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Resistant calcification responses of *Arctica islandica* clams under ocean acidification conditions

Yi-Wei Liu [a,b,*], Alan D. Wanamaker Jr. [c], Sarah M. Aciego [b,d], Ian Searles [c], Thor Arne Hangstad [e], Melissa Chierici [f], Michael L. Carroll [e]

[a] Institute of Earth Sciences, Academia Sinica, 128, Sec. 2, Academia Road, Nangang, Taipei 11529, Taiwan
[b] Department of Earth and Environmental Sciences, University of Michigan, Room 2534, 1100 North University Avenue, Ann Arbor, MI 48109, USA
[c] Department of Geological and Atmospheric Sciences, Iowa State University, Ames, IA 50011, USA
[d] Department of Geology and Geophysics, University of Wyoming, 1000 E. University Ave, Laramie, WY 82071-2000, USA
[e] Akvaplan-Niva, FRAM – High North Research Centre for Climate and the Environment, Tromsø 9296, Norway
[f] Institute of Marine Research, Fram Centre, Tromsø 9007, Norway

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ABSTRACT

Ocean acidification (OA) directly impacts marine calcifying organisms including ecologically and commercially important shellfish species such as *Arctica islandica* (*A. islandica*). To test whether documented growth resilience of *A. islandica* to OA is a general response across ages and populations or a function of adaptation to local habitat, we cultured juvenile and adult clams collected from an environment with little pH variation under four pH levels (7.5, 7.7, 7.9, and 8.1) for three months and integrated our understanding with relevant literature. The average shell growth over the experiment among all (69) individuals was 57 ± 55 μm, and there were no statistically significant differences in growth among pH treatments, including the control treatment, despite the general growth rate differences between juveniles and adults. Our results show that *A. islandica* can maintain its shell growth even in aragonite undersaturated ($\Omega < 1$) conditions (0.65 and 0.83 for pH 7.5 and 7.7 treatments, respectively), supporting the hypothesis that resistance to OA conditions is likely a generalized response across populations. Although the present results show *A. islandica* can maintain their shell growth under short-term OA, long-term impacts of OA on *A. islandica* shell growth and other physical parameters including shell density and microstructure are still needed to better assess the sustainability of *A. islandica* in a more acidified future and to provide guidance on managing this important shellfish stock.

1. Introduction

Atmospheric CO_2 concentrations have increased dramatically since the 20th century and surpassed 415 ppm in May 2019 (Keeling et al., 2001). The ocean has absorbed about 1/3 of the anthropogenic CO_2 from the atmosphere and has resulted in shifting the ocean's chemistry to increase ocean CO_2, reduced calcium carbonate saturation (Ω) and reductions in oceanic pH, so-called ocean acidification (OA) (Sabine et al., 2004). Although seawater pH can vary over a short time period (e.g., daily to monthly), this persistent reduction of seawater pH, known as the OA effect has already been observed in many ocean basins (García-Ibáñez et al., 2016; Ríos et al., 2015). The OA can have multiple impacts on ecosystems including the degradation of habitats and the health of certain marine species, especially calcifying organisms (Anthony et al., 2008; Bednarsek et al., 2012; Chan et al., 2012; Cummings et al., 2019; Mason, 2018; Stumpp et al., 2012; Talmage and Gobler, 2010; Zhan et al., 2016). Recent observations suggest that OA has already impacted ecologically important species and different ocean regions over the past 20 years (Byrne et al., 2010; Feely et al., 2008; Findlay et al., 2010; Hönisch et al., 2012; Krief et al., 2010; Vázquez-Rodrıguez et al., 2012).

Experimental studies have demonstrated varying impacts of OA on calcifying organisms including condition and reproduction success (Kroeker et al., 2010; Langer et al., 2006; Langer et al., 2009; Ries et al., 2009), growth and in survival rates, of which are most prominent in the early stages of development (Dupont et al., 2010; Griffith and Gobler, 2017; Talmage and Gobler, 2010). While some marine bivalve mollusks have certain levels of resilience in their calcification to OA as adults (Ries et al., 2009; Thomsen et al., 2010; Waldbusser et al., 2010), the

* Corresponding author at: Institute of Earth Sciences, Academia Sinica, 128, Sec. 2, Academia Road, Nangang, Taipei 11529, Taiwan.
E-mail address: liuyiwei@earth.sinica.edu.tw (Y.-W. Liu).

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mechanisms by which they are able to tolerate OA are less well known. For example, it has been suggested that bivalve mollusks may have limited capacity to regulate ion and acid-base status (Parker et al., 2013). However, there is consistent evidence supporting relatively high survival of adult bivalve mollusks exposed to low pH/low Ω environments (Kroeker et al., 2010; Kurihara, 2008; Thomsen et al., 2010). The implications of this issue go beyond basic scientific questions because of the economic importance of many commercially harvested shellfish species. In fact, many shellfish farms have been interested in OA impacts and have participated in monitoring programs in concert with government agencies (Capson and Guinotte, 2014; Clements and Chopin, 2017) to better document and understand the risks of OA to shellfish stocks.

Arctica islandica (A. islandica) is a slow growing (Kennish et al., 1994), late maturing (Thompson et al., 1980; Thorarinsdóttir and Steingrímsson, 2000), and long-lived (>500 years (Butler et al., 2013)) bivalve species. It is widely distributed in the temperate-boreal North Atlantic, extending from as far south as Cape Hatteras through the Gulf of Maine to Newfoundland, Iceland, Shetland and the North and western Baltic Seas, and along the Norwegian coast to Porsanger fjord and the White Sea in Russia (Dahlgren et al., 2000; Merrill and Ropes, 1969; Mette et al., 2016). Although this species thrives in relatively cool seawater temperatures (below 16 °C) and prefers near full marine conditions (salinities above 32), their overall tolerances are wider (range between 1 and 20 °C and between 22 and 35 PSU, respectively (Schöne (2013) and references there in). Because of its great longevity and its annual shell increments, it has been used extensively as an environmental archive (Beirne et al., 2012; Butler et al., 2009; Mette et al., 2016; Reynolds et al., 2016; Schöne, 2013; Wanamaker Jr. et al., 2008; Weidman et al., 1994; Witbaard et al., 1994). The ocean quahog can be found just below the surface of mid- to fine-grain size sandy substrates. It is commonly categorized as a suspension feeder on phytoplankton, but it was also suggested to be an infaunal deposit-feeder in shallow water (Cargnelli, 1999; Josefson et al., 1995; Morton, 2011). Ecologically, *A. islandica* is a key species in soft-bottom coastal and shelf habitats, with locally high densities and biomass, thereby channeling much of the system energy flow where it occurs (Brey et al., 1990; Witbaard, 1996; Witbaard et al., 2003). It is food for higher trophic level predators, including cod and other demersal fish, large crustaceans, and diving ducks (Kennish et al., 1994; Kraus et al., 1992; Langton and Bowman, 1980; Stehlik, 1993), and has been historically harvested in Norway by fishermen for bait and remains a widespread commercially harvested species (Fisheries Science Center, 2017; Kennish and Lutz, 1995; Thorarinsdóttir and Jacobson, 2005). Because of its great longevity and late maturation plus evidence of irregular recruitment and low juvenile survival (Lewis et al., 2001), it is listed as a threatened species in European waters (OSPAR Commission, 2008), vulnerable to commercial exploitation and changes in its environment. The main threat to *A. islandica* considered by the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR Convention) stems from disturbances to the seabed, particularly linked to beam trawling, which is known to cause shell damage and direct mortality (Witbaard and Klein, 1994). Sensitivity to OA would be an additional vulnerability for this species.

Toward this end, the impacts of OA on juvenile *A. islandica* shells collected from Kiel Bight, Germany were reported by Stemmer et al. (2013). Notably, there were no detectable changes to the aragonite shell microstructure or shell growth during their experiment. However, it is not fully resolved whether or not the reported resilience to OA on calcification of juvenile *A. islandica* also occurs in adult *A. islandica,* and/or whether this is a result of pre-adaptation to local habitats. To further assess the potential impacts of OA on *A. islandica* shells, we examined shell growth rates in both juvenile (< ~13 years of age) and adult clams collected from northern Norway and cultured in a flowing seawater laboratory under ambient and acidified (low pH and low Ω) conditions.

2. Materials and methods

2.1. Field collection

Living adult and juvenile *A. islandica* were collected from 31 January to 6 February 2014 from Ingøy, northern Norway (see Mette et al. (2016) for a site description). Live samples were dredged nearshore from the bottom in 6 m water depth, temporarily stored in tanks filled with unfiltered seawater from the sampling sites, and then transported to the Research and Innovation Station Kraknes (Forskning -og Innovasjonsstasjonen Kraknes (FISK)), an aquaculture and marine environmental research facility owned by Akvaplan-niva, Tromsø, Norway. During transport, all specimens were housed in coolers with seaweed to maintain temperature and moisture levels. The animals were out of seawater for no more than 4 h during transport. Further, all experimental animals were acclimated in the laboratory (flow-through natural seawater from an intake at 60 m and filtered at 60 μm) for 2 weeks before the start of the growth experiment without extra feeding. Approximately 130 clams were numbered with shellfish tags and pre-acclimated in flow-through tanks with natural seawater and continuously bubbled with aerators. Shell dimensions (height, length, and width) and weight were recorded immediately before and after the pH-controlled experiments.

2.2. Experimental setup

We conducted a three-month of OA experiment at FISK, from February to May 2014. The experiment consisted of clams being cultured at four different targeted pH treatments: 7.5, 7.7, 7.9 and 8.1 (the latter being a control treatment with normal seawater pH) at near constant seawater temperature (7.2 °C ± 0.7 °C), salinity (34.6 ± 0.3 PSU), as well as pH (±0.02 pH unit based on daily pH check on the buffer cooler; 0.04 in EXO2 Sonde) during the experimental period. We used a flow-through system using natural seawater from an intake at 60 m and filtered at 60 μm. To maintain the pH levels in the various treatments, we first injected high-pressure CO_2 into seawater in a 1000 L master tank to achieve a pH level about 7.5. The CO_2-enriched seawater was heated to 7 °C and mixed with different amounts of ambient seawater to achieve different pH levels. The seawater pH of all the experimental treatment tanks was regulated and maintained by adjusting flow rates of ambient and CO_2-enriched seawater in a buffer tank, and flowed through two parallel replication tanks separately for each treatment (Fig. 1). Clams were always housed in sediment in order to mimic their natural habitat to the greatest extent possible. To prevent pH buffering from the dissolution of calcium carbonate substrates, artificial calcium carbonate-free sediment (silica glass beads, 430–850 μm diameter) was used as substrate in the trays. For in situ water monitoring, two multiparameter instruments (EXO2 Sonde, YSI, USA) were mounted in the pH end-member cultivation tanks (pH = 8.1 and 7.5) for continuous data recording (Fig. S1 and S2). Each sonde was equipped with 2 pH, 1 temperature/conductivity, 1 DO and 1 Chlorophyll-total algae sensors. Considering the temperature range of this experiment, the resultant accuracy was 0.1 pH units. Additional pH meters were also mounted in the master header tank and four buffer tanks to monitor the pH levels of the different treatments, and the pH values were recorded every day (Fig. 1). During monthly water sampling, pH in each cultivation tank was checked with a handheld pH meter (826 pH mobile, Metrohm, Switzerland) with a pH accuracy of 0.003 unit. All pH meters were calibrated with NBS buffer solution, and the results are shown in NBS scale. By comparing the independent pH measurements taken by EXO2 Sonde, pH meters housed in buffer tanks, and 826 pH mobile handheld pH meter, we found one of the pH sensors in each sonde drifted during the experiment, and therefore only one pH data set was reported (Fig. S1 and S2). Nevertheless, the data derived from the remaining pH sensor in each EXO2 Sonde, as well as the handheld pH meter, was checked during the experimental period to ensure the pH level in each cultivation tank was consistent with the header tank, and maintained at the level we

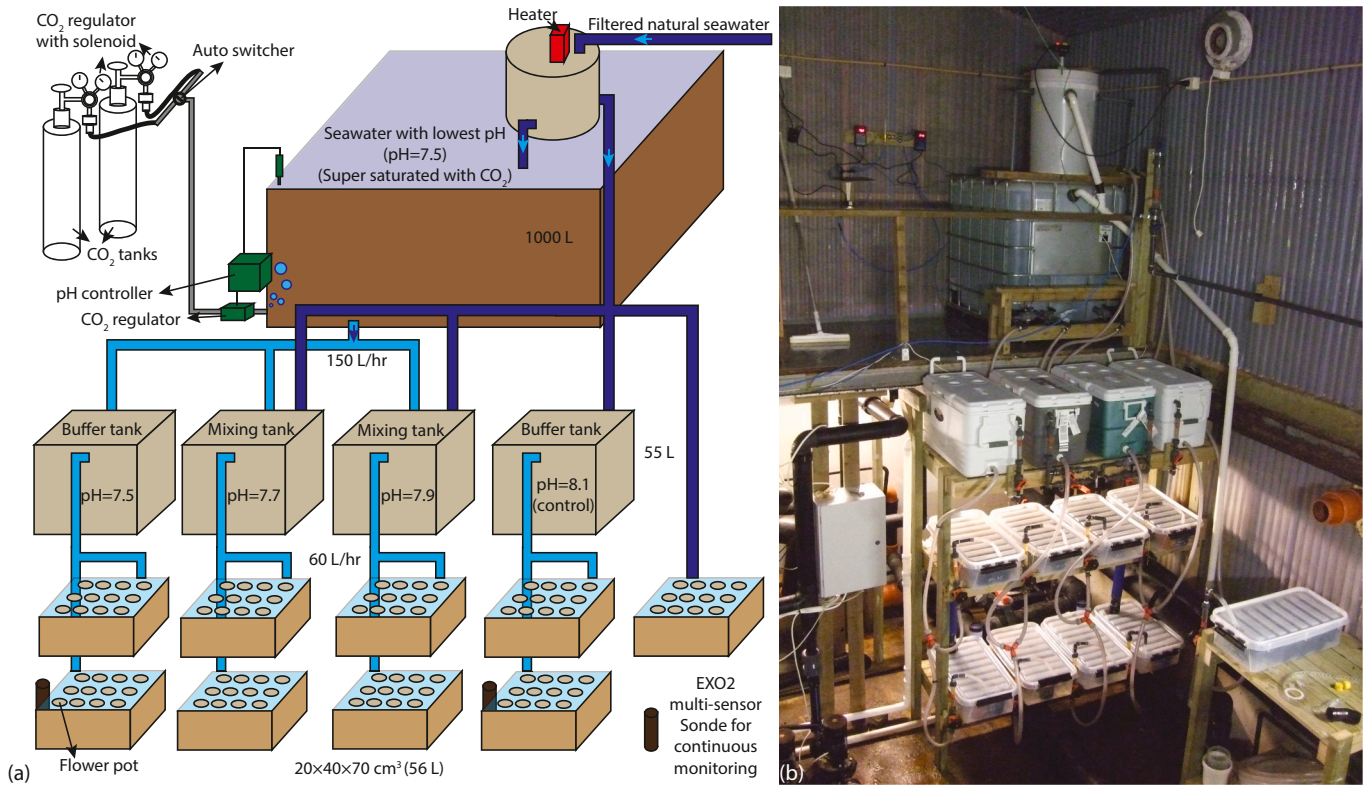

Fig. 1. Experimental setup of pH-controlled culture experiment in Tromsø, Norway. Schematic illustration of the experimental design (a). In brief, natural seawater was acidified to pH 7.5 with the addition of CO_2 in a 1000 L tank. Four pH levels were achieved by mixing of natural seawater and the pre-acidified (pH = 7.5) seawater. The flow rates were maintained in a level that can replace seawater of treatment tanks in an hour to prevent potential variation of the carbonate chemistry by degassing. Picture of the actual experimental setup (b) at the Kraknes Research Station (FISK), Akvaplan-niva, Tromsø, Norway.

targeted. In addition to continuous monitoring of pH, discrete water samples were taken to fully describe the carbonate chemistry in the four treatments (2 replicates each) for analysis of total alkalinity (A_T) and total dissolved inorganic carbon (DIC). A total of 36 seawater samples, covering all nine cultivation tanks and spanning the experimental period, were analyzed following the protocol by Dickson et al. (2007), in the IMR CO_2 laboratory in Tromsø. DIC was analyzed using gas extraction followed by titration and coulometric detection (VINDTA, marianda), and A_T was determined using potentiometric titration with 0.1 N hydrochloric acid (VINDTA, marianda). The precision and accuracy based on replicate analyses of certified reference material (CRM, SIO, USA) were ± 2 μmol/kg, for both DIC and A_T. Other parameters in the carbonate system, such as partial pressure of CO_2 (pCO_2, μatm) and aragonite saturation Ω_{Ar}, were calculated using CO2SYS speciation program (Pierrot and Wallace, 2006) and pair of A_T and DIC and salinity and temperature for the treatments. The carbonate chemistry (pH, A_T, DIC, pCO_2, and Ω_{Ar}) in each treatment is summarized in Table 1. The standard deviation represents the daily variation of seawater

Table 1
Experimental conditions Average value ± one standard deviation was reported for water conditions.

Treatment/ Tray	Temperature (°C)	Salinity (PSU)	pH (NBS)	Total Alkalinity (μmol/kg)	DIC (μmol/ kg)	pCO_2 (μatm)	Ω_{Ar}	Total specimens (N)	Shells with clear growth marks	Dated shells with clear growth marks
1A	7.2 ± 0.7	34.6 ± 0.3	7.49 ± 0.07	2284 ± 13	2288 ± 28	1371 ± 203	0.63 ± 0.10	12	8	8
1B			*(7.56 ± 0.07)	2286 ± 8	2279 ± 19	1264 ± 242	0.68 ± 0.15	12	8	7
2A			7.61 ± 0.06	2285 ± 6	2251 ± 32	1026 ± 306	0.84 ± 0.17	12	11	9
2B				2283 ± 5	2251 ± 34	1049 ± 316	0.82 ± 0.17	12	9	7
3A			7.82 ± 0.06	2281 ± 9	2200 ± 29	701 ± 149	1.14 ± 0.19	12	10	8
3B				2279 ± 7	2199 ± 35	709 ± 167	1.14 ± 0.22	12	7	6
4A			8.06 ± 0.02 *(8.09 ± 0.04)	2278 ± 7	2135 ± 2	438 ± 9	1.64 ± 0.08	12	9	9
4B				2279 ± 7	2159 ± 24	522 ± 96	1.45 ± 0.21	12	12	7
4C				2282 ± 6	2137 ± 4	435 ± 14	1.65 ± 0.09	12	8	8

* Values in parenthesis were measured by EXO2 Sondes.

temperature, salinity, and pH measurements and monthly variation of tank water DIC and TA. A *t*-test confirmed that the carbonate chemistry was statistically significantly different among treatments.

Overall, 108 clams were divided into nine tanks at four pH levels (12 clams/tray, two trays/pH level, plus one extra tray under control pH treatment). The initial size distribution of shells was not significantly different among the tanks (ANOVA, $p = 0.99$). Clams were stained with calcein (125 mg/L of seawater for 24 h) at the start of the experiment (after a one-week acclimation period) and again in May to constrain individual growth rates. Calcein is incorporated into the crystalline structure of the shell that grew during exposure of the staining and fluoresces when exposed to fluorescent light (Hernaman et al., 2000; Kaehler and McQuaid, 1999; Riascos et al., 2007), providing an unambiguous time marker in the shell. The distance between stain marks indicates the new growth and the dates of staining provides an absolute time reference.

Only ambient food was delivered to the clams during acclimation and throughout the experiment. The clams produced fecal pellets throughout the acclimation period and the growth experiment, indicating active feeding and that the level of food naturally occurring in ambient seawater was sufficient to sustain growth (Fig. S4). Two clams died during the experimental period. One of the dead clams was replaced in the third week of the experiment. We kept several reserve animals under the same conditions as the experimental animals. The replacement clam was of similar size as the original (72.5 vs. 72.03 mm in shell height) and was acclimated and calcein stained the same way as the original. Because the replacement occurred early in the experimental period in early March, well prior to the spring bloom and the time of primary shell growth (Beirne et al., 2012), we believe that any effect of the replacement on shell growth was minimal.

2.3. Shell harvest, age determination, and growth analysis

The clams were harvested in September 2014. The soft tissues were removed, and the shells were cleaned by hand, rinsed in deionized (DI) water, and air-dried. The shells were then bagged and labelled for later examination.

Arctica islandica shells were prepared at Iowa State University in order to determine the age of the animal during the experiment based on modified methods outlined by Mette et al. (2016). One valve from each shell was sectioned along the maximum growth direction. Approximately a 7 mm-thick shell section was made for each clam and mounted on a glass plate and polished using a Buehler variable-speed, grinding-polishing wheel with a series of decreasing grit-size silicon carbide grinding paper (P120, P400, P600, P800, and P1200). The shells were thoroughly rinsed with DI water before immersing in an 1% HCl etching bath for approximately 3 min. After etching, shells were rinsed by dipping in DI water and allowed to dry for at least 12 h. A 0.035 mm cellulose acetate film (Electron Microscopy Sciences Catalog No. 50420–30) was carefully laid over the etched shell block surface after coating the surface with a thin layer of acetone, and then allowed to dry completely. The acetate replica peels were removed from the shells and mounted between glass slides for visual analysis of shell growth increments under transmitted light microscopy using $2\times - 20\times$ objectives. Annual increments were counted in the hinge region of the shells and age determinations (± 1 year) were made for each individual clam.

The new growth of *A. islandica* during the experiment was determined under fluorescence microscopy at Research Center for Environmental Changes at Academia Sinica, Taiwan. Because the CO_2 controlling system was not stable during late August, we could not adequately evaluate the growth of the shell under the four pH treatments after this time. We therefore only report and analyze the growth results between the two calcein marks of each shell (February and May). Because the shell morphology at the outer growing margin is highly variable, and sometimes the two calcein marks cannot be clearly focused at the growing margin, measurements were taken from 100 μm from the growing margin of the sectioned shell to minimize potential biases during measurements (Fig. 2). The uncertainty of these measurements was about ± 2 μm. Analysis of variance (ANOVA) was used to test for differences in the size distributions of individuals among treatments. Measurements of all 12 specimens in each tray were included in the test to ensure there was no statistically significant difference in the shell size distribution before the experiment was initiated (Fig. 3 (a)). The initial height data in each tray passed Kolmogorov-Smirnov (Lilliefors) or Shapiro-Wilk W normality tests, except for treatment 1B, which exhibited moderate Skewness (-1.7) and Kurtosis (5.4). Shells with prominent calcein marks in both the beginning (February) and end of the experiment (May) were included in the rest of the statistical analysis. Based on the relevant literature, we conducted exponential regression to find the von Bertalanffy growth curves for this population (Fig. 3 (b)) between shell height and shell age. We used linear, quadradic, and exponential regression to test if there were significant trends between calcein preservation rates and pH treatments, and general pattern between shell growth and shell age. The best fit was determined based on the smallest root mean square error (RMSE) and the largest R^2 among the three regressions (Fig. 3 (c)). We further conduct a mixed effect linear model analysis with full factorial fixed effects between treatment pH and age, and tank as a random effect using JMP software. The significance threshold in all tests was $P < 0.05$. Because it is well-known that *A. islandica* exhibit strong ontogenetic changes in shell growth, we separated shell growth results to two groups (juveniles vs. adults, based on the average age at maturity) to eliminate potential age-based bias. We then applied linear regression analysis of growth at the pH treatments in different age categories (all ages, juveniles, and adults).

3. Results

3.1. Calcein mark preservation and basic shell measurements

Overall, 82 of the shells had distinct calcein marks in both February and May, allowing a determination of shell growth under different pH treatments (Table 1, Fig. 3). Of these shells, 76% had two calcein marks, from the beginning and the end of the experiment, clearly preserved. The preservation rates (i.e., two prominent calcein stains) were 67%, 87%, 71%, and 81% from the lowest to the highest pH, respectively. There was no relationship between the absorption/preservation rates of calcein in shell structure and pH treatments (with respect to the best fit quadratic regression, $P = 0.83$, $F = 0.22$). Of these 82 shells, the ages of 69 clams could be determined. Because the shell growth rate is higher in juveniles, we tested the shell size distribution among treatments at the beginning of the experiment in different tanks to ensure there were no statistically significant differences before the acclimation (1-way ANOVA, $P > 0.05$). The average shell size was 67.1 ± 10.9 and 66.6 ± 8.7 mm in the 7.5 pH treatment, 67.5 ± 8.2 and 65.4 ± 6.6 mm in the 7.7 pH treatment, 67.9 ± 7.0 and 67.7 ± 6.0 mm in the 7.9 pH treatment, and 65.2 ± 7.7, 67.0 ± 8.0, and 67.1 ± 7.5 mm in the 8.1 pH (control) treatment (Fig. 3 (a)). We did find that both initial shell height and shell growth during the experiment were significantly correlated with animal age (Fig. 3 (b, c)).

3.2. Justification of sample replicates and potential tank effect

To test contributions of different effects on the shell growth observed, we conducted a mixed effect model analysis on our dataset with age and pH being account as fixed effects and tank position as a random effect. This analysis showed that age was the primary control on the shell growth ($P < 0.01$; Table 2). Additionally, the tank effect contributed 0% to the total variant of the model, suggesting tank effect is insignificant on the shell growth in the experiment (Table 3). Therefore, we treated individual measurements as biological replicates.

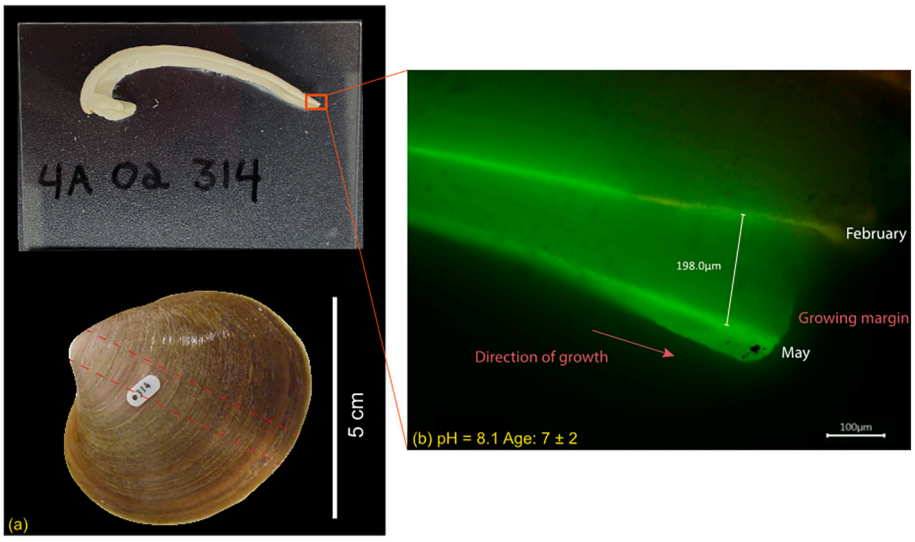

Fig. 2. Examples of calcein marks in the shell sections (a) shows where a shell section was taken and (b) shows an image under a fluorescence microscope. Growth measurements were only taken for the shells with obvious and unambiguous calcein marks. Measurements were consistently taken from the same location, from the shell edge/growing margin, to minimize impacts that may arise from different shell morphologies.

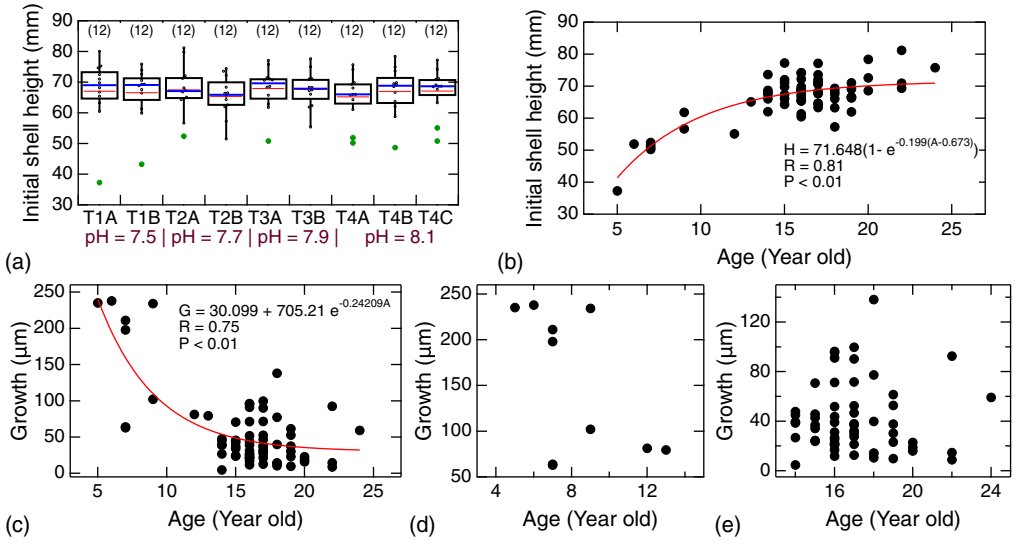

Fig. 3. Age and growth distributions in the four pH treatments. Box plots showing the shell height distributions of all shells in the cultivation tanks. (b) and (c) show the correlations between shell height and the age, and the between shell age and shell growth, respectively. Red and blue lines in (a) show the mean and median of the dataset. Numbers in parenthesis in (a) denote the total number of shells in the cultivation tank. Red curves in (b) and (c) show the exponential regression curves. (d) and (e) show that there is no statistically significant difference ($P > 0.05$) between growth and shell age when examining the juveniles (Age < 13) and the adults separately. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Results of linear mixed effects model.

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	287.15276	172.7381	65	1.66	0.1013
pH	−11.06404	21.91135	65	−0.50	0.6153
Age	−9.187747	1.320329	65	−6.96	<0.0001
(pH -7.78304)*(Age-15.7536)	−7.757938	5.952114	65	−1.30	0.1970

3.3. Evaluation of potential impacts of OA on A. islandica shell growth

To avoid potential confounding of the ontogenetic effects on the final interpretation of the pH control shell growth experiment, we separated the growth results based on the average maturity age (Age > 13 (Rowell et al., 1990; Schöne, 2013)) of the animals (Fig. 3 (d, e)). Although there were relatively few samples in the juvenile category (1, 4, 2, and 5 juveniles in treatments pH = 7.5, 7.7, 7.9, and 8.1, respectively), the correlation between age and growth rate observed in both juvenile and adult groups was not statistically significant (Fig. 3 (d, e); $R^2 < 0.02$, $P > 0.05$ and $R^2 < 0.01$, $P > 0.05$, respectively). However, the youngest two

Table 3
Restricted maximum likelihood variance component estimates.

Random Effect	Var Ratio	Var Component	Std Error	95% Lower	95% Upper	Wald *p*-Value	Percent of Total
Tank	−0.003884	−6.84383	1.2004872	−9.196742	−4.490919	<0.0001	0.000
Residual		1762.0703	309.08758	1284.3489	2567.8674		100.000
Total		1762.0703	309.08758	1284.3489	2567.8674		100.000

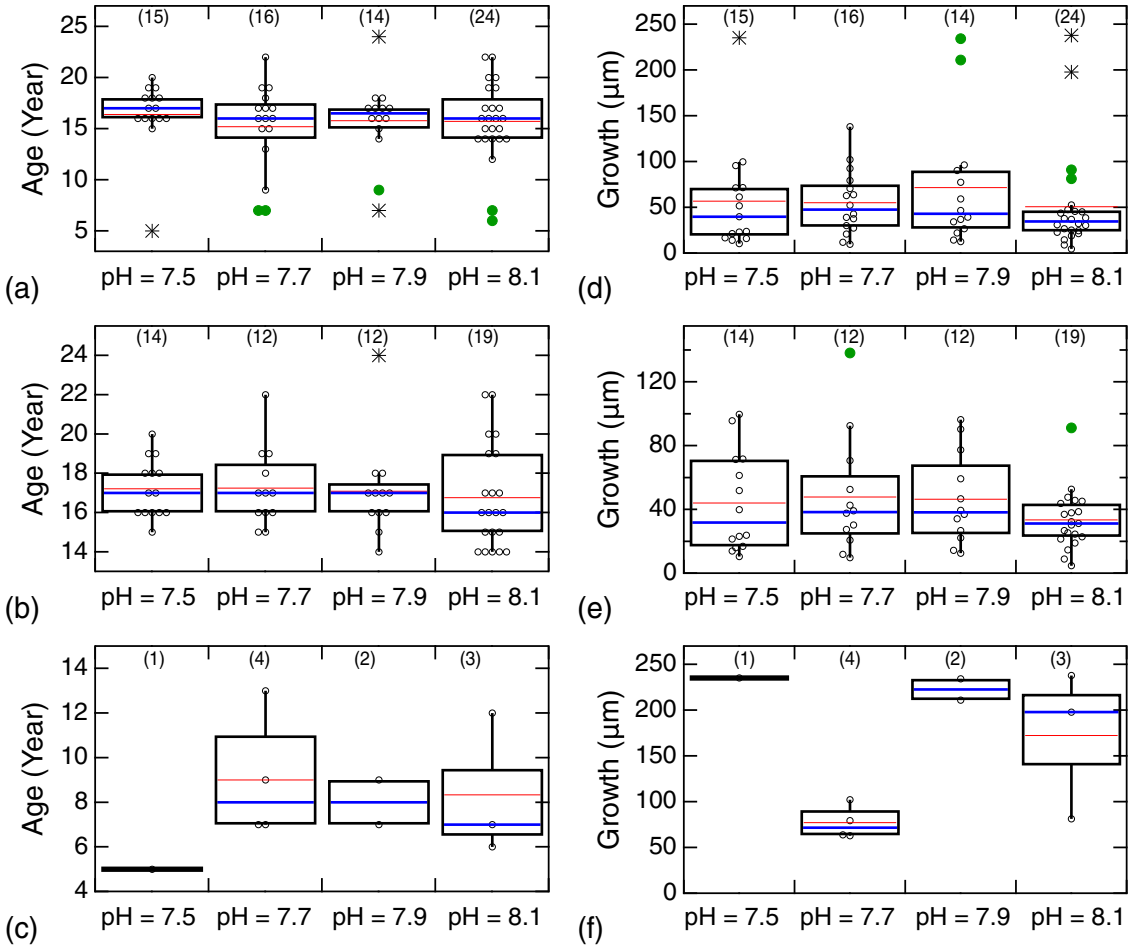

Fig. 4. The age and growth distribution of *A. islandica* shell in each treatment. (a) – (c) show the age distributions in different treatments, with (a) including shells of all ages, only adults (b) and only juveniles (c). (d) – (f) are box plots showing growth in three different age categories: all ages (d), adults (e), and juveniles (f), respectively. Red and blue lines show the mean and median of the dataset. Black circles, green dots, and star signs represent data points, outliers, and far outliers of the dataset, respectively. Numbers in parenthesis denote the total number of shells in each parameter and treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

specimens (Age 5 and 6, Fig. 4 (a)) were found to be in treatments 1 and 4, pH 7.5 and 8.1, respectively, and can therefore help explain the large growth discrepancies observed in the two treatments. Similarly, after rechecking the ages of the shells in different treatments, we found no difference in the age distributions among pH conditions (Fig. 4 (a-c)). Because there were no age-related pH effects on growth, we felt justified to analyze the data without introducing a bias.

Average growth in the control treatment was 50.8 ± 55.5 μm when using all sample ages, whereas in the most acidified treatment (pH = 7.5) the average growth was 56.8 ± 57.7 μm (Fig. 4 (d)). Using only the adults, growth was 33.4 ± 18.5 μm in the control and 44.1 ± 31.1 μm in the most acidified treatment (Fig. 4 (e)). The juveniles grew the fastest, with average growth between 77.1 and 235.2 μm per treatment (Fig. 4 (f)), but the small sample size of two treatments makes formal comparison difficult. Although the average growth in the control treatment was lower than the most acidified treatment, there was no statistically significant difference among treatments ($P > 0.05$) regardless of whether we used the results of all ages (Fig. 4 (d)), adults (Fig. 4 (e)) or juveniles (Fig. 4 (f)).

4. Discussion

4.1. Resistance of A. islandica shell growth to OA is a cross-population feature in culture experiment

Our results suggest that the shell growth in *A. islandica* is not affected by seawater pH in the range between 7.5 ($\Omega < 1$) and 8.1 (Fig. 4). Although growth variations within a treatment were over 50% of the average growth, this could be due to the shell age, and the growth variations were decreased after grouping the shells into two age categories (juveniles and adults, Fig. 3). Therefore, the consistent growth rates among pH treatments should not be a result of large within-treatment growth variations, but indicate that *A. islandica* can regulate shell growth under variable OA conditions.

Earlier work on *A. islandica* has shown that the pCO_2 of the seawater during culture (300–1600 μatm) had little impact on the mortality, shell growth, and shell breaking force (Hiebenthal et al., 2013). Another independent culture experiment also found no observable change in shell growth and mineral structure for the same species cultured in a pCO_2 range of 380–1120 μatm, which corresponds to a pH range between 7.75 and 8.07 (Stemmer et al., 2013). However, both studies used *A. islandica* collected from the Kiel Bight, where the clams were regularly exposed *in situ* to high pCO_2 and hence low pH conditions during the summer months (Hiebenthal et al., 2013). It has been shown that *A. islandica* morphometry and growth are significantly different between the Norwegian coast and the Kiel Bight (Begum et al., 2010). Therefore, the authors of the Kiel Bight study (Stemmer et al., 2013) cautiously mentioned the possibility of local adaptations of this bivalve species with this resistant growth. In the present study, we collected clams from Ingøy in the northern Norway, where the local meteorological records show less temperature and salinity variations at intra- and interannual timescales. The seawater temperature in Northern Norway is typically between 5 and 12 °C, and the salinity is within a range from 33.5 to 35 PSU (Aure et al., 2001). The pH and carbonate chemistry variation in the northern Norwegian coast is influenced by the Norwegian Coastal Current, Atlantic water intrusions as well as local processes such as river runoff. From a station nearby Ingøy at (71°N, 19.9°E) in the upper 10 m, pH and pCO_2 showed relatively small variability between 8.07 and 8.18, and 305 to 374 μatm, respectively (Chierici et al., 2019). Including deeper waters pH is as low as 7.96. (Jones et al., 2020). In contrast, the seasonal pH variation in the Kiel Bight is larger and ranges from 7.49 to 8.23, caused by upwelling of CO_2-rich subsurface water, at reaches at times pCO_2 levels of >2000 μatm (Thomsen et al., 2010). This implies that the carbonate chemistry differs greatly between these sites. Although the habitats of the two *A. islandica* populations (Northern Norway and Kiel Bight) differ, our results support the findings that shell growth under different levels of ocean acidification were similar during the experimental period (~3 months). These consistent resilient growth responses of *A. islandica* populations from two very distinct habitats in contrasting living conditions suggest that this is a cross-population response. Furthermore, this characteristic is not a result of adaptation in their current natural habitats. It has been suggested that the fossil records of *A. islandica* can be traced back to the late Mesozoic (Casey, 1952; Schöne, 2013) with pCO_2 levels potentially several times higher than modern conditions (~400 ppmv) (Berner and Kothavala, 2001); therefore *A. islandica* clams may be genetically pre-adapted to high pCO_2 levels (Stemmer et al., 2013).

Alternatively, *A. islandica* occasionally performs metabolic rate depression for up to several days during periods when food supply is limited or the temperature is too low, for example. Under metabolic rate depression, the animals usually bury in the sediments, which are often hypercapnic due to decomposition of organic matter (Aller, 1982; McNichol et al., 1988). Although food supply was not limited and temperature during the culture experiment was well within the habitable range (1° to 16 °C) for this species (Schöne (2013) and references therein), the lifestyle of burying in sediments may also make *A. islandica* pre-acclimated to exposure to undersaturation with regard to aragonite seawater as well as high pCO_2 levels. Nevertheless, although the metabolic rate depression is common for various bivalve species, not all the species sustained calcium carbonate shell growth under acidified conditions (Ries et al., 2009).

4.2. Potential mechanisms to maintain the shell growth

There are two prevailing hypotheses that could explain the resistance of calcification of an organism to OA: 1) pH regulation of an organism at its calcification site fluid (e.g., McCulloch et al. (2012); Stemmer et al. (2019); Wall et al. (2016)), and 2) biomineralization facilitated by skeletal organic matrix (Addadi et al., 2003; Arias and Fernández, 2008; Cusack and Freer, 2008; Mass et al., 2017; Von Euw et al., 2017). In the first hypothesis, an organism might control the calcification fluid pH through Ca-H-ATPase, Na-K-ATPase, or adjusting its calcification site fluid buffer capacity (Pörtner et al. (2004) and references there in). In the latter hypothesis, amorphous calcium carbonate may be stabilized by the presence of Mg^{2+}, phosphate, and/or organics (Addadi et al., 2003; Arias and Fernández, 2008; Cusack and Freer, 2008; Meibom et al., 2004).

Direct measurements of pH of *A. islandica* show pH spatial variations in the extrapallial fluid, and temporal changes of pH in the extrapallial fluid close to the outer mantle epithelium (Stemmer et al., 2019). In Stemmer et al. (2019) experiment, the observed rapid change of pH from 7.0 to 9.5 show a strong H^+ efflux at outer mantle epithelium, supporting the hypothesis that *A. islandica* actively regulate pH in extrapallial fluid. However, although the rapid pH upregulation around the mantle epithelium helps to increase aragonite saturation state and therefore supports shell precipitation, whether this regulation strategy is equally sustainable under OA conditions is still unknown. Alternatively, a more recent study has applied boron isotope as a pH proxy to assess the pH regulation of bivalve species (Liu et al., 2020). The results also revealed that three other bivalve species performed moderate to strong pH regulation in their calcification site fluid, although the extent of pH regulation did not directly correlate to their net calcification patterns. The authors (Liu et al., 2020) therefore suggest other physiological factors may play more important roles in the bivalves' biomineralization processes. Nevertheless, both the in situ and the proxy records support the hypothesis that many of the bivalves have the ability to regulate pH in their extrapallial fluid.

In contrast to pH regulation in the calcification site fluid, the presence of organic matrix could potentially support the net precipitation of biominerals under acidified conditions. Studies have shown that the amorphous calcium carbonate is a precursor of aragonite in bivalves (Addadi et al., 2003; Weiss et al., 2002). The presence of the amorphous calcium carbonate was thought to be beneficial in molding the various shapes of shell structures (Weiner et al., 2005), and could crystalize rapidly when optimal environmental conditions have been achieved (Jacob et al., 2008). It has been shown that the presence of organic matrix will result in lowered activation energy of nucleation (Mann, 2001), and therefore, facilitate biomineralization. Additionally, many proteins, glycoteins, and polysaccharides are thought to be involved in biomineralization due to their abundant negative charges and repeating motif of their amino sequences that could mimic crystal lattice (Addadi and Weiner, 2014; Marin and Luquet, 2007). With the presence of organic matrix, the unstable form of amorphous calcium carbonate can therefore be stabilized more efficiently. Furthermore, organic matrix (e.g., periostracum) can potentially protect the biominerals from dissolution by shielding against direct exposure of acidic ambient seawater. To date, however, there are no reports of changes in the organic matrix of *A. islandica* in response to OA. We therefore cannot evaluate whether *A. islandica* sustains its growth via control of the organic matrix, and if it is the primary factor controlling the resistant shell growth of *A. islandica* under OA conditions.

Note that the two prevailing mechanisms may not necessarily conflict with each other. The amorphous calcium carbonate can crystalize instantly through a rapid change of pH in the extrapallial fluid, as well as being stabilized and sheltered by organic matrix under OA conditions. Further studies addressing the question may shed lights on the contributions of physiochemical regulation and organic matrix to facilitate the resistance of shell growth of *A. islandica* to OA.

4.3. Resistance of A. islandica shell growth and their survival under future acidified conditions in the field

Although there was no difference in the shell growth during the experimental period (3 months between calcein markings), it is still unclear if the maintenance of growth in response to OA would continue for longer time periods. As discussed above, *A. islandica* can perform

metabolic rate depression when stressed, and therefore might have higher tolerance to acidified conditions during relative short periods before energy deficiency occurs. Additionally, the consistency of shell growth observed in the present study can only reflect the shell extension along the maxima growth axis. Alternatively, shell density or mineral orientation can also affect the ability of shells to protect from external threats (e.g., attacks by predators or other physical damages). For example, a recent study of bivalve mussel *Mytilus edulis* species showed that although the *M. edulis* can continue to calcify at 1000 μatm, this continued growth is compensated by their shell structural integrity (Fitzer et al., 2014). Although earlier studies have revealed that the shell microstructure and hardness were not sensitive to OA (Hiebenthal et al., 2013; Stemmer et al., 2013), all experiments were conducted for about 3 months, including ours. Further changes under longer exposures to acidified conditions (> a year) are warranted and may help to better assess the sustainability of *A. islandica* in a more acidified future and to provide more useful guidance on the ecological and commercial aspects of managing this important shellfish stock.

In addition to OA alone, increasing seawater temperature and associated nutrient changes can impact food availability and quality and have mixed effects on the survival of *A. islandica*. Future studies that also consider multiple stressors are on calcification dynamics and survivability are of *A. islandica* needed. Our collective understanding would improve if future OA work on *A. islandica* (or other mollusks) examines vitality, shell condition, reproductive condition, condition of periostracum, among others.

5. Conclusion

Our results suggest that *A. islandica* has resistance to OA conditions based on sustained shell growth in both juvenile and adult clams in seawater pH range between 7.5 and 8, which corresponds to a pCO_2 range between 465 ± 66 and 1325 ± 209 μatm. Our results are also consistent with previous studies on *A. islandica* from other locations, indicating that this is a general cross-population response, and that habitat type is not likely the dominant factor. This apparent resistance to OA, as evidenced by consistent shell growth, is likely due to physiological control of internal pH in extrapallial fluid. Furthermore, resistance to OA conditions may be mediated by the presence of organic matrix in the outer shell layer (i.e., periostracum) and/or within the shell structure, which acts to protect the biominerals from direct contact with acidic/corrosive ambient seawater conditions. Multiple mechanisms are likely acting simultaneously during biomineralization for these clams to tolerate, and even thrive, in OA conditions. However, future work should include investigations into long-term (> 12–18 months) impacts of OA on *A. islandica* shell growth and other physical parameters (shell density; microstructure, etc.) in combination with full carbonate chemistry from the treatments. More data are needed from calcifying fluids (extrapallial fluid) to better understand the calcification processes during OA conditions. Although the present study indicates resistance to OA, shell growth/survivability may be dampened below a certain pH threshold, or longer exposure in acidified environments.

Author contributions

Yi-Wei Liu: Conceptualization, Methodology, Formal analysis, Writing - Original Draft, Writing - Review & Editing **Alan D. Wanamaker Jr.:** Conceptualization, Resources, Formal analysis, Writing - Review & Editing **Sarah M. Aciego:** Conceptualization, Resources, Writing - Review & Editing **Ian Searles:** Formal analysis, Writing - Review & Editing **Thor Arne Hangstad:** Methodology, Writing - Review & Editing **Melissa Chierici:**Formal analysis, Writing - Review & Editing **Michael L. Carroll:** Resources, Methodology, Writing - Review & Editing

Declaration of Competing Interest

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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