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Contrasting responses of commercially important Northwest Atlantic bivalve species to ocean acidification and temperature conditions

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Abstract

Modern calcifying marine organisms face numerous environmental stressors, including overfishing, deoxygenation, increasing ocean temperatures, and ocean acidification (OA). Coastal marine settings are predicted to become warmer and more acidic in coming decades, heightening the risks of extreme events such as marine heat waves. Given these threats, it is important to understand the vulnerabilities of marine organisms that construct their shells from calcium carbonate, which are particularly susceptible to warming and decreasing pH levels. To investigate the response of four commercially relevant bivalve species to OA and differing temperatures, juvenile *Mercenaria mercenaria* (hard shell clams), juvenile *Mya arenaria* (soft shell clams), adult and juvenile *Arctica islandica* (ocean quahog), and juvenile *Placopecten magellanicus* (Atlantic sea scallops) were grown in varying pH and temperature conditions. Species were exposed to four controlled pH conditions (7.4, 7.6, 7.8, and ambient/8.0) and three controlled temperature conditions (6, 9, and 12˚C) for 20.5 weeks and then shell growth and coloration were analyzed. This research marks the first direct comparison of these species' biological responses to both temperature and OA conditions within the same experiment. The four species exhibited varying responses to temperature and OA conditions. Mortality rates were not significantly associated with pH or temperature conditions for any of the species studied. Growth (measured as change in maximum shell height) was observed to be higher in warmer tanks for all species and was not significantly impacted by pH. Two groups (juvenile *M. arenaria* and juvenile *M. mercenaria*) exhibited lightening in the color of their shells at lower pH levels at all temperatures, attributed to a loss of shell periostracum. The variable responses of the studied bivalve species, despite belonging to the same phylogenetic class and geographic region, highlights the

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need for further study into implications for health and management of bivalves in the face of variable stressors.

1. Introduction

1.1 Present and future stressors on marine calcifying organisms and the Gulf of Maine ecosystem

Around a third of the excess CO_2 released into the atmosphere since the start of the Industrial Era has been taken up by oceans, increasing seawater dissolved inorganic carbon content [1]. This process is termed "ocean acidification" (OA) and has led to a decrease in global ocean pH of 0.1 pH units since ~1800 CE [2–5] with a further reduction of ~0.1–0.3 pH units predicted by 2100 CE under several CO_2 emission scenarios [6–9]. Carbonate saturation states (aragonite and calcite; $\Omega_{aragonite}$ and $\Omega_{calcite}$) are declining along with pH. As Ω (for both aragonite and calcite) approaches a critical level (generally 1.0–2.0), many calcifying organisms (shellfish, corals, coralline algae, etc.) have difficulty biomineralizing calcium carbonate hard parts, such as shells and skeleton [10–12]. Below an Ω of 1, for both aragonite and calcite, $CaCO_3$ starts to dissolve [5]. However, the degree to which marine calcifying organisms are impacted by OA, especially in coastal systems that generally have more variable and lower pH conditions compared to the global ocean, is of importance to both ecological and economic systems. Marine calcifiers have developed diverse calcification mechanisms [13–16] through their distinct evolutionary histories. As a result, differing taxonomic groups exhibit varying resiliency when confronted with low saturation states [17]. Given predictions of future acidification [6, 7], a slight difference in this critical Ω threshold will substantially impact the health of marine calcifiers.

The Gulf of Maine (GoM) is a semi-enclosed body of water in the northwest Atlantic Ocean, bounded by Cape Cod (Massachusetts, USA) and Cape Sable Island (Nova Scotia, Canada), including Georges Bank and Browns Bank. Oceanic mixing facilitates access to vital nutrients necessary for sustaining high productivity and abundant fish populations in the GoM. This is therefore an essential marine habitat for a diverse range of species and has been identified as a climate change hotspot [18, 19]. Recent sea surface temperatures (SSTs) in the GoM are increasing faster than 99% of the global ocean [20]. The Boothbay Harbor SST record (located in central coastal Maine, USA), extends from 1905 CE to present, and provides instrumental evidence of warming rates of up to 0.4˚C/decade, with an increase in annual average temperature of more than 2.1˚C since measurements began in 1905 [21]. In addition to this longer-term trend, marine heat waves have become more frequent with fisheries being particularly sensitive to these heat extremes in this region (e.g. [22] and in other parts of the ocean [23–26]). Analyses of seawater temperature profiles from the GoM and surrounding regions by Seidov et al. [27] indicate that the rate of warming in the past ten years is faster than the previous forty years, and this uptick in warming is partly related to a change in the northern edge of the Gulf Stream, which allows more warm water to enter the Gulf of Maine. Paleoclimate studies using oxygen isotopes in clam shells and climate model simulations suggest that the warming in the Gulf of Maine started in the late 1800s, reversing 900 years of cooling in this region, with the current rate of warming unprecedented in the last millennium [28, 29]. These changes in ocean temperatures are the result of a combination of anthropogenic warming and hydrographic changes associated with regional water mass variability [28–30]. Predictions of climatic temperature rise [19] and marine heat waves [31, 32] demonstrate that marine calcifiers in this region likely will be impacted by warmer ocean temperatures.

While GoM temperatures are documented to be changing dramatically, changes in pH and possibly OA conditions are less clear. Marine pH (and $\Omega_{aragonite}$) is spatially and temporally variable [33]. pH is particularly variable in coastal environments such as the GoM, where freshwater influences, high productivity, and ocean circulation impact carbonate chemistry [2, 34]. These variables are impacted in deeper waters by the interaction of the warm Gulf Stream from the south and the cold Labrador Current from the north, with surface waters composed primarily of waters from the Scotian Shelf [35]. Carbonate system modelling in this region suggests that the full impacts of ocean acidification may have been partially masked by simultaneous ocean warming in this region [2, 34]. As a result, the impacts and rates of ocean acidification in the GoM are highly uncertain. However, models suggest that surface water Ω across the GoM is expected to fall below a critical value of 1.5 by 2050 CE, with the largest impacts in coastal environments impacted by freshening [2]. This interplay of distinct water masses forms a unique marine environment that warrants intensive study of the past, present, and future marine environmental conditions (including temperature and pH) and their impact on the organisms that inhabit the GoM.

1.2 Documented impacts on marine calcifiers to OA and warming conditions

OA negatively impacts marine calcifiers (e.g., [36–43]). These effects of OA vary among species but include decreased survival, calcification, growth, development, and overall population decline [43]. For example, in the *Nucella lapillus* (dogwhelk), OA has caused negative impacts on shell strength, thickness, and size [44]. For some species, the negative impacts are more acute in early life stages. Some species show suppressed growth in larval and juvenile stages and suffer deformities and reduced survivorship in their larval stage [10, 45, 46]. For some bivalves, OA conditions impact the type of mineral precipitated (e.g., dissolution-resistant calcite substituting for aragonite) and whether the mineral has a protective layer of organic periostracum [47–50]. In many bivalves, this periostracum plays a vital role in calcification and protects from disease, predation, and corrosion. For instance, mussels that have lost their periostracum are shown to be more vulnerable to dissolution [51].

The combined effect of elevated temperature and low pH are varied but are potentially exacerbated when combined in some taxa [49, 50, 52]–e.g. *Tripneustes gratilla* (tropical sea urchin) [53]. However, other species experience minimal impacts or could benefit from increasing water temperatures and increases in pCO_2 (e.g. *Pisaster ochraceus* (ochre sea star); [54]). In the bivalve species *Mytilus edulis* (blue mussel) and *Arctica islandica* (ocean quahog), minimal impacts of higher pCO_2 at higher temperatures have been observed in previous controlled experiments (pH range = 7.63–8.01; [55]). In fact, *A. islandica* shell growth continued when living in undersaturated ($\Omega < 1$) conditions during a three-month tank experiment (pH range = 7.5–8.1; [56]). However, whether these results would be maintained under permanent OA conditions is uncertain. For *A. islandic*a, an increase in temperatures led to an increase in growth under experimental conditions, but once temperatures approached 16˚C, growth rates then decreased (temperature range = 7.5–16˚C; [55]). This growth with warming might reflect a change in shell microstructure, as increased temperatures in laboratory-grown *A. islandica* lead to an increase in the size of the largest individual biomineral unit and a larger proportion of material in the crystalline phase [57].

The differing results for the seemingly OA-resistant *M. edulis* and *A. islandica* compared to other taxa indicate that the effects of acidification and temperature are dependent on the species, even for members of the same class (Bivalvia) and characteristics of each species might make them more or less susceptible to the singular or combined effects of increased temperature and acidification [58].

1.3 The response of economically and ecologically important shellfish to OA and temperature conditions in the Gulf of Maine

Collectively, the knowledge gap regarding how various shellfish will respond to persistent or semi-persistent OA conditions and rising temperatures is substantial. Given the varied threats to organisms within the GoM, additional study of their responses is warranted. To address this gap, we cultured four vulnerable, abundant, and commercially important species from the GoM region in a 20.5-week tank experiment.

Specifically, the experiment was performed with adult and juvenile *A*. *islandica* (ocean quahog), juvenile *P*. *magellanicus* (Atlantic sea scallops), juvenile *M*. *mercenaria* (hard shell clams) and juvenile *M*. *arenaria* (soft shell clams) at four controlled pH conditions (~7.4, 7.6, 7.8, 8.0) and three controlled temperature conditions (~6, 9, 12˚C) utilizing a flow-through system. This is the first study that directly compares these species' biological responses to temperature and OA conditions within the same controlled experiment.

2. Methods

2.1 Species studied

A. *islandica* (ocean quahog) are widely distributed along the east coast of the United States and Canada from Cape Hatteras, NC (USA) to Newfoundland (Canada), and along the Atlantic coastlines of northern Europe [59–61]. *A*. *islandica* are a slow-growing animal, living for over 500 years [62, 63]. This species of clam is a filter feeder, buries itself in ocean floor sediment, and has a temperature range of 6 to 16˚C for optimal growth conditions [64, 65] but can survive at temperatures of 1 to 20˚C ([66] and references therein). The U.S. harvest of *A*. *islandica* from 2023 was valued at US$21 million [67].

M. *mercenaria* (hard clam or northern quahog) are also found along the U.S. and Canada Atlantic coastline from Florida (USA) to Nova Scotia (Canada). These bivalves can live up to 40 years and reach a maximum height of 127 mm. *M*. *mercenaria* are sessile, burrowing in sediment until only their siphon is exposed for feeding purposes. They have an ideal temperature for growth of 20˚C [68]. The 2018 estimate of the U.S. economic impact of *M*. *mercenaria* fisheries was US$52 million [69].

M. *arenaria* (soft shelled clam, longnecks, or steamer) are native to the range from Labrador (Canada) to Cape Hatteras, NC (USA). More recently, they were introduced in other regions, including the eastern Pacific coast of the United States and Canada, and in the North Atlantic around Europe, where they burrow in soft sediments in shallow water and intertidal mud flats [70–73]. *M*. *arenaria* have a typical adult size of 75 to 100 mm, a typical lifespan of 15 years [74] and can survive in temperatures up to 28˚C [75]. Commercial landings for the U.S. from 2019 totaled $23.5 million [76].

P. *magellanicus* (Atlantic sea scallops) have a similar range to the other bivalves in this study; from the Mid-Atlantic coast of the United States to the Canadian border along the Atlantic coast [77]. They have a high economic value to the US fisheries industry, with 2022 U. S. commercial landings totaling 32 million pounds valued at US$480 million [76, 78]. A small percentage (5 to 10%) of individuals are albino [79] and, unlike other bivalves in this study, *P*. *magellanicus* can move in the water to escape predation [76, 80]. The optimal growth temperature for this species is 10 to 15˚C [81].

2.2 Field collection

Adult (n = 15; age range ~14 to 62 years) and juvenile (n = 67; less than 1 year old) *A*. *islandica* specimens were collected via a commercial dredge off of Jonesport, Maine, USA (44˚

33.247'N, 67˚ 16.183'W) in ~76–85 m water depth by the vessel FV 3D's in September 2021. Juvenile (1–2 years old) *P. magellanicus* (Atlantic sea scallops, n = 73) were obtained from Marsden Brewer of PenBay Farmed Scallops in Stonington, Maine (USA) and were cultivated from GoM wild spat in East Penobscot Bay. Juvenile *M. mercenaria* (hard shell clams, n = 161) and juvenile *M. arenaria* (soft shell clams, n = 160) were supplied by the Downeast Institute Hatchery in Beals, ME in November 2021 (map of these sites in S1 Fig). All specimens were transported to the Schiller Coastal Studies Center (SCSC; Bowdoin College, Orr's Island, ME) immediately after collection and placed in a common flow-through tank at ambient temperature and pH until the start of the experiment. All specimens were tagged and given alphanumeric identifiers used to track specimens throughout the experiment and subsequent analysis.

2.3 Experimental conditions and design

The 20.5-week tank experiment (January 14 to June 8, 2022) was conducted at the SCSC flowing seawater laboratory. Specimens were cultured in four pH treatments (7.40±0.01, 7.64 ±0.03, 7.84± 0.04, ambient; 8.00±0.04) and three temperature treatments (6.29±0.15, 9.05 ±0.19, and 11.99±0.18˚C) (Fig 1; Table 1; 20 pH and > 196000 temperature measurements). These three temperatures were chosen as they were within the range of ideal conditions for most of the studied species to ensure sufficient growth. The four pH treatments were chosen to span the range of predicted coastal pH values by the end of the century [82]. Coastal seawater was pumped into the flowthrough seawater lab and sixteen experimental tanks where conditions were manipulated to the targeted pH and temperature (Fig 1). All tanks were set to a flow rate of 1 L/min.

The incoming seawater was chilled to <6˚C with an Aqualogic DX Heat Exchanger System before being diverted into two Apex control systems that each controlled and monitored two target pH levels (neptunesystems.com). To achieve the target pH levels, CO_2 from compressed cylinders was bubbled into the water of a diffusion chamber leading to each of the three controlled pH systems. The three Apex systems each delivered CO_2-treated seawater to four experimental tanks via a four-outlet manifold. The Apex control program used a pH-stat system that measured pH in one of the four experimental tanks per pH level every minute and controlled pH by bubbling CO_2 in a diffusion chamber when pH measurement exceeded the target value. For the ambient pH culture conditions, pH was monitored by the Apex system but carbonate chemistry was not altered from the incoming seawater.

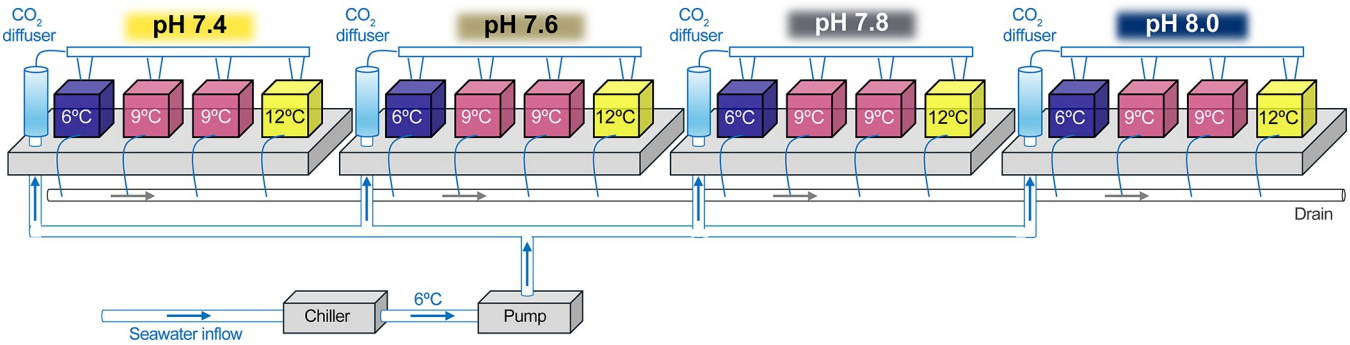


Fig 1. Schematic of the experimental design of the pH and temperature-controlled culture experiment in Schiller Coastal Studies Center (SCSC; Bowdoin College, Orr's Island, ME). Seawater was pumped into a chiller and cooled to <6˚C before being pumped to CO_2 diffusers to achieve the three pH treatments. The ambient pH tanks received no treatment. The water was then heated to the target temperature by heaters within each tank. pH was monitored and adjusted with three Apex systems, temperatures were tracked with two Tidbit probes per tank and both pH and temperature were confirmed with weekly sampling with YSI probes. Colors representing pH and temperature treatments are consistent throughout subsequent figures.

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Table 1. Average experimental conditions (± standard deviation) for each tank. The temperature is the average taken from the two Tidbits within each tank (n>196000). Water samples for total pH (pH_T), TA, DIC, and Ω were collected weekly from each tank (n = 20) and the average values from measured and calculated carbonate system parameters are shown here. The specimen counts are the number of specimens in each tank at the start of the experiment, with deaths (for all species) and missing/lost specimens (for scallops) in parentheses. Note that 22 scallops were removed halfway through the experiment (on April 14, 2022).

pH treatment	Temperature treatment (˚C)	pH_T from water chem (+/-s.d.)	Temperature (˚C) (+/- s.d.)	DIC (μmol/kg) (+/- s.d.)	TA (μmol/kg) (+/- s.d)	$\Omega_{aragonite}$ (+/- s.d)	$\Omega_{calcite}$ (+/- s.d)	pCO_2 equivalent (ppmv)	Total specimens	Juvenile *A. islandica* (deaths)	Adult *A. islandica* (deaths)	Juvenile *M. mercenaria* (deaths)	Juvenile *M. arenaria* (deaths)	Juvenile *P. magellanicus* (deaths, missing)
7.4	6	7.39 +/- 0.08	6.1 +/- 0.6	2182 +/-63	2122 +/-55	0.5±0.1	0.7 ±0.1	1903	31	4 (0)	1 (0)	10 (1)	10 (0)	6 (1,1)
7.4	9	7.40 +/- 0.06	9.0 +/- 0.4	2164 +/-58	2120 +/-55	0.4±0.1	0.7 ±0.1	1862	29	4 (0)	1 (0)	10 (4)	10 (0)	4 (0)
7.4	9	7.41 +/- 0.14	9.3 +/- 0.4	2164 +/-63	2120 +/-55	0.4±0.1	0.6 ±0.1	1908	29	4 (0)	0 (0)	11 (3)	10 (0)	4 (0)
7.4	12	7.40 +/- 0.06	11.6 +/- 0.8	2156 +/-59	2121 +/-56	0.5±0.1	0.7 ±0.2	1892	31	4 (1)	1 (0)	10 (5)	10 (3)	6 (1)
7.6	6	7.67 +/- 0.08	6.3 +/- 0.8	2102 +/-42	2121 +/-59	0.7±0.1	1.1 ±0.2	966	31	4 (0)	1 (1)	10 (1)	10 (0)	6 (0)
7.6	9	7.64 +/- 0.06	9.0 +/- 0.4	2103 +/-48	2123 +/-60	0.7±0.1	1.1 ±0.2	1066	29	4 (0)	1 (1)	10 (3)	10 (1)	4 (0)
7.6	9	7.64 +/- 0.08	9.0 +/- 0.4	2098 +/-42	2119 +/-60	0.7±0.1	1.1 ±0.2	1063	29	4 (0)	1 (1)	10 (3)	10 (0)	4 (0)
7.6	12	7.59 +/- 0.07	12.1 +/- 0.4	2100 +/-46	2119 +/-58	0.7±0.2	1.1 ±0.2	1223	29	4 (0)	1 (0)	10 (1)	10 (1)	4 (1,1)
7.8	6	7.89 +/- 0.07	6.5 +/- 0.7	2038 +/-46	2120 +/-58	1.1±0.2	1.8 ±0.3	558	30	4 (0)	1 (1)	10 (2)	10 (1)	5 (0)
7.8	9	7.84 +/- 0.06	9.2 +/- 0.6	2042 +/-54	2120 +/-60	1.1±0.2	1.8 ±0.2	644	29	4 (0)	1 (0)	10 (2)	10 (0)	4 (0)
7.8	9	7.82 +/- 0.06	9.1 +/- 0.4	2046 +/-55	2118 +/-57	1.1±0.2	1.8 ±0.3	672	29	4 (0)	1 (0)	10 (8)	10 (0)	4 (0)
7.8	12	7.81 +/- 0.07	11.9 +/- 0.4	2034 +/-50	2115 +/-60	1.1±0.1	1.7 ±0.2	707	29	4 (0)	1 (0)	10 (4)	10 (1)	4 (0)
ambient	6	8.05 +/- 0.05	6.3 +/- 0.8	1991 +/-40	2121 +/-56	1.5±0.2	2.4 ±0.3	376	31	4 (0)	1 (1)	10 (1)	10 (0)	6 (0)

(*Continued*)

Table 1. (Continued)

pH treatment	Temperature treatment (˚C)	pH_T from water chem (+/-s.d.)	Temperature (˚C) (+/- s.d.)	DIC (μmol/kg) (+/- s.d.)	TA (μmol/kg) (+/- s.d)	$\Omega_{aragonite}$ (+/- s.d)	$\Omega_{calcite}$ (+/- s.d)	pCO_2 equivalent (ppmv)	Total specimens	Juvenile *A. islandica* (deaths)	Adult *A. islandica* (deaths)	Juvenile *M. mercenaria* (deaths)	Juvenile *M. arenaria* (deaths)	Juvenile *P. magellanicus* (deaths, missing)
ambient	9	8.00 +/- 0.05	9.2 +/- 0.6	1986 +/-44	2115 +/-60	1.5±0.2	2.4 ±0.3	431	29	4 (0)	1 (0)	10 (2)	10 (0)	4 (1)
ambient	9	8.01 +/- 0.05	8.6 +/- 0.3	1989 +/-42	2119 +/-58	1.5±0.2	2.4 ±0.3	419	32	7 (0)	1 (0)	10 (3)	10 (1)	4 (0)
ambient	12	7.964 +/- 0.05	11.9 +/- 0.4	1994 +/-42	2117 +/-57	1.5±0.2	2.4 ±0.3	507	29	4 (0)	1 (0)	10 (3)	10 (3)	4 (2)

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The temperature treatments were individually controlled in each tank with Inkbird controllers and two heaters (500/800W) per tank. The temperature was measured by two Hobo Tidbits (MX2032) in each experimental tank which recorded temperature every minute. Aquarium pumps circulated water and homogenized temperature and pH conditions within each tank. The stability of the experimental conditions in each tank was confirmed with weekly measurements of pH, temperature, salinity, and dissolved oxygen using a YSI Pro-Plus handheld sonde (Xylem Inc.).

Weekly water samples were collected from each tank to determine dissolved inorganic carbon (DIC), total alkalinity (TA) and total pH (pH_T) of the experimental tanks. 250 mL water samples were filtered with a 0.45um in-line filter, stored in borosilicate glass bottles and poisoned immediately after collection. DIC and TA samples were collected in screw-top bottles while pH samples were collected in bottles with greased ground glass stoppers. All samples were analyzed within eight months of the experiment's completion. pH_T, Ω and pCO_2 were also calculated from DIC and TA using Seacarb in R using K1 and K2 values of [83]. Carbonate chemistry was measured directly from the tanks weekly to confirm target pH conditions were met, and to consider temperature dependent pH effects.

Limited food availability can stress marine organisms [84]. To mitigate food limitation, the study tanks were supplemented with Shellfish Diet 1800 five times per week by mixing 1 mL (five different microalgae at a concentration of 2 billion cells/mL) of food with seawater and placing the food mixture into each tank (reedmariculture.com/products/shellfish-diet). Additionally, the tanks were checked weekly for appropriate flow rates, and any visibly dead specimens were removed and recorded.

2.4 Shell morphometrics

All study specimens were measured for maximum height, dry (live) weight and buoyant weight at the start and end of the experiment. Specimen maximum height was obtained using a digital caliper positioned on the maximum growth axis of the shell (Fig 2). Dry (live) and buoyant weight was measured with Mettler Toledo (ME204E) and Ohaus Model Adventurer balances, respectively (S2 Fig; [85, 86]). After dissection at the end of the experiment, shell weight was measured using an Ohaus Model Adventurer (AR1140) balance. Prior to the start of the experiment (January 2022) and ~12 weeks into the experiment (mid-April 2022), all specimens were placed in a calcein bath for 24 hours at ambient seawater conditions. This provides an unambiguous indicator of the start of the experimental period (and the midpoint in April) when shells are viewed under fluorescent light. The calcein stain lines were used to confirm some growth measurements (S3 Fig). Approximately half of the scallops (n = 32) were removed on April 14, 2022, and were measured for maximum height, dry (live) weight and buoyant weight but excluded from analysis as they were not grown in the experiment for a comparable amount of time to the other study groups.

Photos were taken of all specimens at the end of the tank experiment since there were observable visual differences in specimens within the same species group. Shell coloration can indicate differences in the periostracum which is important for calcium carbonate deposition and protection against dissolution and predation [48, 87–93]. Measurements of shell coloration were performed using ImageJ [94]. The shell's outer edge was outlined manually using the polygon tool, and any shell label was excluded from the polygon (Fig 3). The Histogram tool from the Analyze menu displayed a histogram of the distribution of gray values (unitless) from the shell image. From this, the mean value for coloration of each shell was determined. Since color (RGB) images were used, the histogram was calculated by converting each pixel to grayscale using the formula gray = 0.299*red+0.587*green+0.114*blue. Larger values of coloration indicate a more white/less gray image.

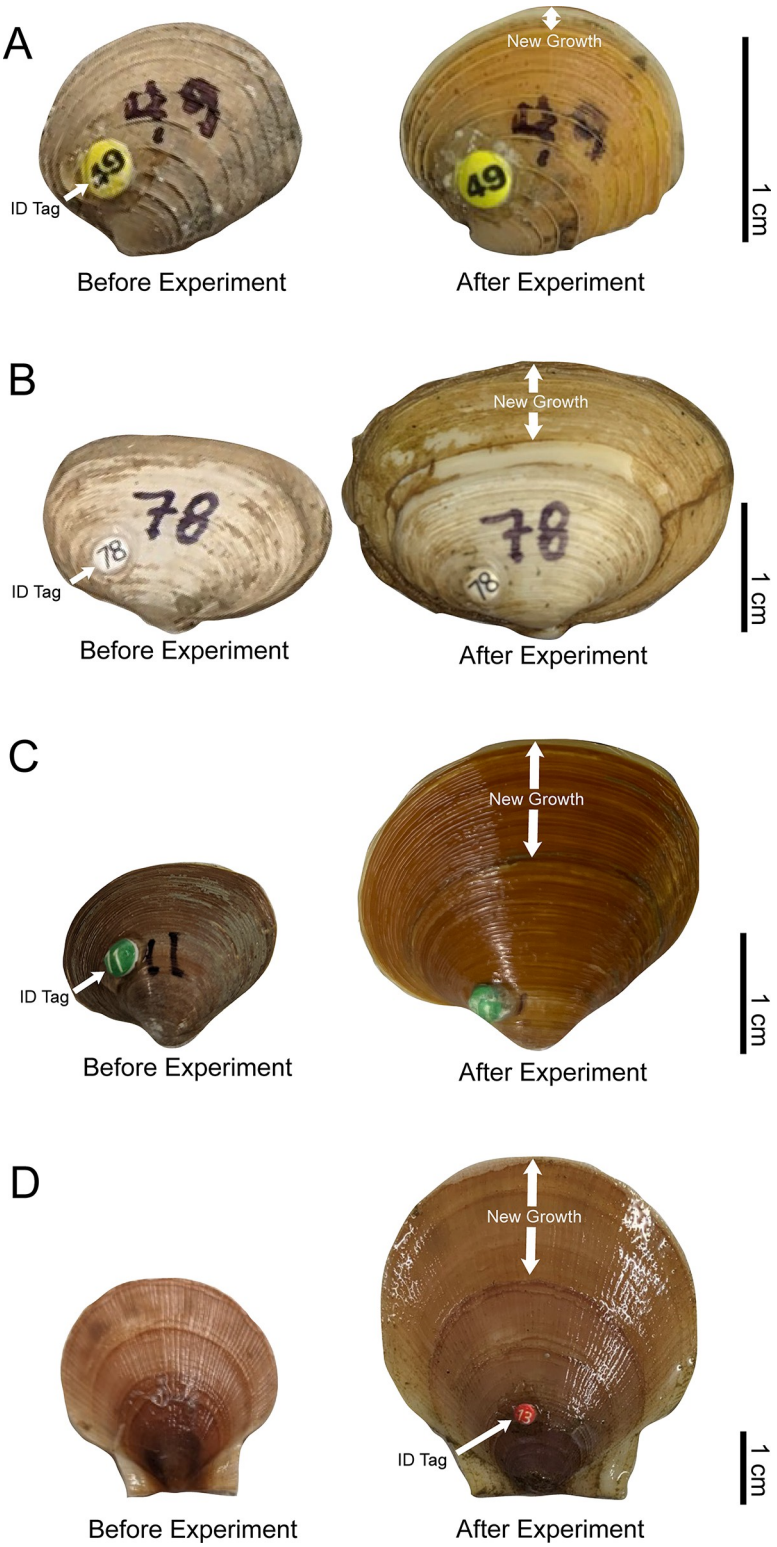


Fig 2. Visual examples of the change in maximum height measure. The left image for all specimens were taken before the start of the tank experiment; the right image is the same specimen after being in the tank experiment for 20.5 weeks. All pictured specimens were grown in 9˚C tanks. A. Juvenile *M. mercenaria*; B. Juvenile *M. arenaria*; C. Juvenile *A. islandica*; D. Juvenile *P. magellanicus*. The scallop image taken before the start of the experiment (in D) was taken after an alphanumeric ID was chosen but before the tag was attached. A. and B. were grown in pH 7.8 tanks and C. and D. were grown in pH 7.6 tanks.

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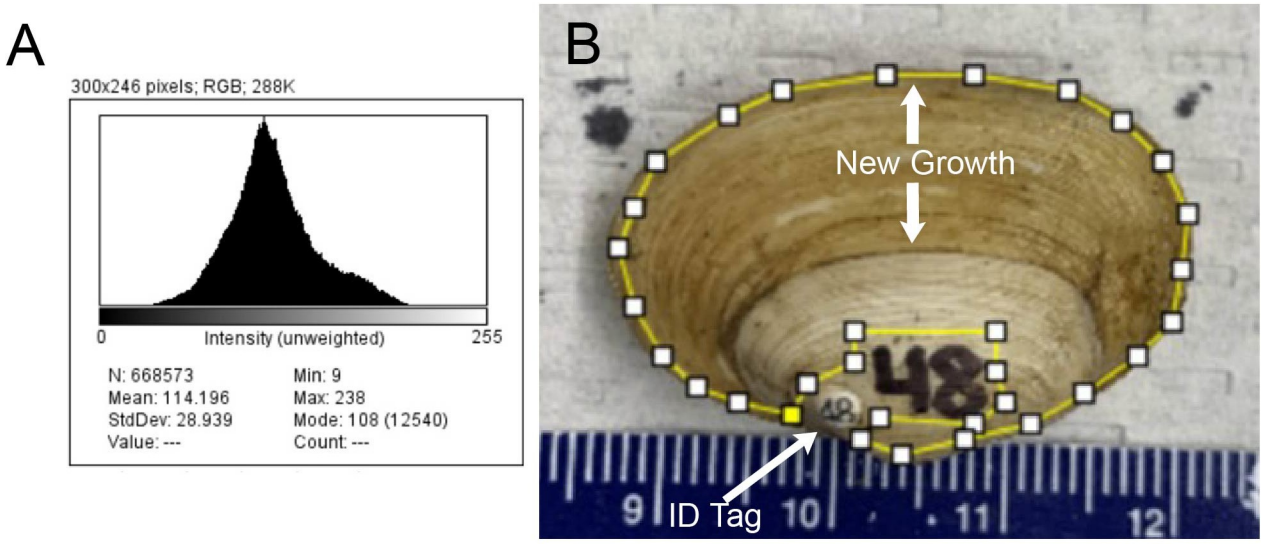


Fig 3. Example of coloration measurement taken in ImageJ for *M. mercenaria* specimen w48 at the conclusion of the 20.5-week experiment. A. ImageJ histogram of color intensity of the defined shell region. B. Polygon outline used for generating histogram in (A) avoiding any ID tags. Note that the two labels were excluded from the polygon.

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2.5 Statistical analysis

Sufficient juvenile *M. mercenaria*, juvenile *M. arenaria*, and juvenile *A. islandica* survived the experiment to explore the effects of the experimental conditions on mortality, growth and shell coloration. The adult *A. islandica* and juvenile *P. magellanicus* groups did not include enough individuals to be evaluated statistically, but data are included for general comparison.

The hypothesis that average tank pH and temperature impacted growth and shell coloration response variables was evaluated using linear mixed effects models for juvenile *M. mercenaria*, juvenile *M. arenaria* and juvenile *A. islandica*. The hypothesis that mortality was influenced by pH and temperature was assessed using a generalized linear mixed-effects model (binomial, logit link). This mortality analysis was only done for juvenile *M. mercenaria* and juvenile *M. arenaria* due to low deaths (juvenile *A. islandica)* and low specimen counts (juvenile *P. magellanicus* and adult *A. islandica*) in the other groups. Tank was included in all models as a random effect. All models were done separately per study species. Using full models, assumptions were verified using visual inspection of residuals versus fitted and Q-Q plots and scaled residuals using the DHARMa package [95] in RStudio.

For each test, a 'full' model was first created, which included average tank pH and temperature and their interaction. If the interaction term was not significant (assessed with a likelihood ratio test), this term was dropped, leaving a model that included only the main effects of the predictors (average tank pH and average tank temperature). Models were fit using the lme4 package [96] and all statistical analysis was done using RStudio (**2022.07.2** Build 576).

3. Results

3.1 Abiotic factors

The actual pH and temperature conditions within each tank were stable throughout the experiment and were close to target values (Table 1; S4 and S5 Figs). For temperature, all tanks were within 2σ of the target value except for one of the 9˚C and ambient pH tanks (8.6 ± 0.3˚C;

Table 2. Parameter estimates from the final models for *M. mercenaria* & *M. arenaria* mortality. All models include the random effect of the tank. The *P* value shown is from lmerTest::drop1 applied to the main effects model, which produces F-tests based on Satterthwaite's method which is equivalent to the summary t-tests using Satterthwaite's method. To make model intercepts interpretable, average pH values were normalized to the lowest measurement (e.g. the most acidic value was 0).

	Covariate	Estimate	SE	*z*	drop1 *P*
Juvenile *M. mercenaria*	(Intercept)	0.0620	0.587	0.105	
	Temperature	-0.0744	0.0595	-1.25	0.210
	pH	0.0668	0.514	0.130	0.896
Juvenile *M. arenaria*	(Intercept)	0.442	0.566	0.781	
	Temperature	-0.0616	0.0567	-1.09	0.276
	pH	-0.0657	0.494	-0.133	0.894

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Table 1). For pH, all tanks were within 2σ of the target value except for the 6˚C and 7.8 pH tank (7.89 ± 0.07; Table 1).

Throughout the experiment, the environmental conditions within the tanks were stable (Table A in S1 Text). Levene's test showed that the variance of tank conditions within treatment groups were equal for DIC (μmol/kg), TA (μmol/kg), temperature (˚C), salinity (psu), DO (%) and pH (S4 and S5 Figs). The variance of tank conditions for treatment groups were not equal for *in situ* pH, $\Omega_{Aragonite}$, $\Omega_{Calcite}$ and CO_3^{2-}. Average (± stdev) pCO_2 equivalents for the four tank pH treatments were 1891±20, 1080±106, 645±64, and 433±54 ppmv for 7.4, 7.6, 7.8, and ambient/8.0 tanks, respectively.

3.2 Mortality

Mortality in tanks was low except for juvenile *M. mercenaria* and adult *A. islandica* which had overall mortality rates of 29% and 33%, respectively. Mortality rates for the other taxa were 1% for juvenile *A. islandica*, 7% for juvenile *M. arenaria*, and 8% for juvenile *P. magellanicus*. We determined that juvenile *M. mercenaria* and juvenile *M. arenaria* mortality were not influenced by pH or temperature (Table 2, S6 and S7 Figs).

3.3 Growth/height

For all species groups that could be analyzed, pH did not influence the proportional change in maximum shell height. Average tank temperature significantly impacted all species groups' proportional change in height. Under all pH conditions, proportional change in maximum shell height increased with increasing temperature, but the slope differed between species (Table 3, Table B in S1 Text, Fig 4).

3.4 Shell coloration

The average shell coloration for juvenile *M. mercenaria* and juvenile *M. arenaria* at the end of the experiment was influenced by pH. *M. mercenaria* shell coloration became lighter, with both increasing temperature and decreasing pH. *M. arenaria* shell color became lighter only with decreasing pH. Juvenile *A. islandica* shell coloration was neither influenced by temperature or pH (Table 4, Table C in S1 Text, Figs 5 and 6, S8 and S9 Figs).

4. Discussion

In this short-term, controlled tank experiment, the growth of juvenile *M. mercenaria*, juvenile *M. arenaria* and juvenile *A. islandica* proved resilient to reduced pH levels (Table 3 and Fig 4).

Table 3. Parameter estimates of linear mixed effects models for juvenile *M. mercenaria*, juvenile *M. arenaria* and juvenile *A. islandica* proportional change in maximum height. All models include the random effect of tank. The *P* value shown is from lmerTest::drop1 applied to the main effects model, which produces F-tests based on Satterthwaite's method which is equivalent to the summary t-tests using Satterthwaite's method. To make model intercepts interpretable, average pH values were normalized to the lowest measurement (ex. the most acidic value was 0).

	Covariate	Estimate	SE	*t*	drop1 *P*
Juvenile *M. mercenaria*	(Intercept)	-0.604	0.106	-5.73	
	Temperature	0.0865	0.0106	8.15	<0.001
	pH	0.0437	0.0931	0.470	0.647
Juvenile *M. arenaria*	(Intercept)	0.0720	0.0628	1.15	
	Temperature	0.0214	0.00635	3.37	0.00476
	pH	-0.0266	0.0549	-0.485	0.636
Juvenile *A. islandica*	(Intercept)	0.117	0.110	1.06	
	Temperature	0.0631	0.0110	5.71	<0.001
	pH	-0.00873	0.0972	-0.090	0.930

https://doi.org/10.1371/journal.pclm.0000509.t003

However, shells of both *M. mercenaria* and *M. arenaria* were lighter in color as the pH of tank conditions decreased, suggesting a loss or bleaching of periostracum in all temperatures (Table 4, Figs 5, 6 and S8). Growth of these three species groups increased with rising temperature (Table 3 and Fig 4) and the shells of *M. mercenaria* exhibited lighter color with increasing temperatures (Table 4, Figs 6 and S8).

Throughout the experiment, mortality rates remained low for most groups, and survival was not significantly related to temperature or pH (for the groups that could be analyzed;

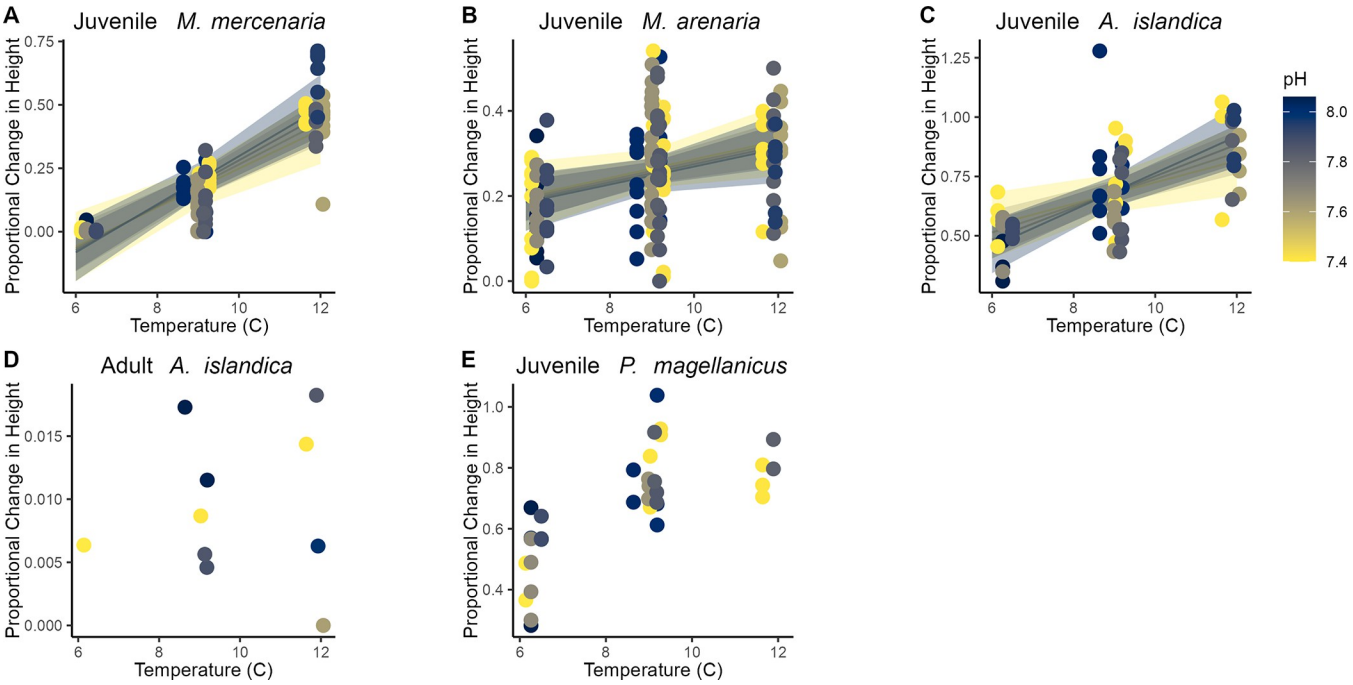


Fig 4. The proportional change in maximum height for the five study groups. For all groups proportional change in maximum height is plotted against average tank temperature on the x-axis and colored by average tank pH, with each point representing an individual study specimen. For the groups with sufficient sample numbers for modeling (juvenile *M. mercenaria* (A), juvenile *M. arenaria* (B) and juvenile *A. islandica* (C)) lines represent linear mixed effects model predictions and shaded regions represent 95% confidence intervals; both are colored by average tank pH. Groups without sufficient sample numbers (adult *A. islandica* (D), juvenile *P. magellanicus* (E)) are included without model predictions or confidence intervals.

https://doi.org/10.1371/journal.pclm.0000509.g004

Table 4. Parameter estimates of linear mixed effects models for juvenile *M. mercenaria*, juvenile *M. arenaria* and juvenile *A. islandica* mean shell coloration. All models include the random effect of tank. The *P* value shown is from lmerTest::drop1 applied to the main effects model, which produces F-tests based on Satterthwaite's method which is equivalent to the summary t-tests using Satterthwaite's method. To make model intercepts interpretable, average pH values were normalized to the lowest measurement (ex. the most acidic value was 0).

	Covariate	Estimate	SE	*t*	drop1 *P*
Juvenile *M. mercenaria*	(Intercept)	103	6.39	16.1	
	Temperature	2.79	0.644	4.33	<0.001
	pH	-19.6	5.66	-3.46	0.00357
Juvenile *M. arenaria*	(Intercept)	162	7.47	21.7	
	Temperature	-1.47	0.752	-1.96	0.0682
	pH	-49.9	6.54	-7.63	<0.001
Juvenile *A. islandica*	(Intercept)	68.3	3.76	18.1	
	Temperature	-0.726	0.381	-1.91	0.0611
	pH	-5.21	3.23	-1.62	0.111

https://doi.org/10.1371/journal.pclm.0000509.t004

Table 2, S6 and S7 Figs). These findings suggest that while study specimens could survive and grow over the 20.5-week experimental duration, disparities in health, indicated by lighting in shell color under more acidifying conditions, are likely present. Despite most bivalves surviving and growing in the experimental conditions, coloration changes suggest that they may not

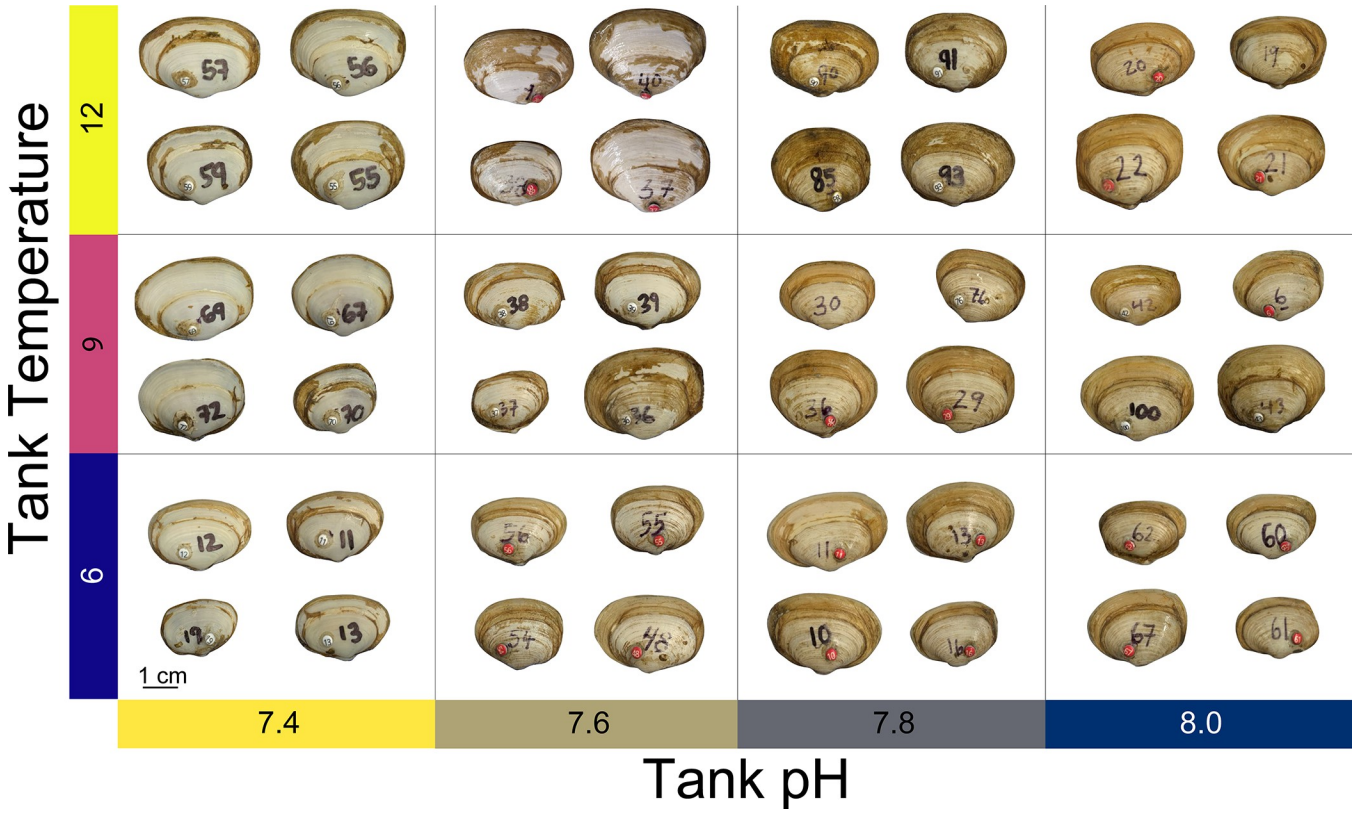


Fig 5. Subset of juvenile *M. arenaria* specimens at the end of the experiment. Specimens are grouped by their controlled temperature and pH conditions. pH increases from left to right across the figure and temperature increases from bottom to top, colors indicate these treatments and are consistent with prior and subsequent figures. Note that shells become lighter to the left of the figure as pH decreases (becoming more acidic). This difference occurs regardless of temperature treatment for *M. arenaria*.

https://doi.org/10.1371/journal.pclm.0000509.g005

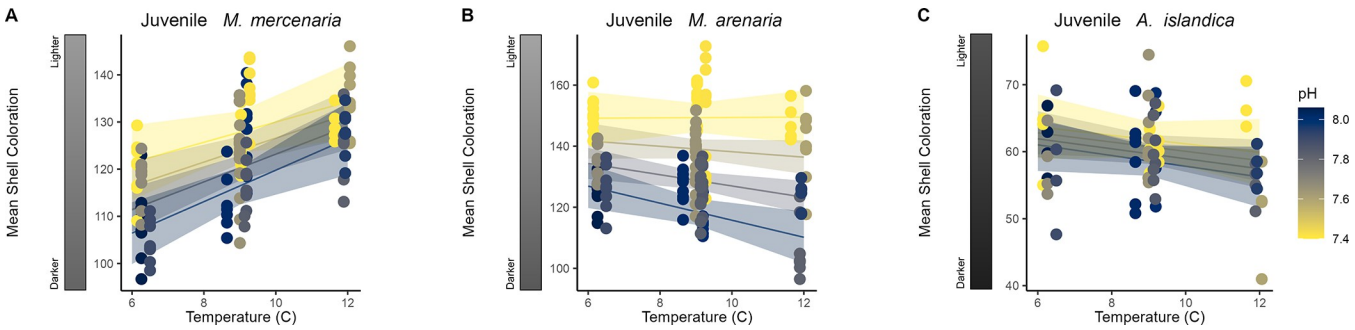


Fig 6. Shell coloration for the three study groups analyzed (*M. mercenaria* (A), *M. arenaria* (B) and juvenile *A. islandica* (C)). Mean shell coloration is plotted against average tank temperature on the x-axis and colored by average tank pH with each point representing an individual study specimen. Lines represent linear mixed effects model predictions and shaded regions represent 95% confidence intervals; both are colored by average tank pH. Larger shell coloration values correspond to lighter or whiter shells, lower values correspond to darker shells. Representative color scales included on the y-axis for each group.

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have all been thriving. Our results underscore the variable response of bivalve species to pH stress. This experiment provides a unique opportunity to directly compare organism responses to identical stressor conditions across species. Despite belonging to the same phylogenic class and geographic region, the species in our study exhibited diverse responses to identical experimental conditions.

4.1 Growth

Optimal growth ranges vary for the studied specimens and range from 6–16˚C for adult *A. islandica* [64, 65], 18–25˚C for adult *M. mercenaria* [97], and ~20˚C for adult *M. arenaria* with a high degree of uncertainty depending on life stage and location [75, 98, 99]. For example, the optimal temperatures are 10–15˚C for adult *P. magellanicus* [81] with larvae viable at 12–18˚C and juveniles able to survive across a larger temperature range (1.5–15˚C; [100]). Given these temperature ranges, the differing growth of species in this experiment is perhaps expected, as the tank temperatures fall below or are consistent with the species' optimal growth temperatures. Increased growth with increased temperature was a striking similarity between juvenile *M. mercenaria*, juvenile *M. arenaria* and juvenile *A. islandica*.

In this experiment, juvenile *M. mercenaria* growth increased with increasing temperature but was not influenced by pH (Table 3, Fig 4). Ansell et al. [68] demonstrated that *M. mercenaria* increase growth with increased temperature, and suggested that growth stops at water temperatures below 9˚C. Jones et al. [101] also established a standardized growth index, which is strongly positively correlated with mean annual water temperature. Our results corroborate this relationship with temperature. The lack of pH influence on *M. mercenaria* growth we find contrasts with previous studies, which found that elevated pCO_2 is associated with decreased growth and changes in other shell properties in *M. mercenaria* [102–105]. This difference might be at least partially related to life stage. Our specimens were exclusively juveniles while Talmage and Gobler [103, 104] included larval clams and Talmage and Gobler [105] included both juveniles and larval clams.

M. arenaria growth in this experiment also increased with increasing temperature but was not influenced by pH (Table 3, Fig 4). This finding supports previous research that shows the growth of *M. arenaria* is impacted by water temperature until a thermal maximum is reached around 20˚C [106]. Although temperature-performance curves for *M. arenaria* are well established, it is likely that adaptation to local temperature may shift these curves throughout the species range. Several studies on *M. arenaria* have shown that lowered pH/elevated pCO_2 can lead to shell dissolution and decrease shell growth, particularly in experiments with lower pH

than used here (7.2) or in the wild in naturally variable environments [48, 107, 108], which contradicts the findings of this study. This contradictory behavior could be the result of life stage ([48] studied adults) and/or the specimen's natural habitat as Zhao et al. [108] studied specimens from a naturally pCO_2-enriched habitat.

A. islandica growth was moderately elevated under warmer temperature treatments but was not influenced by pH (Table 3, Fig 4). The sample size for adult *A. islandica* was too small to evaluate statistically, but the surviving adult specimens showed increased growth in warmer temperatures, a finding similar to that of the juveniles (Figs 4 and S10). Our findings are comparable with previous studies, particularly because our study temperature range was within the optimal temperature range of the species [64, 65]. Others have shown in lab conditions that juvenile *A. islandica* have increased growth with increased temperatures [109] and that rapid growth can occur under conditions similar to those used here [110, 111]. The resilience of growth to acidifying pH here is supported by previous studies of *A. islandica*. For instance, Liu et al. [56] found no statistical differences in growth between pH treatments ranging from 7.5 to 8.1. Other work showed no effect on shell growth at elevated pCO_2 conditions or decreasing aragonite saturation [55, 112]. Although not specifically considering growth, a five-day study by Bamber et al. [113] demonstrated that valve movement did not change significantly until pH conditions decreased to 6.2, a finding that the authors used to conclude that *A. islandica* are a species somewhat tolerant of ocean acidification conditions. Taken together in the context of past work, our results suggest that warming could have a greater influence on survivability, growth, and geographic distribution than OA for *A. islandica* under future climate change scenarios in this region.

The number of scallops in the tanks at the end of the experiment limited our ability to statistically investigate this group. However, results generally indicated that growth increased with increasing temperatures (Figs 4 and S11) and growth was variable between all pH conditions. *P. magellanicus* become thermally stressed at temperatures above 13˚C [78] and are limited geographically to regions below the 20˚C isotherm [114, 115]. Modeling of *P. magellanicus* growth predicts that growth will increase with warmer temperatures and decrease under OA conditions [116]. Utilizing similar experimental conditions to our study, Cameron et al. [117] found growth to be reduced at high pCO_2 conditions for adult specimens of *P. magellanicus*.

The general results of this study indicate that more growth occurs at warmer temperatures across these four species, and, for some species, a warmer future could lead to more growth in certain life stages. However, food availability may impact organisms living in the wild in a warmer ocean. Some studies have shown that as ocean waters warm, the availability of phytoplankton decreases [e.g. 118, 119], which may result in warmer ocean temperatures leading to decreased growth. In the present experiment, the incoming water was identical before being warmed in each tank and thus had consistent concentrations of phytoplankton. Additionally, there is isotopic evidence that the organisms selected to eat phytoplankton in the incoming seawater as opposed to the Shellfish Diet that was supplemented. The stable carbon isotopes of the material found in their stomachs were much closer to that of phytoplankton than the Shellfish Diet (see S12 Fig). Although future warming and associated decreases in phytoplankton might lead to mixed growth results for organisms in the wild, the growth seen in the present experiment is primarily the result of temperature variability.

Although growth in these experimental conditions can be largely attributed to temperature variability and not food availability, the presence of food for these organisms could have buffered stress in the lower pH tanks. Studies involving other bivalves have shown that it is energetically costly to survive in low pH conditions [120] and that adequate food availability could allow organisms to survive. For example, for larval and adult *Crassostrea virginica* (eastern

oysters), with greater food availability, there was less mortality and greater growth [121]. Similarly, juvenile *Pecten maximus* (king scallops) indicated a greater tolerance for OA with sufficient food available [122]. The availability of food in the present experiment (both natural and the Shellfish Diet 1800 supplemental food) may have mitigated some detrimental effects of low pH conditions.

For all species studied, growth was seemingly unaffected by decreased pH and was instead influenced by temperature, with growth enhanced as temperatures increased up to 12˚C. This growth trend with temperature is expected as the tank temperatures generally remained within previously determined optimal growing conditions for the organisms. However, with the exception of juvenile *A*. *islandica*, the resilience of growth to acidifying pH was not expected. Our results suggest that the relationship between shell growth and pH for these species could be more complicated than previously thought. Specifically, differences in regional stocks, life stage and/or food availability could be important.

Prior studies indicating decreased growth in these species at lower pH/higher CO_2 did not evaluate differences in regional stocks and none have been done using populations from the GoM. As all our study specimens originated from the GoM this could, to some degree, explain why we saw the resilience of growth in acidifying pH while other experiments did not. Additionally, juvenile bivalves often prioritize linear extension over increasing shell density until they reach the size of sexual maturity [123]. This could be the case in our study, with these individuals prioritizing increasing shell maximum height over other crucial biological processes. Further study is needed to evaluate the differential growth responses to acidifying pH in species stocks from different regions and life stages.

4.2 Coloration

Variation in specimen coloration resulting from exposure to experimental pH and temperature treatments was an unexpected finding. Notably, the coloration of juvenile *M*. *mercenaria* and juvenile *M*. *arenaria* was strongly influenced by pH, and *M*. *mercenaria* coloration was additionally influenced by temperature (Table 4, Figs 5, 6 and S8). Conversely, the same environmental conditions had almost no influence on juvenile *A*. *islandica* coloration (Table 4, Figs 6 and S9).

Coloration in bivalves often displays considerable variability. In certain species (i.e. *P*. *magellanicus*) a dominant color prevails, while a minority may exhibit minimal pigmentation (i.e. albino specimens; S11 Fig). In others, color serves as a defining trait, utilized for camouflage or communication, and it is believed to bolster shell integrity [124, 125]. For *M*. *arenaria*, shells have been reported to become more translucent with increased metabolic activity [106]. However, for most bivalves, individual coloration is largely influenced by the periostracum, the outer organic layer comprising mucopolysaccharides and lipids, which serves as a matrix for calcium carbonate deposition, a protective barrier against dissolution and to deter predation [48, 87–93]. The loss of periostracum compromises these vital functions.

Our findings reveal that juvenile *M*. *mercenaria* and juvenile *M*. *arenaria* shells were lighter under lower pH conditions, indicating changes or loss of the periostracum and thus possible susceptibility to shell dissolution. For *M*. *mercenaria*, increasing temperature also increased the lightness of the shell. Similar visual changes in shell condition due to stressors have been observed previously; for instance, *M*. *arenaria* shells become more transparent with increased metabolic rates [106], and in the bivalve species *Corbicula fluminea* (Asian freshwater clam), internal shell color differences with stress suggest a redirection of energy from shell building to vital processes [126]. Alternatively, a lighter shell color could indicate increased shell dissolution. In *M*. *edulis*, exposure to unsaturated water ($\Omega_{Ar}<1$) led to visible periostracum loss and

subsequent shell dissolution, whereas areas with intact periostracum remained unaffected [51].

Published data on expected coloration differences in response to changing temperature and pH in *M. mercenaria* are scarce. Our study fills this gap by providing the first evidence that *M. mercenaria* shell color responds to environmental changes. Our results indicate that shell color becomes lighter as pH decreases and as temperatures rise (Table 4, Figs 6 and S8), suggesting a similar stress-induced impact on shell coloration as hypothesized for *M. arenaria*.

In our study, the most robust and significant differences in color were observed in *M. arenaria* specimens across pH treatments (Table 4, Figs 5 and 6). Our results suggest that loss of coloration in *M. arenaria* clams under low pH conditions may be attributed to a loss of periostracum (Fig 5). Anecdotal observations of wild *M. arenaria* also support that shell color may become lighter when clams are exposed to lower pH conditions (A. Strong, personal communication and [107]). While Lewis & Cerrato [106] did not directly investigate the relationship between pH and shell color during their study on metabolic stressors, they did report differences in the translucency of *M. arenaria* shells suggesting that variations in shell coloration may indeed reflect fluctuations in metabolic rates.

By contrast, juvenile *A. islandica* exhibited consistent coloration irrespective of temperature or pH (Table 4, Figs 6 and S9). While previous laboratory-grown shell material has displayed distinct growth checks and differences in periostracum color [57], our study did not yield any notable impacts of pH or temperature on shell color or periostracum. However, a distinct growth check and a slight change in periostracum color were noticeable at the beginning of the experiment (Fig 2).

While differences in bivalve shell condition and coloration have been seen before under environmental stress, we have now established a quantitative relationship between pH and shell coloration for juvenile *M. mercenaria* and juvenile *M. arenaria*. The mechanism for these color changes is poorly understood, but we hypothesize that lighter shells could indicate loss of periostracum, shell dissolution and/or metabolic stress. In the case of *M. mercenaria*, where shells also became lighter at higher temperatures, the pattern cannot be fully explained by shell dissolution alone.

Regarding the differential shell coloration response of these species to identical temperature and pH conditions, it is plausible that this disparity stems from inherent distinctions in shell biology or differences in the species' tolerance to environmental stress. For example, the resilience of juvenile *A. islandica* shell coloration compared to juvenile *M. arenaria* might be attributed to differences in periostracum characteristics or shell composition. Alternatively, the lack of fluctuation in juvenile *A. islandica* shell coloration under varying environmental conditions could suggest that physiological processes in this species are less susceptible under the range of temperatures and pH studied here.

4.3 Mortality

In this experiment, the observed mortality rate did not show a clear relationship with fluctuations in pH or temperature. Mortality rates were notably higher for *M. mercenaria* and adult *A. islandica* across all tank conditions. However, factors beyond the scope of this experiment, including the initial stock quality and the health status of the organisms, may have contributed to these deaths. Mortality can vary significantly depending on species, specific environmental conditions, and even geographic location [127], with bivalves generally proving highly sensitive to temperature variability outside of their optimal range [128, 129]. While some bivalves can endure short-term exposure to extreme temperatures, prolonged exposure often results in death [130]. Throughout our 20.5-week experiment overall mortality for all specimens combined was below 15% but varied between the study groups.

Notably, despite mortality rates exceeding 29% for juvenile *M*. *mercenaria*, we did not see changes in the probability of survival with changing pCO_2/pH or temperature (Table 2, S6 and S7 Figs). This outcome was in line with expectations, as *M*. *mercenaria* is known to be sensitive to fluctuating temperature and pH conditions, especially juveniles in undersaturated waters ($\Omega<1$; [131]). Additionally, lower temperatures, such as those employed in our study, increase the risk of mortality due to the presence of Quahog Parasite Unknown which is more prevalent at temperatures below 13˚C [132]. Since all temperature treatments in the present experiment fell below this critical threshold, we cannot rule out that Quahog Parasite Unknown played a role in the mortality of juvenile *M*. *mercenaria* across treatments.

The low mortality rates in juvenile *M*. *arenaria* and their independence from temperature/pH are perhaps unsurprising given the tested ranges. *M*. *arenaria* mortality from environmental variability is not well established, but temperatures in the range of the present experiment (6 to 12˚C) appear conducive for survival. However, *M*. *arenaria* are more susceptible in warmer temperatures, with waters exceeding 28˚C proving historically lethal for specimens from Chesapeake Bay, USA [75].

Similar to previous findings on juvenile *A*. *islandica* [55], our study showed low mortality rates independent of varying pH and temperature conditions. The ranges of temperatures and pH conditions in our study were similar to those in that of Hiebenthal et al. [55], yet we observed mortality rates around three times lower in juveniles and ten times higher in adults across all treatments.

Although the total number of juvenile *P*. *magellanicus* specimens in our study was limited, a noteworthy trend emerged, with higher mortality rates observed in 12˚C tanks (four individuals) compared to 6˚C and 9˚C treatments (one in each) (S6 Fig). Although it is known that *P*. *magellanicus* are sensitive to handling [133], this trend suggests a potential influence of temperature on *P*. *magellanicus* mortality. Other studies involving *P*. *magellanicus* indicate that this species is perhaps less tolerant to varying temperature and pH conditions compared to other bivalve taxa, with particularly elevated mortality rates observed under combined high pCO_2 (up to 2199 ppm) and temperature (up to 12˚C) conditions [117].

Overall, while the study specimens generally survived (overall mortality <15%) and grew throughout our 20.5-week experiment, differences in shell coloration may hint at underlying differences in organism health. Our results highlight the variable response of bivalve species to pH and thermal stress, indicating that despite coming from the same class of organisms and locality the response of each species to identical experimental conditions was non-uniform.

4.4 Conclusions and implications

Our controlled tank experiment reveals that the growth of juvenile *M*. *mercenaria*, juvenile *M*. *arenaria*, and juvenile *A*. *islandica* was resilient to the low pH levels predicted for surface waters in the GoM by the end of the century. Although shell growth for juvenile *M*. *mercenaria* and juvenile *M*. *arenaria* remained unaffected by changing pH, noticeable differences in shell coloration were observed, with shells appearing lighter in color as pH decreased. Mortality rates remained low for most groups throughout the experiment and survival appeared unrelated to temperature or pH. These findings suggest that while study specimens were able to survive and grow during the experiment, disparities in health, indicated by variations in shell coloration under more acidifying conditions, may exist.

The results presented in this study could be a starting point for assessing the risk of these four bivalve species to OA in the Gulf of Maine. Our results highlight the fact that individual species likely harbor differential vulnerability to OA and that the juvenile stage for these four species is likely not at the greatest risk, at least to exposures like those described here. More

study is needed to disentangle the effects of multiple stressors on differing life stages and stocks of these species. Additionally, shellfish growers may see differential impacts from OA depending on the bivalve species they grow and their site. Considering OA in site leasing and diversifying stocks could be beneficial for bivalve aquaculture.

Our results underscore the variable response of bivalve species to pH stress and provide a unique opportunity to compare organism responses to identical stressor conditions across species. Despite belonging to the same phylogenetic class and geographic region, the species in our study exhibited variable responses to identical experimental conditions. Considering the trajectory of predicted seawater temperatures and pH in the GoM, further research is warranted to explore the mechanisms underlying these differential responses and their implications for bivalve health, resilience, and management in the face of changing environmental conditions.

Supporting information

S1 Fig. Map of collection areas within the Gulf of Maine.
(PDF)

S2 Fig. Correlations of various growth metrics from juvenile *A. islandica*, *M. mercenaria*, *M. arenaria* & *P. magellanicus*.
(PDF)

S3 Fig. Calcein stain lines under fluorescent light from a juvenile *Arctica islandic*a shell.
(PDF)

S4 Fig. Abiotic tank treatment measures throughout the experiment.
(PDF)

S5 Fig. Abiotic tank treatment measures throughout the experiment by date.
(PDF)

S6 Fig. Deaths by species throughout the controlled tank experiment.
(PDF)

S7 Fig. Predicted probabilities of survival for juvenile *M. mercenaria* and juvenile *M. arenaria*.
(PDF)

S8 Fig. Subset of juvenile *M. mercenaria* at the at the end of the experiment.
(PDF)

S9 Fig. Subset of juvenile *A. islandica* at the end of the experiment.
(PDF)

S10 Fig. Subset of adult *A. islandica* at the end of the experiment.
(PDF)

S11 Fig. Subset of juvenile *P. magellanicu*s at the end of the experiment.
(PDF)

S12 Fig. Box plot of carbon isotopes from food, carbon isotopes of stomach/intestine contents, and carbon isotopes of the filters which tank water was filtered each week, representing the phytoplankton naturally available to the organisms.
(PDF)

S1 Data. Growth and color data for all specimens and tank temperature and pH conditions.
(XLSX)

S1 Text. Tables of Levene's test for tank conditions and summary statistics of growth and coloration.
(DOCX)

S2 Text. Links to R code used for statistical analyses.
(DOCX)

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