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The interactive effects of acidification, temperature stress, and food supply on the growth and survival of the forage fish, *Menidia beryllina* and *Cyprinodon variegatus*

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Abstract of the Thesis

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Abstract:

The combustion of fossil fuels is increasing atmospheric CO_2 concentrations and, in turn, acidifying and warming world oceans. A further consequence of warming oceans may be declines in the abundance of plankton. Some of these changes can already be observed in coastal oceans and may affect marine biota including forage fish which play a key role in marine food webs as key consumers of primary production and prey of fishery species. While these climate change stressors have previously been shown to negatively affect the performance of larval fish, the interactive effects of these multiple stressors on fish have yet to be explored. This thesis presents a series of experiments that examined the effects of changing temperature, pCO_2, and food levels

on the growth and survival of early life (embryo and larval) stages of two species of forage fish indigenous to the Northwest Atlantic Ocean, *Menidia beryllina* and *Cyprinodon variegatus*. These two fish species are a useful comparison as they lay pelagic and demersal eggs, respectively, and have been previously shown to be vulnerable and resistant to ocean acidification, respectively. Temperature had the strongest effect on the hatching rate, hatching success, survival, and growth of both fish species with higher temperatures yielding more rapid hatching and higher and lower temperatures leading to reduced growth and survival. In contrast, pCO_2 and food levels had no effects on hatching, but elevated pCO_2 and reduced food supplies significantly reduced the survival of larval *M. beryllina*. There were synergistically negative effects of elevated temperature and elevated pCO_2 as well as low food supply and elevated pCO_2 on larval fish. For example, larvae that were resistant to high pCO_2 experienced elevated mortality when high pCO_2 was experienced outside their thermal optimum. In other cases, elevated pCO_2 resulted in smaller larvae at high, but not optimal, temperatures. Furthermore, larvae that were fed *ad libitum* were resistant to high pCO_2 but experienced elevated mortality when exposed to high pCO_2 and given a restricted diet. Such interactions evidence the importance of simultaneously considering effects of multiple stressors on larval fish rather than their individual effects as these outcomes could not have been predicted by observing each stressor individually and these stressors co-occur in estuaries. Collectively, this thesis demonstrates that the effects of multiple climate change stressors may interact to synergistically suppress the productivity of fisheries shallow coastal ecosystems. It is anticipated these effects will intensify through this century due to the intensification of climate change.

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Introduction:

Climate change is altering multiple aspects of world oceans (Poloczanska et al. 2013). Since the beginning of the industrial revolution atmospheric carbon dioxide levels have progressively risen from ~280 parts per million (ppm) to 410 ppm in May of 2016 (http://scrippsCO2.ucsd.edu/). Concurrently, global temperatures have risen 1°C during the past century and are expected to rise up to 5°C this century (I.P.C.C. 2014). The world oceans are the only significant net sink for atmospheric CO_2 on the planet having absorbed about one third of all anthropogenic CO_2 produced to date (Sabine et al. 2004). As CO_2 enters the ocean it reacts with water to form carbonic acid (H_2CO_3), which quickly disassociates into bicarbonate (HCO_3) releasing a hydrogen ion (H+) that reduces ocean pH and subsequently sequesters carbonate ions, a process commonly known as ocean acidification (Sabine et al. 2004). Since the Industrial Revolution, ocean pH has decreased 0.1 units and it is expected that pH will decrease by another 0.3 - 0.4 units by the year 2100 (Orr et al. 2005) simultaneously causing a 150% decrease in carbonate ion availability.

In addition to future acidification of global oceans caused by the accumulation of anthropogenic CO_2, some coastal systems can experience seasonal and local acidification caused by high levels of microbial respiration (Cai et al. 2011). For example, many estuaries experience extreme acidification (pH<7.2) and high levels of pCO_2 (>2,000 µatm) during summer months (Melzner et al. 2012, Baumann et al. 2014, Wallace et al. 2014). This acidification coincides with the spawning periods of many fish, bivalves, and crustaceans in temperate ecosystems (Kennedy and Krantz 1982, Sherman et al. 1984, Helluy and Beltz 1991) and thus could have negative implications for fisheries whose early life stages are sensitive to acidification (Baumann et al. 2012, Murray et al. 2014).

Declines in carbonate concentrations associated with acidification have been shown to have negative consequences for organisms that synthesize $CaCO_3$ shells or exoskeletons (Doney et al. 2009, Kroeker et al. 2010, Kroeker et al. 2013). For example, acidification negatively affects the growth and survival of the bivalve larvae as well as their ability to form a shell (Talmage and Gobler 2010, Gazeau et al. 2013, Waldbusser et al. 2013). Even organisms with shells only partially comprised of calcium carbonate such as larval crabs can experience reduced survival when exposed to acidification during development (Walther et al. 2010, Long et al. 2013).

While a large number of studies have demonstrated the negative effects of acidification on externally calcifying organisms, careful study of acidification effects on internally calcifying organisms such as marine fish has been comparatively less common. Multiple studies have demonstrated that the ability of some fish to sense predators, locate suitable habitat, and exhibit normally predictable lateralization can all be altered by acidification (Pimentel et al. 2016, Silva et al. 2016) due to interference within the acid-base regulation of GABA-A neural receptors (Munday et al. 2009, Nilsson et al. 2012). It has been suggested that the earliest stages of life for fish, eggs thru larvae, are the most sensitive to ocean acidification (Luckenbach et al. 2001, Ishimatsu et al. 2008, Baumann et al. 2012). Some studies have shown that fish eggs and larvae exposed to low pH suffer declining growth and survival (Baumann et al. 2012, Miller et al. 2012, Chambers et al. 2014, Murray et al. 2014, DePasquale et al. 2015). Others have shown that larvae grown under low pH develop deformed internal organs and improper physiological structure, impairing their ability to swim and function (Frommel et al. 2011, Pimentel et al. 2014). When combined with other stressors such as hypoxia and thermal stress, acidification can both additively and synergistically reduce the survival rate of larvae fish (DePasquale et al. 2015).

Temperature has profound effects on fish populations (Munch and Conover 2002, Munch and Conover 2003, Bian et al. 2015). Many fish populations are shifting poleward or into deeper waters to maintain their preferred thermal niche as water temperatures rise, changes that can influence the productivity of regional fisheries (Perry et al. 2005, Nye et al. 2009, Pinsky et al. 2013). Temperature can have particularly strong effects on fish embryos by controlling the hatching rates with warmer temperatures typically facilitating more rapid hatch times (Gillooly et al. 2002, Martell et al. 2005). For some species of fish, eggs that are exposed to warmer waters develop faster thus shortening the distance traveled within the currents prior to hatching decreasing egg mobility and recruitment in historically suitable areas. Furthermore, eggs incubated at temperatures warmer or colder than optimal for a given fish species can led to decreased hatch rates and yolks that are less nutritious for larvae (Bobe and Labbe 2010). Incubation of fish eggs at sub-optimal temperatures can also result in larvae hatching before embryonic development is complete, enhancing to the mortality of newly hatched larvae (Kucharczyk et al. 1997).

Food supplies for fish can be altered by climate change processes and climate change-induced reductions in food availability can make marine organisms more vulnerable to climate change stressors (Melzner et al. 2011, Pansch et al. 2014, Ramajo et al. 2016). Warming of world oceans during the past century has enhanced water column stratification and, in turn, led to reduced primary productivity and lowered planktonic food availability (Roemmich and McGowan 1995, Behrenfeld et al. 2006, Boyce et al. 2010). Warming has also been shown to alter food availability within specific ecosystems; for example, in the North Sea warmer waters have both increased the metabolism of cod while causing reductions in the size of their primary food source (Beaugrand et al. 2003). Ocean warming has also been shown contribute toward lowered food availability for most post larval zooplanktivores (Roemmich and McGowan 1995, Doney et al. 2009, Piontkovski

and Castellani 2009) including forage fish. Additionally, changes in seawater chemistry or temperature can result in match-mismatch scenarios that separate predators and prey over time during important feeding periods (Durant et al. 2007, Parry et al. 2007, Siddon et al. 2013). Prior research has demonstrated that the susceptibility of marine invertebrates to acidification can be dependent on their food supply (Melzner et al. 2011, Pansch et al. 2014, Ramajo et al. 2016) likely due to smaller energy reserves being available to overcome the stress of acidification during food restriction (Pörtner and Farrell 2008). While similar effects might be expected for fish experiencing acidification, this has not been studied to date.

Forage fish are small pelagic fish that feed at or near the base of marine food webs and are often preyed upon by larger, commercially important fisheries species making them an important component of marine ecosystems. It has been estimated that forage fish contribute 16.9 billion dollars (USD) to the global fisheries economy (Pikitch et al. 2014). Forage fish are one of the few direct links between primary producers and upper trophic level fish (Conover et al. 2005) making their influence on fisheries productivity profound. Forage fish in the U.S. are generally found in coastal ecosystems (Johnson 1974) that can be highly vulnerable to both rapid warming (Nixon et al. 2004, Baumann and Doherty 2013) and acidification (Nixon et al. 2004, Baumann and Doherty 2013, Baumann et al. 2014, Wallace et al. 2014) suggesting that forage fish are likely to experience these stressors simultaneously. A recent study has shown the synergistic effect of temperature and acidification on predation in reef fish combined with antagonistic effects on predatory selectivity (Ferrari et al. 2015) emphasizing the importance of species-specific studies of combined stressors. Further, like other fish (Nye et al. 2009), some forage fish may be shifting their habitat pole-ward, a change that in some cases may expose these fish to differing levels of temperature and/or pH as well as changes in the timing of local production.

The objective for this thesis was to assess the individual and combined effects of varying levels of pCO_2, temperatures, and food supply on egg incubation time, hatch rate, survival, and growth of larval forage fish including *Menidia beryllina* (inland silverside) and *Cyprinodon variegatus* (sheepshead minnow). These two fish species are a useful comparison as they lay pelagic and demersal eggs, respectively (Able, 1994), and have been previously shown to be vulnerable and resistant to ocean acidification alone, respectively (DePasquale et al., 2015). Through manipulation of pCO_2, temperature, and food quantity this thesis demonstrates that changing these climate change-related environmental conditions can cause physiological stress, thermal stress, and additive physiological impairments that can reduce hatch rates, depress growth rates, and an increase in the mortality of larval fish.

Methods:

Experiments were performed at the Stony Brook Southampton Marine Science Center. Fish were reared in 8-liter white plastic buckets filled with 0.2 μ m-filtered, UV sterilized seawater from eastern Shinnecock Bay, conditions that have previously yielded maximal rates of survival of *M. beryllina* and *C. variegatus* (Baumann et al. 2012, DePasquale et al. 2015). These forage fish contrast in their biogeography, reproductive strategies, and preferred estuarine habitats. *M. beryllina's* geographic range spans from Northern Florida to the Gulf of St. Lawrence (Conover et al. 2005) whereas *C. variegatus* have been found in waters from Venezuela to Maine as well as the West Indies (Bennett 1997). Spawning occurs for both species in salt marshes and estuaries with *M. beryllina* depositing eggs on algae and grasses, while *C. variegatus* deposits its eggs on the sea floor. *M. beryllina* live slightly beyond one year; maturing during their first summer, overwintering off shore, and returning in the spring to spawn over the course of one month in the

spring. Spawning is timed around the optimal temperature (24°C) for both the growth of larvae and their food (Lambert 1984). *C. variegatus* can live up to three years entirely in estuaries, burying themselves in the muds during winter (Bennett 1997) and spawning almost continuously; potentially to take advantage of environmental variability a lack of food for larger cohorts and to protect larvae from predation (Lambert 1984).

Experiments were conducted with four replicates per treatment and two levels of CO_2 (Ambient: ~400 µatm and elevated: ~2000 µatm). Two or four temperatures were examined to contrast the optimal temperature for each species (EPA 1978) with temperatures cooler and/or warmer than optimal (Doney et al. 2009, Feely et al. 2010, Gruber et al. 2012). Experimental vessels were maintained in water baths using electronically controlled heat exchangers (Aquatic Eco-systems, Inc., Florida, USA) to maintain individual target temperatures. Temperatures were continuously monitored with *in situ* data loggers (Onset©) and remained within <1°C of target values. Gas proportionators (Cole Parmer flowmeter system) were used to deliver CO_2 gas and ambient air to the experimental buckets at rates that provided desired experimental pCO_2 levels. The control (~400 µatm pCO_2) treatment was attained via bubbling with ambient air whereas the elevated pCO_2 (~2,000 µatm pCO_2) treatment was achieved by bubbling water with a mixture of 5% CO_2 and ambient air (Talmage 2009, Talmage and Gobler 2010, 2011). Each bucket was bubbled via an aquaculture glass-bonded silica air stone attached to a plastic (Tygon) hose that supplied one of the two gas mixtures. All buckets, hoses, and stones were soaked in 10% HCl and liberally rinsed with deionized water prior to use discourage microbial contamination. All experiments were allowed to bubble for one day prior to adding fish eggs in order to achieve stable experimental chemistry (described below).

Once experimental temperature and pCO_2 levels were stable, 80 eggs ≤ 36 h post fertilization (described below) were added to each experimental vessel. *Menidia beryllina* and *Cyprinodon variegatus* eggs were obtained 24 h post-fertilization from broodstock of hundreds of wild fish of each species (Aquatic Research Organisms, Hampton, New Hampshire (Baumann et al. 2012, DePasquale et al. 2015). During the course of experiments, the number of eggs and/or larvae present per experimental vessel was recorded daily along with levels of pH and temperature. For the first five days following hatching, fish larvae were fed a diet of the rotifer, *Brachionus plicatilis* grown on a diet of the alga, *Isochrysis galbana*, at a rate of 400 rotifers individual^{-1} d^{-1}, an amount considered *ad libitum* for fish larvae (EPA 1978). For the remainder of the experiment, fish were fed freshly hatched (<18 hours) *Artemia salina* (San Francisco strain) at a rate of 100 *Artemia* individual^{-1} d^{-1}, again an amount considered *ad libitum* (EPA 1978). A second round of experiments were performed in which the food supply to some of the hatched fish was 20% of optimal levels (EPA 1978). A 50% water change was performed every other day once feeding of the larvae began. Experiments were conducted for at least 10 days post hatch to capture the most vulnerable early life period for larval fish (Murray et al. 2014). A sub-sample of larvae was collected at the conclusion of each experiment and preserved in 10% buffered formalin for length measurements made via digital imaging, a dissecting microscope, and ImageJ software. After 48 h, formalin-preserved fish were transferred to 10% ethanol to minimize degradation of tissues.

Levels of pH in experimental vessels were measured daily with a Honeywell Durafet Ion Sensitive Field Effect Transistor (ISFET)-based pH sensor calibrated with a seawater pH standard (Dickson 1993). Discrete samples were spectrophotometrically analyzed for pH utilizing the dye, m-cresol purple (Dickson 2007) and results were consistent with sensor-based measurements. Concentrations of total dissolved inorganic carbon (DIC) in experimental treatments were

quantified both before and after experiments using an infrared-based Environmental Gas Analyzer (EGM-4, PP Systems) calibrated with sodium bicarbonate standards and quality controlled via the analysis of certified reference material for DIC (University of California San Diego, Scripps Institution of Oceanography certified reference material for DIC, Batches 132-147) which provided 105 ± 5% recoveries. Levels of pCO_2 were calculated based on measured levels of DIC, pH (mol kg seawater^{-1}), temperature, salinity, phosphate, silicate, and known first and second dissociation constants of carbonic acid in seawater (Millero 2010) using the program CO2SYS.

Two-way Analysis of Variances (ANOVA) were employed when temperature and pCO_2 or pCO_2 and food supply were the main treatment effects. A three-way ANOVA was used when temperature, food supply, and pCO_2 were the main treatment effects. Differences among levels within a treatment were assessed using the post-hoc Holm-Sidak multiple comparison test. Variables assessed via ANOVAs included hatch time (the time for all eggs in an experimental vessel to hatch), percent hatch (the percentage of embryos that hatched in an experimental vessel), percent survival (percentage eggs in an experimental vessel surviving to ~10-day post hatch), and larval length in mm. A p-value of 0.05 was used to assess statistical differences among treatments during experiments. Statistical analyses were performed using SigmaPlot™ 11.0.

Results:

Temperature and CO_2 effects on *Menidia beryllina*

Lower temperatures significantly extended the hatching time of *M. beryllina* eggs (p<0.001, ANOVA, Figure 2A, Table 2) by five days in the cooler treatment (19 ± 0.53°C) compared to the warmer treatment (29 ± 0.29°C). The hatching success in the colder treatment was 80 ± 12%, significantly lower than the warmer treatment (96 ± 3.1%; p<0.005, Two-Way

ANOVA; Figure 2B, Table 3). At ~10 days post-hatch, elevated levels of pCO_2 significantly reduced larval survival in the higher temperature treatment (29ºC) to 6.0 ± 2.0% compared to 35 ± 7.9% in the ambient treatment ($p<0.01$, Two-Way ANOVA; Figure 2C, Table 4). The fish in the colder treatment were significantly shorter (4.3 ± 0.14 mm) compared to larvae the warmer treatment (5.9 ± 0.68 mm; $p=<0.001$, Two-way ANOVA, Figure 2D, Table 5). Levels of CO_2 also significantly affected length within the 29°C treatment, with fish exposed to elevated pCO_2 being 15±0.1% smaller than in the ambient CO_2 treatment ($p<0.001$, Holm-Sidak, Figure 2). There was, however, a significant interaction between temperature and pCO_2 level as the effect of CO_2 on lengths was not present at the preferred optimal temperature of 19°C ($p<0.001$, Holm-Sidak).

In a subsequent experiment with *M. beryllina*, an expanded temperature range (20 ± 0.15°C, 23± 0.28°C, 27 ± 0.3°C, and 30 ± 0.6°C) was considered in tandem with normal and elevated levels of pCO_2 (~400 and ~2000 μatm). Temperature significantly altered the duration of time required for fish to hatch (Two-way ANOVA, Figure 4A, Table 7). Larvae in the coldest 20°C treatment took significantly longer to hatch (11.0 ± 0.0 days) compared to fish embryos incubated at 23°C (8.4 ± 1.2 days), 27°C (6.1 ± 0.4 days) and 30°C (7.3 ± 0.65 days; $p<0.001$ all, Holm-Sidak, Figure 4). Temperature had a significant effect on hatch success with the percent of embryos hatching at 20°C (61 ± 5.6%) being significantly lower than at 27°C (94 ± 0.9%; $p<0.001$), the percent hatching at 23°C (67% ± 1.0) being significantly lower than at 27°C ($p<0.01$), and the percent hatching at 20°C being significantly lower than at 30° (85% ± 4.3; $p<0.01$, Holm-Sidak, Figure 4B, Table 8). Temperature significantly altered the 10 day post-hatch survival of larvae ($p=0.001$, Two-way ANOVA, Figure 4C, Table 9). Survival at 27°C was 80 ± 11%, significantly higher than survival at 20ºC and the 23°C treatments (60 ± 8.6% and 71 ± 6.0% respectively; $p<0.001$, $p<0.01$ respectively, Holm-Sidak, Figure 4C). There was a significant

interaction between temperature and pCO_2 level regarding larval survival as elevated levels of pCO_2 significantly depressed larval survival only within the 20°C and the 27°C treatments with elevated pCO_2 treatments showing 54 ± 6.0% and 60 ± 18% survival, respectively, compared to 67 ± 4.1% and 87 ± 11% survival at 400 µatm pCO_2 ($p<0.05$ and $p<0.001$ respectively; Holm-Sidak, Figure 4C, Table 11). Temperature significantly altered fish lengths ($p<0.001$, Two-way ANOVA, Figure 4D, Table 10) with individuals in the 20°C treatment being 16 ± 0.07% smaller than those at 23°C, 21 ± 0.05% smaller than those at 27°C, and 18 ± 0.08% smaller than those at 30°C ($p<0.001$, Holm-Sidak, Figure 4D). The fish reared at 23°C were also 6 ± 0.07% smaller than fish at 27°C ($p<0.01$, Holm-Sidak, Figure 4D).

Diet and CO_2 effects on *Menidia beryllina*

Diet and pCO_2 levels did not alter embryo hatch time nor hatch success. Larval survival at 10 days post-hatch under optimal conditions (low pCO_2, *ad libitum* feeding) was 84 ± 5% but was significantly reduced in the elevated pCO_2, *ad libitum* fed larvae to 14 ± 13% and further reduced to 4 ± 5% in the elevated pCO_2, food limited treatment ($p<0.001$ all, Holm-Sidak, Figure 6C). While feeding rate was not a significant treatment effect for survival, there was a significant synergistic interaction between food level and pCO_2 as the percent survival in the combined treatment was significantly lower than would have been predicted by either individual variable ($p<0.05$, Two-way ANOVA, Figure 6C, Table 15). Levels of pCO_2 and diet both had a significant effect on fish length ($p<0.001$, Two-way ANOVA, Figure 6D, Table 16). Control fish (*ad libitum* food, control pCO_2 levels) were 11 ± 0.5% larger than the elevated pCO_2 treatment and were 14 ± 0.6% larger than the starved fish ($p<0.01$, Holm-Sidak, Figure 6D). There was also a significant interaction between pCO_2 and diet regarding fish lengths whereby the lengths in the combined

treatment were longer than would be been predicted by the individual treatments (p<0.05, Two-Way ANOVA, Figure 6D, Table 16).

Temperature, food, and CO_2 effects on *Menidia beryllina*

Higher temperatures significantly shortened incubation time with the 22°C averaging 9.6 ± 0.8 days to hatch compared to 6.3 ± 1.2 days at 30°C (p<0.001, Three-way ANOVA, Figure 8A, Table 18). Survival 10 days post-hatch was significantly lower (7.4 ± 14%) under the low food treatment relative to *ad libitum* feeding 52 ± 17% (p<0.001, Three-way ANOVA, Figure 8C, Table 20) and was significantly lower at higher temperatures (23 ± 7.9%) compared to lower temperatures 37 ± 15%; *(* p<0.05, Holm-Sidak, Figure 8C). Levels of pCO_2 were also a significant treatment effect as elevated CO_2 significantly reduced their survival to 44 ± 3% from 59 ± 5% in the control (p<0.001, Holm-Sidak, Figure 8C). The length of fish at the end of the experiment was significantly affected by food supply with larvae fed a limited diet being 25 ± 0.4% shorter than the fed ad libitum larvae (p<0.001, Three-way ANOVA, Figure 8D, Table 21). Temperature, food and CO_2 did not significantly alter hatch success in this experiment.

Temperature, food, and CO_2 effects on *Cyprinodon variegatus*

Temperature significantly affected hatch rate of *C. variegatus* (p<0.001, Two-way ANOVA) with fish in the warmest treatment (30°C) hatching in 5.8 ± 0.9 days, fish at 23°C hatching in 12 ± 4.1 days, and fish at 16°C hatching in 34 ± 5.9 days. (p<0.001, Holm-Sidak, Figure 10A, Table 23). Hatch success was affected by temperature (p<0.001, Two-way ANOVA) with more fish hatching at 30°C (87 ± 7.3%) than at 16°C and 23°C (59 ± 3.5% and 77 ± 7.5%, respectively; p<0.001, Holm-Sidak, Figure 10B, Table 24). Larval survival 10 days post-hatch

was significantly affected by temperature ($p<0.001$, Two-Way ANOVA, Figure 10C, Table 25). Fish grown at 16°C had the highest survival rates (94 ± 0.2%) while survival for fish at 23°C was 87 ± 0.1%, and fish at 30°C had only 38 ± 0.1% survival ($p<0.01$ and $p<0.001$, respectively, Holm-Sidak method, Figure 10C). There was a significant interaction between temperature and pCO_2 for fish survival as elevated levels of pCO_2 depressed the survival of larval *C. variegatus* at the highest and lowest temperatures only ($p<0.05$, Two-Way ANOVA, Figure 10C, Table 25). Temperature had a significant effect on fish length ($p<0.05$, Two-way ANOVA, Figure 10D, Table 26) with fish grown at 16°C being 4 ± 0.2% larger than fish in the 23°C treatment ($p=0.01$, Holm-Sidak, Figure 10D). Food was also significant within the 23°C treatments as the *ad libitum* group was 9 ± 0.4% larger than the food limited group and the *ad libitum* fish in the elevated pCO_2 treatment were 12 ± 0.1% larger than the starved elevated pCO_2 fish ($p<0.05$ and $p<0.001$, respectively, Holm-Sidak, Figure 10D).

Discussion:

Temperate estuaries represent important breeding and nursery grounds for forage fish (Pikitch et al. 2014) but are prone to elevated levels of pCO_2, temperature, and large fluctuations in plankton levels during fish spawning seasons (Nixon et al. 2004, Cai et al. 2011, Wallace et al. 2014). Climate change is expected to intensify extremes in warming and acidification in the near future (Doney et al., 2012). This study has demonstrated that changing temperatures, elevated levels of pCO_2, and reduced food supplies can act and interact to significantly reduce the survival of forage fish. These findings provide new insight regarding the understanding of how multiple stressors associated with climate change can effect fisheries.

Temperature plays a central role in the development of embryonic and larval fish (Houde 1989, Pepin 1991). Forage fish lack the ability to regulate their internal body temperature and are generally found inhabiting waters at or near the limits of their thermal niche (Sunday et al. 2012) a common trend among marine organisms. Temperature can increase or decrease enzymatic functions in organisms and thus alter nutritional requirements (Sherman et al. 1984, Kucharczyk et al. 1997, Bobe and Labbe 2010). During experiments, higher temperatures accelerated hatching time and improved hatch success of embryos, outcomes that would be beneficial on a population level for these species (Lasker 1981, Sissenwine 1984). However, these benefits were short lived as larval survival at 10 days post-hatch at elevated temperatures were the same as survival at optimal temperatures for *M. beryllina* and was significantly lower for *C. variegatus*, perhaps due to temperature-induced, accelerated larval hatching occurring before embryonic development was complete (Kucharczyk et al. 1997). The rapid shift in outcomes for fish at elevated temperatures from positive for embryos to neutral or negative at the larval stage suggests high temperatures that accelerate development results in unstainable rate of metabolism for larvae. This further suggests that outcomes for larval fish at higher temperatures could worsen at time horizons exceeding 10-days.

As ocean waters warm, the fish are migrating deeper or towards to higher latitudes (Nye et al. 2009) and in some cases could migrate to a new, cooler habitat outside of their optimal thermal range (Sunday et al. 2012). During experiments, fish developing at the coolest temperatures had decreased survival for both species and for *M. beryllina*, the fish larvae were also smaller likely due to a slower metabolism and a reduced ability to convert energy into growth (Kucharczyk et al. 1997). In contrast to *M. beryllina*, *C. variegatus* seemed to thrive in cooler temperatures with survival and length being maximal at 16°C despite an extended incubation time for eggs. This

outcome could be related to life history traits as these fish deposit eggs on the seabed (Able and Fahay 1998) where temperatures are often cooler and thus perhaps making them better adapted to lower temperatures.

Rising ocean temperatures are increasing ocean stratification, decreasing the availability of nutrients in surface oceans and in some cases, fostering plankton communities that are less abundant (Roemmich and McGowan 1995, Behrenfeld et al. 2006, Boyce et al. 2010). Such reduced plankton inventories have been shown to make some bivalves more vulnerable to ocean acidification (Melzner et al. 2011, Pansch et al. 2014, Ramajo et al. 2016). During this study, restricted food supplies increased mortality rates and resulted in smaller *M. beryllina* larvae. There were also complex interactions between food supply and pCO_2 levels for larval *M. beryllina.* In a manner somewhat consistent with prior studies of bivalves, there was a synergistic interaction between food supply and pCO_2 level for larval fish. Specifically, *M. beryllina* survival was depressed by restricted food when pCO_2 levels were elevated, but not when they were normal, suggesting that larval fish vulnerability to a restricted diet was enhanced by the additional stress of acidification. Physiologically, this is intuitive, as the stress of acidification likely enhanced the energy requirements of the larvae and makes them less tolerant of a restricted diet. In contrast to the patterns in fish survival that were synergistically suppressed by low food and high pCO_2, fish lengths that were depressed by food restriction or pCO_2 were not further depressed by both stressors perhaps due to the newly hatched fish already being at their minimal size possible for these larvae.

Larval stage fish in general (Miller et al. 2012, Chambers et al. 2014) and *M. beryllina* in particular have been shown to be sensitive to acidification (Baumann et al. 2012, DePasquale et al. 2015). During this study, increased pCO_2 reduced the numbers of *M. beryllina* larvae surviving

to 10 days in every experiment performed ($n = 4$). This 10 day mark is important in the life cycle of fish larvae as it represents the point at which larvae have transitioned from reliance on yolk and are able to utilize their internal systems to mitigate the effects of external environmental stress (Mangor-Jensen 1987, Perry and Gilmour 2006, Ishimatsu et al. 2008, Baumann et al. 2012). *M. beryllina* also exhibited shorter lengths when exposed to increased pCO_2 levels, a consequence that could lead to enhanced mortality within an ecosystem setting (Sogard 1997). Beyond the effects measured here, predator avoidance and detection of sensory cues may also be impacted by high CO_2 and could further decrease a species' ability to survive and reproduce successfully (Munday et al. 2009, Nilsson et al. 2012).

In contrast to *M. beryllina*, elevated pCO_2 alone had no effect on *C. variegatus*, a finding consistent with a prior study of this species (DePasquale et al. 2015) and perhaps with its preferred habitat as this species lays eggs on the seabed (Chitty and Able 2004), which can regularly exhibit elevated pCO_2 levels in estuaries (Wallace et al. 2014). Importantly, however, larval *C. variegatus* became vulnerable at high pCO_2 when it occurred in tandem with temperatures above or below its thermal optimum (16°C or 30°C), conditions that yielded significantly higher mortality for this species. This outcome facilitated by multiple stressors demonstrates a key mechanism by which fisheries may be impacted by climate change. Embryos and larval fish exposed to temperatures outside of their thermal optimum may expend more energy maintaining their general metabolism and thus may be less capable of resisting addition stressors such as acidification (Pörtner and Farrell 2008). Similarly, acidification can cause the narrowing of an organism's thermal tolerance (Knust 2007, Pörtner and Farrell 2008). In future oceans, fish are expected to experience an intensification of warming and acidification (Doney et al., 2012). The results presented here

demonstrate that even fish that are tolerant to acidification may be negatively impacted by it if they are concurrently exposed to elevated temperatures.

The synergistic effects of temperature and CO_2 were also often apparent for the inland silverside, *M. beryllina*. For example, in the experiment examining only one temperature, elevated pCO_2 resulted in smaller larvae but only at high, and not optimal, temperatures. When four temperatures were examined, the ability of elevated pCO_2 to caused elevated mortality in larval fish occurred at the highest and lowest temperatures, but not at optimal temperatures. In the same experiment, hatching success was inhibited by pCO_2 at low but not high temperature. This result as well as depressed mortality at lower temperature and elevated pCO_2 are not surprising given that prior research has found that the negative effects of high CO_2 on *M. beryllina* are largely the result of embryonic rather than larval exposure of elevated pCO_2 (Baumann et al. 2012). Hence, cooler temperatures that extend egg hatching times and thus lengthen the time during which embryos experience the negative effects of acidification ultimately cause greater rates of embryonic and larval mortality. The synergistic negative effects at higher temperatures also fit within a theoretical framework of organismal physiology given that acidification depresses thermal tolerance (Pörtner, 2008, 2010). These outcomes demonstrate the importance of considering the combined effects of multiple climate change stressors on marine life, as in each case, the effects of both stressors were unexpected compared to the individual effects of each stressor.

Conclusions:

Acidification, thermal extremes, and sub-optimal food supplies are predicted to be caused by future climate change and are common phenomenon in coastal ecosystems, but the interactive effects of these stressors on most marine organisms are unknown. While *p*CO2 levels have

increased by more than 40% since the Industrial Revolution the complete implications of this change for marine life are not fully understood (Kroeker et al., 2013). My thesis demonstrates how elevated *p*CO2 coupled with changing temperatures and limited food supplies can lead to significant reductions in growth and survival for forage fish that incubate, hatch, and remain in estuaries where they are likely to experience these conditions under extreme scenarios today and will experience them more commonly in the future. Given that forage fish are key components of coastal marine food webs and effect the productivity of many marine fisheries, this study has important implications for managing coastal fisheries in modern day, eutrophic ecosystems and future oceans experiencing to climate change. Considering most fish species, forage or not, start from similarly sized eggs and larvae I feel this work is relevant to a broader range of fish outside the classification of forage fish.

Fish have been adapting to climate change and climate variability for millions of years (Brander 2007). Some fish, such *C. variegatus*, have developed reproductive strategies to offset differences in growth, survival, and predation rates based on environmental variability (Lambert 1984). Significant efforts have been spent on predicting and modeling what a changing environment might mean to fish in terms of reproductive strategies, timing of maturity, and growth rates (Beverton 1992, Winemiller 1992, Hutchings 1993). Less is known regarding the adaptability of fish to climate change (Malvezzi et al. 2015). Survival of fish populations experiencing climate change will depend on precise evolutionary responses.

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Figures and Tables

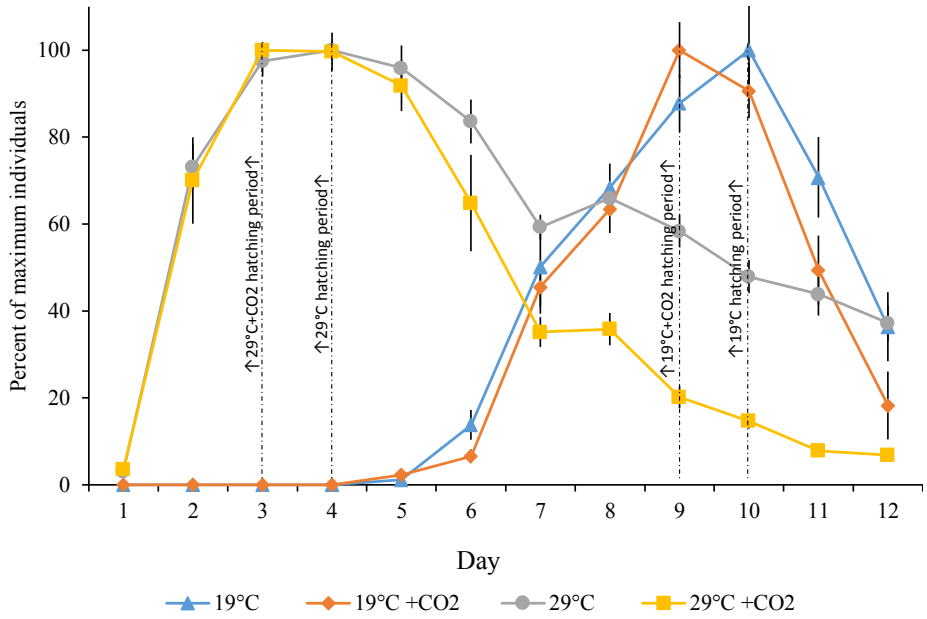

Figure 1: Larval survival during the experiment on the effects of temperature and CO_2 on *Menidia beryllina*. Points represent the mean (n=4) and error bars represent the standard deviation.

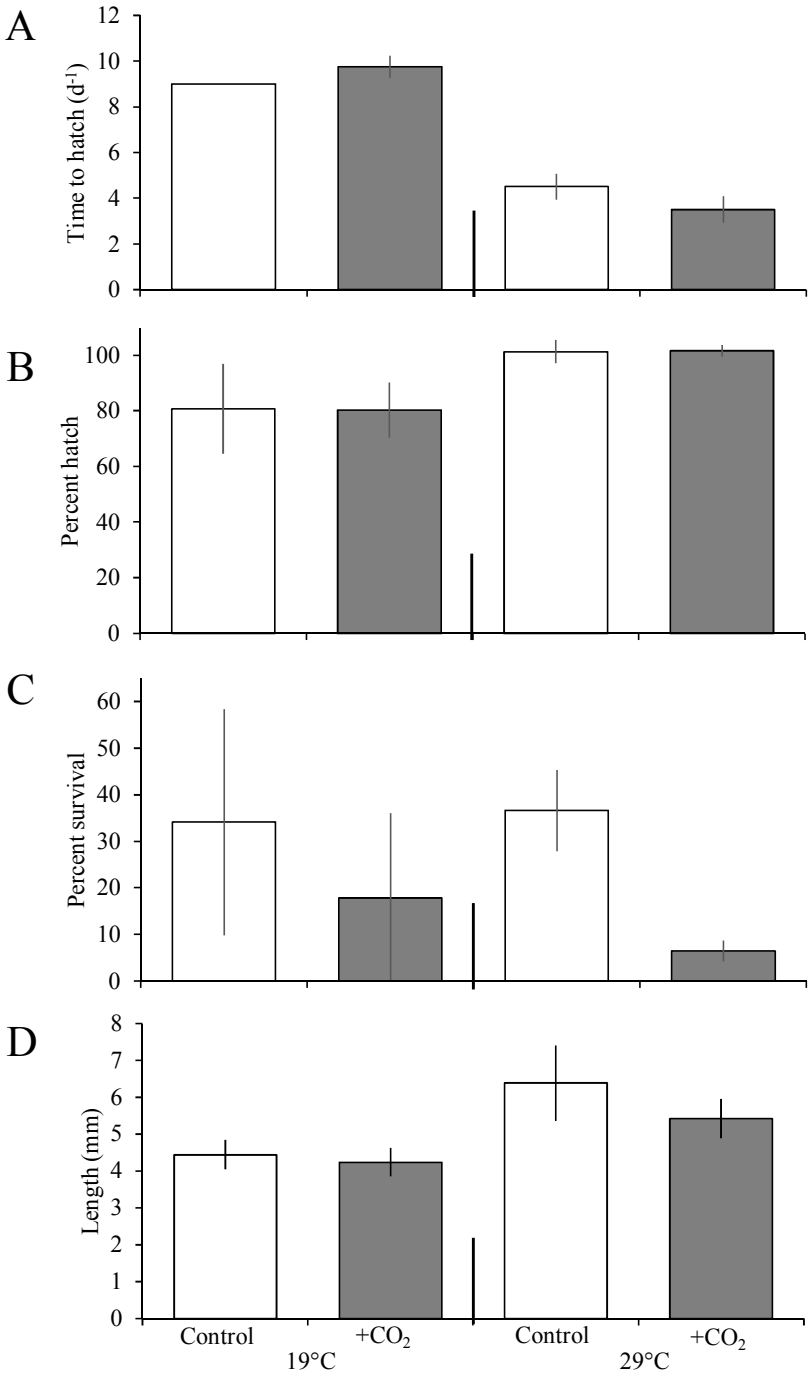

Figure 2 : Temperature and CO_2 effects on *Menidia beryllina*. A) Days to hatch. B) Percent hatched. C) Percent survival. D) Length. Bars represent the mean (n=4) and error bars represent the standard deviation.

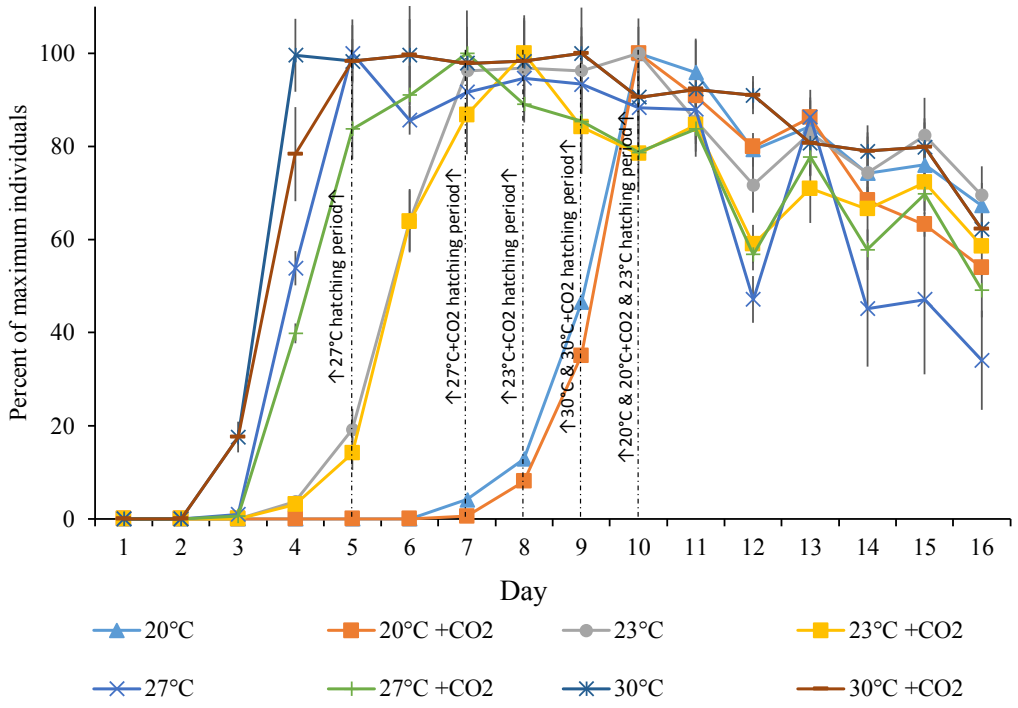

Figure 3: Larval survival during the expanded temperature and CO_2 effects on *Menidia beryllina* experiment. Points represent the mean (n=4) and the error bars represent the standard deviation.

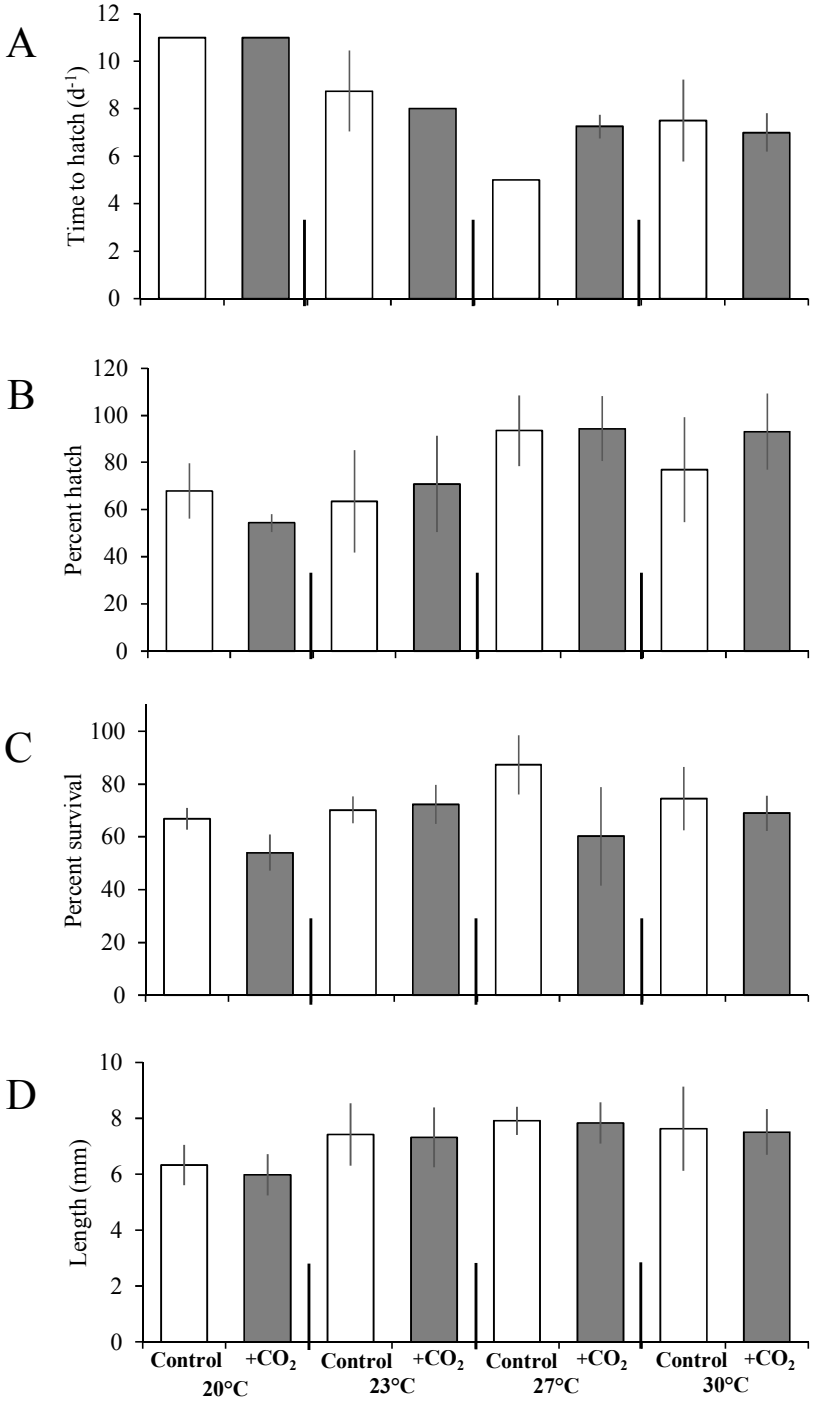

Figure 4: Expanded effects of temperature and CO_2 on *Menidia beryllina.* A) Days to hatch. B) Percent hatch. C) Percent survival. D) Length. Bars represent the mean (n=4) and error bars represent the standard deviation.

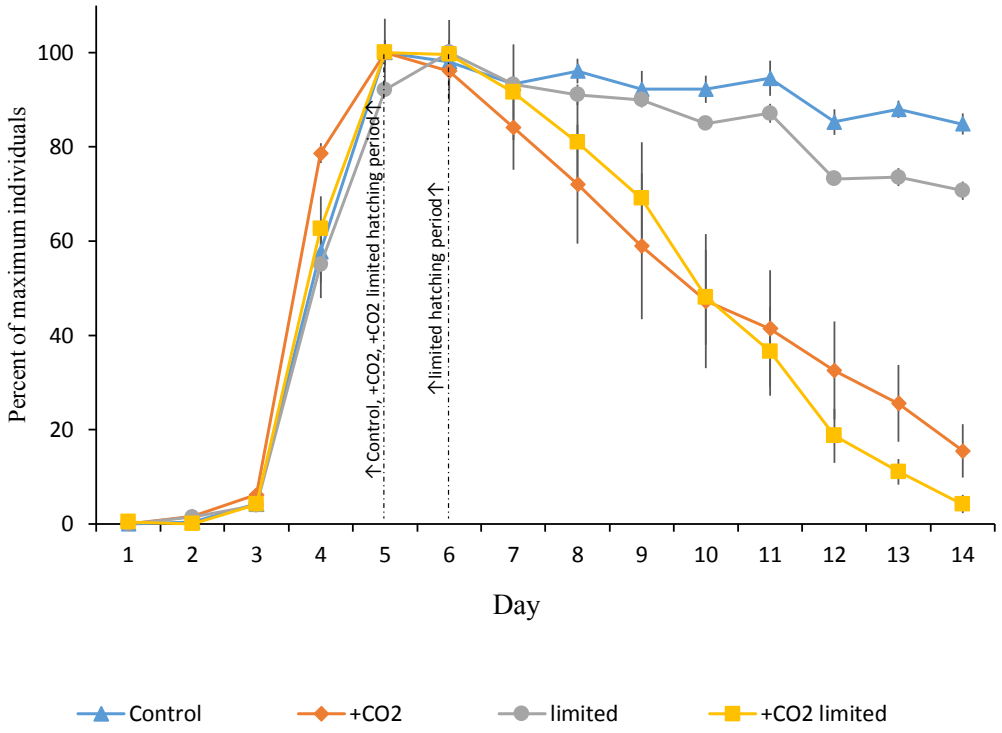

Figure 5: Larval survival throughout the diet and CO_2 effects on *Menidia beryllina* experiment. Points represent the mean (n=4) and error bars represent the standard deviation.

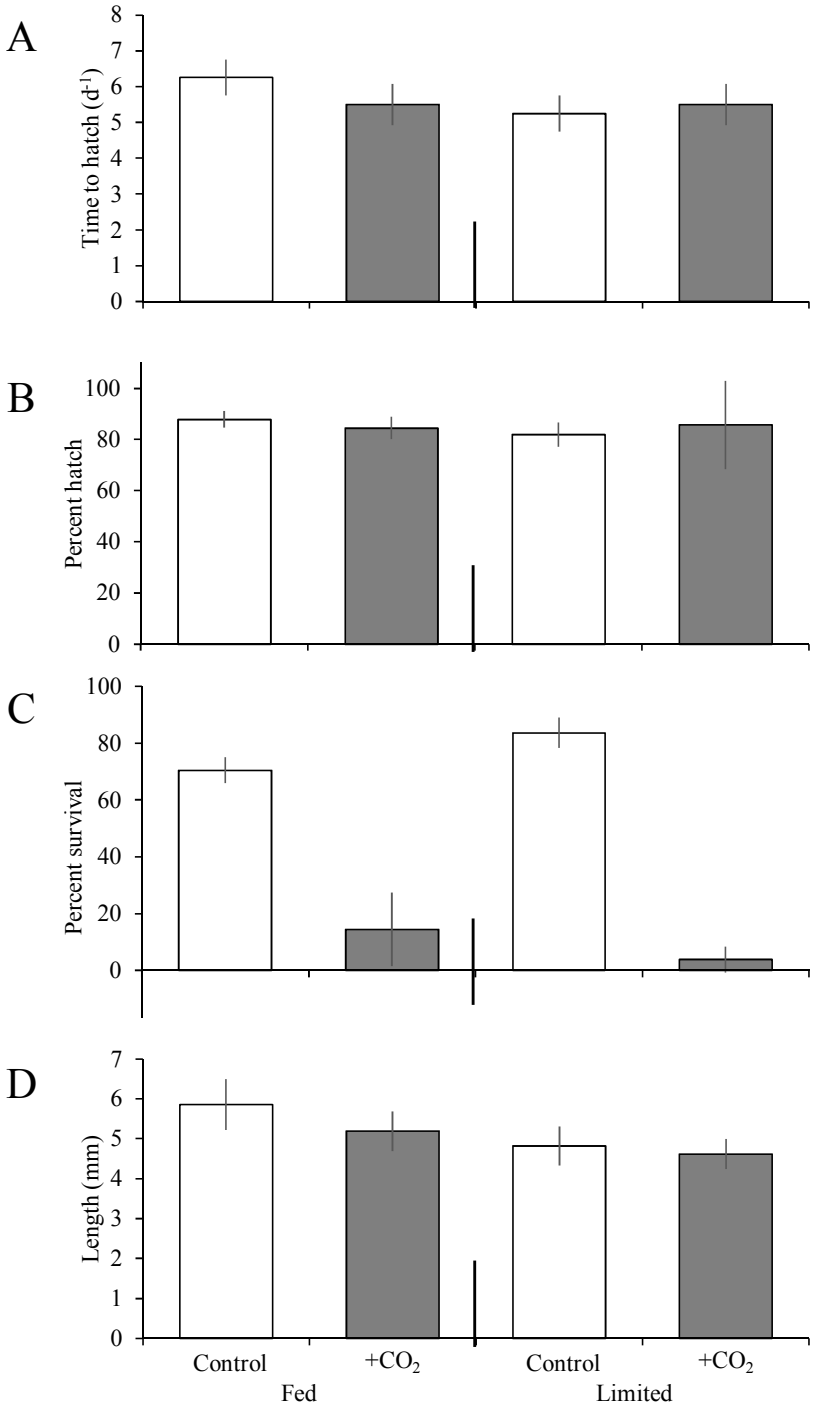

Figure 6: Diet and CO_2 effects on *Menidia beryllina*. A) Days to hatch B) Percent hatch C) Percent survival D) Length. Bars represent the mean (n=4) and error bars represent the standard deviation.

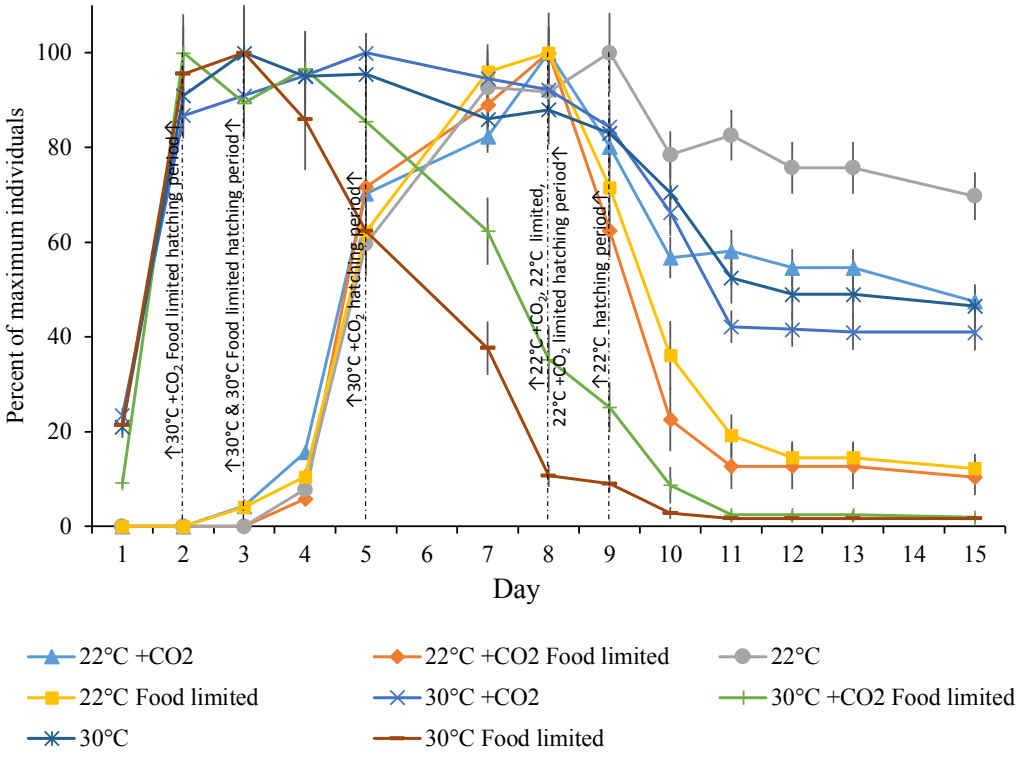

Figure 7: Larval survival throughout the temperature, food and CO_2 effects on *Menidia beryllina* experiment. Points represent the mean (n=4) and error bars represent the standard deviation.

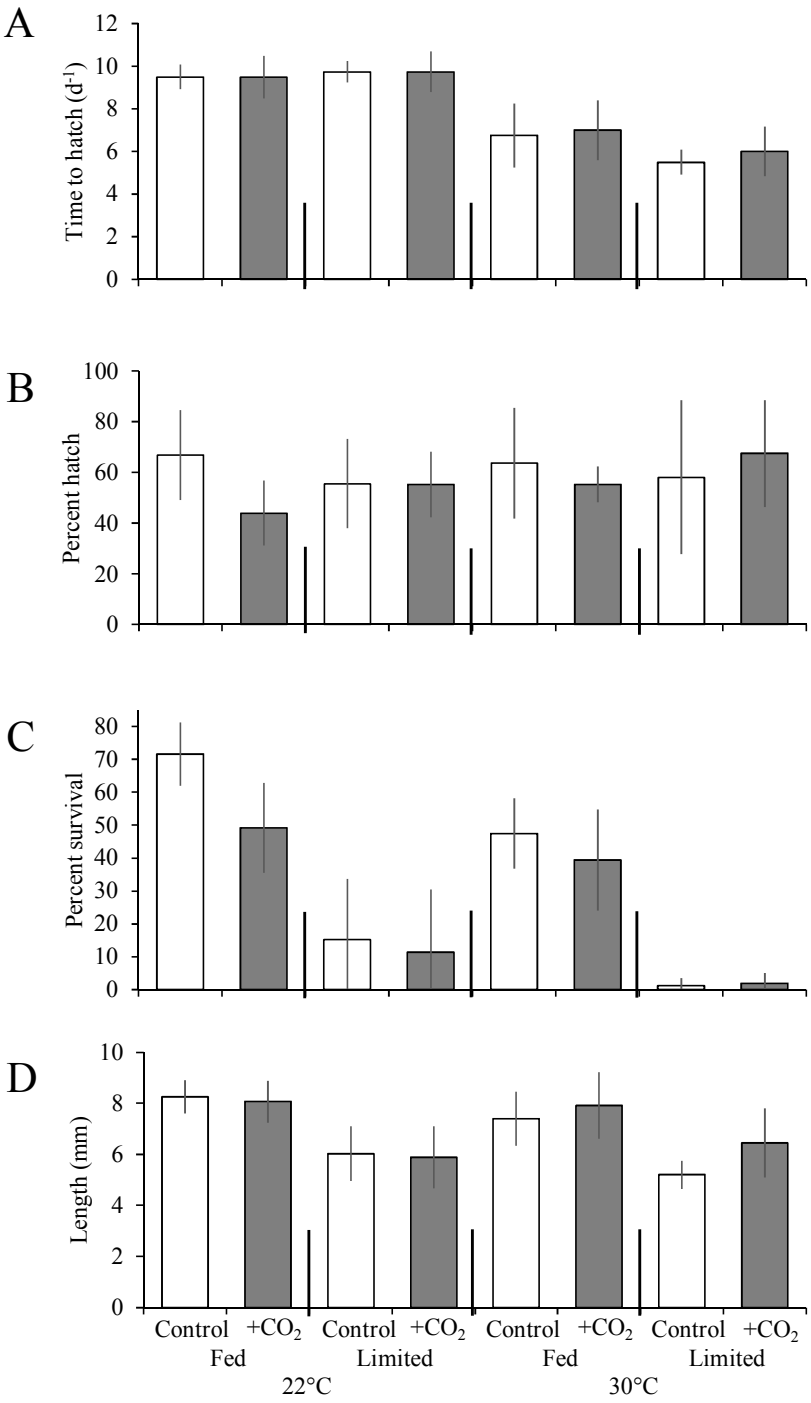

Figure 8: Temperature, food and CO_2 effects on *Menidia beryllina.* A) Days to hatch. B) Percent Hatch. C) Percent survival. D) Length. Bars represent the mean (n=4) and error bars represent the standard deviation.

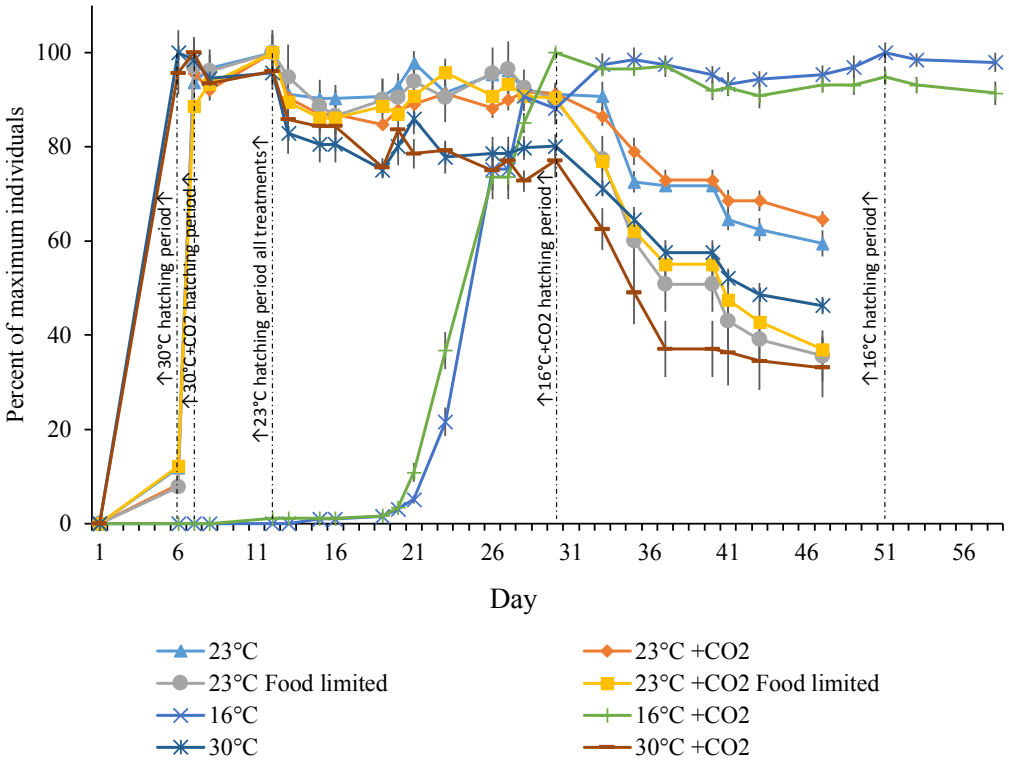

Figure 9: Larval survival throughout the temperature, food and CO_2 effects on *Cyprinodon variegatus* experiment. Points represent the mean (n=4) and error bars represent the standard deviation.

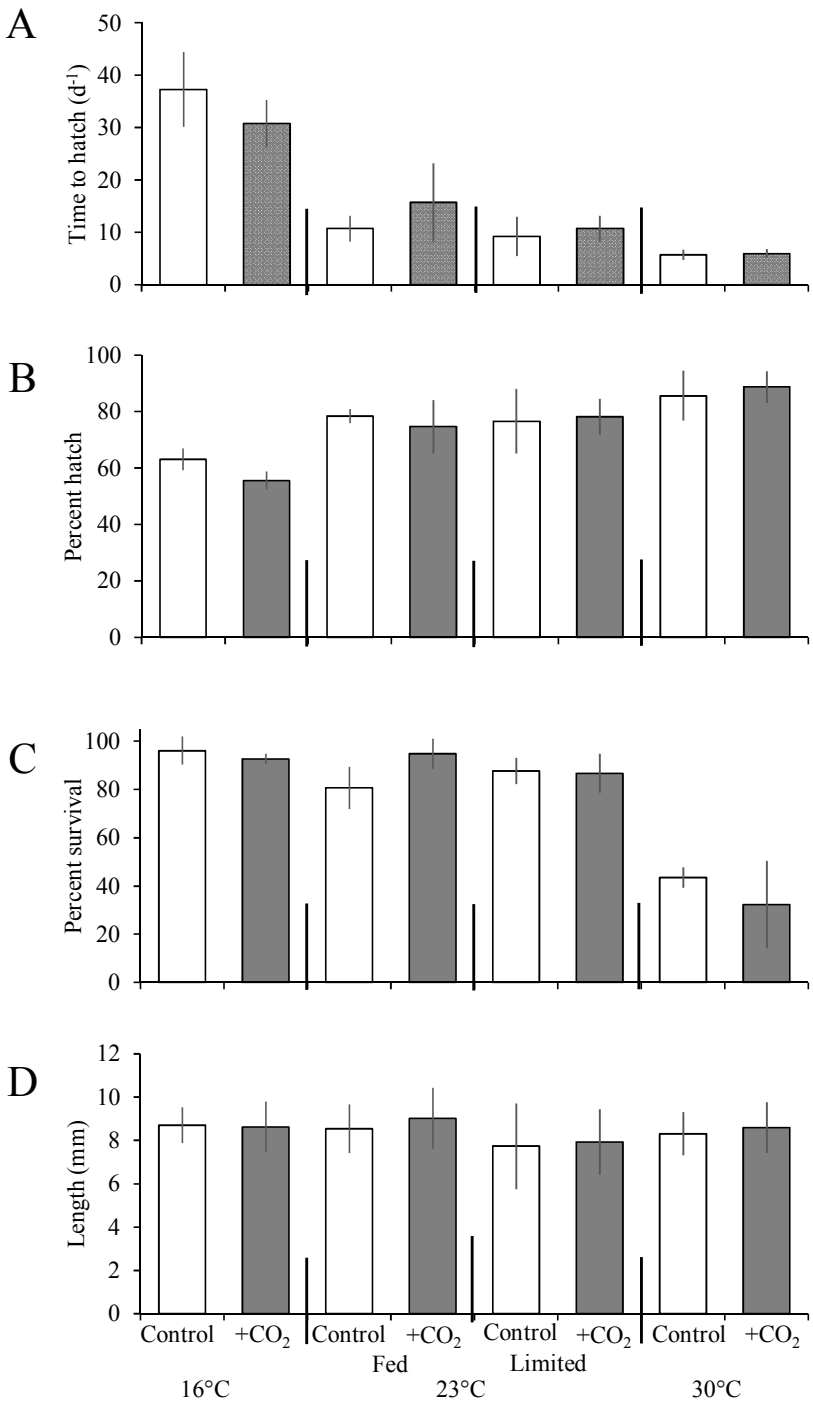

Figure 10: Temperature, food and CO_2 effects on *Cyprinodon variegatus.* A) Days to hatch. B) Percent hatch. C) Percent survival. D) Length. Bars represent the mean (n=4) and error bars represent standard deviation.

Tables

Table 1: Seawater chemistry for the experiment investigating temperature and CO_2 effects on *Menidia beryllina*. Mean and standard deviation in parentheses for temperature (°C), pH (total scale), *p*CO2 (µatm), total alkalinity (µmol kg^{-1}), CO_3^{2-} (µmol kg^{-1}), and total dissolved inorganic carbon (µmol kg^{-1}).

	Control, 19°C	**+CO_2, 19°C**	**Control, 29°C**	**+CO_2, 29°C**
Temperature	19 (0.53)	19 (0.53)	29 (0.29)	29 (0.29)
pH_T	7.93 (0.03)	7.34 (0.05)	7.91 (0.06)	7.40 (0.05)
pCO_2	389 (18.1)	1750 (244)	3723 (23.7)	1630 (366)
Ω calcite	4.33 (0.15)	1.42 (0.12)	5.40 (1.05)	1.76 (0.64)
Ω aragonite	2.81 (0.10)	0.92 (0.08)	3.54 (0.72)	1.15 (0.43)
TA	2200 (67.1)	2280 (50.8)	2280 (111)	2240 (112)
CO_3^{2-}	175 (6.41)	57.6 (4.83)	216 (40.3)	40.6 (25.0)
DIC	1950 (65.9)	2250 (55.8)	1980 (61.0)	2190 (90.1)
Salinity	32.0 (0.5)	32.0 (0.5)	32.0 (0.5)	32.0 (0.5)

Table 2: Two-way ANOVA for the number of days required for 100% of eggs to hatch in the temperature and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	DF	SS	MS	F	P
Temp	1	115.563	115.563	504.273	<0.**001**
CO2	1	3.063	3.063	13.364	0.**003**
Temp x CO2	1	0.0625	0.0625	0.273	0.611
Residual	12	2.750	0.229		
Total	15	121.438	8.096		

Table 3: Two-way ANOVA of the hatching success of eggs in each treatment of the temperature and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	DF	SS	MS	F	P
Temp	1	1753.516	1753.516	11.746	**0.005**
CO2	1	0.000	0.000	0.000	1.000
Temp x CO2	1	0.391	0.391	0.00262	0.960
Residual	12	1791.406	149.284		
Total	15	3545.313	236.354		

Table 4: Two-way ANOVA for the survival of larvae in each treatment at 9 days post hatch in the temperature and CO_2 effects on *Menidia beryllina* experiment

Source of Variation	DF	SS	MS	F	P
Temp	1	0.000	0.000	0.000	1.000
CO2	1	1260.250	1260.250	9.520	**0.009**
Temp x CO2	1	169.000	169.000	1.277	0.281
Residual	12	1588.500	132.375		
Total	15	3017.750	201.183		

Table 5: Two-way ANOVA for the analysis of fish length of preserved fish taken on the final day of the temperature and CO_2 effects on *Menidia beryllina* experiment

Source of Variation	DF	SS		MS	F	P
CO2	1	12.971	12.971	21.838	**<0.001**	
Treatment	1	93.422	93.422	157.286	**<0.001**	
CO2 x Treatment	1	5.548	5.548	9.340	**0.003**	
Residual	227	134.829	0.594			
Total	230	355.679		1.546		

Table 6: Seawater chemistry for the experiment investigating expanded temperature and CO_2 effects on *Menidia beryllina.* Mean and standard deviation in parentheses for temperature (°C), pH (total scale), *p*CO2 (µatm), total alkalinity (µmol kg^{-1}), CO_3^{2-} (µmol kg^{-1}), and total dissolved inorganic carbon (µmol kg^{-1}).

	Control, 20°C	**+CO_2, 20°C**	**Control, 23°C**	**+CO_2, 23°C**
Temperature	20 (0.15)	20 (0.15)	23 (0.28)	23 (0.28)
pH_T	7.82 (0.06)	7.21 (0.05)	7.82 (0.05)	7.26 (0.06)
pCO_2	392 (39.1)	2164 (368)	368 (35.5)	1610 (438)
Ω calcite	2.9 (0.13)	0.7 (0.12)	3.2 (0.23)	1.0 (0.18)
Ω aragonite	1.9 (0.08)	0.5 (0.08)	2.1 (0.15)	0.6 (0.12)
TA	1721 (50.4)	1718 (92.0)	1766 (119)	1711 (122)
CO_3^{2-}	116 (5.20)	28.8 (4.70)	129 (9.20)	38.8 (7.31)
DIC	1545 (49.1)	1737 (93.7)	1571 (112)	1697 (141)
Salinity	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)

	Control 27°	**+CO_2 27°**	**Control 30°**	**+CO_2 30°**
Temperature	27 (0.28)	27 (0.28)	30 (0.55)	30 (0.55)
pH_T	7.81 (0.06)	7.23 (0.09)	7.82 (0.06)	7.21 (0.06)
pCO_2	333 (21.0)	1740 (301)	327 (4.79)	1402 (289)
Ω calcite	3.4 (0.21)	0.9 (0.13)	3.6 (0.10)	1.1 (0.22)
Ω aragonite	2.2 (0.13)	0.6 (0.08)	2.4 (0.07)	0.7 (0.15)
TA	1756 (85.3)	1753 (116)	1794 (46.8)	1728 (91.0)
CO_3^{2-}	138 (8.44)	37.7 (5.27)	146 (4.28)	45.4 (9.04)
DIC	1545 (78.0)	1744 (121)	1572 (43.1)	1697 (90.8)
Salinity	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)

Table 7: Two-way ANOVA for the number of days required for 100% of eggs to hatch in the expanded temperature and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**		**MS**	**F**	**P**
Temp	3	104.625	34.875	40.829	**<0.001**	
CO_2	1	0.500	0.500	0.585	0.452	
Temp x CO_2	3	11.250	3.750	4.390	**0.013**	
Residual	24	20.500	0.854			
Total	31	136.875	4.415			

Table 8: Two-way ANOVA of the hatching success of eggs in each treatment of the expanded temperature and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Temp	3	5591.602	1863.867	6.738	**0.002**
CO_2	1	63.281	63.281	0.229	0.637
Temp x CO_2	3	940.234	313.411	1.133	0.356
Residual	24	6639.063	276.628		
Total	31	13234.180	426.909		

Table 9: Two-way ANOVA for the survival of larvae in each treatment at 10 days post 100% hatched in the expanded temperature and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Temp	3	2712.844	904.281	9.511	**<0.001**
CO_2	1	247.531	247.531	2.604	0.120
Temp x CO_2	3	1059.094	353.031	3.713	**0.025**
Residual	24	2281.750	95.073		
Total	31	6301.219	203.265		

Table 10: Two-way ANOVA for the analysis of fish length of preserved fish taken on the final day of the expanded temperature and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Temp	3	130.077	43.359	47.702	**<0.001**
CO2	1	1.862	1.862	2.048	0.153
Temp x CO2	3	0.894	0.298	0.328	0.805
Residual	294	267.230	0.909		
Total	301	399.415	1.327		

Table 11: Two-way ANOVA for analysis of the survival of larvae on day 10 in the 19° and 27° treatments in the expanded temperature and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Temp	1	2352.250	2352.250	40.267	**<0.001**
CO_2	1	1122.250	1122.250	19.211	**<0.001**
Temp x CO_2	1	56.250	56.250	0.963	0.346
Residual	12	701.000	58.417		
Total	15	4231.750	282.117		

Table 12: Seawater chemistry for the diet and CO2 effects on *Menidia beryllina* experiment. Mean and standard deviation in parentheses for temperature (°C), pH (total scale), pCO_2 (µatm), total alkalinity (µmol kg^{-1}), CO_3^{2-} (µmol kg^{-1}), and total dissolved inorganic carbon (µmol kg^{-1}).

	Control, 24°C	**+CO_2, 24°C**	**Control Starved, 24°C**	**+CO_2 Starved, 24°C**
Temperature	24 (0.43)	24 (0.43)	24 (0.43)	24 (0.43)
pH_T	7.92 (0.03)	7.38 (0.05)	7.92 (0.03)	7.39 (0.05)
pCO_2	494 (16.4)	2088 (343)	500 (14.9)	2068 (376)
Ω calcite	3.1 (0.07)	1.0 (0.13)	3.1 (0.18)	1.0 (0.15)
Ω aragonite	2.0 (0.04)	0.6 (0.08)	2.0 (0.12)	0.7 (0.09)
TA	2056 (16.9)	2042 (10.6)	2064 (46.3)	2102 (64.2)
CO_3^{2-}	129 (3.02)	39.0 (5.08)	128 (8.29)	41.8 (5.84)
DIC	1895 (16.3)	2049 (16.6)	1884 (34.7)	2105 (74.9)
Salinity	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)

Table 13: Two-way ANOVA for the number of days required for 100% of eggs to hatch in the diet and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Food	1	1.000	1.000	3.429	**0.089**
CO_2	1	0.250	0.250	0.857	0.373
Food x CO_2	1	1.000	1.000	3.429	0.089
Residual	12	3.500	0.292		
Total	15	5.750	0.383		

Table 14: Two-way ANOVA of the hatching success of eggs in each treatment of the diet and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Food	1	21.973	21.973	0.252	0.625
CO_2	1	0.0977	0.0977	0.00112	0.974
Food x CO_2	1	51.660	51.660	0.592	0.456
Residual	12	1046.484	87.207		
Total	15	1120.215	74.681		

Table 15: Two-way ANOVA for the survival of larvae in each treatment at 9 days post hatch in the diet and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Food	1	4.000	4.000	0.140	0.715
CO_2	1	8372.250	8372.250	293.334	**<0.001**
Food x CO_2	1	156.250	156.250	5.474	**0.037**
Residual	12	342.500	28.542		
Total	15	8875.000	591.667		

Table 16: Two-way ANOVA for the analysis of fish length of preserved fish taken on the final day of the diet and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**		**MS**	**F**	**P**
Food	1	20.989	20.989	67.297	**<0.001**	
CO_2	1	6.073	6.073	19.472	**<0.001**	
Food x CO_2	1	1.833	1.833	5.878	**0.016**	
Residual	468	145.964	0.312			
Total	471	267.910	0.569			

Table 17: Seawater chemistry in the temperature, food and CO2 effects on *Menidia beryllina* experiment. Mean and standard deviation in parentheses for temperature (°C), pH (total scale), *p*CO2 (µatm), total alkalinity (µmol kg^{-1}), CO_3^{2-} (µmol kg^{-1}), and total dissolved inorganic carbon (µmol kg^{-1}).

	Control, 22°C	**+CO_2, 22°C**	**Control Starved, 22°C**	**+CO_2 Starved, 22°C**
Temperature	22 (0.26)	22 (0.26)	22 (0.26)	22 (0.26)
pH_T	7.92 (0.02)	7.26 (0.09)	7.93 (0.02)	7.20 (0.07)
pCO_2	381 (49.0)	2410 (250)	358 (15.4)	2170 (112)
Ω calcite	4.2 (0.28)	0.8 (0.05)	4.3 (0.18)	0.8 (0.04)
Ω aragonite	2.7 (0.18)	0.5 (0.03)	2.8 (0.12)	0.5 (0.02)
TA	2120 (24.0)	1978 (37.7)	2111 (86.9)	1873 (1.31)
CO_3^{2-}	169 (11.2)	32.9 (1.93)	174 (7.48)	32.5 (1.49)
DIC	1879 (43.3)	2002 (48.8)	1862 (79.9)	1889 (7.14)
Salinity	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)
	Control, 30°C	**+CO_2, 30°C**	**Control Starved, 30°C**	**+CO_2 Starved, 30°C**
Temperature	30 (0.24)	30 (0.24)	30 (0.24)	30 (0.24)
pH_T	7.95 (0.01)	7.33 (0.06)	7.96 (0.02)	7.31 (0.06)
pCO_2	351 (11.8)	2576 (190)	364 (15.8)	2340 (30.1)
Ω calcite	5.8 (0.76)	1.3 (0.12)	6.4 (0.14)	1.4 (0.06)
Ω aragonite	3.9 (0.50)	0.9 (0.08)	4.3 (0.09)	0.9 (0.04)
TA	2211 (200)	2267 (27.4)	2381 (5.20)	2220 (66.2)
CO_3^{2-}	231 (30.3)	53.3 (4.74)	256 (5.56)	55.6 (2.53)
DIC	1881 (166)	2257 (15.9)	2024 (13.9)	2199 (64.0)
Salinity	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)

Table 18: Three-way ANOVA for the number of days required for 100% of eggs to hatch in the temperature, food and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**		**MS**	**F**	**P**
Food	1	1.531	1.531	1.455	0.239	
Temp	1	87.781	87.781	83.436	**<0.001**	
CO_2	1	0.281	0.281	0.267	0.610	
Food x Temp	1	3.781	3.781	3.594	0.070	
Food x CO_2	1	0.0313	0.0313	0.0297	0.865	
Temp x CO_2	1	0.281	0.281	0.267	0.610	
Food x Temp x CO_2	1	0.0313	0.0313	0.0297	0.865	
Residual	24	25.250	1.052			
Total	31	118.969	3.838			

Table 19: Three-way ANOVA of the hatching success of eggs in each treatment of the temperature, food and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Food	1	21.533	21.533	0.0604	0.808
Temp	1	260.205	260.205	0.730	0.401
CO_2	1	246.143	246.143	0.690	0.414
Food x Temp	1	21.533	21.533	0.0604	0.808
Food x CO_2	1	812.549	812.549	2.279	0.144
Temp x CO_2	1	289.502	289.502	0.812	0.377
Food x Temp x CO_2	1	10.986	10.986	0.0308	0.862
Residual	24	8558.203	356.592		
Total	31	10220.654	329.699		

Table 20: Three-way ANOVA for the survival of larvae in each treatment at 10 days post 100% hatched in the temperature, food and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**	**MS**	**F**	**P**
Food	1	3894.031	3894.031	63.500	**<0.001**
Temperature	1	413.281	413.281	6.739	**0.016**
CO_2	1	442.531	442.531	7.216	**0.013**
Food x Temp	1	42.781	42.781	0.698	0.412
Food x CO_2	1	427.781	427.781	6.976	**0.014**
Temp x CO_2	1	124.031	124.031	2.023	0.168
Food x Temp x CO_2	1	87.781	87.781	1.431	0.243
Residual	24	1471.750	61.323		
Total	31	6903.969	222.709		

Table 21: Three-way ANOVA for the analysis of fish length of preserved fish taken on the final day of the temperature, food and CO_2 effects on *Menidia beryllina* experiment.

Source of Variation	**DF**	**SS**		**MS**	**F**	**P**
Food	1	50.995	50.995	48.719	**<0.001**	
Temp	1	1.284	1.284	1.227	0.271	
CO_2	1	1.619	1.619	1.547	0.217	
Food x Temp	1	0.429	0.429	0.409	0.524	
Food x CO_2	1	0.480	0.480	0.458	0.500	
Temp x CO_2	1	3.426	3.426	3.273	0.074	
Food x Temp x CO_2	1	0.354	0.354	0.338	0.562	
Residual	94	98.392	1.047			
Total	101	174.960	1.732			

Table 22: Seawater chemistry in the temperature, food and CO2 effects on *Cyprinodon variegatus* experiment. Mean and standard deviation in parentheses for temperature (°C), pH (total scale), pCO_2 (µatm), total alkalinity (µmol kg^{-1}), CO_3^{2-} (µmol kg^{-1}), and total dissolved inorganic carbon (µmol kg^{-1}).

	Control, 23°C	**+CO_2, 23°C**	**Control Starved, 23°C**	**+CO_2 Starved, 23°C**
Temperature	23 (0.29)	23 (0.29)	23 (0.29)	23 (0.29)
pH_T	7.86 (0.06)	7.31 (0.08)	7.87 (0.07)	7.31 (0.07)
pCO_2	397 (7.13)	2057 (168)	404 (10.2)	2191 (156)
Ω calcite	2.2 (0.04)	0.5 (0.05)	2.2 (0.07)	0.5 (0.02)
Ω aragonite	1.4 (0.03)	0.3 (0.03)	1.4 (0.05)	0.3 (0.01)
TA	1519 (16.5)	1429 (15.9)	1505 (16.7)	1431 (45.4)
CO_3^{2-}	90.4 (1.63)	20.1 (1.86)	88.5 (3.00)	19.0 (0.88)
DIC	1378 (15.4)	1458 (13.4)	1366 (12.3)	1466 (49.5)
Salinity	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)
	Control 16°	**+CO_2 16°**	**Control 30°**	**+CO_2 30°**
Temperature	16 (0.09)	16 (0.09)	30 (0.16)	30 (0.16)
pH_T	7.88 (0.04)	7.30 (0.09)	7.90 (0.06)	7.45 (0.10)
pCO_2	519 (11.4)	2356 (17.4)	417 (4.00)	1365 (98.1)
Ω calcite	1.7 (0.03)	0.5 (0.04)	2.6 (0.06)	1.2 (0.13)
Ω aragonite	1.1 (0.02)	0.3 (0.02)	1.7 (0.04)	0.8 (0.08)
TA	1487 (5.56)	1481 (59.3)	1676 (15.1)	1873 (164)
CO_3^{2-}	69.5 (1.19)	18.6 (1.43)	103 (2.60)	48.8 (5.09)
DIC	1384 (8.20)	1524 (57.7)	1520 (11.3)	1893 (162)
Salinity	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)	32.5 (0.5)

Table 23: Two-way ANOVA for the number of days required for 100% of eggs to hatch in the temperature, food and CO_2 effects on *Cyprinodon variegatus* experiment.

Source of Variation	DF	SS	MS	F	P
Temp	2	3716.844	1858.422	92.256	**<0.001**
CO_2	1	7.200	7.200	0.357	0.555
Temp x CO_2	2	126.844	63.422	3.148	0.060
Residual	26	523.750	20.144		
Total	31	4367.469	140.886		

Table 24: Two-way ANOVA of the hatching success of eggs in each treatment of the temperature, food and CO_2 effects on *Cyprinodon variegatus* experiment.

Source of Variation	DF	SS	MS	F	P
Temp	2	3202.002	1601.001	33.306	**<0.001**
CO_2	1	23.926	23.926	0.498	0.487
Temp x CO_2	2	115.283	57.642	1.199	0.318
Residual	26	1249.805	48.069		
Total	31	4588.623	148.020		

Table 25: Two-way ANOVA for the survival of larvae in each treatment at 9 days post hatch in the temperature, food and CO_2 effects on *Cyprinodon variegatus* experiment.

Source of Variation	DF	SS	MS	F	P
Temp	2	2.439	1.220	57.650	**<0.001**
CO_2	1	0.0118	0.0118	0.556	0.463
Temp x CO_2	2	0.147	0.0733	3.463	**0.046**
Residual	26	0.550	0.0212		
Total	31	3.136	0.101		

Table 26: Two-way ANOVA for the analysis of fish length of preserved fish taken on the final day of the temperature, food and CO_2 effects on *Cyprinodon variegatus* experiment.

Source of Variation	DF	SS	MS	F	P
Temp	2	12.025	6.012	3.569	**0.029**
CO2	1	0.420	0.420	0.250	0.618
Temp x CO2	2	0.955	0.478	0.283	0.753
Residual	305	513.829	1.685		
Total	310	527.118	1.700		