employed to test simultaneously three temperature and two pH treatments (N = 5; all sample sizes refer to replicate tanks, unless otherwise stated). Temperature treatments (17, 20 and 23°C) were chosen to represent past (1970), current (2020) and future (2100) mean summer whole-water column temperatures, respectively, in New York Bight inner-shelf waters (Alexander et al., 2018; Thorne et al., 2020). Two pH treatments (7.7 and 7.3) were chosen to represent current (2020) and future (2100) mean summer whole-water column pH, respectively, in the same habitat (Thorne et al., 2020; Wright-Fairbanks et al., 2020). Treatments were chosen with the New York Bight as a target region. The range of temperatures within the New York Bight also overlaps with northern and southern latitudes across the surfclam distribution. For example, 17°C represents a temperature that surfclam larvae may experience present day in Southern New England, and 23°C represents a temperature that surfclam larvae may experience present day in the southern MAB (Ropes, 1968; Mann, 1985; Weissberger and Grassle, 2003). Therefore, these temperature treatments have implications for spatial variability throughout the distribution of the surfclam. Additionally, Czaja Jr et al. (2023)found that in the New York Bight, summer temperature negatively affects

recruitment, suggesting a potential mechanism where larvae respond negatively to ocean warming.

Broodstock (100-150 mm) were collected during low tide from an intertidal flat on Deer Isle, Maine (44°16'40.5"N, 68°40'48.9"W) on 15 November 2020 and conditioned at DEI until spawning on 16 June 2021. Spawning and fertilization were conducted according to standard commercial hatchery procedures. Briefly, spawning was induced via heat shock (25°C) after adults were held at 12°C overnight. Sperm from six males were combined and added to eggs from one female. Therefore, maternal effects on different replicates should be nonexistent. After approximately 30 minutes of gamete incubation, during which gametes were gently mixed every five minutes to resuspend eggs, fertilized eggs were removed from remaining male gametes, as approximately 95% of the eggs showed the presence of polar bodies. Larvae were stocked in experimental tanks at densities of 10 larvae ml^{-1} and were at ambient conditions (21°C, pH of 7.8) for four hours before acclimation to treatment conditions. Acclimation occurred by adjusting temperature 1°C and the pH 0.1 units every three hours in each tank until treatment conditions were met. Holding tanks were stocked with filtered seawater (FSW) pumped into the lab from nearby Black Duck Cove and filtered to 1 µm. Larvae were fed ad libitum (algal concentrations were maintained at 20,000 cells ml^{-1} for the first 15 days and 50,000 cells ml^{-1} for the last 15 days) using a 1:1 mix of the haptophyte *Tisochyrsis lutea* (Tahitian strain) and the diatom *Chaetoceros muelleri* (Hawaiian strain). Tanks were cleaned, water replaced and larvae graded on a three-day cycle such that ten tanks were cleaned daily. Salinity was measured from the holding tank every 1-2 days. Experiments were terminated after 30 days.

Throughout the experimental trial, seawater pH_T and temperature measurements from each tank were logged once per minute using a custom VBA (Visual Basic for Applications)

macro that scraped real-time sensor data from the Apex. Seawater samples (50 ml) were collected weekly from each tank and preserved using mercuric chloride for later total alkalinity (TA) analysis. Upon conclusion of the trial, erroneous log data (i.e., sensor readouts during calibrations or water changes) were removed and pH_T and temperature were averaged by tank. Salinity was measured in the preserved seawater samples using a digital refractometer (Sper Scientific 300035, accurate/precise to 1 ppt). TA was measured using a spectrophotometrically-guided titration method accurate/precise near 1 µmol kg^{-1} SW (Yao and Byrne, 1998; Liu et al., 2015) adapted to the Cary 60 UV/Vis spectrophotometer (Agilent Technologies, Santa Clara, CA) using a custom ADL (Applications Development Language) script. Measurements were averaged by tank. TA method accuracy was verified using CO_2 in seawater certified reference material (CRM, batch #162) supplied by the Dickson lab (Scripps Institution of Oceanography, UC San Diego). Remaining seawater carbonate chemistry parameters (partial pressure of CO_2, pCO_2; dissolved inorganic carbon, DIC; saturation state of aragonite, Ω_{Ar}) were calculated for each tank from pH_T, temperature, salinity, and TA using CO2Sys v2.1 (Pierrot et al., 2006) (K_1, K_2 from Lueker et al. (2000); K_{HSO4} from Dickson (1990); B_T from Uppstrom (1974)).

2.2 Developmental Responses

Mortality was quantified by estimating total number of remaining live larvae (via ciliary movement, swimming and shell contents) on day 30 relative to the initial number of live larvae in each tank, and is presented as a percent by subtracting the percent remaining alive on day 30 from 100. On days 5, 11, 16, 23 and 30, larvae were preserved via glutaraldehyde (0.5%) fixation and stored at -20°C for growth rate and biomineralization analyses. Growth rate was measured (50 larvae per tank) by the increase in larvae length through time via image analysis (ImageJ) on microscope-captured photos. Biomineralization was measured (20 larvae per tank) via cross-

polarized light microscopy similar to Wessel et al. (2018) on larvae preserved on days 16, 23 and 30. Briefly, biomineralization was quantified by calculating the mean grey scale value (hereafter referred to as biomineralization index) for individual larva in ImageJ, such that a grey scale value of zero represents black shell material (no biomineralization) and a grey scale value of 255 represents white shell material (100% biomineralization). This approach quantifies biomineralization via observed birefringence and is based on the principle that more crystalline calcium carbonate yield more (i.e., brighter) birefringence (Weiss et al., 2002). Photos for growth rate and biomineralization analyses were measured on a Nikon Eclipse TE2000-S inverted compound microscope (100x magnification).

2.3 Physiological Responses

Respiration rate assays were conducted on larvae (day 20-24) after modifying the approach of Waldbusser et al. (2015). Approximately 75 larvae were placed in a 4.5 ml capped cuvette with the respective treatment seawater. Each cuvette contained a Pyroscience oxygen-sensor spot that used a fiber-optic cable to transmit real-time oxygen concentration measurements to a computer. Assays lasted three hours and were kept at room temperature (21°C). Three controls were conducted without larvae to estimate background oxygen loss. Background oxygen loss was subtracted to estimate the total loss in oxygen due to larvae respiration from time zero to time three hours. Oxygen loss per hour^{-} was standardized to exact larvae counts per cuvette and to estimated larvae biovolume. Biovolume was estimated using the equation for the volume of a sphere where each radius is represented by half the larval length, width, and height.

Clearance rate assays were conducted on day 14 larvae via modifying the approach of Ginger et al. (2013). Approximately 200 larvae were placed in a 50 ml centrifuge tube with 30

ml of FSW and 50,000 cells ml^{-1} of *T. lutea*. Tubes were placed in a temperature-controlled water bath (for the appropriate temperature treatment) for six hours. At the start and end of the assay, 2 ml from each tube were fixed with 0.5% glutaraldehyde to estimate algae concentrations. Algae concentrations were estimated via FlowCam, a continuous imaging flow cytometer. Approximately 0.1 ml of sample were analyzed with auto-image mode, a 300 µm FlowCell, a 4x objective, a minimum cell diameter of 1 µm and a speed dial setting of 10 at fast mode. Clearance rate as particle loss $hour^{-1}$ was standardized to exact larvae counts per tube and to estimated larvae biovolumes. Scope for growth was then calculated as [(clearance rate x absorption efficiency) - (respiration rate)]. Assumptions and values used for scope for growth calculations were based on Gray et al. (2017). Briefly, clearance rate and respiration rate were standardized to biovolume, clearance rate was converted into µJoules h^{-1} assuming an energetic cell content of 0.61 µJoules algae $cell^{-1}$ (Sprung, 1983), absorption efficiency was assumed to be 0.38 (Sprung, 1983) and respiration rate was converted into µJoules h^{-1} assuming 1 nL of O_2 to be 20.1 µJoules of respired energy (Crisp, 1971). Scope for growth calculations were performed on clearance and respiration rate values that came from the same tank, with two exceptions. In these two exceptions, clearance rate and respiration rate were measured from closely matched individuals from the same treatment but from different tanks. This was necessary because time limitations prohibited a complete set of measurements from all tanks. Clearance rate was measured for Tank 10 (17°C & 7.3) and Tank 25 (17°C & 7.7), but respiration rate was not measured from these tanks. These were combined with respiration rate measurements from Tank 22 (17°C & 7.3) and Tank 14 (17°C & 7.7), respectively, where no clearance rate measurements were taken. It was felt that including these two exceptions would beneficially reduce the effects of unbalanced treatment replicates for the data analysis.

Immune performance assays began on day 13 after modifying the approach of Schwaner et al. (2020). Larvae were exposed to bacteria (*Vibrio* spp.) cocktails (sensu Schwaner et al., 2020) for five days in 16.8 ml 6-well microplates with no aeration but in temperature controlled water bath (for the appropriate temperature treatment). Each well contained 100 larvae and 12 mL of FSW. Wells were dosed with 10,000 colony forming units per ml (CFU ml^{-1}) at the beginning of the assay and then 100,000 CFU ml^{-1} halfway through the assay. For each treatment, three control wells without bacteria were used. Immune performance was assessed as a function of percent mortality on the final day on ~50 larvae per well via ciliary movement, swimming and shell contents (high mortality equating low immune performance).

2.4 Behavioral Responses

Swimming responses were measured on days 5, 11, 16 and 23 via microscope video (Accu-scope Excelis HD camera attached to a Nikon SMZ745T dissection microscope) recording analysis software in ImageJ via the wrMTrck plugin (see Gamain et al. 2020 for details regarding the image analysis technique used by wrMTrck). For each well in a 6 well plate, 15 ml of FSW was added with ~50 larvae. Preliminary analyses showed that swimming responses were unaffected by densities between 20 and 100 larvae ml^{-1}, whereas for densities of 100 larvae ml^{-1} or greater, the software yielded biased (higher) swimming speeds (likely due to double counting of larvae and increased larval collisions). Preliminary analyses also showed no significant differences in swimming responses when using video durations between 5 and 45 seconds. However, a video duration of 15 seconds was chosen as a conservative approach to minimize potential measurement error associated with shorter videos. When running wrMTrck, program settings were adjusted from Gamain et al. (2020) to improve analyses at the video resolution used (Table S1). Swimming speed was calculated as mm $second^{-1}$, and percent swimming was

calculated as the percent of larvae, from each tank, that swam at any time point for each 15-second video. Swimming speed was also analyzed when standardized to larval length, however, outcomes did not change. Therefore, raw swimming speeds were reported and analyzed. PLD was determined by the presence of a 'searching foot' (Rodriguez-Perez et al. 2019; Fig. S1), as this stage of development indicates the larva is nearing settlement and seeking substrate for attachment. Every day from day 21 to 30, approximately 50 larvae from each tank were analyzed for the presence of a searching foot. Settlement percent was calculated as the percent of larvae from each tank that displayed settlement behavior at any time point for a 15-second period. Analyzing settlement percent through time allows for quantifying PLD as the day on which 50% of the larvae were ready to settle. All behavioral metrics were measured within one hour of removing larvae from holding tanks at room temperature (21°C). For all assays on live larvae (i.e., physiological and behavioral responses), larvae were removed from tanks by gently pipetting (via 50 ml serological pipettes) the appropriate amount of tank water (i.e., if 50 larvae were needed, 500 ml of tank were pipetted) into an appropriately sized beaker. Larvae were then concentrated using a 40 μm sieve after which, larval counts were performed to determine if the needed number of larvae were obtained for the assay.

2.5 Statistics: Analyses of Variance and PLD

All statistical testing was performed in R version 4.0.2 (base packages for ANOVAs). Two-way ANOVAs with pH and temperature as fixed, categorical factors were used to compare mortality (N = 5), immune performance (N = 3), respiration rate (N = 3), clearance rate (unbalanced with N = 3-4) and scope for growth (N = 3) among treatments. Sample sizes for individual responses are lower than the total number of tanks as some responses contained replicates that yielded extreme outliers (values outside three times the interquartile range) and/or

replicates with too few larvae to yield a reliable signal. For growth rate, heterogeneity of regression slopes with time precluded application of a two-way factorial ANCOVA of the variables of interest (pH and temperature). Therefore, growth rate differences were analyzed via a two-way ANOVA on calculated, linear growth rates ($\frac{L30-L5}{25}$), where L30 is larval length on day 30, L5 is larval length on day 5 and N=4. A three-way ANOVA, including two-way and three-way interactions, with pH, temperature and time (continuous) was initially used to compare biomineralization (N = 4), swimming speed (N = 4-5) and percent swimming (N = 4-5) among treatments. A repeated measures design to remove temporal pseudoreplication was used by including tank as a random effect (i.e., because the same tanks were measured through time). When time interactions were present, a two-way ANOVA was used for individual time points. Three separate time points were used and a Bonferroni correction was applied, using an adjusted alpha (α') of 0.0167. For clearance rate, swimming speed and percent swimming ANOVAs, a Type II Sum of Squares was used because of the unbalanced experimental design (Underwood, 1997). Multiple comparisons were carried out using Tukey's test for balanced designs and a Dunnett-Tukey-Kramer (DTK package) test for unbalanced designs. For DTK multiple comparisons, the outcome was considered significant if confidence intervals of estimated differences did not contain zero. An extended Box-Cox analysis (Sokal and Rohlf, 1981) was used to identify the best power transformation of the data to meet normality and homogeneity of variance assumptions of the ANOVAs.

It was expected that settlement rate through time should yield a logistic function, with an asymptote of zero percent settlement at time zero (day 21) and an asymptote of 100 percent settlement at time final (day 30). However, data for multiple treatments would not converge to a logistic function because of high variance and because settlement rates were not high enough at

the end of the experiment (day 30) (i.e., asymptotes were not achieved). Therefore, because the data were unable to be appropriately modelled as an autoregressive logistic function, settlement percent through time was analyzed as a line plot time series. Settlement percent for each treatment was averaged across tanks and in two-day bins to minimize variability (e.g., settlement percent on day 21 was averaged with day 22). PLD was then quantified as the first day on which the median surpassed 50% settlement.

2.6 Statistics: Multivariate Analyses

Principal component analysis (PCA) was used via the R packages 'vegan' (Oksanen et al., 2013), 'ggbiplot' (Vu, 2011) , 'cluster' (Maechler et al., 2013) and 'factoextra' (Kassambara and Mundt, 2017) to examine potential relationships between responses related to energy use including growth rate, clearance rate, respiration rate, swimming speed, scope for growth and biomineralization. These responses (hereafter referred to as the energy budget profile) allowed for 16 tanks to be used in PCA, as not all tanks were used for every response metric. Swimming speed and biomineralization data on the oldest larvae available (day 23 and day 30, respectively) were used for PCA. All data were standardized and made unitless by subtracting by the mean and dividing by the standard deviation (for each individual response). Biplots were examined visually to assess relationships between different response metrics. K-means cluster analysis was used to identify groups of data points (and their associated treatments) which were most alike. To determine the appropriate numbers of clusters, the 'average silhouette method' was used via the 'fviz_nbclust' function.

3. Results

3.1 Water Chemistry

All water chemistry parameters remained reasonably stable (within pH treatments), with TA exhibiting the highest variance (Table 1, Fig. S2). Increasing pCO_2 successfully reduced pH, increased DIC and reduced Ω_{Ar} (Table 1, Fig. S2). The highest pCO_2 occurred in the 23°C & 7.3 treatment (Table 1, Fig. S2). The lowest Ω_{Ar} occurred in the 17°C & 7.3 treatment and the highest Ω_{Ar} occurred in the 23°C & 7.7 treatment (Table 1, Fig. S2).

3.2 Developmental Responses

Larvae did not experience any significant difference in mortality between treatment groups (Table 2, Fig. 1). Although mortality was highly variable and seemingly high, average larval mortality (82.5%) was similar to other studies on surfclam larvae (Hurley and Walker, 1997; Meseck et al., 2021). Larvae experienced significantly higher growth rates (i.e., increase in larval length through time) at 23°C & 7.7 than all other treatments, but did not experience differences in growth rates between any other treatment groups (Fig. 2). Larvae experienced no difference in biomineralization between treatment groups on days 16 and 23 (Fig. 3, Table S2). However, on day 30, larvae experienced significantly lower biomineralization at pH 7.3 than 7.7 and at 17°C than 20°C ($p = 0.006$) and 23°C ($p = 0.015$) (Fig. 3, Table S2).

3.3 Physiological Responses

Respiration rate was significantly impacted by pH only, but clearance rate and scope for growth were significantly impacted by temperature only (Table 2). Larvae experienced a significantly higher respiration rate at pH 7.3 than pH 7.7 (Table 2, Fig. 4). Larvae experienced a significantly higher clearance rate at 23°C than at 20°C ($p = 0.0472$) and 17°C ($p = 0.0051$) (Fig. 5). Larval scope for growth was significantly higher at 23°C than at 17°C ($p = 0.0143$, Fig. 6). Larvae at pH 7.3 experienced significantly higher mortality when challenged with *Vibrio* spp. (lower immune performance) than larvae at pH 7.7 (Table 2, Fig. 7). For immune performance

assays, larvae exposed to bacteria generally had higher mortality than controls (Fig. S3), with an average of 5.34% of observed mortality being due to bacteria exposure.

3.4 Behavioral Responses

The percent of larvae swimming was not significantly different between treatment groups, but did significantly increase after day 5 (Table 3, Fig. 8A). Larval swimming speed also increased through time (Table 3, Fig. 8B). Larvae also swam significantly slower at 23°C, than at 20°C and 17°C (Table 3, Fig. 8B). Larvae from the 23°C & 7.7 treatment experienced a PLD of just above 25.5 days (Fig. 9). Larvae from all other treatment did not achieve 50% settlement by day 29.5 and therefore have a PLD of longer than 29.5 days (Fig. 9).

3.5 Multivariate Statistics

The silhouette plot showed that the optimal number of clusters was two (Fig. S4). The first cluster contained all three data points from the 23°C & 7.7 treatment, where larvae experienced increased growth and biomineralization. All other data points (and treatments) were contained in the second cluster, where larvae experienced decreased growth and suppressed physiological responses (clearance and respiration rate). The two dimensions represented by the biplot explain ~66% of the variance (Fig. 10). Within this biplot, vector directions show that within the first two principal components, three pairs of responses were changing in the same direction. The first pair was biomineralization and growth rate, the second pair was swimming speed and respiration rate, and the third pair was clearance rate and scope for growth rate (Fig. 10). Vector directions also showed that within the first two principal components, biomineralization and growth rate were changing in the opposite direction as swimming speed and respiration rate (Fig. 10).

Discussion

4.1 General Larval Performance

Previous studies have demonstrated that ocean warming and/or OA may not significantly affect mortality rates of mollusk larvae (Gobler and Talmage, 2014; Fonseca et al., 2020), but may significantly affect other responses (i.e., non-lethal responses) including immune performance (Schwaner et al., 2020), clearance rate (Cole et al., 2016), respiration rate (Gray et al., 2017), biomineralization (Wessel et al., 2018), growth rate (Meseck et al., 2021) and behavior (Fonseca et al., 2020). For example, Gobler and Talmage (2014) found that for larval Eastern oysters, *Crassostrea virginica*, projected OA did not significantly affect mortality but did significantly affect biomineralization, and Fonseca et al. (2020) found that for larval Netted whelk, *Tritia reticula*, projected ocean warming and OA did not affect mortality but did significantly affect swimming behavior. Such variable responses may be due to experimental design decisions, as more severe OA scenarios and lower pH may more likely lead to higher mortality. For example, Gobler and Talmage (2014) found that a moderate OA scenario (pH of 7.68) did not yield increased mortality for larval oysters, but Barros et al. (2013) found that a severe OA scenario (pH of 7.37) did increase mortality for larval oysters. These variable responses also highlight the importance if considering a suite of responses. More specifically, considering a suite of responses is important as survival under environmental changes may come at the cost of immune functioning (Rauw, 2012), growth (Harrington et al., 2019) and normal behavior (Holt and Jørgensen, 2014). The present study supports this idea as ocean warming and OA did not affect surfclam larval mortality, but ocean warming affected growth rate, clearance rate, biomineralization and behavioral responses and OA affected growth rate, immune performance, respiration rate and biomineralization. Although it should be noted that while

ocean warming and OA did not directly lead to increased mortality, decreased fitness in the natural environment and delayed metamorphosis (i.e., longer PLD) caused by climate change stress may indirectly lead to increased mortality via mechanisms such as increased susceptibility to predators (Jackson and Strathmann, 1981; Sponaugle et al., 2006).

4.2 Physiological Responses and the Energy Budget

pH had no significant effect on growth at 17°C and 20°C, but at 23°C, a pH of 7.3 yielded lower larval growth than at 7.7 (Fig. 2). Additionally, at ambient pH levels, temperature increased larval growth. These results suggest that surfclam larvae respond positively to ocean warming at specific pH levels. It should be noted that while increased food availability may offset climate change stress, the algae concentrations used in the present study (20,000-50,000 cells/ml) fall within the range of other studies, including OA studies on surfclam larvae (Talmage and Gobler, 2011; Meseck et al., 2021). Therefore, there is little evidence to suggest food availability artifacts in the present study. The positive impacts of increased temperature contrasts previous findings in a general context, as a meta-analysis of climate change impacts on marine larvae found that calcifying larvae typically respond negatively to ocean warming (Przeslawski et al., 2015). This contrast is particularly noteworthy, as the present study used a relatively large temperature change of +6°C total, or +3°C of projected warming, and still found that such an OW scenario increased growth. Although, it should be noted that in an aquaculture setting, 20-22°C has been identified as the optimal temperature range for rearing larval surfclams (Loosanoff and Davis, 1963; Fay et al., 1983). Therefore, this temperature increase does not appear stressful for surfclam larvae. Nevertheless, the outcome of increased growth under projected warming, contrasts some previous findings, as Munroe et al. (2016) found increases in

current bottom water temperature of >1°C can lead to decreased adult surfclam growth. Furthermore, the present study found that temperature increased mean larval scope for growth, but Hornstein et al. (2018) found that adult surfclam scope for growth was lower at 23°C than 19°C, providing further contrast. However, other studies have found that adult bivalves exhibit stronger, more negative responses to ocean warming than juveniles or larvae (Pörtner and Farrell, 2008; Stevens and Gobler, 2018), potentially because larvae and juveniles have lower total metabolic rates. In further contrast to the present findings, sea scallops, *P. magellanicus*, which also occupy cool, MAB continental shelf waters, had lower larval growth at 19°C than lower temperatures (Culliney, 1974). While both surfclams and sea scallops occupy continental shelf waters, surfclams are found more inshore, exposing them (and presumably their larvae) to warmer waters than sea scallops and potentially yielding increased adaptive ocean warming responses. Indeed, other studies have found that larval bivalves in shallow and/or warmer habitats may be tolerant to ocean warming (Cole et al., 2016; Lawlor and Arellano, 2020). Providing further support for this hypothesis, Loosanoff and Davis (1963) found that 20-22°C is the optimal temperature range for culturing larval surfclams, compared to 10-15°C for larval ocean quahogs, *Arctica islandica*, which also occupy deeper MAB continental shelf waters. These results also suggest that the temperature-induced recruitment failure observed for surfclams in the New York Bight by Czaja Jr et al. (2023) is not likely due to harmful ocean warming effects on larval fitness. As another relevant comparison, Pousse et al. (2022) found that OA alone decreased simulated growth of juvenile surfclams near the end of the century, but combined OA and ocean warming increased simulated growth. The present study found potential opposite trends were ocean warming alone increased growth, but combined OA and ocean

warming decreased growth. This contrast highlights the importance of life stage specific responses to additive, if not synergistic stressors.

The interactive ocean warming and OA effects on larval surfclam larval development have been documented for other bivalve larvae including brooding flat oysters, *Ostrea angasi* (Cole et al., 2016), and northern bay scallops, *Argopecten irradians irradians* (Talmage and Gobler, 2011). Growth rate differences may have been observed between pH treatments only at 23°C because Ω_{Ar} was the highest (1.50) for the 23°C and 7.7 treatment, potentially yielding the most ideal conditions for development. Carbonate chemistry parameters and temperature interact via a negative relationship where warmer water retains a higher Ω_{Ar} (relative to cooler waters) under increased CO_2 due to decreased solubility (Millero, 2007). Such interactions have been observed in the field where ocean warming is slowing the OA-induced Ω_{Ar} decline in the Arctic (Yamamoto- Kawai et al., 2011) and in previous lab experiments where warmer water reduced dissolution processes for adult mollusks under high pCO_2 (Noisette et al., 2016). Furthermore, Gray et al. (2017), found that Ω_{Ar} may best predict bivalve larvae responses to OA, thereby potentially explaining why the 23°C and 7.7 pH treatment yielded the highest growth rate. While the underlying cellular mechanisms are outside of the scope this study, other OA-marine invertebrate studies have found that OA-induced hemolymph pH decreases can lead to disrupted ion regulation and altered enzyme activity, thereby decreasing growth (Pörtner et al., 2004).

Further potential explanations regarding growth rate responses to ocean warming and OA may involve physiological responses that affect the energy budget. For example, larvae experienced lower respiration rate under OA conditions (Fig. 4), suggesting larvae were coping with abiotic stress (potentially via energy conservation). Therefore, less energy was available for growth. Additionally, growth and clearance rate were highest at 23°C (Fig. 5), suggesting

potential metabolic depression at lower temperatures. PCA suggested that growth rate and respiration rate show opposite response profiles (Fig. 10). Therefore, it is possible that for the 23°C and pH 7.7 treatment, more energy was available to increase growth rate because less energy was used for maintenance, as represented by respiration. Counter to expectation, PCA did not show a link between growth rate and clearance rate, but larvae experienced higher clearance rates under ocean warming conditions. This difference in clearance rate may explain why larvae grew faster at 23°C than 17°C, but does not explain why larvae grew faster at 23°C and 7.7 than the 20°C treatments, as larval clearance rates did not significantly differ between 23°C and 20°C. However, the opposite responses profiles for growth rate and swimming speed leads to the possibility of an energy trade-off such that when more energy is allocated to growth, less energy is allocated to locomotion. Therefore, larvae may have grown faster at 23°C than 20°C because less energy is used for locomotion at 23°C and therefore more energy remains available for growth. Such a hypothesis has never been investigated in marine invertebrate larvae, but support for an energy trade-off between growth and locomotion when faced with environmental variability (e.g., temperature and food availability) has been found for marine fish (Billerbeck et al., 2001; Killen et al., 2014). PCA also suggested that growth rate may be linked with biomineralization (Fig. 10). This relationship aligns with previous studies that found when under the influence of ocean warming and/or OA, larval shell properties respond similarly to growth (Miller et al., 2009). This suggests that when more energy is allocated to linear growth, more energy may be simultaneously allocated to shell development (e.g., increasing shell thickness or different crystalline structure). This distinction is specified because increased linear growth does not always lead to increased shell development. For example Talmage and Gobler (2010) found that shell length increased in larval hard clams (*Mercenaria mercenaria*) grown at a pCO_2 of 750

as compared 1500 ppm, while shell thickness did not change. However, it should be noted that other studies have found a growth-calcification trade-off where reduced pH increases calcification costs, which then decreases shell length (Ramesh et al., 2017; Sanders et al., 2018). The potential lack of this trade-off for surfclam larvae suggests that they may be relatively tolerant to OA.

While many studies have documented negative impacts of climate change on adult bivalve immune performance (Wang et al., 2016; Nardi et al., 2018; Huang et al., 2022), surprisingly few studies have analyzed impacts of climate on larval bivalve immune performance. However, Schwaner et al. (2020) found that larval hard clams (*M. mercenaria*) experienced higher pathogen-induced mortality under OA, aligning with the results of the present study. Such higher mortality may be due to increased bacterial growth under OA conditions or decreased host immunity (e.g., hemocyte activity), as Schwaner et al. (2020) indeed found higher *Vibrio*. spp. concentrations under acidified conditions. Additionally, Elston et al. (2008) found that larval oyster and clam mortality events on the west coast of North America were linked to *Vibrio* spp. blooms and warmer waters, suggesting increased temperature may worsen pathogen-induced mortality. This contrast results of the present study where temperature had no significant impact on bacteria-induced mortality (Fig. 7). Furthermore, while adult bivalve immune responses may differ than those of larvae, Hornstein et al. (2018) showed that adult surfclam immune performance responds negatively to ocean warming. This further highlights that larval surfclams are more resilient to ocean warming than adult surfclams. While it is unknown if pathogens such as *Vibrio* spp. regularly leads to mortality of wild population surfclams, the results of the present study suggest that OA may exacerbate *Vibrio* spp. risks for surfclams, at least during the larval stage. Furthermore, while temperature did not affect

pathogen-induced mortality in the present study, warming coastal waters may support larger *Vibrio* spp. populations, providing an avenue by which ocean warming may increase surfclam risks to pathogens (Le Roux et al., 2016).

4.3 Behavioral Responses

A vast literature exists regarding the effects of ocean warming and OA on swimming behavior of larvae of fauna other than mollusks (e.g., fish, echinoderms and crustaceans) (Chan et al., 2015; Cominassi et al., 2019; Gravinese et al., 2020); however, few studies have focused on the combined effects of ocean warming and OA on larval mollusk swimming behavior. Four studies investigating OA impacts on mollusk swimming behavior revealed either decreased swimming speeds at projected pH levels (Zhang et al., 2014; Fonseca et al., 2020), or no change in swimming speed (Meyer-Kaiser et al., 2019; Kavousi et al., 2021), the latter aligning with the results of the present study. Additionally, studies on swimming behavior of non-mollusk marine invertebrates have found that echinoderm larvae do not change swimming behavior in response to OA; (Chan et al., 2015), aligning with the results of the present study.

The present study found that surfclam larvae decrease swimming speed in response to ocean warming (Fig. 8), contrasting previous studies that found that bivalve larvae typically increase swimming speed in response to ocean warming, due to increased metabolic activity, (Hidu and Haskin, 1978). While decreased swimming speed in response to ocean warming would be expected if upper thermal tolerance thresholds are passed, surfclam larval growth increased in response to ocean warming, suggesting thermal tolerance thresholds were not passed. Therefore, the observed decreased swimming speed in response to ocean warming may be considered unexpected. While decreasing swimming speed in response to ocean warming is unexpected, it is not unprecedented in marine larvae. For example, Cominassi et al. (2019) found

that for European sea bass larvae, swimming speed was lower at 20°C than 15°C even though growth increased at 20°C. Therefore, it is possible that different optimal thermal windows exist for growth and swimming behavioral responses. Additionally, Mann and Wolf (1983) reported presence or absence of swimming *A. islandica* larvae in an artificial stratified water column and found that later stage larvae did not swim in temperatures above 20°C. Therefore, inner continental shelf bivalves such as *S. solidissima* and *A. islandica* may produce larvae with constrained upper limit thermal sensitivities, as these bivalves experience lower temperatures relative to estuarine bivalves.

It is well documented that changes in bivalve larvae swimming behavior may lead to dispersal changes (North et al., 2008; Burgess et al., 2021). This occurs due to swimming behavior controlling vertical position, such that larvae higher in the water column in the surface mixed layer may experience greater advection than those at depth (Garland et al., 2002; North et al., 2008; Daigle et al., 2016). For example, Chen et al. (2021) used biophysical models to test the relative impact of diel swimming behavior against thermocline-seeking swimming behavior for sea scallop (*Placopecten magellanicus*) larvae in the MAB and found that such differences in swimming behavior can lead to ~10% changes in settlement success in different regions (e.g., Georges Bank vs. Southern New England). However, it is not known if decreases in swimming speed documented in the present study may lead to significant dispersal pattern changes. Additional biophysical modelling studies are needed to assess if swimming speed differences of less than 0.5 mm^{-s} changes will lead to negligible or large changes in dispersal patterns for surfclam larvae in the MAB.

Under ocean warming-only conditions, our results suggest a low PLD of ~26 days, however under combined ocean warming and OA conditions, as well as present conditions, our

results suggest a PLD greater than ~30 days (Fig. 9). These results align well with previous studies that document a PLD for surfclam larvae from 19-36 days (Loosanoff and Davis, 1963; Ropes, 1980). A longer PLD can be considered analogous to delayed metamorphosis, as both concepts highlight extended time in the water column before settlement, and therefore may affect dispersal patterns (Pechenik, 1990). Delayed metamorphosis for mollusk larvae under stressful conditions is well established in the literature (Bayne, 1965; Pechenik, 1984; Talmage and Gobler, 2009), but directly linking climate changed-induced longer PLD (i.e., delayed metamorphosis), to altered larval dispersal patterns is not as well documented. Under ocean warming conditions, a shorter PLD may compound the effects of decreased swimming speeds, as previous studies have also found that shorter PLDs can lead to shorter dispersal distances (Shanks et al., 2003; Phelps et al., 2015; Ospina-Alvarez et al., 2018). Of particular geographic relevance, Gilbert et al. (2010) found that for sea scallop (*P. magellanicus*) larvae in the MAB, changes in PLD of 5 days may lead to significant changes in dispersal patterns, more specifically, connectivity between subpopulations, by up to a factor of 10. Such dispersal changes corresponded with decreases in settlement of up to 81%. Therefore, ocean warming-induced decreases in PLD and swimming speed may decrease dispersal distances of surfclam larvae. Previous studies have examined climate change impacts on marine larvae dispersal (Andrello et al., 2015; Lacroix et al., 2018). For example, Figueiredo et al. (2022) found that ocean warming-induced (increase to 29°C from 27°C) changes in larval coral (*Acropora* spp.) survival and PLD led to an average 7% decrease in dispersal distance and a 20% decrease in larval retention. To the knowledge of these authors, this is the only study that has examined how climate change induced alterations in PLD will affect marine invertebrate larval dispersal. Such studies are needed for different phyla and in different systems.

4.4 Conclusions

These results suggest that OA may have moderate, negative effects on surfclam larvae physiology and development, as lone OA effects were only observed for immune performance and biomineralization. However, ocean warming may have stronger but positive effects on surfclam larvae physiology, development and behavior. More specifically, ocean warming may increase clearance rate and biomineralization, thereby positively affecting growth and development (assuming that the structural defense associated with a more mineralized shell outweighs any swimming behavior cost associated with a denser shell). Additionally, ocean warming may decrease PLD and swimming speed, potentially affecting dispersal patterns (see references in previous paragraphs, as well as O'Connor et al., 2007). Interactive ocean warming and OA effects were observed on larval growth where under ocean warming only, larval growth is higher, but under ocean warming and OA, larvae growth is lower. These results can be used by resource managers to make projections affecting the surfclam fishery. To make projections more accurate, however, additional studies are needed to investigate how food availability may impact surfclam larvae responses to ocean warming and OA. Food availability may not only be affected by climate change, but has the potential to interact with ocean warming and OA effects on bivalve larvae (Cole et al., 2016). Nevertheless, results from this study not only provide insight regarding climate change impacts on a declining and valuable continental shelf bivalve fishery, but may also encourage other scientists to conduct additional experimental climate change work that examines multiple fisheries-relevant responses. Such approaches provide holistic insights regarding climate change impact on bivalve fisheries and therefore may allow for more informed management decisions.

Tables:

Table 1: Mean seawater carbonate chemistry parameters (± SD) for each treatment. * denotes combined mean, pooled standard deviation (where parameters were first averaged by tank and then averaged again by treatment).

Treatment	Salinity (PSS)*	Temp (°C)*	pH*	TA (umol kg^{-1})*	DIC (umol kg^{-1})	pCO_2 (uatm)	Ω_{Ar}
17°C & 7.7	35.00 ± 0.00	17.05 ± 0.02	7.70 ± 0.01	2145.31 ± 33.76	2057.088± 34.90	910.78 ± 30.38	1.22 ± 0.02
17°C & 7.3	35.00 ± 0.00	17.07 ± 0.04	7.34± 0.01	2120.13 ± 5.70	2142.60 ± 5.78	2185.36 ± 42.65	0.56 ± 0.01
20°C & 7.7	34.80 ± 0.45	20.00 ± 0.02	7.71 ± 0.00	2161.98 ± 22.72	2060.04 ± 21.68	921.92 ± 12.31	1.38 ± 0.01
20°C & 7.3	34.40 ± 0.55	19.96 ± 0.09	7.33 ± 0.02	2154.84 ± 32.18	2175.48 ± 40.88	2382.84 ± 128.15	0.61 ± 0.03
23°C & 7.7	35.00 ± 0.00	22.99 ± 0.04	7.70 ± 0.01	2130.61 ± 40.85	2015.98 ± 42.32	928.10 ± 41.21	1.50 ± 0.04
23°C & 7.3	35.00 ± 0.00	22.96 ± 0.03	7.31 ± 0.02	2158.41 ± 35.17	2168.40 ± 32.80	2447.20 ± 89.23	0.67 ± 0.03

Table 2: Two-way ANOVA output and power exponent (per extended Box-Cox transformation procedure) for mortality, clearance rate, respiration rate, scope for growth, immune performance and growth rate (displayed as change in larval length through time). Bolded *p*-values denote a significant difference.

Variable	Value	Mortality	Clearance Rate	Respiration Rate	Scope for Growth	Immune Performance	Growth Rate
Temperature	df	2	2	2	2	2	2
	F	0.873	6.992	1.34	6.06	0.405	19.13
	p	0.431	**0.007**	0.230	**0.015**	0.676	**3.51e-05**
pH	df	1	1	1	1	1	1
	F	0.840	2.779	4.918	0.021	5.272	23.48
	p	0.368	0.114	**0.047**	0.888	**0.041**	**0.0001**
Temperature * pH	df	2	2	2	1	2	2
	F	0.184	0.642	0.868	1.327	0.376	19.11
	p	0.833	0.539	0.444	0.302	0.695	**3.53e-05**
Total	df	29	17	17	17	17	23
Residual	df	24	12	12	12	12	18
Power Transformation Exponent		n/a	0.40	0.23	n/a	0.22	n/a

Table 3: Three-way ANOVA output and power exponent (per extended Box-Cox transformation procedure) for biomineralization, swimming speed and percent swimming. Bolded *p*-values denote a statistically significant difference.

Variable	Value	Biomineralization	Swimming Speed	Percent Swimming
Temperature	df	2	2	2
	F	2.581	12.098	0.953
	p	0.098	**0.001**	0.403
pH	df	1	1	1
	F	13.971	0.002	0.759
	p	**0.001**	0.968	0.394
Time	df	1	1	1
	F	215.626	31.458	52.508
	p	**< 2e-16**	**4.844-07**	**6.377e-10**
Temperature * pH	df	2	2	2
	F	0.533	0.540	0.461
	p	0.594	0.591	0.637
Temperature * Time	df	2	2	2
	F	5.015	1.903	0.062
	p	**0.011**	0.158	0.940
pH * Time	df	1	1	1
	F	7.427	0.234	0.008
	p	**0.009**	0.630	0.932
Temperature * pH * Time	df	2	2	2
	F	1.774	0.743	0.404
	p	0.181	0.480	0.669
Total	df	71	95	95
Residual	df	60	77	77
Power Transformation Exponent		n/a	n/a	1.49

Figure Captions:

Fig. 1 Bar plot displaying percent mortality of larvae on day 30 (N = 5, ± SE). No significant differences were detected. pH of 7.7 can be considered the control.

Fig. 2: Bar plot displaying growth rate (N = 4, ± SE) in microns per day. Growth rate was calculated from larvae length measurements on days 5 and 30. Different letters (a, b) indicate significant differences within each pH treatment. pH 0f 7.7 can be considered the control.

Fig. 3: Line plot displaying mean biomineralization (N = 4, ± SE) on days 16, 23 and 30. Triangles and solid lines denote treatments of pH 7.7 (control), whereas circle and dashed lines denotes treatments of pH 7.3. Biomineralization is displayed as biomineralization index (i.e., grey scale value). Different letters (a, b, c and d) indicate significant differences on day 30 measurements, as no significant differences were detected for other days.

Fig. 4: Bar plot displaying respiration rate, standardized to individual larva biovolume, (N = 3, ± SE) on day 20-24 larvae. Respiration rate is displayed as picomoles (pmol) of oxygen consumed $hour^{-1}$. Different letters (a, b) indicate significant differences between pH treatments, as no significant temperature (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 5: Bar plot displaying clearance rate, standardized to individual larva biovolume, (unbalanced, N = 3-4, ± SE) on day 14 larvae. Clearance rate is displayed as number of algae cells consumed $hour^{-1}$. Different letters (a, b) above color shadings for temperature indicate significant differences, as no significant pH (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 6: Bar plot displaying scope for growth (N = 3, ± SE) as microjoules h^{-1} standardized to individual larvae (day 21). Different letters (a, b) above color shadings for temperature indicate significant differences, as no significant pH (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 7: Bar plot displaying immune performance (N = 3, ± SE) (represented as percent mortality in response to *Vibrio* spp. exposure), on day 18 larvae. Different letters (a, b) indicate significant differences between pH treatments, as no significant temperature (or interaction) effect was detected. pH of 7.7 can be considered the control.

Fig. 8: Line plot displaying mean (± SE) percent swimming (A) and swimming speed (B) (both unbalanced, N = 4-5) for each treatment on days 5, 11, 16 and 23. Triangles and solid lines denote treatments of pH 7.7 (control), whereas circle and dashed lines denotes treatments of pH 7.3. Different letters (a, b) above color shadings for temperature indicate significant differences, as no significant pH (or interaction) effect was detected. No significant differences were observed for percent swimming (A). Analyses for A are separate from B.

Fig. 9: Line plot displaying PLD (N = 6, ± SE) as the percent of larvae settled for each treatment from day 21.5 to 29.5 Triangles and solid lines denote treatments of pH 7.7 (control), whereas

circle and dashed lines denotes treatments of pH 7.3. The black dashed line denotes 50% settlement.

Fig. 10: Principal component biplot displaying relationships between energy budget profile response metrics including biomineralization, respiration rate, clearance rate, swimming speed, scope for growth and growth rate. Biplot also displays k-mean cluster analysis results where similar data points are grouped in one of four clusters (grey ovals). pH of 7.7 can be considered the control.

Figures:

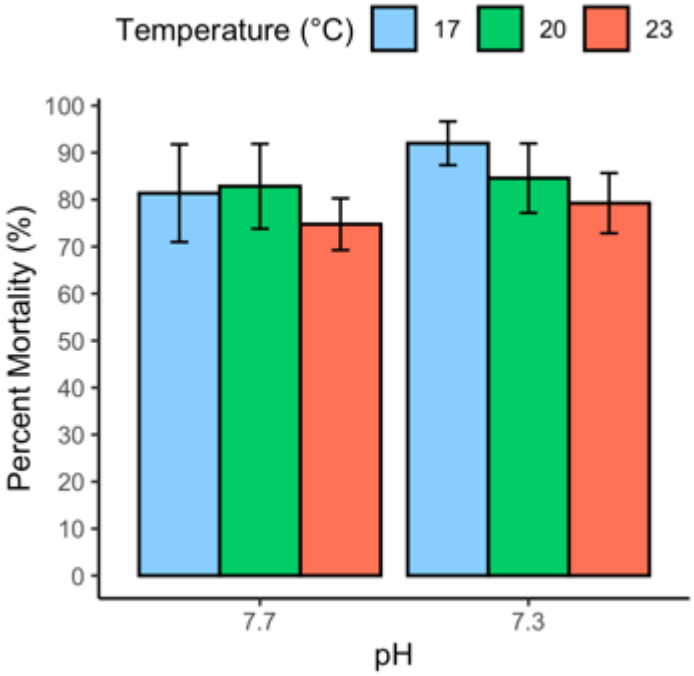

Fig. 1

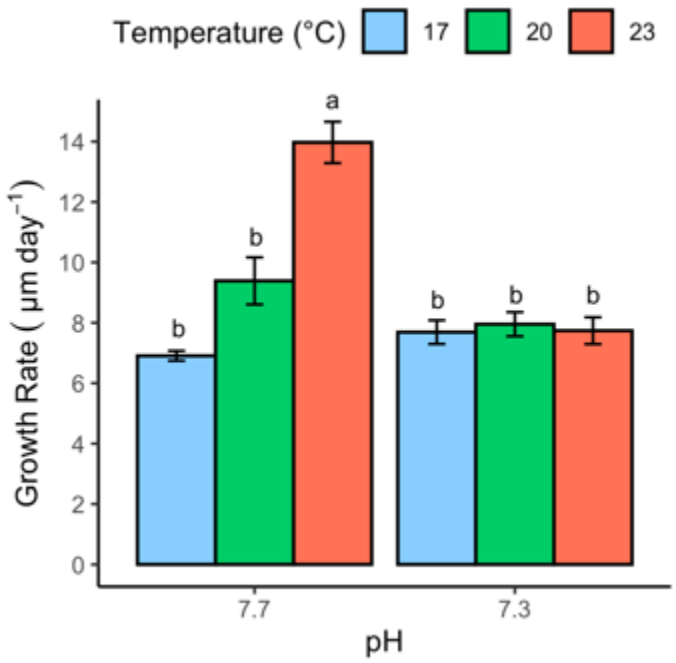
Temperature (°C)
17
20
23
Growth Rate (µm day⁻¹)
0
2
4
6
8
10
12
14
a
b
b
b
b
b
7.7
7.3
pH

Fig. 2

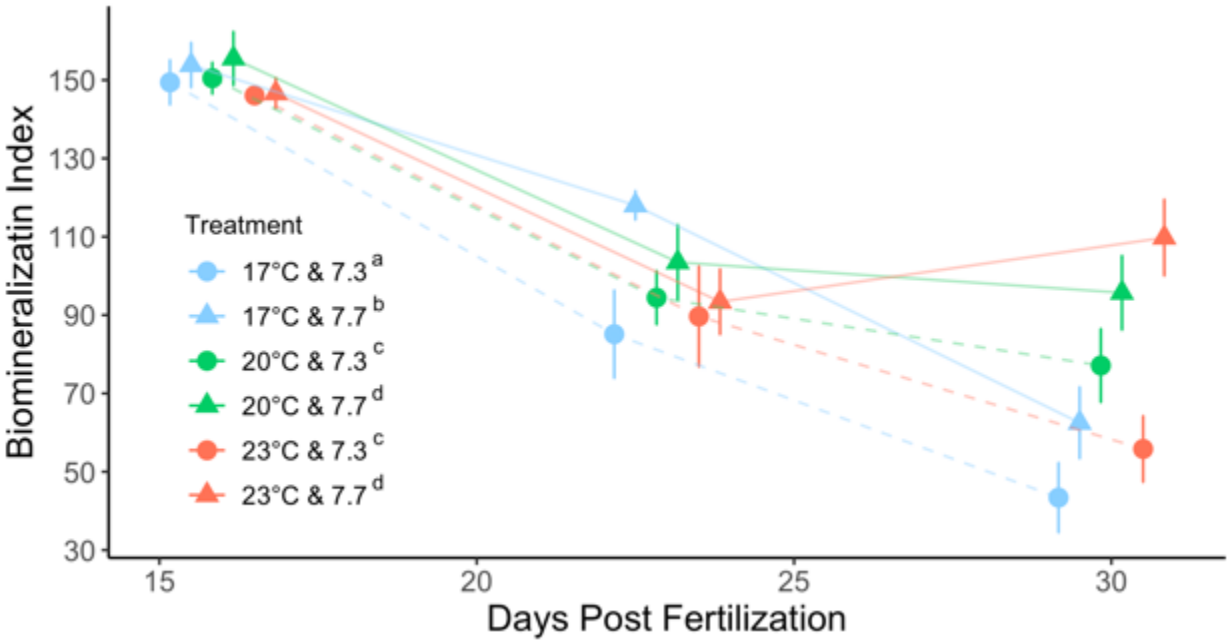
Biomineralizatin Index
30
50
70
90
110
130
150
15
20
25
30
Days Post Fertilization
Treatment
17°C & 7.3 a
17°C & 7.7 b
20°C & 7.3 c
20°C & 7.7 d
23°C & 7.3 c
23°C & 7.7 d

Fig. 3

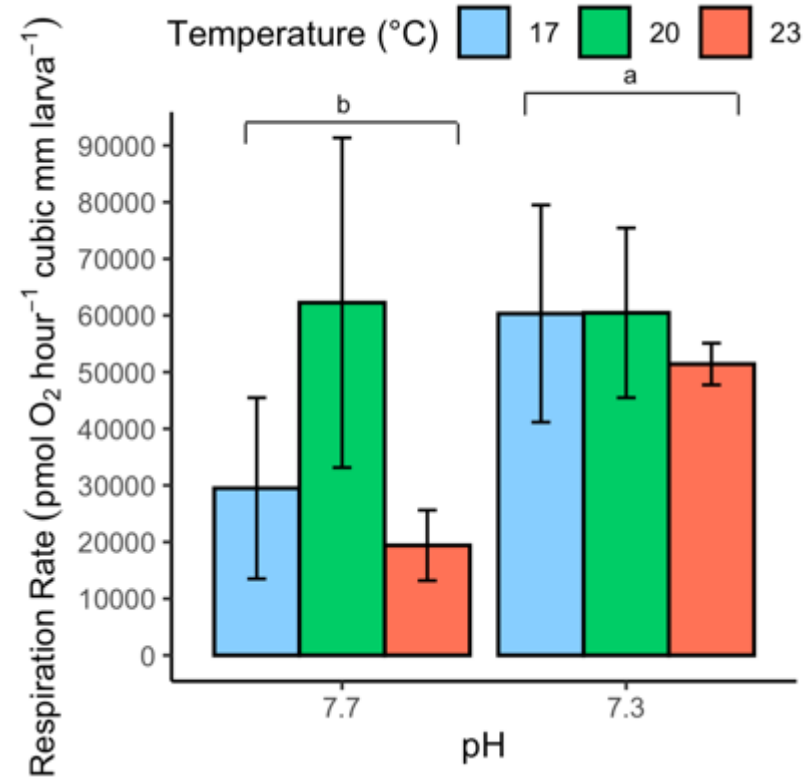
Temperature (°C)
17
20
23
b
a
Respiration Rate (pmol O_2 hour^{-1} cubic mm larva^{-1})
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
7.7
7.3
pH

Fig. 4

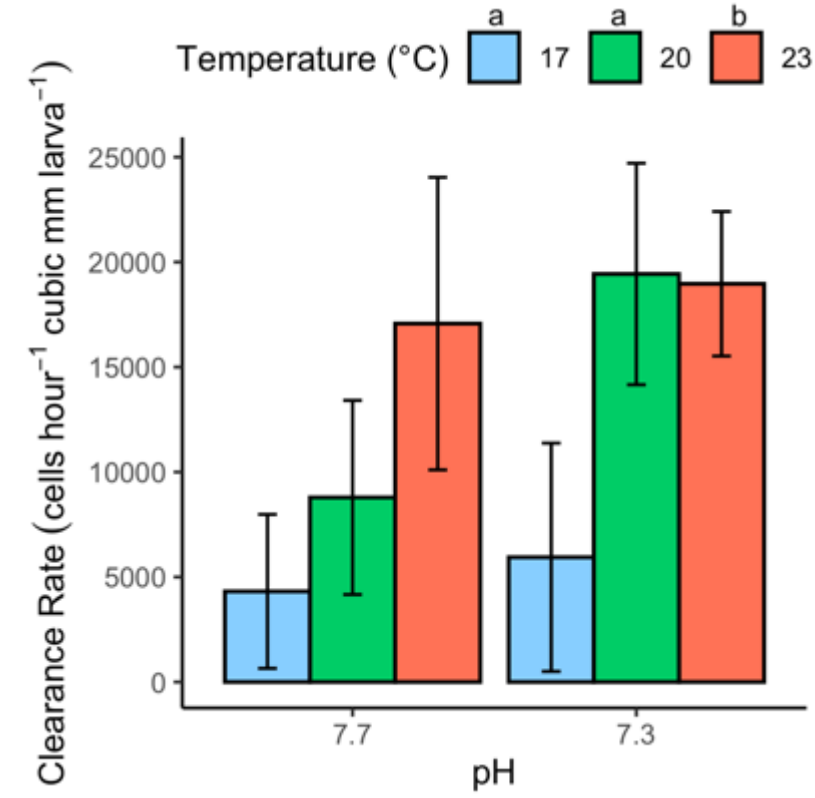
Temperature (°C)
17
20
23
a
a
b
Clearance Rate (cells hour^{-1} cubic mm larva^{-1})
25000
20000
15000
10000
5000
0
7.7
7.3
pH

Fig 5

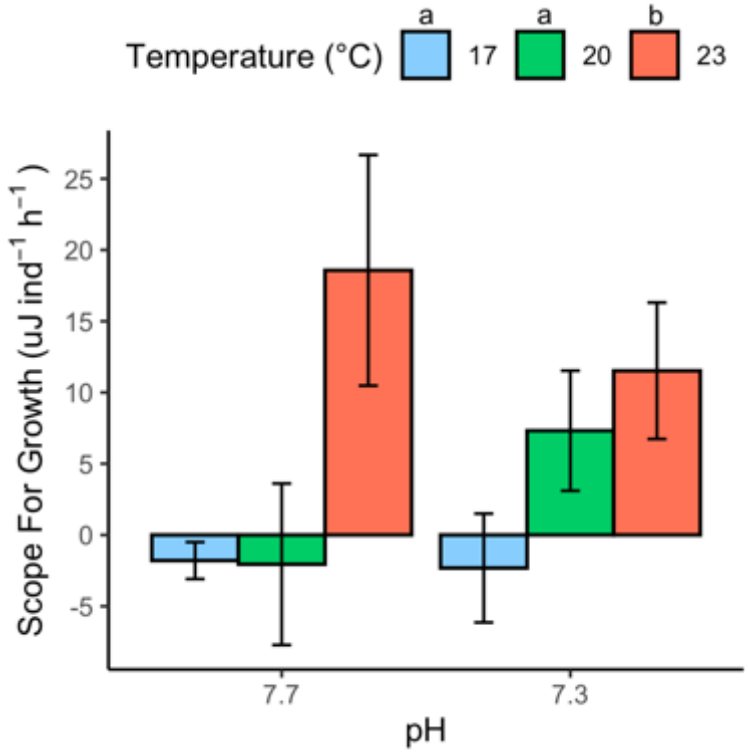
a
a
b
Temperature (°C)
17
20
23
Scope For Growth (uJ ind^{-1} h^{-1})
25
20
15
10
5
0
-5
7.7
7.3
pH

Fig. 6

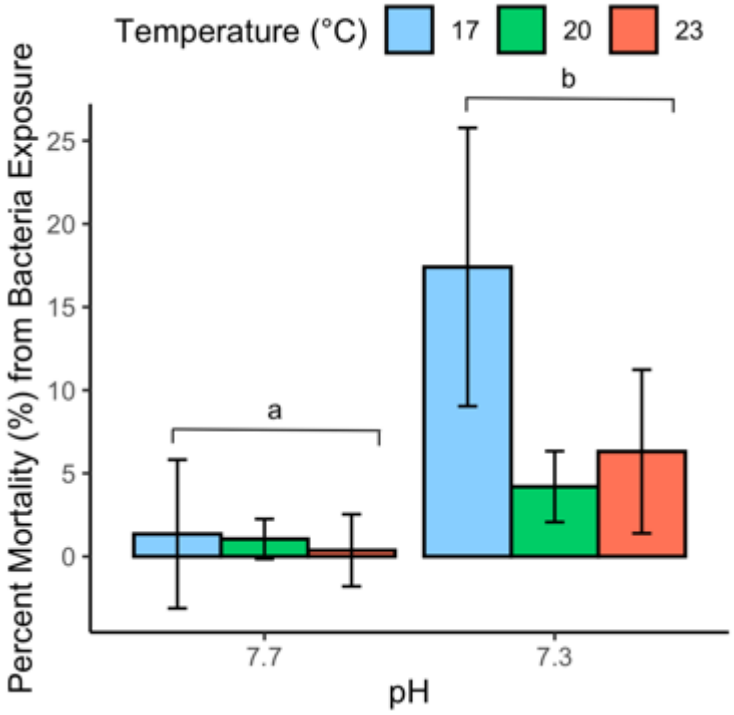
Temperature (°C)
17
20
23
b
a
Percent Mortality (%) from Bacteria Exposure
25
20
15
10
5
0
7.7
7.3
pH

Fig. 7

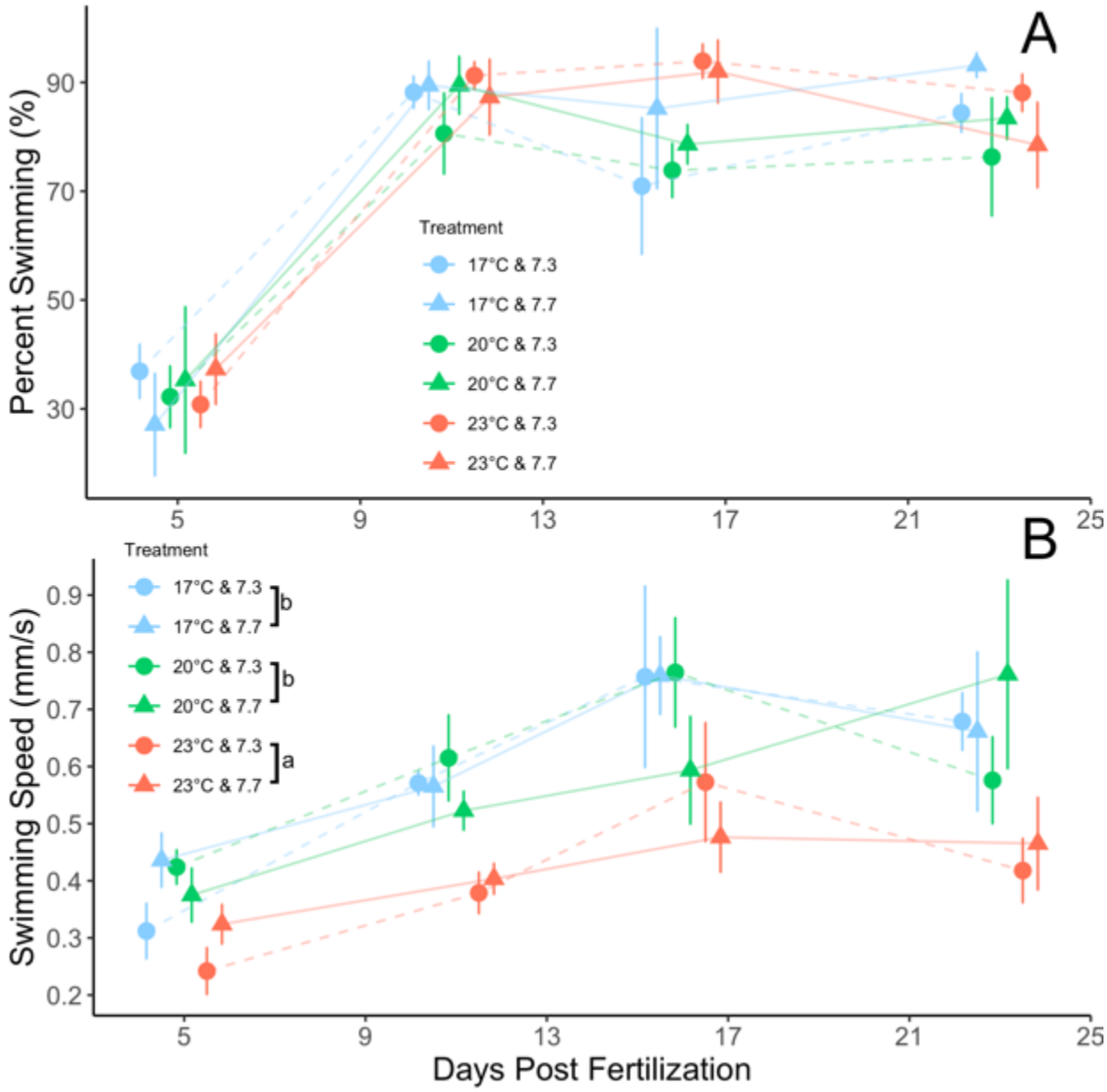
A
Percent Swimming (%)
90
70
50
30
5
9
13
17
21
25
Treatment
17°C & 7.3
17°C & 7.7
20°C & 7.3
20°C & 7.7
23°C & 7.3
23°C & 7.7
B
Swimming Speed (mm/s)
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
Treatment
17°C & 7.3
17°C & 7.7
20°C & 7.3
20°C & 7.7
23°C & 7.3
23°C & 7.7
b
b
a
Days Post Fertilization

Fig. 8

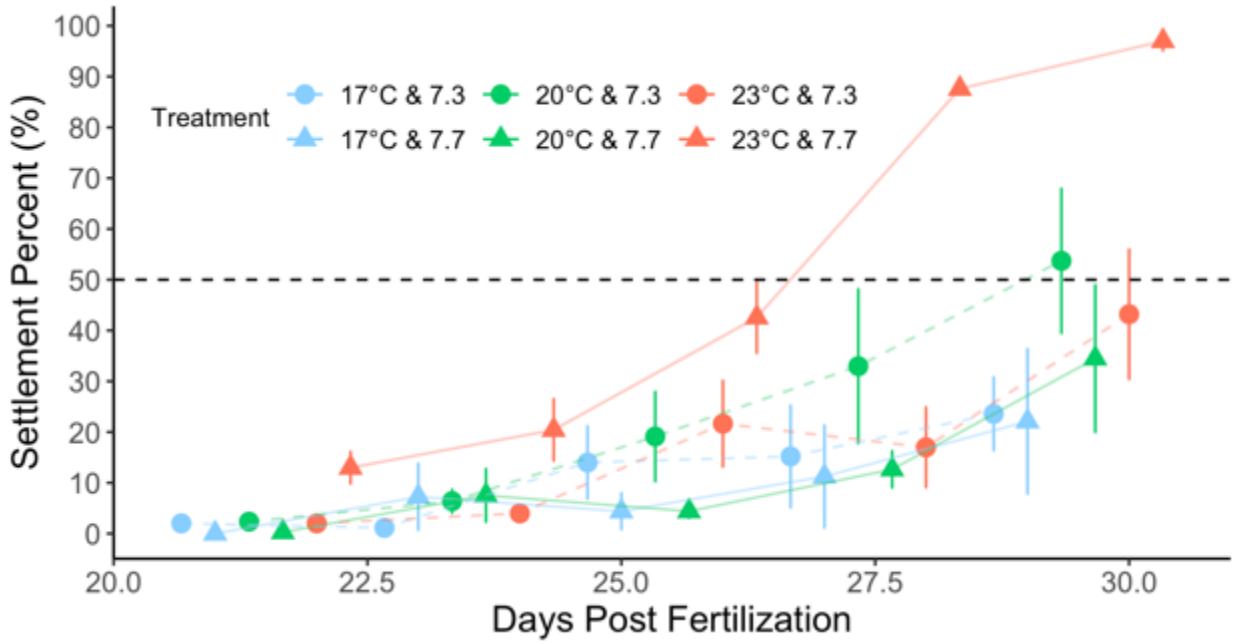
Settlement Percent (%)
Days Post Fertilization
Treatment
17°C & 7.3
20°C & 7.3
23°C & 7.3
17°C & 7.7
20°C & 7.7
23°C & 7.7
100
90
80
70
60
50
40
30
20
10
0
20.0
22.5
25.0
27.5
30.0

Fig. 9

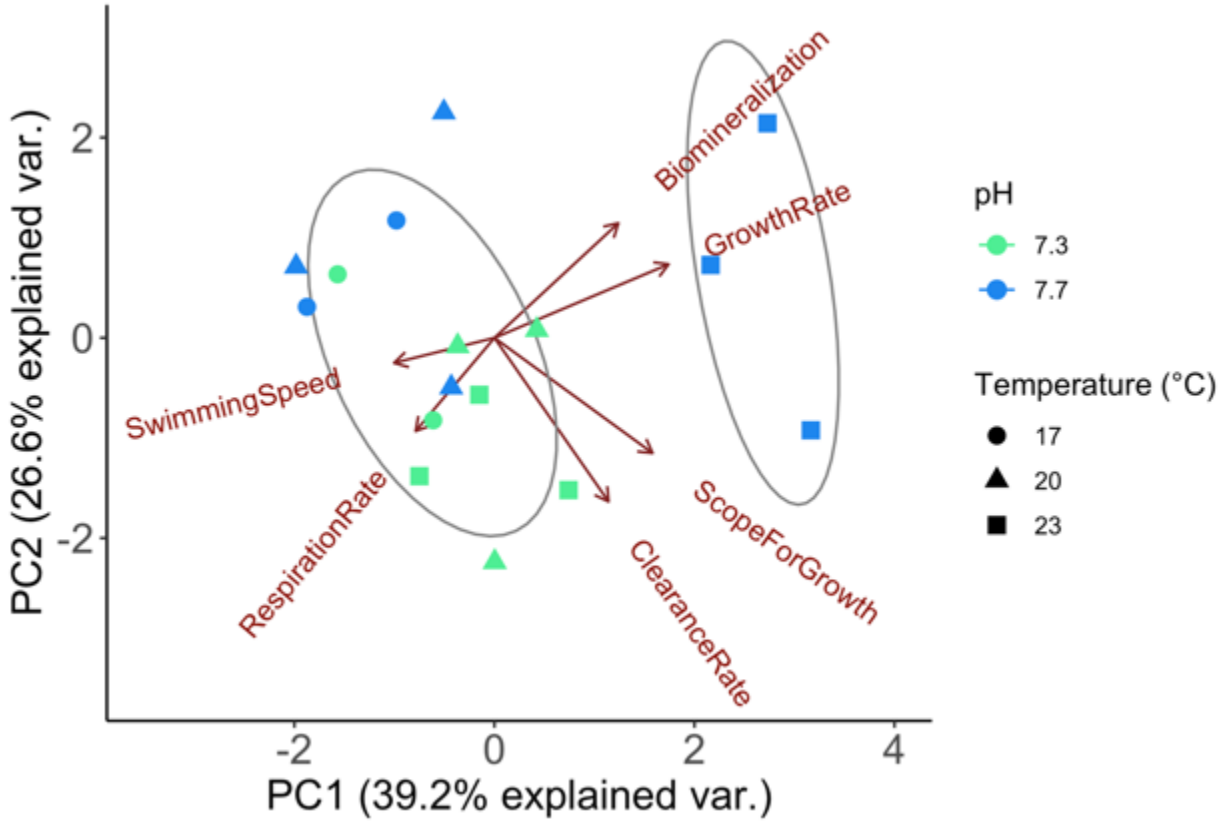
PC2 (26.6% explained var.)
PC1 (39.2% explained var.)
Biomineralization
GrowthRate
SwimmingSpeed
RespirationRate
ClearanceRate
ScopeForGrowth
pH
7.3
7.7
Temperature (°C)
17
20
23
2
0
-2
-2
0
2
4

Fig. 10

Supplementary Material

Table S1: Settings used in wrMTrck plugin for tracking larvae.

Program Setting	Value
Minimum size (pixel^2)	50
Maximum size (pixel^2)	10,000
Maximum velocity (pixels/frame)	300
Maximum area change (%)	99
Minimum track length	50
Threshold for turn	2.0
Size of bin for speed histogram (pixels/frame)	0.0
Show path length	Yes
Show labels	Yes
Show positions	Yes
Show paths	No
Show summary	Yes
Smoothing	Yes
Raw data (0: off, 3: AR + histogram)	0
Frame per second	2
Background subtraction (0: off)	25
Threshold method	0
Size of labelling font	16

Table S2: Two-way ANOVA output for multiple timepoints for biomineralization. Bolded *p*-values denote a statistically significant difference.

Variable	Value	Day 16	Day 23	Day 30
Temperature	df	2	2	2
	F	0.614	0.628	7.609
	p	0.552	0.545	**0.004**
pH	df	1	1	1
	F	2.597	4.019	18.392
	p	0.124	0.060	**0.0004**
Temperature * pH	df	2	2	2
	F	0.074	1.391	3.352
	p	0.929	0.274	0.058
Total	df	23	23	23
Residual	df	18	18	18

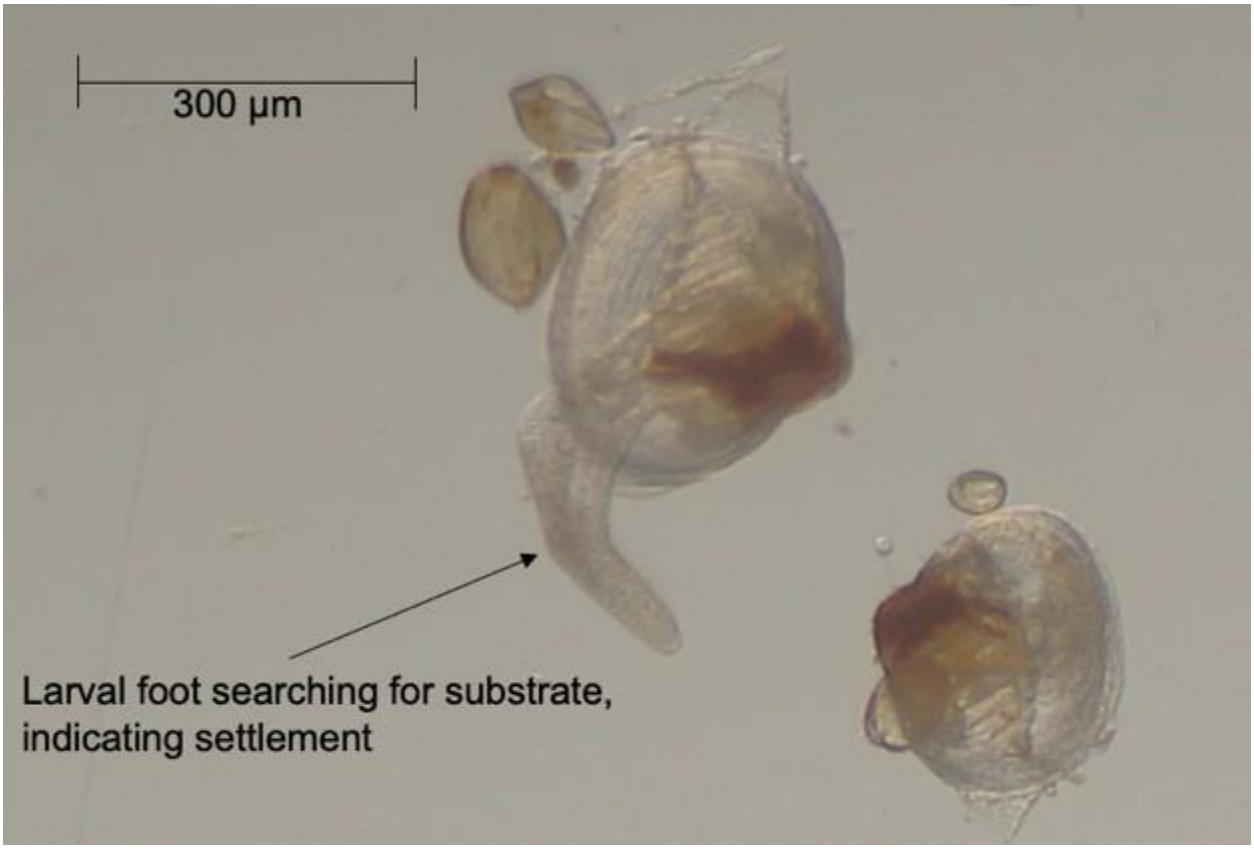

Figure S1: Two live, day 25 old larvae, one of which is displaying settlement behavior by using its foot to search for substrate to attach.

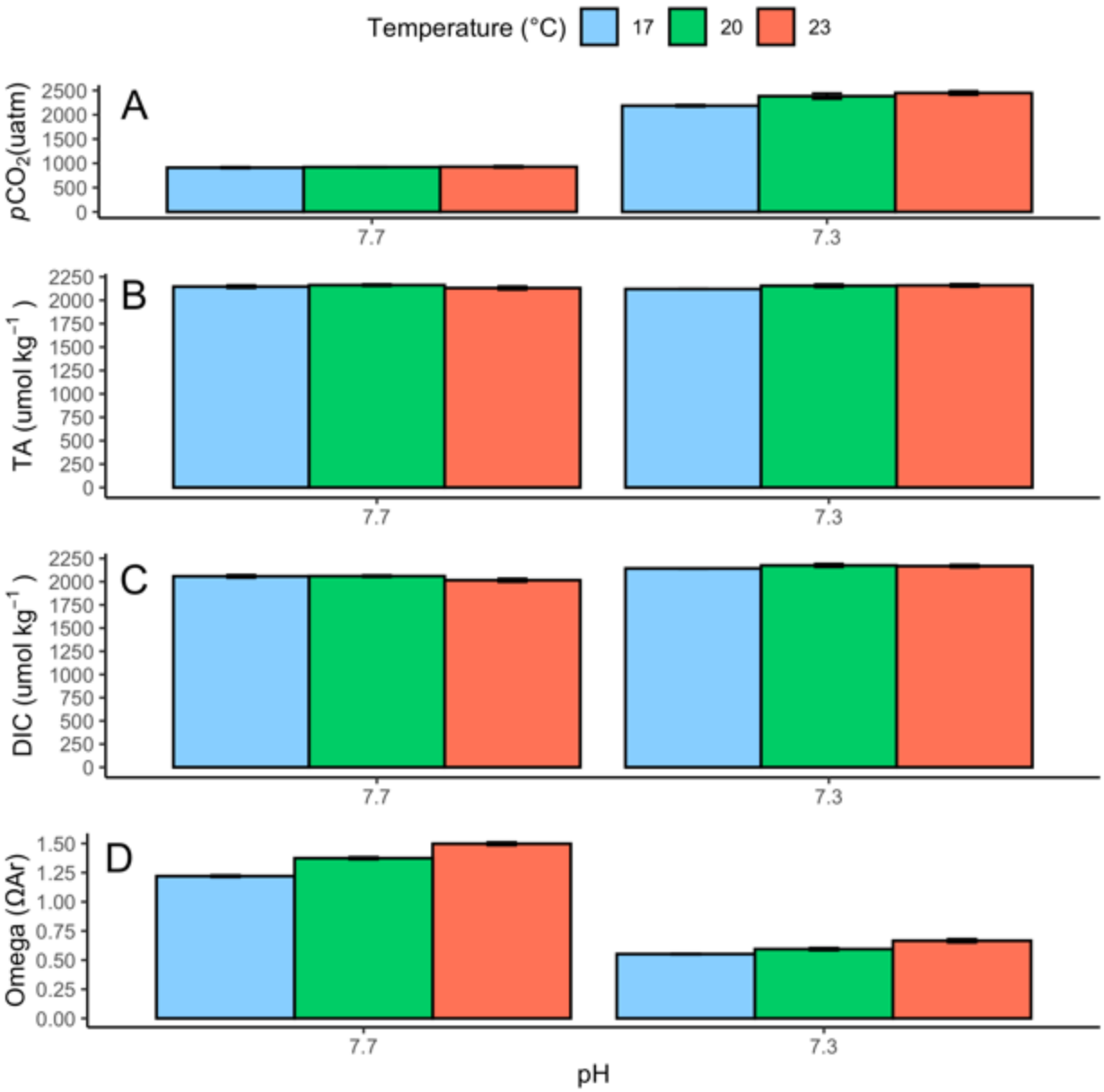

Figure S2: displaying carbonate chemistry parameters including pCO_2 (A), TA (B), DIC (C) and Omega (D) for each tank from each treatment Legend applies to A, B, C and D.

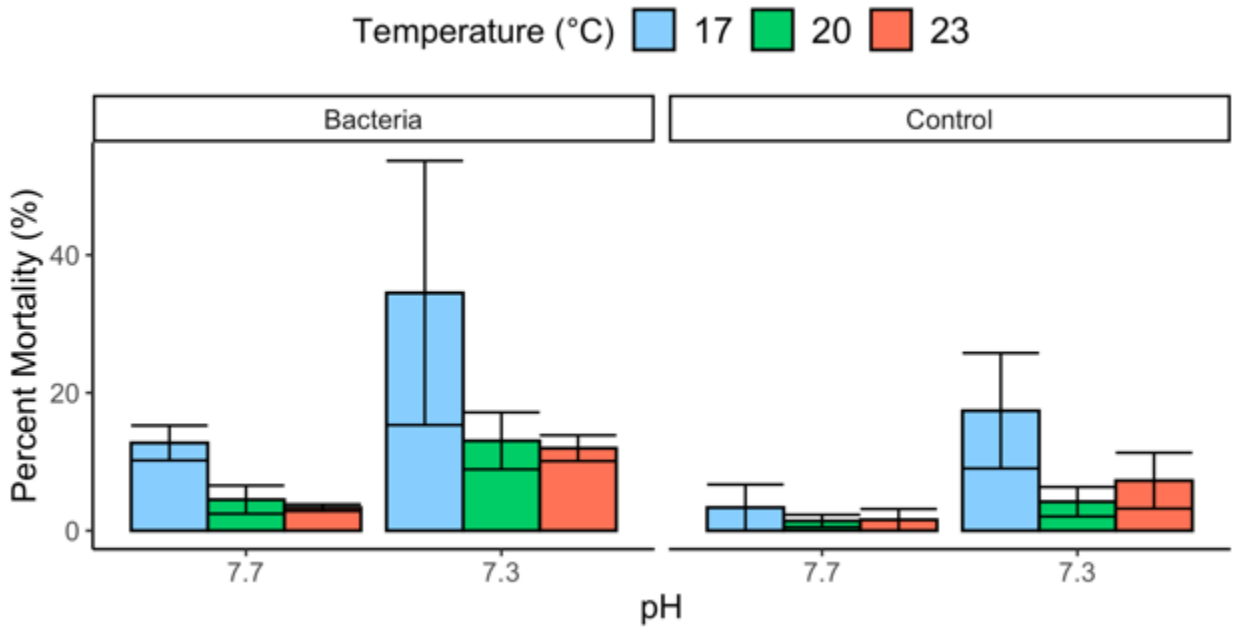

Figure S3: displaying raw immune performance assay mortality values for larvae in both wells with bacteria and wells without bacteria (i.e., controls).

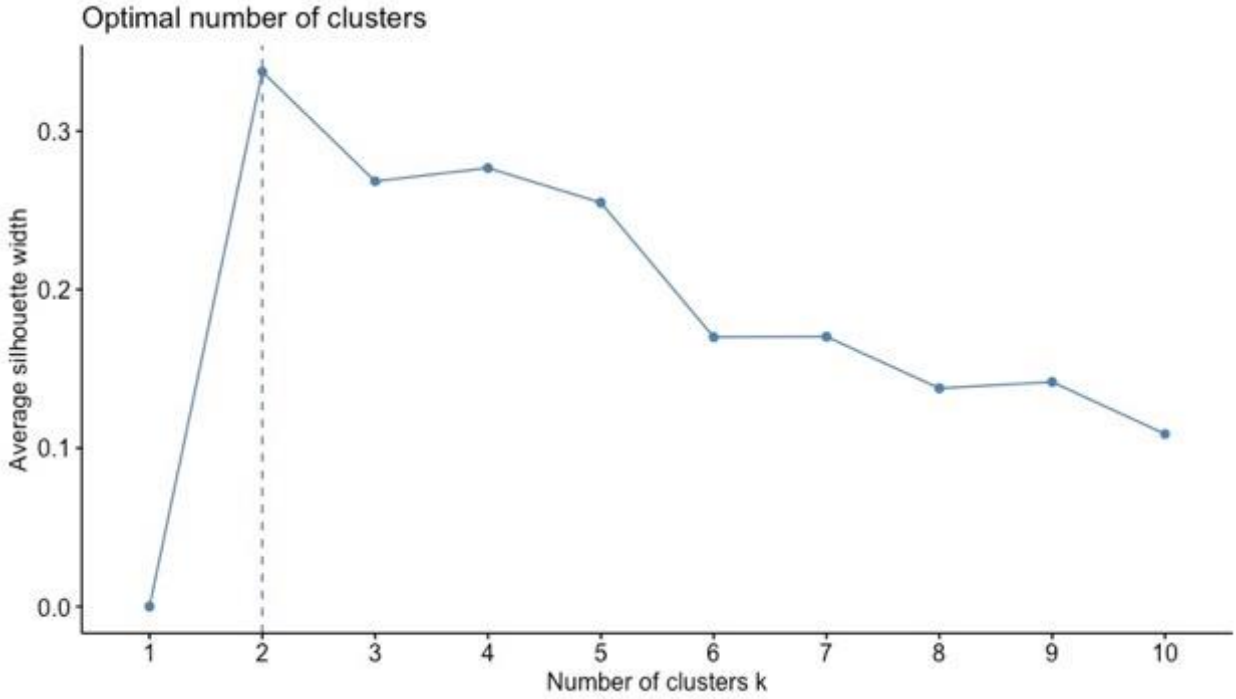

Figure S4: Average silhouette plot displaying the optimal number of clusters for k-means cluster analysis.

Author Statements

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Competing interests: the authors declare there are no competing interests. Data are available upon request. Author roles are as follows Conceptualization (BA, EPE, RH, BB, KL), Data curation (RCJ, RB, HZ, KR), Formal analysis (RCJ, RC, BB, DH), Funding Acquisition (BA, EPE, RC, BB, RH, JO), Investigation (RCJ, RH, HZ, KR), Methodology (RCJ, BA, EPE, RH), Resources (BA, EPE, BB, RH), Software (RCJ, DH, RC), Visualization (RCJ, RC, DH), Writing-original draft (RCJ), Writing-reviewer and editing (RCJ, RH, DH, RC, KL, EPE, JO, BB, HZ, KR, BA).

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Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Author Statement:

Conceptualization (BA, EPE, RH, BB, KL), Data curation (RCJ, RB, HZ, KR), Formal analysis (RCJ, RC, BB, DH), Funding Acquisition (BA, EPE, RC, BB, RH, JO), Investigation (RCJ, RH, HZ, KR), Methodology (RCJ, BA, EPE, RH), Resources (BA, EPE, BB, RH), Software (RCJ, DH, RC), Visualization (RCJ, RC, DH), Writing-original draft (RCJ), Writing-reviewer and editing (RCJ, RH, DH, RC, KL, EPE, JO, BB, HZ, KR, BA).