THE EFFECTS OF OCEAN ACIDIFICATION AND SEA SURFACE WARMING ON THE EMBRYONIC DEVELOPMENT OF THE OPISTHOBRANCH GASTROPOD *STYLOCHEILUS STRIATUS*

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Abstract. Anthropogenic increases in atmospheric CO_2 compound the rates of long-term changes in the abiotic conditions of the Earth's oceans. Because many physiological processes, including calcification rate, depend on these physical factors, there is mounting concern over how changes in temperature (T) and the $CaCO_3$ saturation of seawater will affect marine organisms. These effects may be particularly relevant during development— many organisms produce protective calcified structures critical for pelagic dispersal and larval survivability. I investigated how unmitigated increases in oceanic pCO_2 and temperature consistent with climate change predictions affect the embryonic development rate, hatching success, and veliger morphology of the opisthobranch gastropod, *Stylocheilus striatus*. Embryos were reared in four seawater treatments: 1) control (pH=8.02, T=27°C), 2) high-temperature (pH=8.02, T=31°C), 3) acidified (pH=7.67, T=27°C), 4) acidified high-temperature (pH=7.67, T=31°C). Development times increased under reduced pH conditions, but substantially decreased under high-temperature and acidified high-temperature treatments, with significant interaction between temperature and pH. The percentage of embryos that hatched into veligers significantly decreased in all three treatments (<70% reductions in acidified high-temperature conditions). Larval shell size decreased in all three treatments— effects of acidification and temperature were synergistic, causing greater decreases in shell size with significant interaction in acidified high-temperature treatments. Additionally, there were observable deformities in the shell morphology of hatchlings incubated in decreased pH treatments— these deformities were exacerbated by temperature increases. Thus, my results indicate that oceanic conditions congruent with climate change predictions ca. 2100 suppress successful development in encapsulating gastropod embryos, potentially reducing their viability as pelagic larvae.

Key words: Climate change, ocean acidification, sea surface warming, marine gastropod, embryonic development, Stylocheilus, Anaspidea, Aplysiomorpha, Mo'orea, French Polynesia

INTRODUCTION

Anthropogenic increases in atmospheric CO_2 compound the rates of long-term changes in both the Earth's atmosphere and oceans. These changes exert considerable control over biotic systems and their composition through a number of variables, especially sea surface temperature (SST), oceanic pH and its consequent alterations of the mineral saturation state of seawater [Ω] (Pandolfi 2011). These changes, caused by the increased uptake of CO_2 at the ocean surface, are expected to have profound and potentially irreversible effects on marine ecosystems (Kleypas, 2006, Fabry et al. 2008, Hoegh-Guldberg et al. 2011, Kimura et al. 2011). More than 30% of the industrial CO_2 released into the atmosphere in the past 200 years has been absorbed by our oceans causing oceanic pH to decline at a rate about 100 times faster than at any time in the past 650,000 years (Kleypas 2006, Fabry et al. 2008). Global ocean pH is estimated to have dropped 0.1 units below preindustrial levels and, by current CO_2 emission rates, is projected to fall another 0.3–0.4 units by the end of the century, more than doubling current oceanic acidity (Caldeira and Wickett 2005, Munday et al. 2009, Orr et al. 2005). These changes are projected to be accompanied by a congruent +4°C increase in SST.

There is mounting concern over how these changes, including reductions in the calcium carbonate ($CaCO_3$) saturation state of seawater caused by ocean acidification, will

effect marine organisms, particularly corals and other invertebrates that precipitate calcium skeletons (Munday et al. 2009, Ries et al. 2009, Ries 2011, Watson et al. 2012). These effects may be particularly relevant during development— many organisms produce protective calcified structures critical for pelagic dispersal and larval survivability (Byrne 2012). Consequently, considerable research efforts have focused on predicting the effects of both ocean warming and acidification on larval development— there is paucity, however, in efforts investigating how the these climate change components and their interaction will effect embryonic development (Parker *et al.* 2010, Sheppard Brennand *et al.* 2010, Walther *et al.* 2010, Byrne *et al.* 2011). This is especially true for opisthobranch gastropods, whose larval and developmental fate in near future oceanic conditions remains almost entirely unknown.

I investigated how increasing oceanic pCO_2 and temperature consistent with climate change predictions affect the developing embryos of the opisthobranch gastropod, *Stylocheilus striatus* in Mo'orea, French Polynesia. Although it has been observed that the embryonic shell development of gastropods is negatively affected by low pH, such effects have not been studied in encapsulated embryos, where intracapsular fluids may buffer against environmental effects caused by extracapsular seawater (Ellis et al. 2009; Fernandes et al. 2012). Furthermore, effects have only been observed in direct developing gastropods that maintain an aragonitic shell through adult life-stages (Ellis et al. 2009); the opisthobranch, *S. striatus,* provides a unique perspective in that its embryonic shell (protoconch) is only maintained for its most vulnerable pelagic larval period, and is entirely absent in post settlement morphologies/life stages (Switzer-Dunlap et al. 1977).

To infer the potential effects of climate change on the embryonic development of the opisthobranch, *S. striatus*, I tested the following hypotheses in this study: 1) that, despite individual embryo encapsulation, the predicted reductions in oceanic pH ca. 2100 will have deleterious effects on the embryonic viability of the opisthobranch gastropod, *S. striatus*, via potential reductions in development rate, net embryonic calcification, and hatching success; 2) that high-temperature treatment congruent with SST increase predictions ca. 2100 will have negative developmental effects comparable to acidification treatments; 3) that increases in temperature and acidification will interact synergistically and exacerbate the effects of observed in reduced pH and increased-temperature treatments respectively.

METHODS

Collection, maintenance and mating of adult S. Striatus

Adult *Stylocheilus striatus* (N=56; Appendix A) were either collected from three sites at the fringing reef surrounding the northern coast of Mo'orea, French Polynesia, or collected after being pulled into the seawater intake system at the University of California Gump Field Research Station (Fig. 1; Table 1) (Thompson et al. 1976, Thompson 1977, Thompson et al. 1984). One collection site was located on a degraded reef flat on the fringing reef of Cook's Bay (Table 1, Site 1).

Figure 1: Map depicting the location of the 5 collection sites for *S. striatus* on Mo'orea, French Polynesia. Numbers correspond to the GPS coordinates and site names listed in Table 1.

Table 1: Collection site names and GPS Coordinates

Collection Site:	Site Number:	GPS Coordinates:
Gump Field Station	1	-17°48'99.8"S -149°82'59.2"W
Ta'ahiamanu Public Beach	2	-17°58'78.8"S -149°84'83.6"W
	3	-17°48'60.3"S -149°84'79.8"W
Temae Public Beach	4	-17°49'91.4"S -149°76'00.4"W

The remaining three sampling sites were located at public beaches of primarily white sand substrate featuring both live and dead coral heads scattered throughout (Table 1, Sites 2-4). Opisthobranchs obtained from the field were collected from either the canopies of opportunistic alga (*Sargassum spp.*) colonizing damaged coral heads, or large cyanobacterial mats (*Lyngbya majuscula*) present along the white sand substrate (Harvey 1833). Specimens were kept in temporary tanks (volume=16L, stocking density=10 ind. aquarium^{-1}) during transportation to the University of California Gump Field Research Station and were transferred within 5 hours of harvest into an outdoor constant flow-through aquarium (volume=668 L, stocking density=58 ind. aquarium^{-1}) containing aerated seawater from Pao Pao Bay (salinity=35±1 PSU, temperature=27±1.5°C). All adult *S. Striatus* were collected between September 1, 2012-October 3, 2012. In captivity, slugs were both freely permitted to graze on the cyanobacteria colonizing the sides of the tank and fed ad libitum the cyanobacteria *L. majuscula*. All adult specimens paired for mating in this study were housed in a single tank, irrespective of collection date; however, all snails experienced a minimum acclimation period of three weeks before any mating/oviposition was induced for experimental purposes.

For mating, 20 adults were selected and paired randomly from the total pool of adult slugs. Mating pairs (N = 10) were immediately distributed after selection between 10 aquaria (volume=36L, stocking density=2 ind. aquarium^{-1} [1 mating pair aquarium^{-1}]). Mating aquaria were housed within the primary holding tank. Lateral openings were cut in each mating aquarium to allow flow and equilibrate contained water with the aerated seawater from the primary holding tank. To induce oviposition, mating

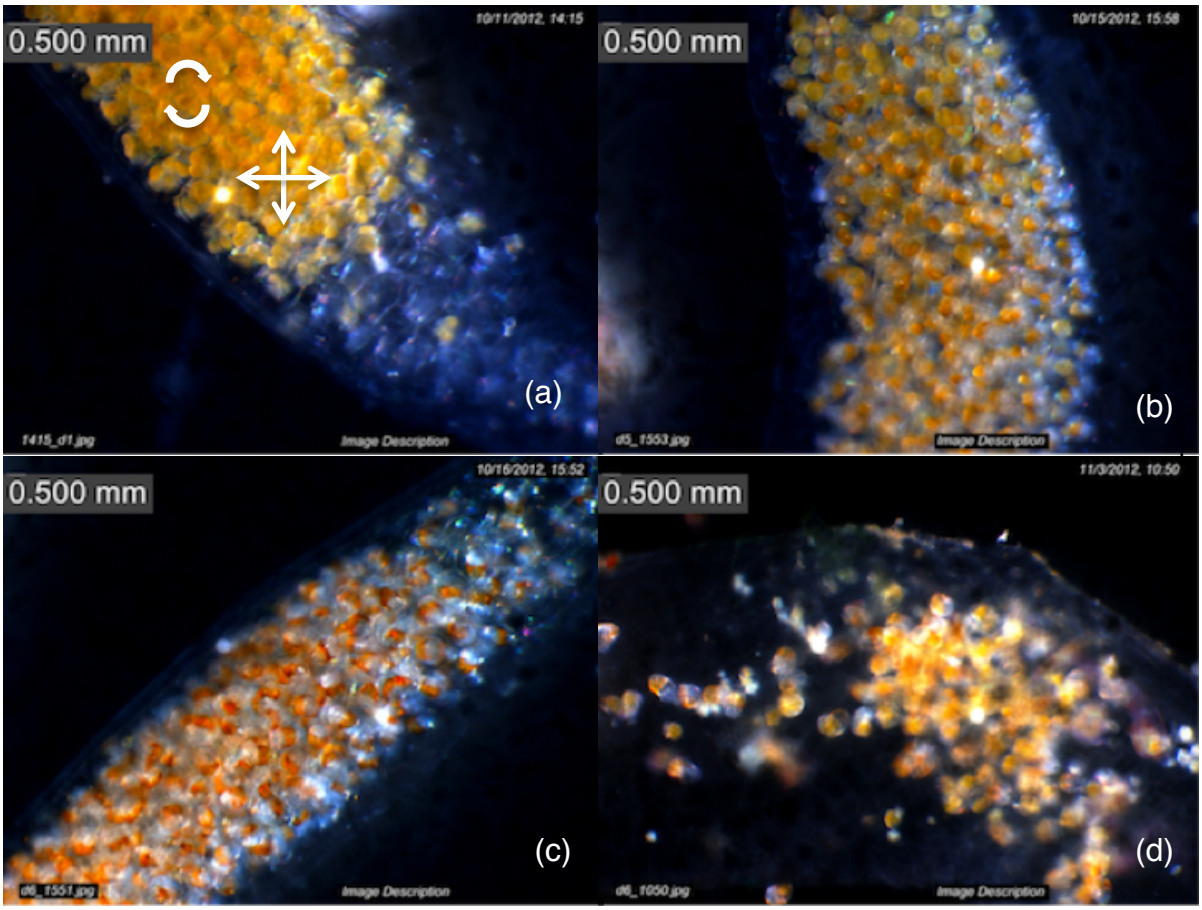

Figure 2: *Stylocheilus striatus*. Recorded embryological development events: (a) embryonic movement [defined as the first time individual embryos were observed moving or rotating inside egg capsule], (b) protoconch separation [clear division and color differentiation of an aragonite protoconch in individual embryos], (c) eye spot formation, (d) hatching. Scale bar = 0.5 mm

pairs were starved and provided algal masses (*Sargassum spp.*) as a substrate to protect egg ribbons.

Egg ribbon maintenance and treatment

Each mating aquaria was scanned every few hours for egg ribbons. Clutches were removed within 12 hours of oviposition. Upon observation, each egg ribbon (N = 11) was detached from the algal mass using a razor blade and divided with a scalpel into 2 equal halves (each half will hitherto be termed a batch)[1]. Each batch was then transferred to a sealed experimental petri dish (volume=96 mL) filled with either acidified seawater enriched with CO_2 (pCO_2 = 1113.6914 μatm, pH=7.67), or untreated water as a control (pCO_2 = 420.8915 μatm, pH=8.02), with a half from each egg mass being allocated to 1 of each of the 2 treatments. Petri dishes were stored at ambient temperature (27±1°C) in a covered water tray to reduce light exposure. Every 24 hours each egg batch was imaged using a dissecting microscope and optical light microscope camera (Leica MZ16; Leica DFC420). Throughout the progression of embryonic development, a minimum of three images were taken of each batch daily. Post hatching, egg ribbons were maintained at experimental conditions for an additional 24 hours to allow for full hatchling emigration before the hollow remains of the empty egg ribbon were photographed, sealed and stored in treatment water at –20°C for subsequent imaging and morphological analysis (see description below).!

After completion of the first incubation period (10 days) adult *S. Striatus* were allowed an additional 5 days of recovery and re-acclimation before the aforementioned procedures were fully replicated with manipulations in treated egg batch storage to test the added effects of increased temperature. Rather than cultivating batches at an ambient 27±1°C as in the previous trial, petri dishes containing individual batches were incubated in a constant, covered 31±1°C water bath and were only removed for a short period each day (<15 minutes day^{-1}) to be imaged. Thus, embryos were reared in four seawater treatments: 1) control (pH=8.02, T=27°C), 2) high-temperature (pH=8.02, T=31°C), 3) acidified (pH=7.67, T=27°C), 4) acidified high-temperature (pH=7.67, T=31°C).

Embryonic development

The time to reach four key visible events of embryonic development and morphology (*1)* individual embryonic movement [defined as the first time individual embryos were observed moving inside egg capsule], *2)* protoconch separation [clear division and color differentiation of an embryonic aragonite protoconch in individual embryos], *3)* eye spot formation, and *4)* hatching) were recorded for each batch as they were observed during imaging, and subsequently confirmed from photographs (Fig. 2). For each batch, data on developmental stage for each day was standardized by translation to occurrence at days pre-hatching event, then divided by the total number days the batch was incubated before hatching to infer developmental rate. Two-way analyses of variance (ANOVAs), using the general linear model, were used to investigate the effects of pH and temperature on the time to reach each development event. percent embryo viability, percent hatch rate, and all morphological traits of veligers. All data were analyzed using JMP (JMP 1989-2007).

Hatching success and embryo viability

An egg was considered viable if the veliger hatched out of the egg ribbon or if the embryo was able to swim/crawl freely inside the empty ribbon prior to the storage at –20°C. Eggs remaining in the tube post storage were not considered viable if their development lagged significantly behind the rest of the clutch (i.e. did not develop past the formation

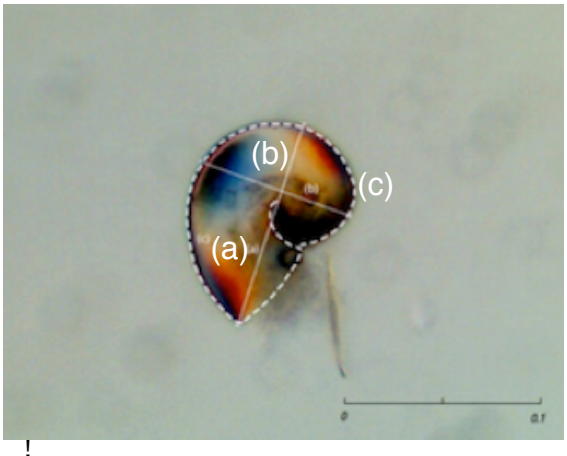

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Figure 3. *Stylocheilus striatus*. Measured veliger morphological measures: (a) lateral shell length, (b) lateral shell width (spiral height), (c) lateral 2-dimenshional shell area. Scale bar = 0.1 mm

of a protoconch or development of eye spots), if the embryo was unable to move and/or swim (generally due to visible deformities of the embryo) or if 2+ embryos were conjoined within a single capsule. Hatching success was measured as percent hatch rate estimated by subtracting the total number of unviable embryos of a batch by the total eggs in that batch and dividing the sum by the total number of eggs of within that batch. The number of unviable embryos within a batch was estimated by randomly selecting 5 0.50mm sections of the batch 24 hours post-hatching, counting the total number of unviable embryos for each section and then averaging the 5 before extrapolating that mean across the entire length of the empty batch. The total number of eggs in a batch was calculated by counting every egg (irrespective of embryonic viability) in a 0.50mm section of 5 different egg ribbons, (selected randomly, each at a different stage of development), averaging the total number of eggs between the 5 and then extrapolating that number across the entire length of the batch. Two-way analyses of variance (ANOVAs), using the general linear model, were used to investigate the effects of pH and temperature on the percent hatch rate of each egg batch. All data were analyzed using JMP (JMP 1989-2007).

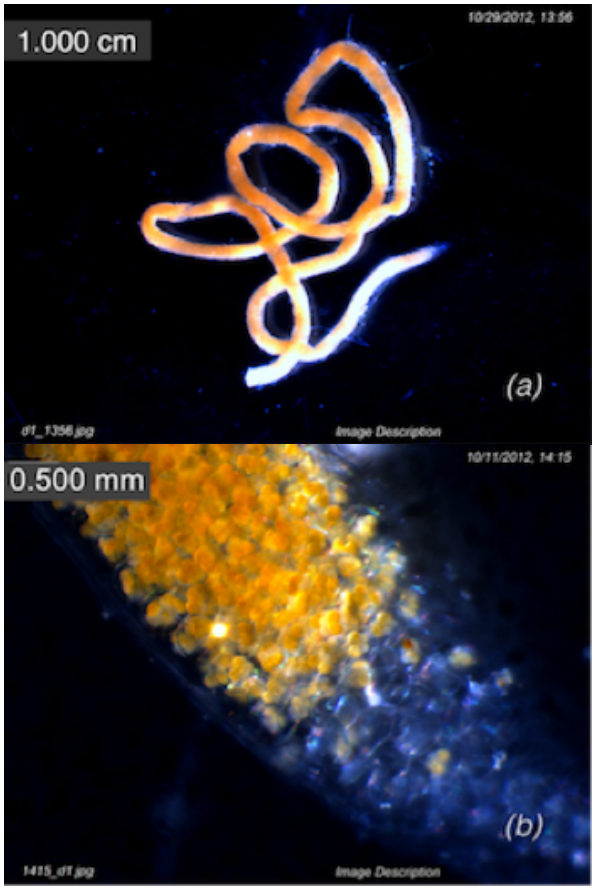

Figure 4: Egg ribbon of *Stylocheilus striatus*, 1 hour post oviposition. (a) The entire string of the tangeled mass. Scale bar = 1.000 cm. (b) Short portion of the egg ribbon illustrating individual egg capsules. Scale bar = 0.500 mm.

Hatchling morphology

Hatchling morphology was measured in 10 randomly selected individuals from each egg batch. Post removal from –20°C storage, batches were thawed and visualized using a compound light microscope and optical light microscope camera (Leica DM2500; Leica DFC420). Three measures were obtained for each veliger using ImageJ64 (Rasband 1997-2012): lateral shell length, lateral shell width (spiral height) and 2-dimensional lateral shell area (Fig. 3). Measurements were then averaged across the 10 individuals in each batch to get a mean value of each measure for each batch. Two-way analyses of variance (ANOVAs), using the general linear model, were used to investigate the effects of pH and temperature on all morphological traits of veligers. All data were analyzed using JMP (JMP 1989-2007).

Seawater acidification and carbonate chemistry

Seawater was supplied from a depth of 12 m in Cook's Bay, filtered through a sand filter (mesh size ~100 μm), and stored in a header tank. Elevated CO_2 treatments were obtained by bubbling tanks with CO_2-enriched air. CO_2-enriched air was created using a solenoid-controlled gas regulation system (Model A352, Qubit Systems Inc.) that mixed pure CO_2 with ambient air to create gas mixtures with known pCO_2 (as detected by an Infrared Gas Analyzer [IRGA], Model S151, Qubit Systems Inc.) The flow of CO_2-enriched air into each tank was adjusted using needle valves to correct deviations detected by pH measurements of tank seawater from the targeted values. The flow of seawater into each tank was similarly adjusted using ball valves to correct deviations from targeted values and to ensure that A_T remained constant throughout the experiment. Before treating egg batches for each experimental treatment, acidified and control water was then collected from tanks and stored for no more than 48 hours in sealed 1 L containers at 5°C.

Samples for pH_T were collected and measured immediately with an automatic titrator (T50, Mettler-Toledo) using a pH scale using 2-amino-2-hydroxymethyl-1,3-propanediol (TRIS) buffer (Andrew Dickson) at a salinity of 35.0. Calibrations were done every third day based on the rationale that the pH cell was demonstrably stable as it drifted an average of 0.2 mV between 2 calibrations, which corresponded to a pH_T variation of <0.005 unit. The determination of pH using a pH cell was preferred over the use of the indicator dye m-cresol because the pH cell allowed for greater replication with only a trivial reduction in accuracy. Determination of pH using a pH cell yields uncertainty for pH measurements of <0.02 unit for seawater (Dickson 2010). pH_T also was measured spectrophotometrically using the indicator dye m-cresol (Standard operating procedure 6b, Dickson et al. 2007). pH_T measured by the two methods provided similar values with a mean difference of ≤ XXX pH units. Salinity was measured using a conductivity meter (YSI 63) and total alkalinity (A_T) was measured in duplicate. Analyses of A_T were performed on the day of seawater sampling using an open-cell potentiometric titration using an automatic titrator (T50, Mettler-Toledo). Measurements of A_T were conducted on duplicate 50 mL samples at room temperature (~23 C) and A_T was calculated using a Gran function applied to pH values ranging from 3.5 to 3.0 as described by Dickson et al. (2007). Titrations of certified reference materials (CRM) provided by A. G. Dickson (batch 105) yielded A_T values within 4 mol kg -1 of the nominal value (SD=4.1 mol kg -1; n=12).

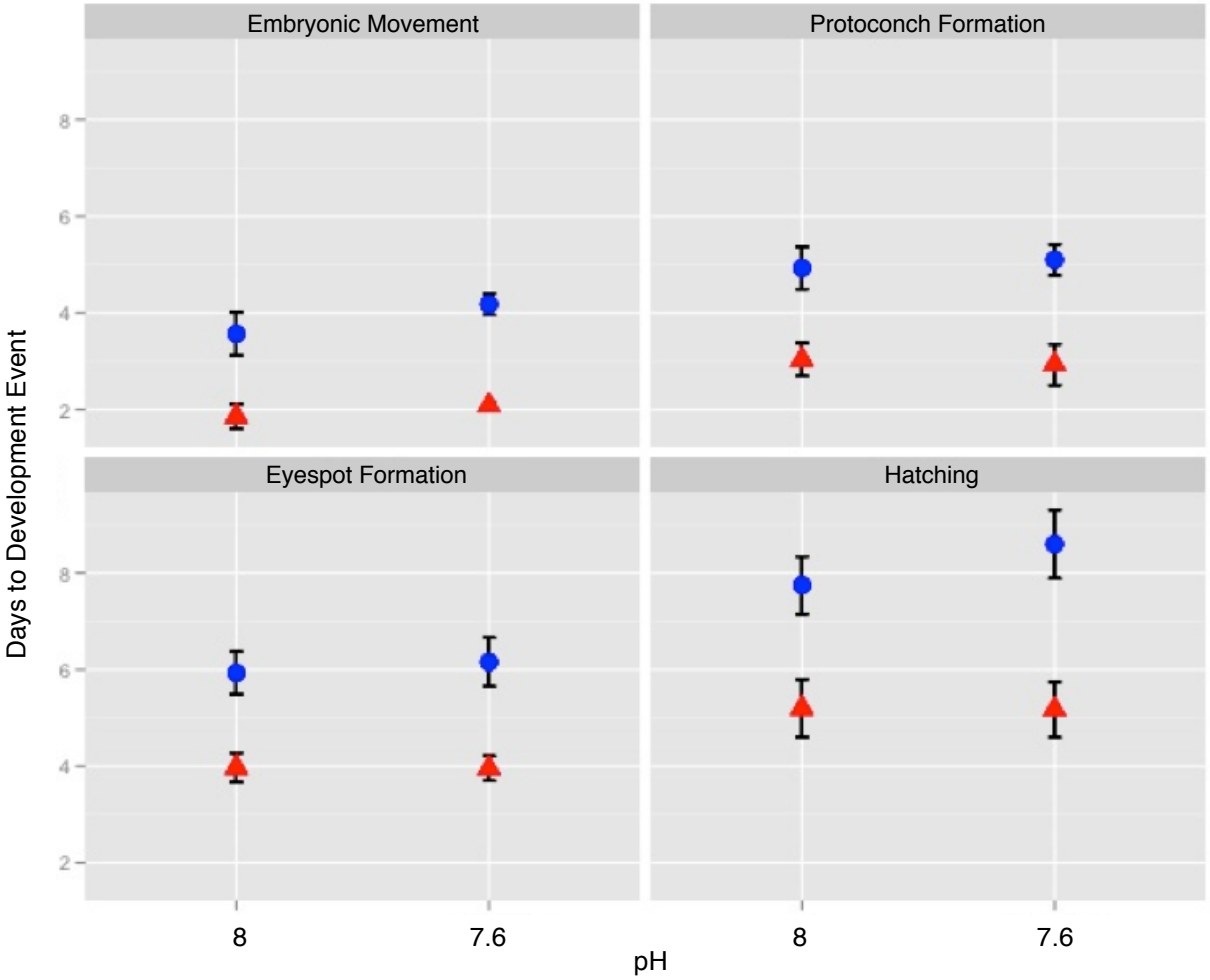

Figure 5: *Stylocheilus striatus*. The developmental rates of batches incubated in control (pH=8.02) and reduced pH (7.67) seawater at both control (27°C) and increased (31°C) temperature treatments. Values represent the mean of the number days to reach each developmental stage (embryonic movement, protoconch formation, eyespot formation, hatching) within each of the four seawater treatments. ● : control temperature (27°C) ▲ : increased temperature (31°C). Error bars were constructed using one standard deviation from the mean.

Parameters of the seawater carbonate system were calculated from salinity, temperature, A_T and pH_T using the R package seacarb (Lavigne and Gattuso 2011) (Appendix D).

RESULTS

Description of egg masses

In captivity, *S. striatus* egg clutches were oviposited to both the fronds and stems of the algae *Sargassum spp.* *S. striatus* egg clutches are produced as a continuous, 1.0 mm wide in diameter egg ribbon averaging 6 cm in length (Fig. 4). Each mm of the ribbon contains approximately 145 round, 0.15 mm wide capsules, each holding between 1-4 eggs. Only one embryo developed per egg capsule. Egg ribbons were initially pale yellow in color and slowly changed to pale amber before veligers hatch from the ribbon. Incubated in untreated seawater conditions, an average of about 8,500 veligers hatched per egg batch after a 7-9 day duration of development.

Embryonic development

Both increased temperature and decreased pH treatments had significant effects on the embryonic development of *S. striatus* (Appendix D, Fig. 5). Increased temperature treatment (31±1°C) significantly decreased the time to reach each of the embryonic stages as well as the total development duration before hatching.

At ambient seawater temperature (27±1°C), acidified seawater slowed the embryonic development of *S. striatus* (Fig.5). Total development duration before hatching as well as the number of days before embryonic movement was observed were both significantly greater in acidified treatments than control treatments. Although embryos in egg batches treated with acidified seawater trended to take longer to clearly develop both a protoconch and eyespots than control batches, differences between the two were not statistically significant.

At elevated temperature treatments, acidification did not decrease developmental rates as was observed at ambient temperature. There was significant interaction between reduced pH and elevated temperature treatments in two of the development variables: the total duration of embryonic development and the time to observe embryonic movement.

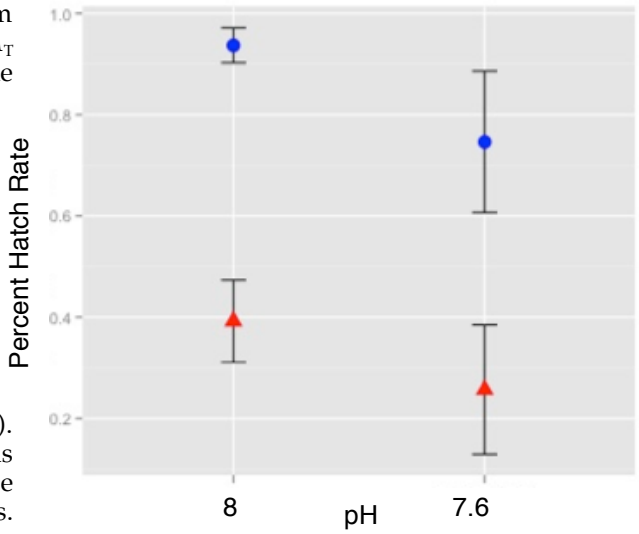

Figure 6: *Stylocheilus striatus*. Percent hatch rate batches incubated in control (pH=8.02) and reduced pH (7.67) seawater at both control (27°C) and increased (31°C) temperature treatments. Values represent the mean percent of embryos that hatched out of each batch as a percentage of the total number of eggs in that batch. : control temperature (27°C) : increased temperature (31°C). Error bars were constructed using one standard deviation from the mean.

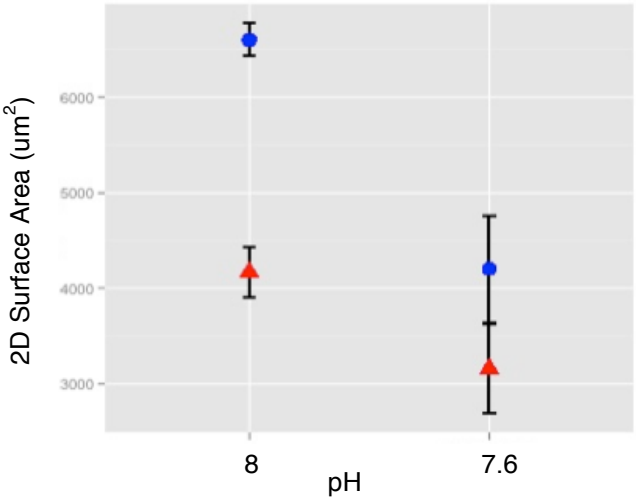

Figure 8: *Stylocheilus striatus*. The 2-dimensional lateral protoconch surface area of hatchlings from batches incubated in control (pH=8.02) and reduced pH (pH=7.67) seawater at both control (27°C) and increased (31°C) temperature treatments. Values represent the mean 2-dimensional lateral protoconch surface area of 10 individuals within each batch, averaged across all batches within each of the four seawater treatments. : control temperature (27°C) : increased temperature (31°C). Error bars were constructed using one standard deviation from the mean.

Hatching success and embryo viability

There was a clear effect of both acidified seawater and increased seawater temperature on the viability of *S. striatus* embryos (Appendix B). The cumulative number of unviable embryos was significantly higher in heated treatments when compared to batches incubated at ambient temperature (Appendix D; Fig. 6). The percentage of viable embryos was also significantly lower in batches incubated in acidified seawater when compared to batches incubated in control seawater. There was not significant interaction between increased temperature and reduced pH treatments.

Veliger morphology

All three measured morphological characteristics of the hatchling shells were significantly affected by reduced pH and elevated temperature treatments (Appendix D; Fig. 7; Fig. 8). The lateral shell length, lateral shell width (spiral height) and lateral 2-

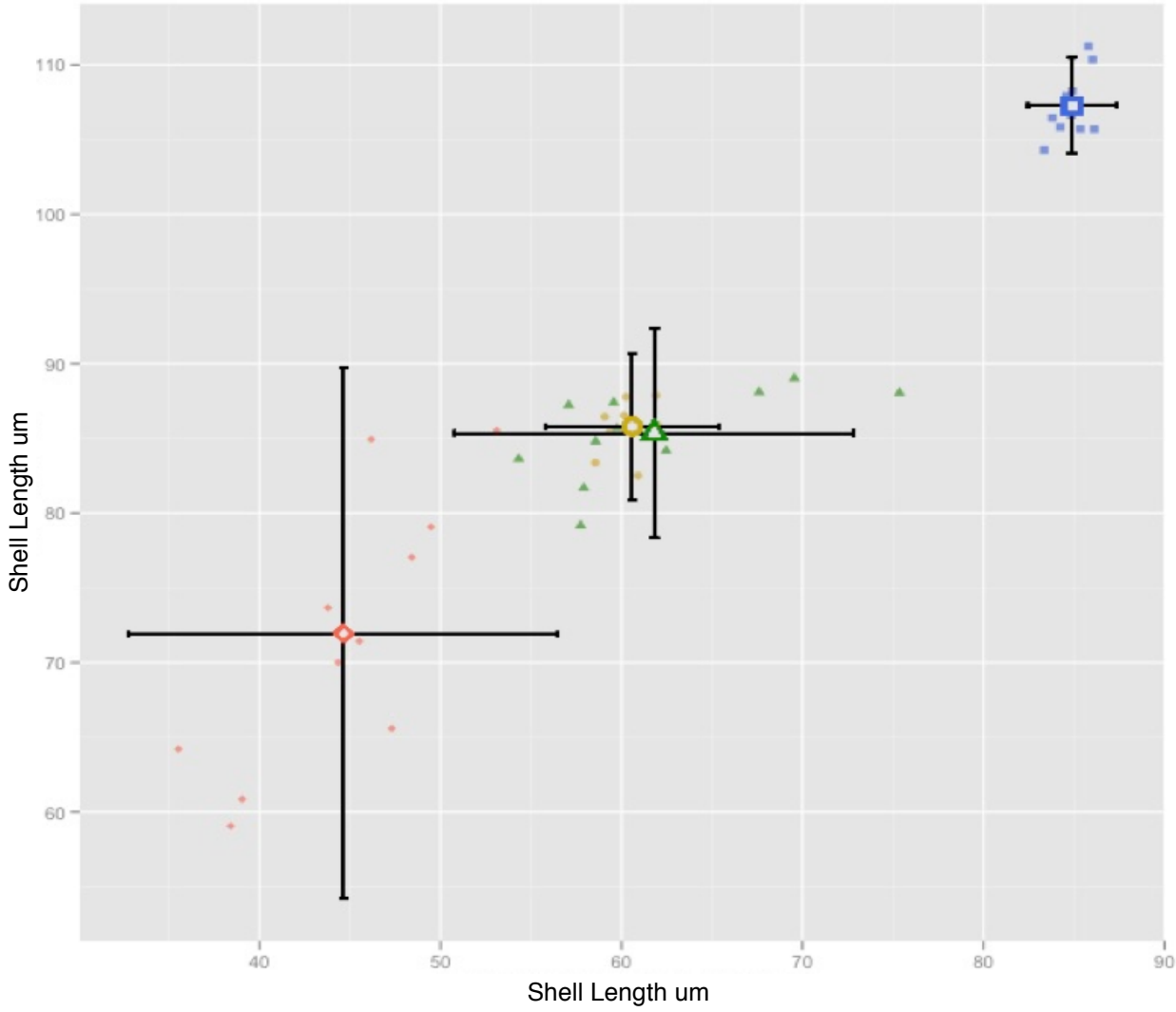

Figure 7: *Stylocheilus striatus*. The lateral shell length and width (spiral height) of hatchlings from batches incubated in control (pH=8.02) and reduced pH (pH=7.67) seawater at both control (27°C) and increased (31°C) temperature treatments. Solid points represent the mean lateral shell length and width averaged across 10 individuals within each batch for each of the four seawater treatments. Hollow points represent the mean lateral shell length and width averaged across all batches within each of the four seawater treatments. ▫ control treatment (pH=8.02, T=27°C) ○ high-temperature treatment (pH=8.02, T=31°C) △ acidified treatment (pH=7.67, T=27°C) ◇ acidified high-temperature treatment (pH=7.67, T=31°C). Error bars were constructed using one standard deviation from the mean.

dimensional shell surface area were all significantly reduced in both decreased pH and elevated temperature treatments (Appendix C). Reduced pH treatment (at both ambient and elevated temperatures) afforded a marked increase in variance among replicates (egg batches) in all three morphological characteristics.

DISCUSSION

Embryonic development

Both acidification and increased temperature significantly affected development duration of *S. Striatus* embryos. The overall embryonic development time of embryos increased in acidification seawater, supporting research showing that larval development is suppressed under reduced pH conditions in other gastropods, in sea urchins and in oysters (Kurihara and Shirayama 2004, Kurihara et al. 2007, Ellis et al. 2009). There was significant interaction, however, between pH and temperature—total development time substantially decreased under acidified high-temperature treatment to rates comparable to high-temperature treatment at ambient pH. This finding both reiterates the results of past studies that identified temperature over acidification as the dominant stressor on embryonic development rates, as well as emphasizes the necessity to consider both major oceanic changes, warming and acidification, in the investigation of life history responses to climate change (Nguyen et al. 2012).

Hatching success and embryo viability

Both warming and acidification procured significant declines in the hatching ability of treated embryos. While the percentage of embryos that hatched into veligers significantly decreased under both acidified and high-temperature conditions, temperature changes had more than twice the hatch rate abatement induced by acidification (<40% and 20% decreases in percent hatch rate, respectively, compared to control treatments). The adverse effects of acidification and temperature elevation were independent and additive—hatch rates were reduced by <70% under acidified high-temperature conditions.

Moreover, although morphological measures were applied to estimate effects of seawater treatments on hatched veligers, potential disparities in larval fitness and/or survivability were not investigated in this study; consequently, the detrimental effects of elevated temperature and acidification on embryonic viability observed therein may be seriously understated.

Veliger morphology

Veligers that hatched from treated seawater conditions exhibited significant alterations in every protoconch characteristic employed in this study as a proxy for veliger morphology. Both acidification and temperature increases significantly reduced larval shell size. Their effects were synergistic, causing greater decreases in shell size with significant interaction in acidified high-temperature treatments. Additionally, there were observable deformities in the shell morphology of hatchlings incubated in decreased pH treatment. Observations were similar to the results of previous studies that report highly elongated larval gastropod shells—the relative spiral height being shorter and the lateral shell length longer when compared to non-acidified treatments (Ellis et al. 2009). Furthermore, these deformities were substantially exacerbated by elevated temperature, increasing both the degree of deformity as well as variation in abnormality and its intensity. This supports the synergistic interaction of temperature and acidification in the inhibition of gastropod shell deposition.

Conclusion

The potential for acclimation or adaptation of taxa are important considerations when assessing the vulnerability of organisms to climate change (Munday et al. 2009). Although the potential for the vast majority of marine organisms to adapt to the rapid changes associated with climate change has not been extensively studied, oceanic pH and SST have changed little over the past 650,000 years (Kleypas, 2006, Fabry et al. 2008, Hoegh-Guldberg et al. 2011). Because increases in atmospheric-CO_2 are causing changes at a rate more than 100 times faster than at any point within that timeframe, it is highly unlikely that many marine species possess the genetic variation required to adapt to these changes at the comparable rates necessary to retain their current viability (Kleypas, 2006, Fabry et al. 2008, Munday et al. 2009, Hoegh-Guldberg et al. 2011).

In conclusion, this study clearly demonstrates that future oceanic conditions congruent with climate change projections have detrimental effects on the developmental success of encapsulated gastropod embryos, and thereby potentially decrease their viability as pelagic larvae. Early larval stages in gastropods are critically important and may serve as a bottleneck for both their population numbers and distribution patterns (Harley et al. 2006, Fabry et al. 2008, Brierley and Kingsford 2009). It is very unlikely that these effects are limited to gastropods, and if we extrapolate said reductions in dispersal capacity and larval survivability to other calcifying organisms, they potentiate severe detriments to marine biodiversity as well as the extirpation of marine ecosystems as they currently exist (Edwards and Richardson 2004, Brierley and Kingsford 2009, Hofmann and Todgham 2010).

Thus, results from this study further substantiate the mounting concern for marine ecosystems and their fate in the face of oceanic changes induced by anthropogenic increases in atmospheric CO_2. As such, and in order to mitigate the extent of marine bios degradation by these processes, I believe the results of this study reiterate the importance of two modes of action and exacts their administration: 1) research efforts to further delineate the potential effects of climate change on oceanic flora and fauna. 2) congruent integration of this growing knowledge into policy and action instantiated to reduce CO_2 emissions, stabilize the atmospheric concentration of CO_2, and limit the extent of future ocean acidification and global warming as well as its consequent effects on marine biota.

Future Research

While this study has demonstrated that the predicted increases in SST and oceanic pH within this century will have considerable deleterious impacts on the embryology of *S. Striatus*, many questions regarding the mechanisms of these effects remain unanswered. How observed larval deformities will affect the viability of *S. striatus* and its ecology is not known; such information would provide invaluable in linking the embryological detriments observed in this study to the organism's life history, ecology and ecosystemal significances. I have three suggestions for future research directions that would be especially informative in the context of this study: 1) Because of the limitations of this study, only the highest predicted changes in both SST and oceanic pH were applied to egg batches. Investigating the specific responses of *S. striatus* embryos to gradual increases in both temperature and acidity may provide more relevant information in regard to the effect climate change over time. 2) For this study, seawater treatments were only applied to egg batches post-oviposition and not to adults selected for mating. Studies that acclimate adults before mating and/or treat sample populations over multiple generation times may better predict the adaptability these organisms and their larval fate in the face of rapid climate change. 3) Past studies have also found significant effects of increased temperature and oceanic hydrogen-ion concentration on the physiology of gastropod embryos. Moreover, physiological effects associated with either factor may exacerbate the morphological detriments observed in this study, and are of key importance in regard to understanding the effects of climate change on the viability of calcifying organisms.

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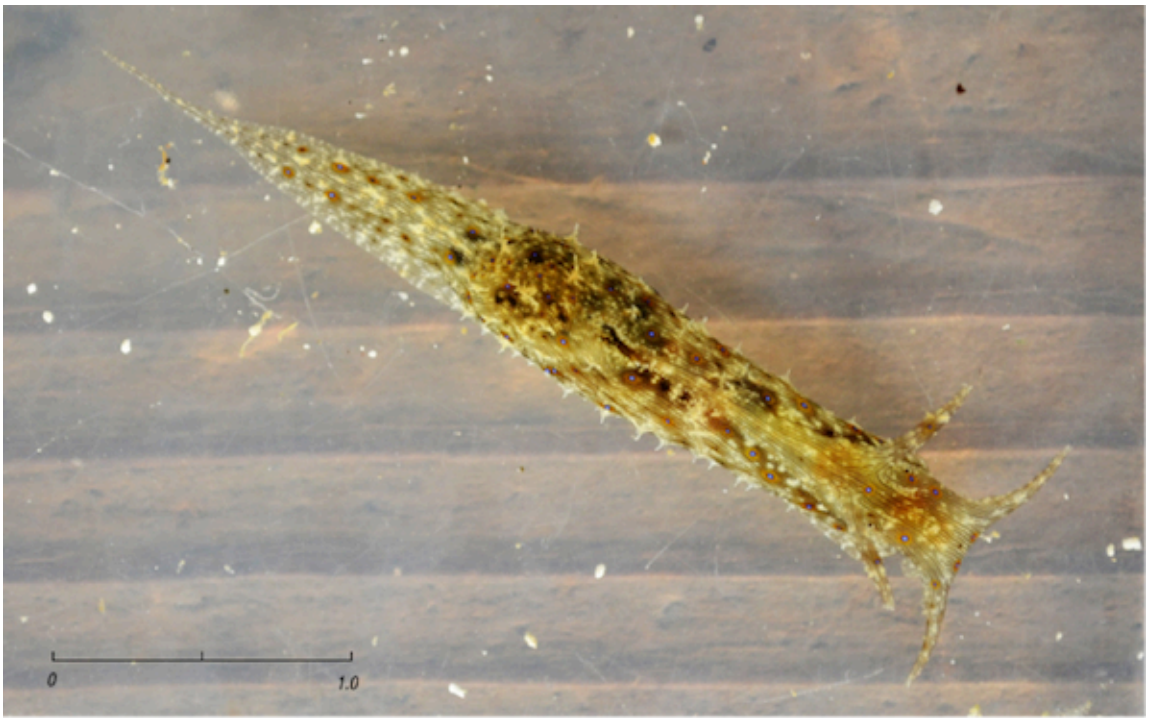
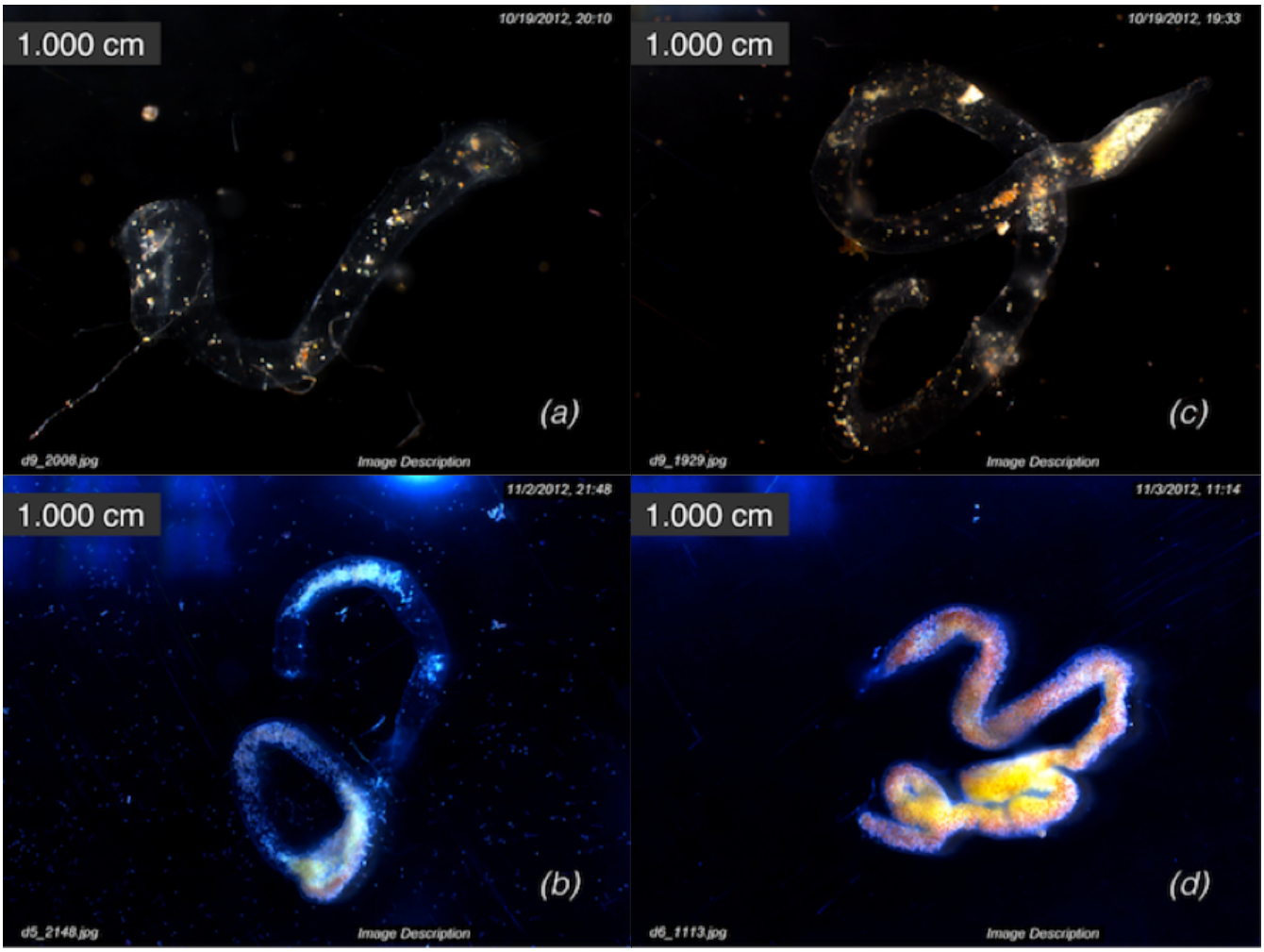
APPENDIX A

Adult *Stylocheilus striatus* collected on September 19, 2012— post three weeks acclimation in captivity and pre induction of mating/oviposition for experimental purposes. Scale bar = 1.0 cm



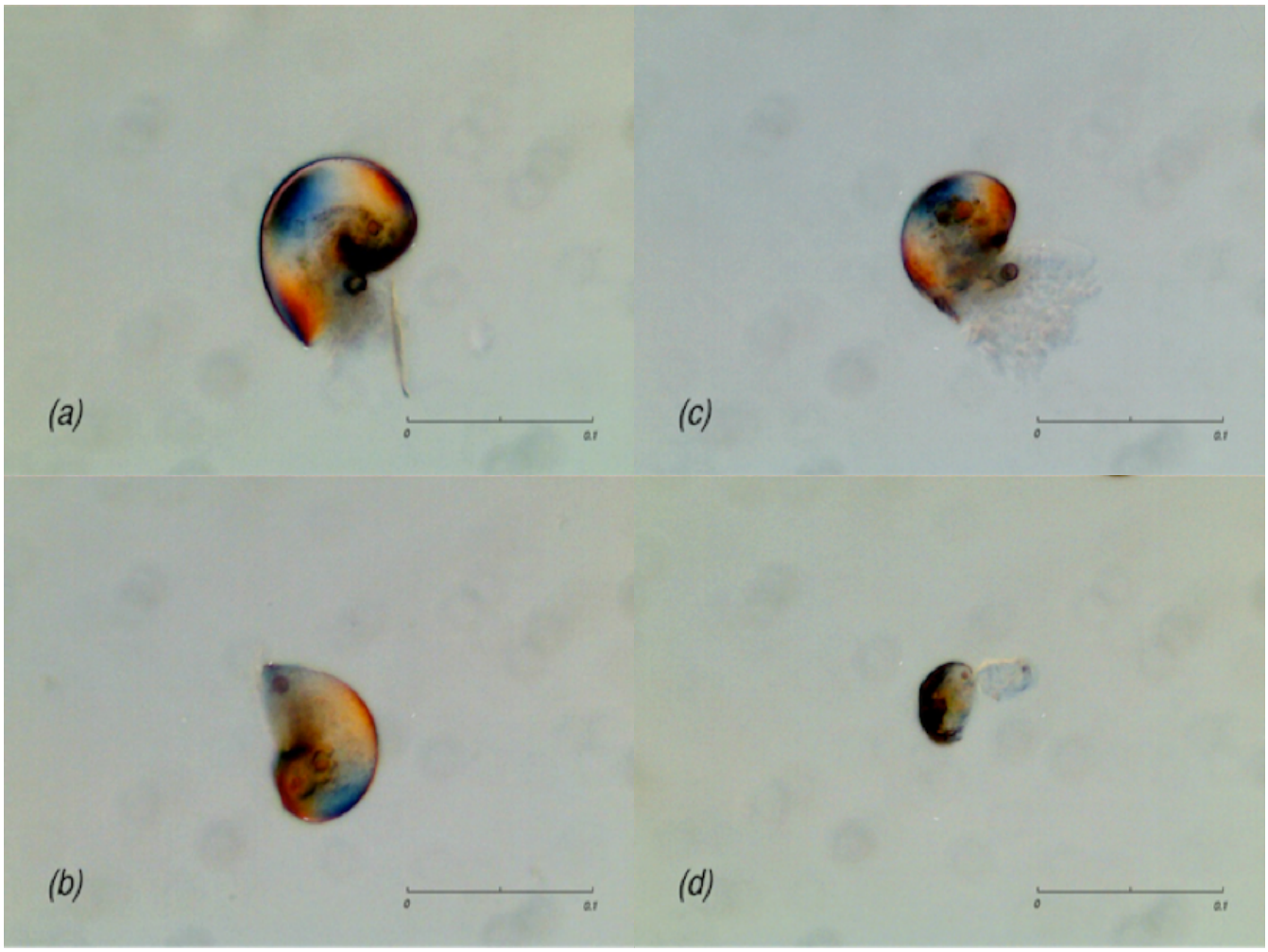
APPENDIX B

Images of *Stylocheilus striatus* egg batches incubated in control (pH=8.02) and reduced pH (pH=7.67) seawater at both control (27°C) and increased (31°C)— egg batches were imaged 48 hours post-hatching event: *(a)* control treatment (pH=8.02, T=27°C), *(b)* high-temperature treatment (pH=8.02, T=31°C), *(c)* acidified treatment (pH=7.67, T=27°C), *(d)* acidified high-temperature treatment (pH=7.67, T=31°C). Scale bar = 1.00 cm

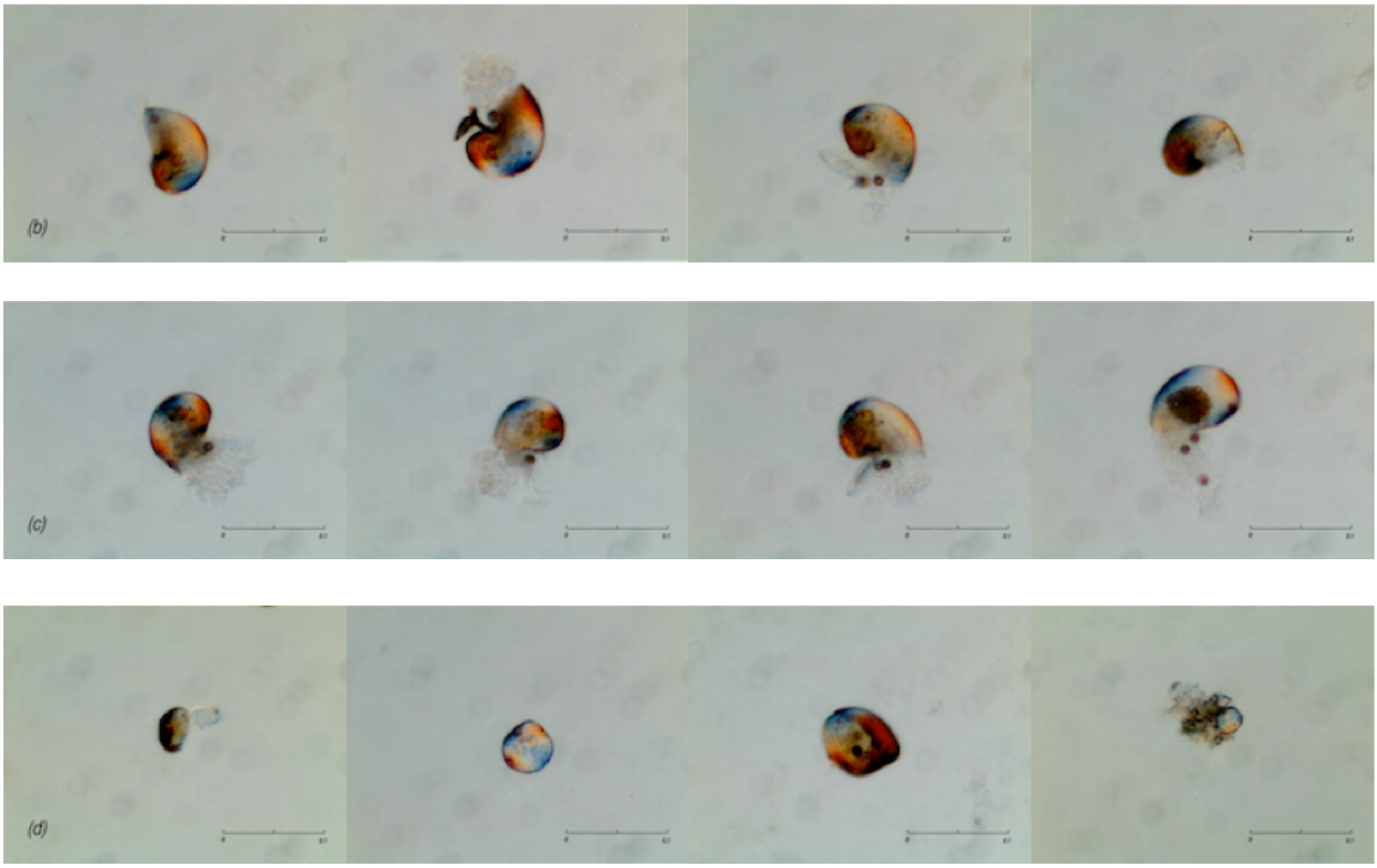


APPENDIX C

Images of *Stylocheilus striatus* hatchlings incubated in control (pH=8.02) and reduced pH (pH=7.67) seawater at both ambient (27°C) and increased (31°C) temperature seawater treatments displaying disparities both between all four treatment groups as well as among groups under treated seawater conditions—veligers imaged after being frozen at -20°C and thawed for analysis: *(a)* control treatment (pH=8.02, T=27°C), *(b)* high-temperature treatment (pH=8.02, T=31°C), *(c)* acidified treatment (pH=7.67, T=27°C), *(d)* acidified high-temperature treatment (pH=7.67, T=31°C). Scale bar = 0.1 mm.



APPENDIX C



APPENDIX D

Results of statistical analyses. All response variables were analyzed by two-way analysis of variance under pH and temperature parameters. *Indicates statistical significance.

Response Variable	Source	DF	Sum of Squares	F Ratio	Prob > F
Days to Movement	Temperature	1	16.051221	200.6669	<0.0001*
	pH	1	2.077366	25.9705	<0.0001*
	Temperature*pH	1	0.414584	5.1830	0.0282*
Days to Protoconch	Temperature	1	19.831067	134.226	<0.0001*
	pH	1	0.155602	1.0532	0.3109
	Temperature*pH	1	0.198348	1.3425	0.2535
Days to Eyespot	Temperature	1	21.041283	139.1846	<0.0001*
	pH	1	0.282888	1.8713	0.1790
	Temperature*pH	1	0.166259	1.0998	0.3006
Days to Hatch	Temperature	1	35.654439	92.0709	<0.0001*
	pH	1	3.980468	10.2788	0.0026*
	Temperature*pH	1	2.126477	5.4912	0.0242*
% Viable Embryos	Temperature	1	1.6327447	149.6732	<0.0001*
	pH	1	0.2000862	18.3418	0.0001*
	Temperature*pH	1	0.0085099	0.7801	0.3824
Lateral Shell Length	Temperature	1	2535.5713	102.5883	<0.0001*
	pH	1	2638.2368	106.7421	<0.0001*
	Temperature*pH	1	178.7044	7.2302	0.0104*
Lateral Shell Width (Spiral Height)	Temperature	1	3249.4577	184.5203	<0.0001*
	pH	1	2931.9363	166.4898	<0.0001*
	Temperature*pH	1	140.0651	7.9536	0.0074*
2D-Lateral Shell Area	Temperature	1	32519886	190.8857	<0.0001*
	pH	1	31516191	184.9942	<0.0001*
	Temperature*pH	1	5288329	31.0415	<0.0001*

APPENDIX E

Abiotic seawater treatment conditions either measured or calculated during experiments. Parameters of the seawater carbonate system were calculated from salinity, temperature, A_T and pH_T using the R package seacarb (Lavigne and Gattuso 2011).

	VARIABLE	AMBIENT pH ± SD	LOW pH ± SD
Measured	pH	8.02 ±0.06	7.67 ±0.06
	Ambient Temperature	27±0.5	27±0.5
	Increased Temperature	31±0.5	31±0.5
	Salinity	36 ±0.9	35.7 ±1.0
	Alkalinity (µmol Kg^-)	2354.8 ±98	2337.9 ±105
Calculated[1]	pCO_2 (µatm)	420.89 ±105	1113.69 ±199
	DIC (µmol Kg^-)	2033.7 ±95	2200.1 ±104
	HCO_3^- (µmol Kg^-)	1796.4 ±122	2056.01 ±124
	CO_3^- (µmol Kg^-)	225.9 ±14	114.5 ±9