

EARLY LIFE HISTORY TRAITS INFLUENCE THE EFFECTS OF OCEAN
ACIDIFICATION ON THE BEHAVIOR AND PHYSIOLOGY OF JUVENILE
ROCKFISHES IN CENTRAL CALIFORNIA

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EARLY LIFE HISTORY TRAITS INFLUENCE THE EFFECTS OF OCEAN
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ROCKFISHES IN CENTRAL CALIFORNIA

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ABSTRACT

Early life history traits influence the effects of ocean acidification on the behavior and physiology of juvenile rockfishes in central California

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The rapid increase of anthropogenic carbon dioxide (CO₂) emissions to the atmosphere is altering seawater chemistry at an alarming rate. Many studies have shown that elevated dissolved seawater CO₂ concentrations and associated decreases in seawater pH (termed ocean acidification) will result in negative impacts on marine organisms that accrete calcium carbonate structures. Recent research has also suggested that ocean acidification has the potential to negatively affect marine fishes by modifying behaviors and physiological processes; however, some species appear to be resilient to projected future pH changes. In this thesis, I investigated the potential for ocean acidification to impair the behavior and physiology of six closely related species of nearshore rockfishes (*Sebastes* spp.) in central California and tested whether the susceptibility or tolerance of each species to elevated CO₂ was associated with their early life history strategy. Two life history syndromes were selected based on the conditions larvae and juveniles experience: canopy recruiting and benthic recruiting rockfish ($n=3$ species of each syndrome). Canopy recruiting species inhabit shallower water and experience relatively low CO₂ conditions during development, whereas benthic recruiting species inhabit deeper water and experience higher CO₂ levels during development. Individuals from each of these 6 species were reared in 4 CO₂ treatments (540 ppm, 820 ppm, 2100 ppm, and 3300 ppm) for 3 months. During that time, I examined the effect of elevated CO₂ on a suite of behavioral and physiological responses: behavioral lateralization, escape time, critical swimming speed, aerobic scope, and growth rate. Meta-analysis techniques were used to synthesize results across multiple experiments and to permit comparisons of the responses of the canopy recruiting group and benthic recruiting group to ocean acidification. I found that benthic recruiting species appear resistant to CO₂-mediated impairments, whereas canopy recruiting species experienced altered brain function via a reversal of behavioral lateralization in two species and increased escape time in all three species. Additionally, all three canopy recruiting species experienced decreased swimming endurance in elevated CO₂. These findings indicate that kelp forest rockfishes have life history-specific responses to ocean acidification. Either early exposure to high CO₂ water during development allows benthic recruiting species to acclimate to OA conditions, or the benthic recruiting group has previously adapted to these conditions. If juvenile canopy species cannot acclimate or adapt to future OA conditions, their behavioral and physiological impairments may translate into increased mortality, as has been found in settlement stages of tropical species, and recruitment of canopy species may decline as climate change progresses. The divergent responses of these two groups of rockfish may indicate future changes in the species composition of kelp forest rockfish assemblages.

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Introduction:

Burning of fossil fuels since the industrial revolution has increased the partial pressure of atmospheric CO₂ (*p*CO₂) levels from 280 ppm to over 400 ppm, and carbon dioxide emissions continue to rise (Meinshausen et al., 2011). Atmospheric CO₂ traps heat from reflected solar radiation, which elevates air and ocean temperatures, accelerates polar ice cap melting, and alters teleconnections in land and sea weather patterns. This rapid increase in atmospheric carbon dioxide (CO₂) is also expected to profoundly impact ocean chemistry and marine organisms (Orr et al., 2005). As CO₂ dissolves into the oceans it reacts with water to form carbonic acid, which quickly dissociates to bicarbonate (HCO₃⁻) and a hydrogen ion (H⁺). Depending on pH, HCO₃⁻ can further deprotonate to carbonate (CO₃²⁻) and an additional hydrogen ion. The equilibrium $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CO}_3^{2-} + 2\text{H}^+$ is sensitive to pH. Currently pH is high enough (~8) that as CO₂ dissolves into the ocean it increases bicarbonate and hydrogen ion concentrations, while decreasing carbonate ion concentrations. These additional hydrogen ions lower the pH of ocean surface waters and contribute to ocean acidification (OA) (Caldeira and Wickett 2003). The ocean surface waters have absorbed nearly 50% of the anthropogenic CO₂ emitted since 1750, resulting in a 0.1 pH unit decrease in seawater pH (Sabine et al., 2004). Ocean pH is currently lower than it has been at any time in the last 400,000 years and is expected to decrease by another 0.3 to 0.4 pH units by 2100 (Feely et al., 2004; Orr et al., 2005).

OA is predicted to have negative impacts on a variety of organisms (Fabry et al., 2008; Kroeker et al., 2013). Previous research has predominantly focused on the deleterious effects to calcifying organisms that occur following reductions in the saturation states of biogenic carbonates (i.e., the forms of calcium carbonate used to make shells and coral skeletons) due to changes in pH and the availability of carbonate ions (Kleypas et al., 2006; Kleypas and Langdon

2006; Anthony et al., 2008; Doney et al., 2009). Until recently, little work has explored the effects of elevated CO₂ and thus lowered pH on fishes (Hayashi et al., 2004; Barry & Drazen 2007, Checkley et al. 2009; Munday et al., 2009a, b, c; Dixson et al., 2010; Ferrari et al., 2011 Munday et al., 2011a, b; Domenici et al., 2012; Bignami et al. 2013), especially in commercially important temperate species (Frommel et al., 2012; Hurst et al., 2012; Frommel et al., 2013; Hurst et al., 2013; Frommel et al., 2014; Hamilton et al., 2014; Hurst et al., 2015). A recent review concluded that the early life stages of fishes appear to be more sensitive than most invertebrate taxa to projected pH changes by the year 2100 (72.7% of species responded negatively to elevated CO₂) (Wittman and Portner, 2013).

Increases in seawater CO₂ have been shown to alter brain chemistry and function of fishes (Domenici et al, 2012, Nilsson et al., 2012; Domenici et al., 2014). These CO₂-induced chemical changes negatively affect the behavior of tropical marine fishes, leading to impairment of predator detection via odor cues (Munday et al., 2009a; Dixson et al., 2010), and increased mortality of larval (Munday et al., 2010) and settlement stage fishes (Ferrari et al., 2011). Enlarged otoliths (i.e., earstones) of some fish species in response to elevated CO₂ indicate that OA may cause changes in hearing abilities (with potential improvements) and sensory equilibrium (Bignami et al., 2013a). In addition, increased CO₂ can alter the physiology of fishes by increasing larval growth (Munday et al., 2009b) and reducing aerobic performance (Munday et al., 2009c) in some species, but not others (Munday et al., 2011a, b; Bignami et al., 2013a). Because elevated CO₂ has variable effects on different fish species, it is critical to understand the mechanisms driving behavioral and physiological changes in fish in order to predict how different species will fare in the face of changing ocean chemistry.

Behavioral and physiological impairments in fish following exposure to elevated CO_2 may be a result of changes in blood and tissue chemistry. As $p\text{CO}_2$ in the ocean rises, so does the internal $p\text{CO}_2$ of fishes. This increase in internal $p\text{CO}_2$ can decrease the pH of fish blood and tissues. Fish initially compensate for decreased internal pH by using chemical buffering proteins and active ion transport to accumulate acid-base compensatory ions. The accumulation of compensatory ions, such as HCO_3^- , requires the excretion of Cl^- through energy intensive ion transport (Heisler 1986). The alteration of extracellular ion concentrations to compensate for decreases in internal pH leads to a reversal in Cl^- and HCO_3^- concentration gradients across the GABA_A neural transmitter receptor. The consequence of a reversal in transmembrane ion concentration gradients is depolarization of a major inhibitory neurotransmitter receptor and transmission of inappropriate excitatory signals, which precipitate behavior abnormalities (Nilsson et al., 2012).

Additionally, initial changes in extracellular pH can reduce the binding efficiency of hemoglobin and limit delivery of oxygen to different tissues, however there is some evidence that acid-base compensation can buffer blood pH, allowing it to normalize over a period of hours to days (Heisler 1986). As blood chemistry shifts to balance the changes in pH, acid-base and ion equilibria reach new steady states that can affect processes such as growth and reproduction (Pörtner 2005). The energetic costs of maintaining acid-base balance with CO_2 enrichment can result in shifts in the metabolic processes of marine organisms (Wittman and Pörtner 2013). Some marine invertebrates depress their metabolic rates by reducing anaerobic respiration in response to elevated CO_2 (Guppy and Withers 1999). Tropical marine fishes exhibit similar metabolic depression (Munday et al., 2009) and changes in respiration rates (Stiller et al., 2015) with increased CO_2 , while other species are unaffected (Melzner et al., 2009). The multitude of

potential compensatory responses to changes in ocean chemistry may affect the energy budgets, performance, and overall fitness of many marine fishes.

Initially, tropical coral reefs were the main focus of OA research because pH was assumed to be relatively stable and thus organisms were thought to be inexperienced at coping with pH fluctuations. However, recent research has shown that seawater pH on tropical reefs is highly variable, fluctuating nearly 0.3 pH units a day, due to biological activity such as photosynthesis and respiration (Anthony et al., 2011). Many tropical species may already be experiencing extremely low pH for short durations, but the consequences of long term exposure and the ability of organisms to acclimate and adapt to reduced pH are still not well understood. Because many temperate fish species inhabiting eastern boundary current zones are naturally exposed to highly variable pH, especially in regions affected by upwelling (Feely et al. 2008), it has been assumed that they are better adapted to respond to changing seawater pH than tropical species. Given the new evidence of highly variable pH levels in some tropical settings, this assumption requires further evaluation.

The California Current Large Marine Ecosystem (CCLME) on the west coast of the United States naturally experiences highly variable CO_2 in nearshore regions due to seasonal weather patterns. In spring and summer, strong northwest winds combined with the Coriolis force result in Ekman transport that drives surface waters offshore and cause deep, cold, nutrient rich, and high CO_2 water to shoal in nearshore habitats. This upwelled water is naturally high in CO_2 because the remineralization of organic matter and respiration by organisms below the thermocline enriches these deep-water masses in CO_2 (Feely et al., 2008). The strength and duration of seasonal upwelling winds determines how close to the surface upwelled water shoals and for how long coastal environments are exposed to these corrosive waters. Many nearshore

habitats in the CCLME are already experiencing CO₂ levels that are higher than levels predicted for the average surface waters in 2100 (Feely et al., 2008). Annual mean seawater pH off the central California coast in 2005 was 7.96, and is projected to decrease to 7.82, and much lower during upwelling season, in nearshore habitats by 2050 (Gruber et al., 2012). Areas that experience a combination of strong upwelling and proximity to submarine canyons, such as Monterey and Carmel Bay in central California, already experience pH as low as 7.4 (Fig. 1). Due in part to the high nutrient content of upwelled water, the CCLME supports a highly productive ecosystem (Block et al., 2011), and is host to many important fishery species (Costanza et al., 1997).

Organisms that inhabit the CCLME and other temperate ecosystems were initially found to be tolerant to high CO₂ levels (Kikkawa et al., 2003; Ishimatsu et al., 2004), perhaps because of their natural exposure to variable and high CO₂ levels during upwelling events (Munday et al., 2011). However, there is mounting evidence that some temperate fishes (Melzner et al., 2009; Franke and Clemmesen 2011; Moran and Støttrup 2011; Frommel et al., 2012; Jutfelt et al., 2013; Kim et al., 2013; Hamilton et al., 2014; Hamilton et al., 2015 in prep) respond negatively to elevated CO₂, while others appear to be resistant to future levels of OA (Hurst et al., 2012; Hurst et al., 2013; Frommel et al., 2013). Identifying the mechanisms responsible for determining species-specific susceptibility to OA remains a major gap in our knowledge. Differences in evolutionary history and early life history traits, which influence exposure to elevated CO₂ in the marine environment, are both potential drivers of the sensitivity of different fish species to future changes in ocean chemistry. To predict how fish stocks will fare in the future, it is necessary to determine which factors influence differential susceptibility to climate change stressors and to identify what traits may confer resistance.

Disentangling the influence of adaptation and acclimation to high CO₂ levels in fishes is a critical step in understanding how fish will cope with climate change. If evolutionary history determines species-specific susceptibility to OA, then species that have not previously adapted to high CO₂ levels may not be able to keep pace with rapidly progressing climate change. However, if early life history exposure facilitates acclimation to high CO₂, many if not all fish species have the potential to acclimate to future CO₂ levels. One study demonstrates divergent responses to OA of two tropical fish species that co-occur as adults, but differ in exposure to high CO₂ during development (Munday et al., 2011). This indicates that early exposure to high CO₂ may provide larval and juvenile fish the opportunity to acclimate to OA conditions. However, because these species aren't closely related, evolutionary history may also be responsible for their divergent responses. A single temperate species with two distinct populations that differ in exposure to high CO₂ during development was found to differ in its tolerance of OA conditions. The stock exposed to high CO₂ during development was tolerant of OA conditions, while the stock that does not experience high CO₂ during development experienced organ damage (Frommel et al., 2012; Frommel et al., 2013). These studies demonstrate that one temperate species acclimation potential to OA, depends on early life history exposure to high CO₂, or early exposure to high CO₂ lead to local adaption of resistance to OA of this species. An ideal test the importance of life history traits and shared evolutionary history in determining resistance to future CO₂ levels, requires a group of closely related fishes with shared evolutionary history, but differing life history traits (i.e. life history syndromes). Rockfish, genus *Sebastes*, provide an excellent opportunity to test the influence of different life history syndromes (i.e. different exposure to high CO₂ during development) among species that co-occur as adults and have a well-studied

phylogenetic history (Moser and Boehlert, 1991; Love et al., 2002; Hyde and Vetter, 2007; Pavoine et al., 2009).

In this study, I investigate the behavioral and physiological responses to chronic elevated CO₂ exposure in six closely related species of rockfish, grouped into two shared early life history syndromes. One group composed of kelp, gopher, and copper rockfish (*Sebastes atrovirens*, *S. carnatus*, and *S. caurinus* respectively) shares early development habitats and commonly recruit to kelp forest canopy. This group will be referred to as the “canopy recruiting” group. The canopy recruiting group only very recently diverged from a common ancestor 1.5 million years ago (mya) (Hyde and Vetter, 2007; Pavoine et al., 2009). The second group is composed of stripetail, olive, and blue rockfish (*S. saxicola*, *S. serranoides*, and *S. mystinus* respectively), which share early life development habitats and recruit to the benthos in kelp forests. This second group will be referred to as the “benthic recruiting” group. Within the benthic recruiting group are two closely related species (olive and blue rockfish), which diverged from each other 4 mya. The third benthic species (stripetail rockfish) is more closely related to the canopy recruiting species (diverged from the canopy species 4 mya vs. diverging from the two other benthic species 6 mya) despite sharing common early life history characteristics with the other two benthic species (see below). All species in this study diverged from a common ancestor within the last 6.5 million years (Hyde and Vetter, 2007; Pavoine et al., 2009).

Juvenile fish with early life stages that differ in exposure to elevated CO₂ as larvae and juveniles may differ in their response to OA. The canopy recruiting group is characterized by releasing young in spring (Echeverria, 1987), having a short pelagic larval duration (1-2 months), small size at recruitment (<2 cm), having larvae that inhabit shallow depths (Moser and Boehlert 1991), recruit well during El Niño periods (i.e. weak upwelling and low CO₂) (Lenarz et al.,

1995), and recruit to kelp forest canopies (Carr 1991). During their larval and early juvenile stages, the canopy recruiting group inhabits water masses that are relatively low in CO₂ because they live near the surface and co-occur with many photosynthetic organisms (i.e., phytoplankton and kelp) that absorb CO₂. The benthic recruiting group is characterized by releasing young in winter (Echeverria, 1987), having a long pelagic larval duration (3-4 months) with recruits arriving to nearshore habitats at relatively large sizes (> 2.5 cm), having larvae that inhabit deeper depths (Moser and Boehlert 1991), exhibiting strong recruitment during La Niña periods (i.e. strong upwelling, high CO₂) (Lenarz et al., 1995), and settling to the benthos of rocky reefs (Carr 1991). Additionally, benthic recruiting species inhabit water masses where respiration greatly exceeds photosynthesis and thus experience much higher CO₂ from both allochthonous and autochthonous sources than their canopy recruiting relatives.

To determine if early life history syndromes dictate susceptibility of rockfish to CO₂-mediated behavioral and physiological impairments, the six species of rockfish from two life history syndromes (canopy vs. benthic recruiting) were exposed to one of four CO₂ treatments for a period of 3-4 months and tested in a suite of behavioral and physiological experiments. I hypothesized that the canopy recruiters are naïve to elevated CO₂ water before the settlement transition and as such are predicted to be more susceptible to OA conditions. In contrast, benthic recruiting species are predicted to be exposed to higher CO₂ levels during the larval, pelagic juvenile, and settlement periods; therefore, they may have developed mechanisms to compensate for higher CO₂ water and are hypothesized to be tolerant of projected future ocean conditions.

Methods:*Study Species, Collections, and Husbandry:*

Field collections of juvenile rockfishes occurred during April – July of 2013 and 2014 at Stillwater Cove, Carmel Bay, CA (36.5605° N, 121.9458°W), the Monterey Harbor, Monterey Bay, CA (36.6046°N, 121.8931W), and at Otter Point, Monterey Bay, CA (36.6295°N, 121.8913°W). Fish were collected from shallow rocky reefs in large kelp beds. Fish were collected using hand nets on SCUBA. Collections focused on species from two life history syndromes: canopy recruiting and benthic recruiting. These groups were selected because they are predicted to differ in their exposure history to elevated CO₂ during the larval and early juvenile phases. Six species were collected; three canopy recruiting species: kelp, gopher, and copper rockfish (*Sebastes atrovirens*, *S. carnatus*, and *S. caurinus* respectively) and three benthic recruiting species: stripetail, olive, and blue rockfish (*S. saxicola*, *S. serranoides*, and *S. mystinus* respectively). Copper and blue rockfish were collected in 2013 and kelp, gopher, stripetail, and olive rockfish were collected in 2014. All the juvenile rockfishes were transferred to one-gallon zip lock bags and placed in coolers for transport to the Moss Landing Marine Laboratories (MLML) aquarium facility (IACUC protocol # 986). Fish were acclimated to the lab setting for two weeks in flow through seawater at ambient conditions, while being fed mysid shrimp daily *ad libitum* before being transferred to the experimental treatment tanks.

In 2013, $n = 40$ copper and blue rockfish, and in 2014, $n = 48$ kelp, gopher, stripetail, and olive were randomly assigned to the four CO₂ treatments. The addition of each species to the experimental treatments occurred at two week intervals because of differences in timing of recruitment and to offset the use of rate limiting experimental apparatuses. In the experimental

aquaria, fish were fed frozen mysid shrimp daily ad libitum. Excess food and waste was removed from aquaria weekly. Every fish was used in each experiment. If a fish died before the end of the experiments, it was replaced by a new individual as available. Replacement individuals were run in every experiment and had the same acclimation period before the first experiment and resting periods between experiments as all other individuals.

To distinguish between individual fishes that were housed in shared tanks, visual implant elastomer tags (Northwest Marine Technology Inc.) were injected under the skin. A combination of 8 different colors and five tag locations (both sides of the body near the head, both sides of the dorsal musculature, and the caudal peduncle) were used to create individual identification patterns that could be used to track an individual's performance throughout the experiments.

CO₂ manipulation:

Following their two-week acclimation to captivity, experimental fishes were transferred to the seawater lab at the Monterey Bay Aquarium Research Institute (MBARI). Fish were placed into one of four target pCO₂ treatments in a flow through system: ambient pH ~7.9 (pCO₂~540 ppm), pH = 7.75 (pCO₂~820 ppm), pH = 7.5 (pCO₂~2100 ppm), and pH = 7.3 (pCO₂~3300 ppm). These treatments were created and maintained by adding 100% CO₂ gas through Liquid-Cel membrane contactors to four 500 L header tanks to simulate a suite of possible future ocean conditions for the next 100 years and beyond (Meinshausen et al., 2011; Gruber et al., 2012). The most extreme treatment reflects CO₂ levels that occur for short durations at Stillwater Cove during intense periods of upwelling (Fig. 1). In 2013, the pH treatments were unreplicated and fish were housed in 100 L aquaria containing $n = 8-10$ rockfish of each species; in 2014, $n = 3$ fish of each species were housed in replicate 10 L tanks arranged

in a 4x4 (4 treatments x 4 replicate tanks) Latin Squares design to account for any variability in the treatment tanks (Fig. 2). In both years, water flow rates from the header tanks to the treatment tanks were set at 0.5 L min^{-1} . Treatment water was maintained at $10 \text{ }^\circ\text{C}$ using a heater and chiller connected with a counter current exchanger. Dissolved oxygen (DO) was maintained near 100% saturation ($\sim 250\text{-}280 \mu\text{M}$) using a similar membrane contactor and O_2 gas. Flow of CO_2 and O_2 into the header tanks was controlled by solenoid valves and mass flow controllers (Sierra Instruments, SmartTrak 100) based on feedback from changes in pH levels measured using a Honeywell Durafet pH sensor and dissolved oxygen levels using Aanderaa AADI oxygen optodes (model 3830) connected to a computer monitoring and control system running LabView software. A porthole over each rearing tank was used to deliver food (frozen mysids) and provide access for daily measurements of temperature, pH and DO in each tank using a Hach HQ40d portable multi-parameter meter. Every three weeks, water samples were collected from three random tanks in each treatment and analyzed for dissolved inorganic carbon (DIC) on a custom-build DIC analyzer (1), total alkalinity (A_T) using an alkalinity titrator (SI Analytics, TitroLine 7000), and pH using a pH spectrophotometer (Shimadzu, UV-1601), following standard protocols (Riebesell et al., 2010). These metrics were used to verify pH measurements and calculate $p\text{CO}_2$ using the program CO2SYS (Lewis and Wallace 1998).

All experiments, except aerobic scope in 2013, were run in control water due to logistical constraints on treatment water supply. Due to the need to maintain consistent water temperature and limitation of control water production, the 2013 aerobic scope experiments were run in chilled control water ($\sim 8.2 \text{ }^\circ\text{C}$) set aside for another experiment. This was remedied in 2014. Similar studies have found that behavioral impairments in fish remain several days after return to control conditions (Munday et al., 2010; Nilsson et al., 2012). In addition, previous studies

examining the effects of OA on swimming physiology have used control water (Bignami et al., 2013), showing that CO₂ concentration of the water in the treatment chamber for a short-term trial does not affect critical swimming speed (Munday et al., 2009).

Escape Time Experiment

To test for differences in exploratory behavior in response to elevated CO₂, an escape chamber was used (Jutfelt et al., 2013). Fish were acclimated to experimental pCO₂ treatments for three weeks of cumulative exposure (in 2014) or 12 weeks exposure to pCO₂ treatments and a minimum of one week recovery from behavioral lateralization experiments (in 2013). The escape chamber was constructed of a 28 cm tall × 9 cm diameter white PVC tube with a 5 cm diameter whole cut in the side (Fig. 2B). A slit was cut 8 cm from the top of the PVC tube to allow insertion of a clear Plexiglas divider. This allowed the fish to acclimate in the top of the escape chamber before the trial, and standardized how each fish entered the lower portion of the chamber. The escape chamber was placed in a 100 L aquarium with the sides of the aquarium covered in black plastic. Fish were placed into the top of the escape chamber with the divider in place and allowed to acclimate for fifteen minutes (2014) or three minutes (2013). After acclimation, the divider was removed and the time it took each fish to exit the escape chamber was measured. If a fish did not exit the chamber after 15 minutes, the trial ended and the fish was assigned an escape time of 15 minutes. Blue rockfish were not run in this experiment because pilot studies failed to identify a set of conditions where blue rockfish would behave consistently in the escape chamber.

Behavioral Lateralization

Behavioral lateralization arises from asymmetrical brain function, where specific brain hemispheres are responsible for processing certain types of information. Behavioral lateralization is an expression of these brain asymmetries and is reflected in how organisms behave in response to certain stimuli. The degree of an individual's lateralization (bias for left vs. right turning decisions) can affect performance in cognitive tasks, schooling behavior, spatial orientation, and escape reactions from predators. The degree of an individual's lateralization (bias for left vs. right turning decisions) can affect performance in cognitive tasks, schooling behavior, spatial orientation, and escape reactions from predators, and has been shown to decrease in response to elevated CO₂ (Domenici et al., 2012; Jutfelt et al., 2013). Thus, behavioral lateralization provides a powerful test of brain function for different decision-making tasks involving left versus right responses to environmental stimuli. A double T-Maze (Bisazza et al., 1998; Domenici et al., 2012; Jutfelt et al., 2013) was used to test for changes in brain functional asymmetry in response to elevated CO₂. The T-maze was constructed inside a 50 cm long × 30 cm wide × 25 cm tall aquarium (Fig. 2C). The T-maze consisted of a 30 cm long × 10 cm wide channel with 10cm wide T-junctions at the ends of the tank. The aquarium was filled with seawater to a height of 10 cm.

Behavioral lateralization experiments took place after fish had acclimated to their pH treatment for five weeks and had two weeks recovery from the escape time experiment (2014) or nine weeks acclimation to treatment conditions and a minimum of one week recovery from the critical swimming speed experiment (2013). Fish were placed in the T-Maze and allowed to acclimate for three minutes. After acclimation, a small hand net was used to coax the fish towards the end of the channel of the maze. Once a fish passed the mid point of the channel,

coaxing ceased so the fish had no outside influence on its turn direction when it reached the T-junction. A fish was considered to have made a turn once it rotated more than 45° in a certain direction. The turn direction was recorded and then the fish was guided back to the middle channel and coaxed in the opposite direction to account for any potential directional bias. Each fish was run for a total of 10 turns in the T-Maze (five at each end).

To examine the degree of behavioral lateralization of each individual fish, two indices were calculated, following Domenici et al. (2012). Relative lateralization quantifies the propensity of a fish to turn left versus right over the 10 trials, based on the equation:

$$\frac{(\#Right\ Turns - \#Left\ Turns)}{(\#Right\ Turns + \#Left\ Turns)} * 100$$

Positive values indicate a right turn bias, while negative values indicate a left turn bias. Relative lateralization indicates if an individual or group has a specific side bias and is useful in demonstrating changes in strength of a turning bias of a group that share the same bias. However, if a group of fishes is comprised of an equal number of right biased individuals and left biased individuals, relative lateralization becomes a poor indicator of changes in bias as averaging will result in a mean index of 0, suggesting that the group as a whole is unbiased. Absolute lateralization, the absolute value of the relative lateralization index, provides a directionless metric that measures the strength of an individual fish's bias, and the strength of a group's bias. Absolute lateralization has been previously shown to decrease in fishes with increased CO₂ conditions (Domenici et al., 2012, Jutfelt et al., 2013).

Critical Swimming Speed

To measure the effect of OA on swimming speed and endurance of juvenile rockfishes, the critical swimming speed (U_{crit}) of fish in the different pH treatments was measured following methodology in Kashef et al. (2014). U_{crit} measures a fish's maximum swimming performance in a short-term sustained swimming exercise and, if scaled to body length, allows for comparisons of relative swimming endurance and maximum aerobic capacity among fish of different lengths and forms (Plaut 2011). In 2014, critical swimming speed experiments took place after the fish had spent eight weeks in the pH treatments and had a minimum of one week to recover from the behavioral lateralization experiment. In 2013, U_{crit} experiments began after copper rockfish had acclimated to the treatment conditions for 4-7 weeks and after blue rockfish had acclimated for 7-9 weeks. U_{crit} was measured using a Loligo 10 L swimming tunnel with a two-way impeller to create a directional current for the fishes to swim against. First the total length of each fish was measured to the nearest 0.1 mm. Total length was used to standardize the current velocity in the flume in terms of body lengths per second ($bl\ s^{-1}$). Fish with damaged caudal fins were excluded from the experiment. Each juvenile rockfish was placed in the swimming flume and allowed to acclimate for 15 minutes at a flow speed of $0\ bl\ s^{-1}$. After the acclimation period, flume current velocity was increased in 1 body length per second increments at two-minute time intervals (Kashef et al. 2014). The swimming trial was stopped once the fish could no longer maintain its position in the swimming channel for the full duration of the two minute period. If a fish consistently swam along the side of the swim tunnel or hunkered into corners, it was excluded from the experiment. U_{crit} was calculated following Brett (1964):

$$U_{crit} = U_i + U_{ii} (t_i/t_{ii})$$

where, U_i is the highest velocity maintained for the entire interval (penultimate velocity), U_{ii} is the velocity increment (e.g., 1 body length per second), t_i is the time a fish swam in the highest velocity before failure, and t_{ii} is the time increment (2 minutes). To standardize U_{crit} for comparisons of rockfishes of different sizes, relative U_{crit} with units in body lengths per second ($bl\ s^{-1}$) was calculated.

Aerobic Performance

Aerobic scope is a measure of the range of an organism's oxygen consumption and is defined as the difference between maximum oxygen consumption rate and resting oxygen consumption. To compare the range of aerobic scope in response to elevated CO_2 , swimming respirometry trials were conducted using methodology adapted from Munday et al. (2009). Respiration experiments took place after the fish had spent 10 weeks (2014) or 16 weeks (2013) in the pH treatments and had two weeks to recover from U_{crit} experiments (2014) or two weeks recovery from escape experiment (2013). First fish were measured to the nearest mm using calipers, and then wet weight was measured by patting the fish dry with a paper towel before being weighed to the nearest .0001g on a microbalance. To measure resting oxygen consumption in 2013, fish were placed in the Loligo swimming flume with minimal current velocity (to slowly circulate water but allowing the fish to remain motionless) and allowed to acclimate for two hours with the chamber open to exchange with fresh seawater. Preliminary experiments showed that that juvenile rockfish acclimated to the flume rapidly because their oxygen consumption rate stabilized within 2 hours. The chamber was then closed off to prevent circulation of oxygenated water and an oxygen optode (FireSting, PyroScience) was used to measure the decline in oxygen levels in the sealed chamber for 60 minutes. In 2014, juvenile rockfish were placed in 243 mL

Loligo respiration chambers inside of a 100 L water bath. The bath was filled with flow through control water. Resting respiration rates of the 2014 species were measured using intermittent flow respirometry. Intermittent flow respirometry measures respiration rates in cycles of flushing the chamber with oxygenated water, then closing the chamber off from new control water and recirculating water within the chamber to measure changes in oxygen concentration. A cycle was comprised of three different parts. First a 300 s flush cycle, where a flush pump moved oxygenated water from the water bath into the chamber. This ensured that oxygenated water from the water bath completely flushed through the respiration chamber and brought oxygen levels back to saturation. Next, a 180 s waiting period where the flush pump ceased running and a recirculation pump cycled water through a closed loop including the chamber and across an oxygen optode. This period allowed the fish and instruments to adjust to the new oxygen levels. Finally, a 780 s measuring period where water was recirculated within the closed loop and respiration rates were calculated. Oxygen concentration was measured throughout all cycles, but oxygen consumption (MO_2) was calculated during the measuring phase. Five cycles of intermittent flow respirometry were run for each fish. The lowest MO_2 value was used as their resting oxygen consumption rate. To measure maximum oxygen consumption rates at peak aerobic performance, juvenile rockfish were placed in the Loligo swim flume and current velocity was slowly increased from 0-3 $bl\ s^{-1}$ over a two minute period. Next, flume velocity was adjusted according to each species' previously determined average U_{crit} value and then adjusted according to each individual's performance such that a fish was swimming at the highest velocity it could maintain for 20 minutes. Oxygen concentration was measured over the course of the twenty minute experiment and maximum oxygen consumption was calculated by the equation:

$$\frac{(Slope * 3600s)}{W} * V$$

Where Slope is the change in oxygen concentration in the resting respiration trial of a particular fish in ppm/s, W is the weight of the fish in kg and V is the volume of the respiration chamber in L. To calculate aerobic scope of each fish, the resting oxygen consumption was subtracted from maximum oxygen consumption.

Somatic Growth

To measure the effect of OA on growth of juvenile rockfishes, pre-treatment total length of each fish was measured using calipers with a resolution of 0.1 mm. Fish were re-measured at the end of all the experiments (~20 weeks). The difference in length was divided by the number of days the fish were exposed to experimental conditions. Fish weight was measured for kelp, gopher, stripetail, and olive rockfish. Change in biomass was calculated by subtracting initial wet weight from final wet weight and dividing by the total number of days in the experimental conditions.

Data Analysis

A One-Way Blocked ANOVA was used to test for the effect of pH and treatment tank on the different experimental responses of kelp, gopher, stripetail, and olive rockfish. Because treatment tank was not replicated for the 2013 experiments, copper and blue rockfish's experimental responses were tested using a One-Way ANOVA with pH as the fixed factor.

To compare the pooled response of the two groups with different life history syndromes, and to account for running the experiments in different years with different acclimation periods, individual and group mean effect sizes were calculated between the control and the extreme CO₂

treatment. To calculate the effect size of each species' response to CO₂ in a particular experiment, ln transformed response ratios were calculated as

$$LnRR = \ln(\bar{X}_E) - \ln(\bar{X}_C),$$

where \bar{X}_E and \bar{X}_C are the mean response of the experimental and control treatments respectively.

These response ratios quantify proportional changes in effect size in response to experimental manipulation of pH. A ln-transformed response ratio provides easily interpretable biological information as well as robust statistical properties (Hedges et al., 1999; Kroeker et al., 2010). A ln-transformed response ratio of zero indicates no difference between control and experimental treatments, while positive and negative values indicate positive and negative effects of the experimental treatment respectively. Variance was calculated as

$$v = \frac{(S_E)^2}{n_E \bar{X}_E^2} + \frac{(S_C)^2}{n_C \bar{X}_C^2},$$

where S is the standard deviation and n is the sample size of the control and experimental treatments (denoted by their subscripts). Using software developed by Dietz et al. (2015), a Hedges-Olkin random effects model was used to calculate the mean effect of each response variable for each recruitment group. This random effects model was chosen because it accounted for both sampling variation and between experiment variation. Because of the lower pH of central California coastal waters (Fig. 1; Hauri et al., 2009) relative to the average ocean surface water pH, we ran the effect size analysis with both pH 8.0 and 7.8 as control treatments and compared them to the experimental treatment of 7.2. The 95% confidence intervals were calculated and used to test whether the mean effects of each of the different life history syndromes were significantly different from 0 in a given experiment. Two-sample t-tests were

also used to test whether the average response of each experiment differed between the two life history groups.

Additional data manipulation was used to clarify the effect size of relative lateralization. First, 100 was added to all relative lateralization values to eliminate negative values from the data set because log transformation of negative values is not possible. Next the absolute value of the effect size was calculated and used for statistical analysis as negative $\ln RR$ are indicative of changes in turn direction and not strength of response. In this analysis the magnitude of the difference between the control and the experimental treatment is of interest, not the direction of this magnitude.

Results

Behavioral Responses

The average time required for fish to discover the exit hole of the escape chamber did not differ significantly for canopy recruiting kelp, copper, and gopher rockfish as a function of pCO_2 exposure history. However, all three species exhibited a consistent trend for increasing escape time with increasing pCO_2 (Fig. 3 A-C, Table 1 A). Canopy escape times at the highest pCO_2 treatment were 297%, 131%, and 126% longer than at the control treatment for kelp, gopher, and copper rockfish respectively. Benthic recruiting stripetail rockfish also exhibited a non-significant trend for escape to increase with increasing CO_2 . Interestingly, stripetail rockfish exhibited an order of magnitude longer escape time across all treatments than the canopy recruiting species (Fig. 3D, Table 1A). Olive rockfish also had an order of magnitude longer escape time than the canopy recruiting species in all treatments except in the second highest pCO_2 treatment, where they exhibited significantly shorter escape time compared to the control

treatment based on Tukey post hoc analysis (Fig. 3E, Table 1A). Olive rockfish experienced a 10% decrease in escape time between the highest $p\text{CO}_2$ treatments and control treatment. No significant effects were found for the factor of tank location for any of the species. Blue rockfish were not run in the escape time experiment.

Two of the three canopy recruiting species experienced significant changes in relative lateralization as a function of increasing CO_2 . Kelp rockfish showed a significant shift from being right turn biased to left turn biased as CO_2 increased (Fig. 4A, Table 1B). Gopher rockfish were unbiased in all $p\text{CO}_2$ treatments except 2100 ppm, where they showed a non-significant trend for being right turn biased (Fig. 4B, Table 1B). Copper rockfish exhibited a significant shift from being unbiased or left turn biased in their turn direction at low CO_2 to becoming significantly right turn biased as CO_2 increased (Fig. 4C, Table 1B), a pattern opposite that of gopher rockfish. The benthic recruiting species did not have significant changes in relative lateralization by treatment (Fig. 4D-F, Table 1B). There was no effect of treatment tank location for any species. The statistically significant changes in brain asymmetry observed in the lateralization tests for kelp and copper rockfish indicate that elevated CO_2 induced detectable changes in brain function.

Changes in CO_2 did not affect the absolute lateralization index of these six rockfish species (Fig. 5A-F, Table 1C).

Physiological Responses

Increasing CO_2 was significantly associated with a reduction in the critical swimming speed of all three canopy recruiting species. The U_{crit} of kelp, gopher, and copper rockfish decreased from the control to the highest $p\text{CO}_2$ treatment by 16.7%, 9.7%, and 16.8%

respectively (Fig. 6A-C, Table 2A). Gopher rockfish experienced a significant treatment tank effect, with slower swimming individuals across all treatments in block C compared to the other three blocks. There was no tank effect for kelp or copper rockfish. In contrast to the canopy recruiting species, there was no significant difference in the critical swimming speed of the benthic recruiting species as a function of CO₂ exposure history or treatment tank (Fig. 6D-E, Table 2A).

Experimental water temperatures in the aerobic scope experiments differed between species and years. In 2013 chilled control water was used due to logistical constraints, whereas control water from the experimental treatments was used in 2014 experiments. Copper and blue rockfish were run in water with an average temperature of 8.12 and 8.27°C water respectively, and kelp, gopher, stripetail, and olive rockfish were run in 11.90, 12.05, 12.40, and 12.67 °C water, respectively.

Increased CO₂ did not have a consistent effect on the aerobic scope of the different rockfish species. Increased CO₂ significantly impacted the copper rockfish, decreasing the aerobic scope by 52.4% and 40.1% at the second highest and highest CO₂ treatments respectively (Fig. 7C, Table 2B). Increased CO₂ did not affect the other canopy recruiting species (Fig. 7A-B, Table 2). This decrease in aerobic scope was potentially driven by decreased maximum oxygen consumption at the highest CO₂ levels (ANOVA, $F_{3,28}=2.574$, $P=0.074$) with relatively constant resting oxygen consumption rates (ANOVA, $F_{3,28}=0.850$, $P=0.479$) across treatments. Gopher rockfish experienced a significant tank effect where the block with the highest average aerobic scope across treatments was significantly higher than the block with the lowest average aerobic scope across all treatments. There was no significant effect of CO₂ treatment on the aerobic scope of the benthic recruiting species (Fig. 7D-F, Table 2B).

There was no significant difference in the initial lengths of rockfish among $p\text{CO}_2$ treatments for any of the species tested, and no difference in the starting weight of kelp, gopher, stripetail, and olive rockfish (copper and blue rockfish were not weighed prior to experiments), nor were there any treatment tank effects on starting length and weight (Table 3A and B respectively). There were no significant effects of CO_2 on growth rate in length (Fig. 8A-B, Table 3C) except for blue rockfish, which had significantly lower growth rate in length at $p\text{CO}_2$ of 2100 ppm compared to the other treatments (Fig. 8F, Table 3C). Growth in biomass as a function of CO_2 did not differ except for stripetail rockfish, which declined significantly as CO_2 increased (Fig. 9, Table 3).

Comparative Analysis by Life History Syndrome

When the responses of the six species of rockfish to OA were pooled by recruitment syndrome, it was evident that elevated CO_2 differentially affected the behavior and physiology of the canopy recruiting group compared to the benthic recruiting group. The Hedges-Olkin random effects model was used to calculate 95% confidence intervals around the mean effect size for each group, which produced a statistical test for whether the mean effect was different from zero. The effect size analysis indicated that increased CO_2 significantly affected the escape time, relative lateralization, and critical swimming speed of canopy recruiting species, but CO_2 did not significantly affect any of the response metrics for benthic recruiting species (Fig. 10). Canopy recruiting species experienced a 100.9% increase in escape time, while benthic recruiting species experience a non significant 10.4% increase in escape time at the high $p\text{CO}_2$ treatment compared to the control (Fig. 10A, Table 4A). Elevated CO_2 resulted in a significant change in effect size

of the relative lateralization index of canopy recruiting species (by 24.1%), with no significant effect detected for the benthic recruiting group (Fig. 10B, Table 4B). Exposure to elevated CO₂ did not have a significant impact on the absolute lateralization of either life history syndromes (Fig. 10C, Table 4C). Increased CO₂ resulted in a significant decrease in the critical swimming speed (by 15.4%) of the canopy recruiting group, but no significant change in the U_{crit} of the benthic recruiting group (Fig. 10D, Table 4D). Neither aerobic scope, resting metabolic rate, or maximum oxygen consumption exhibited a significant change to increased CO₂ for either rockfish early life history syndrome (Fig. 10E, Table 4E). Ocean acidification did not appear to influence the growth rate (Fig. 10F, Table 4F) or change in biomass (Fig. 10G, Table 4G) of species from either life history syndrome category.

Average effect size in each experiment in response to OA was directly compared between the two life history syndromes to determine if the mean response of canopy or benthic recruiting species were significantly different from each other. The pattern was similar to the effect size analysis comparing each species group's effect size relative to 0. Mean effect size of escape time for the canopy recruiting group was significantly greater than the benthic recruiting group ($t_3 = 3.27, P = 0.046$). There was no difference in mean effect size of absolute lateralization ($t_4 = 1.72, P = 0.26$) or relative lateralization between the two life history syndromes ($t_4 = 0.84, P = 0.45$). Elevated CO₂ decreased the U_{crit} of both groups, but canopy recruiting species had a significantly lower mean effect size than benthic recruiting species ($t_4 = -2.88, P = 0.045$). Elevated CO₂ increased mean effect sizes for aerobic scope of both groups, but these effect sizes were not significantly different from each other ($t_4 = 0.22, P = 0.84$). Ocean acidification did not differentially affect the mean effect sizes for growth rate in length ($t_4 = -0.43, P = 0.69$) or mass ($t_4 = -0.06, P = 0.96$) of the two life history syndromes.

Discussion

The results of this study demonstrate that rockfish exhibit species-specific responses to OA and that early life history traits appear to influence how juvenile rockfish will respond to increasing CO₂ in the future. Species possessing early life histories characterized by larval and early juvenile development in water masses naturally depleted in CO₂ (canopy recruiting group) appear to be strongly affected by future elevated CO₂ levels, while species that undergo early development in water masses characterized by naturally elevated CO₂ (benthic recruiting group) appear resistant to CO₂-induced behavioral and physiological impairments. When analyzed as a group, the canopy recruiting species exhibited significant shifts in relative behavioral lateralization and increased escape time, although absolute lateralization was not affected. In contrast, no behavioral impairments in response to elevated CO₂ conditions were observed in the benthic recruiting species. Interestingly, olive rockfish showed a positive response to OA with decreased escape time in the second highest pCO₂ level. Canopy recruiting species all experienced reductions in U_{crit} at elevated CO₂ when analyzed at both the single species and group levels. In contrast, increased CO₂ did not have an effect on U_{crit} of the benthic recruiting species. Increased CO₂ negatively impacted the aerobic scope of copper rockfish but did not affect this physiological measure of the canopy recruiting species as a group or for any of the benthic recruiting species. Elevated CO₂ resulted in a decrease in growth of blue rockfish at the second highest pCO₂ level, and a decrease in biomass growth of stripetail rockfish at the highest pCO₂ level; however, the effects of increased CO₂ on growth were inconsistent and non-significant for most species.

Tolerance of future ocean conditions may depend on rockfish either having previously adapted to compensate for high CO₂ conditions during their early life stages, or possessing the capacity to acclimate to OA conditions after being exposed to high CO₂ water during development. Unfortunately, the larval dispersal history of the individuals used in these experiments and thus their CO₂ exposure prior to capture are unknown. However, the typical depth and cross-shore distributions of larval rockfish (Moser and Boehlert, 1991, Love et al., 2002) and corresponding seawater chemistry of those water masses indicate that species with different life history syndromes are likely to encounter distinct CO₂ and pH conditions during development. Thus, certain species may produce larvae and juveniles that are either pre-adapted to elevated CO₂ and lowered pH, or acclimate to high CO₂ and low pH conditions during development. The findings from this study suggest that inhabiting an ecosystem that is naturally high in CO₂ is not sufficient for rockfish to develop resistance to future OA conditions. As adults, the canopy recruiting species frequently experience low pH levels in central California (Fig. 1), because they make an ontogenetic shift from living in the kelp canopy as juveniles to becoming benthic (where CO₂ is higher and pH is lower) as adults. Despite these species inhabiting naturally high CO₂ and low pH water as adults, their juveniles are susceptible to CO₂-mediated impairments. Because the six rockfish species used in this study have recently diverged (6.5 mya) it is unlikely that shared evolutionary history is responsible for the differences in susceptibility to OA conditions. The fact that stripetail rockfish are more closely related to the canopy recruiting species than the benthic recruiting species they share life history characteristics with, supports the hypothesis that early life history exposure to high CO₂ determines rockfishes' response to OA.

The species in the canopy recruiting group showed similar behavioral impairments compared to *Neopomacentrus azysron* and *Gasterosteus aculeatus* (Domenici et al., 2012; Jutfelt et al., 2013). Though high CO₂ did not cause shifts in absolute lateralization of any rockfish species, as has been shown in several tropical and temperate species (Domenici et al., 2012; Nilsson et al., 2012; Jutfelt et al., 2013; Welch et al., 2014), the brains of kelp and copper rockfish exhibited dramatic shifts in relative lateralization. Kelp and copper rockfish switch from using one hemisphere in low CO₂ conditions to using the opposite hemisphere in OA conditions. Similar CO₂-mediated reversals of relative lateralization have been found in *G. aculeatus* (Jutfelt et al., 2013) and *Pomacentrus wardi* (Domenici et al., 2014). As a group, the canopy recruiting species also experienced increased escape time, similar to responses seen in *G. aculeatus* (Jutfelt et al., 2013). While this study did not directly test the link between impaired behavior and depolarization of the GABA_A neurotransmitter receptor, depolarization was responsible for changes in behavior of splitnose rockfish (*S. diploproa*), which experienced increased anxiety under OA conditions (Hamilton et al., 2014). In tropical damselfish, the consequences of altered neurotransmitter function are: attraction to predator odor cues that are normally avoided (Munday et al., 2009; Welch et al., 2014) and increased predation in lab (Ferrari et al., 2011) and field (Munday et al., 2010) conditions. Because two of the canopy recruiting species experienced altered relative lateralization and the canopy group experienced increased escape time, it is clear that increased CO₂ impacts the behavior of this suite of species. While not tested, it is possible that canopy recruiting species may experience similar increases in mortality in the wild. The benthic recruiting species did not experience CO₂-mediated behavioral abnormalities, which suggests they possess blood pH compensation mechanisms that do not alter ion concentration

gradients across neuronal membranes, or the CO₂ levels in this study did not sufficiently alter the blood pH of these species to require blood pH compensation.

This is the first study to detect a relationship between OA and the swimming endurance in fishes, though other studies have reported no effect (McKenzie et al., 2003; Melzner et al., 2009; Munday et al., 2009; Bignami et al., 2013). All three canopy recruiting species experienced precipitous declines in U_{crit} with increased CO₂, whereas CO₂ did not have an effect on the benthic recruiting species. Because the U_{crit} experiment took place after 4-8 weeks of treatment exposure, compensation for short term blood and plasma pH drops and subsequent decreases in hemoglobin binding efficiency should already have occurred. Fish can rapidly (3-24 hrs.) buffer blood pH by active ion exchange at the gill epithelia by excreting Cl⁻ and taking up HCO₃⁻ (Heisler, 1986). Decreases in swimming performance are likely due to altered ion concentration steady states and increased energy expenditure involved in maintaining these new equilibria (Cameron and Iwama 1988). In addition, ventilation is much more costly in water breathers (10% at rest and 70% during exercise) than air breathers (Gilmour 1998) and ventilation rates have been observed to increase for marine fishes exposed to high CO₂ conditions (Gilmour 2001), leading to an increased energy expenditure during active metabolism. Furthermore, larvae of two temperate fishes were found to experience tissue damage to the kidneys, liver, and pancreas when reared in elevated CO₂ conditions (Frommel et al., 2012; Frommel et al., 2014), potentially as a result of protein breakdown, decreased protein biosynthesis, or membrane damage. Though not tested in this study, increased energy expended during active metabolism and damaged metabolic regulatory organs would impair active metabolism and could explain the decreased swimming performance of canopy recruiting rockfishes in high CO₂ conditions.

Despite the consistent effects of OA on behavior and U_{crit} of canopy recruiting species, increased CO_2 did not consistently impact their aerobic performance. Copper rockfish exhibited a depression of aerobic performance under elevated CO_2 conditions, while kelp and gopher rockfish increased their aerobic scope, although this increase was not statistically significant. Aerobic performance has been shown to decrease following exposure to elevated CO_2 in several species (Munday et al., 2009b; Jordan and Steffensen, 2007; Methling et al., 2013; Tirsgaard et al., 2015), but increase in others (Rummer, et al., 2013; Grans, et al. 2014). The variability in responses of aerobic scope among the canopy recruiting rockfish species appeared to be driven by changes in maximum oxygen consumption. Increased CO_2 resulted in decreased maximum oxygen consumption in copper rockfish, while the maximum oxygen consumption rate of kelp and gopher rockfish showed an increasing trend with increasing CO_2 . Among the benthic recruiting species, striptail rockfish experienced a combination of increased resting oxygen consumption and decreased maximum oxygen consumption at the second lowest pCO_2 level. Olive rockfish experienced increased aerobic scope at the highest pCO_2 levels, driven by lower resting metabolic rates. Resting metabolic rates and associated resting oxygen consumption rates are generally unaffected by increased CO_2 (reviewed in Ishimatsu et al., 2005; Ishimatsu et al., 2008). Reduced resting metabolic rate at this high CO_2 level may indicate a sublethal stress response of olive rockfish to this treatment. The decreasing trends in aerobic scope of copper and blue rockfish were detected in 2013, while the 2014 species had either an increasing trend or no trend with respect to increasing CO_2 . Colder experimental water temperatures in 2013 in conjunction with increased CO_2 may explain the depressed metabolic activity in both copper and blue rockfishes. Studies on Atlantic salmon smolt have shown a 125% increase in ventilation rates to compensate for increased CO_2 (Fivelstad et al., 1999; Hosfeld et al., 2008) and

ventilation and metabolism are strongly temperature dependent. In general, teleost fish increase ventilation and oxygen consumption rates with increasing temperature (Fry 1971). A combination of warmer water elevating ventilation and oxygen consumption rates in 2014 versus 2013 may be responsible for the different responses of canopy recruiting rockfishes between these two years.

Increased CO₂ did not affect the growth in length or weight of any of the canopy recruiting species in this study despite causing other negative impacts. These results are similar to larval herring, which experienced decreased RNA:DNA ratios with increased CO₂ but no change in growth (Franke and Clemmesen 2011), and larval northern rock sole, which experienced declining condition factor but no change in length with increased CO₂ (Hurst et al., 2015). The benthic recruiting species experienced a decline in growth rate for blue rockfish and a decrease in weight gain of stripetail rockfish. The canopy recruiting species may be partitioning energy towards growth while suffering other sublethal consequences of altered ion concentration steady states that lead to altered behavior and decreased swimming ability. The decreased growth rate of blue rockfish and decrease in weight gain of stripetail rockfish may indicate some benthic species partition energy towards maintaining behavioral and physiological function in high CO₂ conditions.

Some species of fish are able to prepare their young to tolerate some of the negative consequences of ocean acidification via a process called transgenerational plasticity (TGP). For example, experiments have shown in two species of fish (one tropical and one temperate) that adults experiencing high CO₂ produced offspring with greater resistance to high CO₂ conditions (Miller et al., 2012; Murray et al., 2014). TGP appeared to prevent the high CO₂-induced increases in mortality and depressed growth that larvae exhibited when their parents were reared

in ambient or low CO₂ conditions. However, TGP did not confer resistance to altered olfactory discrimination of predator cues nor did TGP prevent changes in behavioral lateralization due to elevated CO₂ in another tropical species (Welch et al., 2014). While TGP may allow fish to cope with future changes in seawater chemistry in terms of growth and mortality, it appears that aspects of behavior are not influenced by transgenerational acclimation to OA conditions. Currently it is unclear whether TGP will affect the swimming physiology of fishes.

It is unlikely that TGP is responsible for the divergent responses of the rockfishes in this study. The canopy recruiting rockfishes inhabit shallower depth ranges as adults compared to the benthic recruiting species. However, the canopy recruiting species are also strongly associated with the benthos (where community respiration is higher) as adults and live in an ecosystem where high CO₂ water is frequently advected to the depths they inhabit (Love et al., 2002, Gruber et al., 2012). Thus the adults of the canopy recruiting species experience high CO₂ conditions, but do not appear to be able to transfer their acclimation to their offspring. In contrast, the adults of the benthic recruiting species (blue rockfish and olive rockfish) swim more actively throughout the water column and may more commonly experience lower CO₂ conditions characteristic of the surface and mid-water column, yet their juveniles are more tolerant of high CO₂. In addition, adult splitnose rockfish, which share early life history characteristics with the canopy recruiting species used in this study, but inhabit much deeper water (and likely higher CO₂) than any of the species used in this study (Love et al., 2002), experience CO₂-mediated behavioral impairments as juveniles (Hamilton et al., 2014). Therefore, the adults of both recruitment groups likely experience high CO₂ water and should have the potential to transfer that experience to their offspring via TGP, but only the benthic recruiting species are resistant to both behavioral and physiological impairments caused by exposure to conditions simulating the effects of OA.

Because TGP does not appear to be a factor in rockfish tolerance of high CO₂ conditions, the benthic recruiting species either have previously evolved resistance (i.e., local adaptation) to high CO₂ levels, or they acclimate to high CO₂ after early life history exposure to high CO₂ conditions. Adaptation to high CO₂ has been shown in a number of phytoplankton and invertebrate taxa (reviewed in Sunday et al., 2013). In experimental conditions, species with short generation times evolved tolerance for high CO₂ after several hundred generations. However, rockfishes have orders of magnitude longer generation times than phytoplankton and may not adapt on the same timescales on which seawater CO₂ concentrations are increasing. The purple sea urchin along the California coast was demonstrated to have location-specific tolerance for CO₂. Larvae of individuals from northern California, where CO₂ is relatively high, had much lower sensitivity to OA conditions than larvae from urchins collected from southern California, where CO₂ is relatively low (Kelley et al., 2013). Studies on Atlantic and Baltic cod found that the early life stages of the same species respond differently to OA conditions depending on their early life history exposure to high CO₂ levels. Atlantic cod larvae experience low CO₂ levels during development and suffered severe tissue damage when reared in OA conditions (Frommel et al., 2012). In contrast, young Baltic cod develop in highly variable CO₂, similar to rockfish in the CCLME, and their eggs and larvae were tolerant of future CO₂ conditions (Frommel et al., 2013). Because adult rockfishes of both groups experience high CO₂ in their natural habitat (Hauri et al., 2009; Gruber et al., 2012) and are closely related (Hyde and Vetter, 2007; Pavoine et al., 2009), the major differences in their responses to OA are likely due to differences in their early life history syndromes. The larger size and greater age of benthic recruiting species during recruitment means they also may be more developed, and thus better able to compensate for changes in CO₂, than the canopy recruiting species. Additionally, if the benthic recruiting species

are experiencing increased metabolic costs of compensating for high CO_2 , as suggested in Munday et al., 2009, especially in critical early life stages, these costs may have acted as a selective pressure to develop early life history-specific compensation mechanisms.

One aspect of OA that has not been well studied among fishes is the genomic response between fish reared in control and simulated future CO_2 conditions. Hamilton et al. (*in prep.*) examined the transcriptomes of copper and blue rockfish and observed dramatic species specific differences in gene expression in response to elevated CO_2 . Copper rockfish exhibited elevated expression of regulatory genes in the high CO_2 treatments. The up-regulation of these genes may be an attempt to cope with the stress of altered internal pH and indicate a sustained cellular stress response. Maintaining this stress response is another energetic cost the canopy recruiting species incur when exposed to elevated CO_2 and could account for decreases in swimming endurance. In contrast, blue rockfish had increased expression of muscle contractile genes in $p\text{CO}_2$ levels of 700 and 1200 ppm, though this expression was relatively down-regulated at the extreme $p\text{CO}_2$ level of 2000 ppm. These results suggest that blue rockfish exhibit a physiological response to elevated CO_2 that may explain this species' tolerance of OA conditions. The increased expression of muscle contractile proteins may indicate that blue rockfish muscle tissue undergoes structural remodeling to maintain physiological function in increased CO_2 conditions. However, the down-regulation of these same genes at the highest CO_2 treatment could indicate that blue rockfish are beginning to experience sublethal effects of OA, but this was not captured in the response variables tested. The differential gene expression between these two species is another way these species differed in their response to OA conditions and may explain a mechanism for tolerance in blue rockfish and heightened sensitivity of copper rockfish to changes in ocean chemistry. The shared behavioral and physiological responses to OA among the canopy

recruiting species suggest that kelp and gopher rockfish may also share gene expression responses to OA with copper rockfish. Additionally, the shared resistance of the benthic recruiting species may indicate a shared genomic response with blue rockfish that results in increased tolerance to OA conditions.

In the face of the projected rapid progression of OA conditions in the CCLME (Gruber et al., 2012), it is important to understand the mechanisms that determine which species will be resistant to and which species will suffer from OA. This is especially important because rockfish are part of the groundfish fishery that is the backbone of west coast commercial and recreational fisheries. Despite the rockfish in this study being closely related, they experienced dramatically different responses to increased CO₂. Because striptail rockfish are more closely related to the canopy recruiting species than the other benthic recruiting species (Hyde and Vetter, 2007; Pavoine et al., 2009), their tolerance of OA conditions suggests that exposure to high CO₂ during development and not genetic relatedness dictate the response of rockfish species to elevated CO₂. The only other OA study on rockfishes found that splitnose rockfish (*S. diploproa*) experience increased anxiety at elevated CO₂. This altered behavior was ameliorated when splitnose rockfish were treated with gabazine (a GABA_A receptor antagonist), establishing the link between altered behavior and improper signal transduction due to depolarization of the GABA_A neurotransmitter receptor. Splitnose rockfish are distantly related to both groups used in this study, diverging from them more than 6.5 mya (Hyde and Vetter, 2007). However, splitnose rockfish share early life history characteristics with the canopy recruiting species because splitnose rockfish recruit to floating kelp paddies and do not experience high CO₂ water during development (Love et al., 2002). The results of the splitnose rockfish experiment provide further evidence that rockfishes

with early life history syndromes that do not experience high CO₂ during development will be susceptible to OA.

These findings indicate that there are species specific and life history syndrome specific responses of juvenile rockfishes to chronic exposure of increased CO₂. Species with larvae and juveniles that inhabit water masses naturally exposed to high CO₂ conditions may be acclimated or adapted to these conditions and therefore resistant to behavioral and physiological impairments of future CO₂ levels. In contrast, species whose early life stages inhabit water masses that are naturally low in CO₂ may not be acclimated or adapted to dealing with the stresses of future CO₂ levels and may suffer both behavioral and physiological impairments that are associated with decreased survival in tropical fishes (Munday et al., 2010). Should these impairments lead to decreased fitness of affected species, early life history strategy may be used to predict how kelp forest rockfish assemblages may change in the face of future ocean conditions.

The progression of climate change may dramatically alter the recruitment success and assemblage of kelp forest recruiting rockfishes. Upwelling is anticipated to increase in strength and duration as atmospheric CO₂ levels rise (Bakun 1995; Bakun et al., 2010). The timing of upwelling may also be delayed by a month and peak upwelling intensity delayed until later in the summer (Snyder et al., 2003). Additionally, upwelled water in central California is depleted in oxygen, and the combined effects of OA and hypoxia have been reported to exacerbate physiological impairments and decrease survival in some fishes (Depasquale et al., 2015). The combination of increased upwelling intensity (especially during the timing of recruitment of the canopy recruiting species), and CO₂-mediated behavioral and physiological impairments may limit the recruitment success of canopy recruiting rockfishes (Lenarz et al., 1995). If canopy

recruiting species cannot adapt or acclimate to increased CO₂, diminished recruitment and impaired performance of recruited individuals at this critical life stage could lead to population declines. Conversely, benthic recruiting species may experience enhanced recruitment and population growth in these same conditions because strong upwelling favors their recruitment (Lenarz et al., 1995), potentially resulting in a shift in the rockfish assemblage in kelp forest ecosystems. Further examination of the effects of climate change on the larval stage of rockfishes, as well as the mechanism (early life history experience, or genetic adaptation) driving the different responses of these early life history groups to OA is required to determine whether rockfish have the potential to acclimate and adapt to future ocean conditions.

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Table I. One way ANOVA results for the effects of elevated CO₂ on each rockfish species' (A) Escape time, (B) Relative Lateralization, and (C) Absolute Lateralization. Experiments with both pH treatment and Tank effects were blocked one way blocked ANOVAs. Significant p-values are in bold.

| Experiment | Effect | DF | F | P-value |
|--|---------------|-----------|----------|----------------|
| <u>A. Escape Time</u> | | | | |
| Kelp RF | pH treatment | 3, 39 | 1.18 | 0.45 |
| | Tank | 3, 39 | | 0.25 |
| Gopher RF | pH treatment | 3, 40 | 1.53 | 0.08 |
| | Tank | 3, 40 | | 0.53 |
| Copper RF | pH treatment | 3, 28 | 0.78 | 0.52 |
| Stripetail RF | pH treatment | 3, 41 | 0.77 | 0.22 |
| | Tank | 3, 41 | | 0.38 |
| OYT RF | pH treatment | 3, 38 | 3.65 | 0.02 |
| | Tank | | | 0.31 |
| Blue RF | pH treatment | NA | NA | NA |
| <u>B. Relative Lateralization</u> | | | | |
| Kelp RF | pH treatment | 3, 39 | 2.22 | 0.02 |
| | Tank | 3, 39 | | 0.37 |
| Gopher RF | pH treatment | 3, 40 | 1.41 | 0.26 |
| | Tank | 3, 40 | | 0.24 |
| Copper RF | pH treatment | 3, 25 | 4.54 | 0.01 |
| Stripetail RF | pH treatment | 3, 41 | 0.29 | 0.86 |
| | Tank | 3, 41 | | 0.81 |
| OYT RF | pH treatment | 3, 36 | 0.41 | 0.78 |
| | Tank | | | 0.71 |
| Blue RF | pH treatment | 3, 29 | 1.22 | 0.32 |
| <u>C. Absolute Lateralization</u> | | | | |
| Kelp RF | pH treatment | 3, 40 | 1.15 | 0.67 |
| | Tank | 3, 40 | | 0.16 |

| | | | | |
|---------------|--------------|-------|------|------|
| Gopher RF | pH treatment | 3, 40 | 0.74 | 0.41 |
| | Tank | 3, 40 | | 0.67 |
| Copper RF | pH treatment | 3, 25 | 2.49 | 0.08 |
| Stripetail RF | pH treatment | 3, 41 | 1.04 | 0.52 |
| | Tank | 3, 41 | | 0.28 |
| OYT RF | pH treatment | 3, 36 | 1.01 | 0.91 |
| | Tank | 3, 36 | | 0.17 |
| Blue RF | pH treatment | 3, 29 | 0.83 | 0.49 |

Table II. One way ANOVA results for the effect of elevated CO₂ on each rockfish species' (A) U_{crit}, and (B) Aerobic Scope. Experiments with both pH treatment and Tank effects were blocked one way blocked ANOVAs. Significant p-values are in bold.

| Experiment | Effect | DF | F | P-value |
|--|---------------|-----------|----------|----------------|
| <u>A. Critical Swimming Speed</u> | | | | |
| Kelp RF | pH treatment | 3, 37 | 2.25 | 0.01 |
| | Tank | 3, 37 | | 0.85 |
| Gopher RF | pH treatment | 3, 39 | 3.20 | 0.05 |
| | Tank | | | 0.02 |
| Copper RF | pH treatment | 3, 21 | 3.79 | 0.03 |
| Stripetail RF | pH treatment | 3, 39 | 1.93 | 0.10 |
| | Tank | 3, 39 | | 0.21 |
| OYT RF | pH treatment | 3, 35 | 1.11 | 0.55 |
| | Tank | 3, 35 | | 0.18 |
| Blue RF | pH treatment | 3, 26 | 1.71 | 0.19 |
| <u>B. Aerobic Scope</u> | | | | |
| Kelp RF | pH treatment | 3, 38 | 1.58 | 0.26 |
| | Tank | 3, 38 | | 0.22 |
| Gopher RF | pH treatment | 3, 37 | 2.30 | 0.22 |
| | Tank | 3, 37 | | 0.04 |
| Copper RF | pH treatment | 3, 25 | 4.57 | 0.01 |
| Stripetail RF | pH treatment | 3, 41 | 1.72 | 0.14 |
| | Tank | 3, 41 | | 0.27 |
| OYT RF | pH treatment | 3, 40 | 1.29 | 0.39 |
| | Tank | 3, 40 | | 0.22 |
| Blue RF | pH treatment | 3, 30 | 0.45 | 0.72 |

Table III. One way ANOVA for each rockfish species' (A) starting length, (B) starting weight, (C) growth rate length, and (D) growth rate of weight in response to elevated CO₂. Experiments with both pH treatment and Tank effects were blocked one way blocked ANOVAs. Significant p-values are in bold.

| Experiment | Effect | DF | F | P-value |
|-------------------------------------|---------------|-----------|----------|----------------|
| <u>A. Starting Length</u> | | | | |
| Kelp RF | pH treatment | 3, 41 | 1.32 | 0.11 |
| | Tank | 3, 41 | | 0.64 |
| Gopher RF | pH treatment | 3, 41 | 0.67 | 0.62 |
| | Tank | 3, 41 | | 0.53 |
| Copper RF | pH treatment | 3, 27 | 0.29 | 0.83 |
| Stripetail RF | pH treatment | 3, 50 | 0.62 | 0.47 |
| | Tank | 3, 50 | | 0.78 |
| OYT RF | pH treatment | 3, 45 | 0.73 | 0.45 |
| | Tank | 3, 45 | | 0.63 |
| Blue RF | pH treatment | 3, 30 | 2.11 | 0.12 |
| <u>B. Starting Weight</u> | | | | |
| Kelp RF | pH treatment | 3, 41 | 1.74 | 0.06 |
| | Tank | 3, 41 | | 0.52 |
| Gopher RF | pH treatment | 3, 41 | 0.51 | 0.67 |
| | Tank | 3, 41 | | 0.68 |
| Copper RF | NA | NA | NA | NA |
| Stripetail RF | pH treatment | 3, 50 | 0.71 | 0.48 |
| | Tank | 3, 50 | | 0.62 |
| OYT RF | pH treatment | 3, 45 | 0.73 | 0.52 |
| | Tank | 3, 45 | | 0.56 |
| Blue RF | NA | NA | NA | NA |
| <u>C. Growth Rate Length</u> | | | | |
| Kelp RF | pH treatment | 3, 39 | 0.56 | 0.36 |
| | Tank | 3, 39 | | 0.98 |

| | | | | |
|-------------------------------------|--------------|-------|------|-------------|
| Gopher RF | pH treatment | 3, 40 | 0.76 | 0.98 |
| | Tank | 3, 40 | | 0.24 |
| Copper RF | pH treatment | 3, 23 | 1.27 | 0.30 |
| Stripetail RF | pH treatment | 3, 41 | 1.50 | 0.08 |
| | Tank | 3, 41 | | 0.60 |
| OYT RF | pH treatment | 3, 39 | 0.88 | 0.53 |
| | Tank | 3, 39 | | 0.39 |
| Blue RF | pH treatment | 3, 28 | 4.55 | 0.01 |
| <u>D. Growth Rate Weight</u> | | | | |
| Kelp RF | pH treatment | 3, 39 | 0.87 | 0.28 |
| | Tank | 3, 39 | | 0.78 |
| Gopher RF | pH treatment | 3, 40 | 1.46 | 0.38 |
| | Tank | 3, 40 | | 0.14 |
| Copper RF | NA | NA | NA | NA |
| Stripetail RF | pH treatment | 3, 37 | 1.69 | 0.04 |
| | Tank | 3, 37 | | 0.68 |
| OYT RF | pH treatment | 3, 39 | 1.89 | 0.12 |
| | Tank | 3, 39 | | 0.17 |
| Blue RF | NA | NA | NA | NA |

Table IV. Results and 95 % confidence intervals of effect size analysis on (A) Escape Time, (B) Relative Lateralization, (C) Absolute Lateralization, (D) U_{crit} , (E) Aerobic Scope, (F) Growth Rate in Length, and (G) Growth Rate in Weight of canopy and benthic recruiting rockfish groups. Significant p-values are in bold.

| Experiment | Estimate | Lower Bound | Upper Bound | SE | P-Value |
|--|-----------------|--------------------|--------------------|-----------|------------------|
| <u>A. Escape Time</u> | | | | | |
| Canopy | 1.009 | 0.650 | 1.367 | 0.183 | <0.001 |
| Benthic | 0.104 | -0.302 | 0.510 | 0.207 | 0.62 |
| <u>B. Relative Lateralization</u> | | | | | |
| Canopy | 0.241 | 0.09 | 0.473 | 0.118 | 0.04 |
| Benthic | 0.155 | -0.054 | 0.363 | 0.106 | 0.15 |
| <u>C. Absolute Lateralization</u> | | | | | |
| Canopy | 0.423 | -0.440 | 1.286 | 0.440 | 0.33 |
| Benthic | -0.144 | -0.312 | 0.025 | 0.086 | 0.10 |
| <u>D. Critical Swimming Speed</u> | | | | | |
| Canopy | -0.154 | -0.207 | -0.101 | 0.027 | <0.001 |
| Benthic | -0.042 | -0.097 | 0.013 | 0.028 | 0.13 |
| <u>E. Aerobic Scope</u> | | | | | |
| Canopy | 0.131 | -0.544 | 0.807 | 0.345 | 0.70 |
| Benthic | 0.050 | -0.178 | 0.278 | 0.116 | 0.67 |
| <u>G. Growth Rate Length</u> | | | | | |
| Canopy | -0.077 | -0.177 | 0.023 | 0.051 | 0.13 |
| Benthic | -0.032 | -0.236 | 0.172 | 0.104 | 0.758 |
| <u>H. Growth Rate Weight</u> | | | | | |
| Canopy | 0.012 | -0.024 | 0.048 | 0.018 | 0.51 |
| Benthic | -0.023 | -0.496 | 0.542 | 0.265 | 0.93 |

Table 5. Mean carbonate chemistry conditions (\pm standard error) in the experimental system. Shown are mean values of pH (total scale), $p\text{CO}_2$, dissolved organic carbon (DIC), total alkalinity, and temperature.

| Treatment | pH | $p\text{CO}_2$ (ppm) | DIC ($\mu\text{mol/kg}$) | Total Alkalinity ($\mu\text{eq/kg}$) | Temp. ($^{\circ}\text{C}$) |
|------------------|-----------------|--|--|--|--|
| Ambient | 7.87 ± 0.01 | 536.4 ± 47.1 | 2101.8 ± 4 | 2223.0 ± 6 | 10.82 ± 0.29 |
| Moderate | 7.74 ± 0.04 | 817.1 ± 26.7 | 2157.3 ± 7 | 2223.1 ± 6 | 10.62 ± 0.04 |
| High | 7.49 ± 0.01 | 2060.3 ± 139.1 | 2259.2 ± 5 | 2223.3 ± 5 | 10.58 ± 0.13 |
| Extreme | 7.32 ± 0.02 | 3281.8 ± 192.6 | 2338.0 ± 6 | 2236.1 ± 17 | 10.7 ± 0.04 |

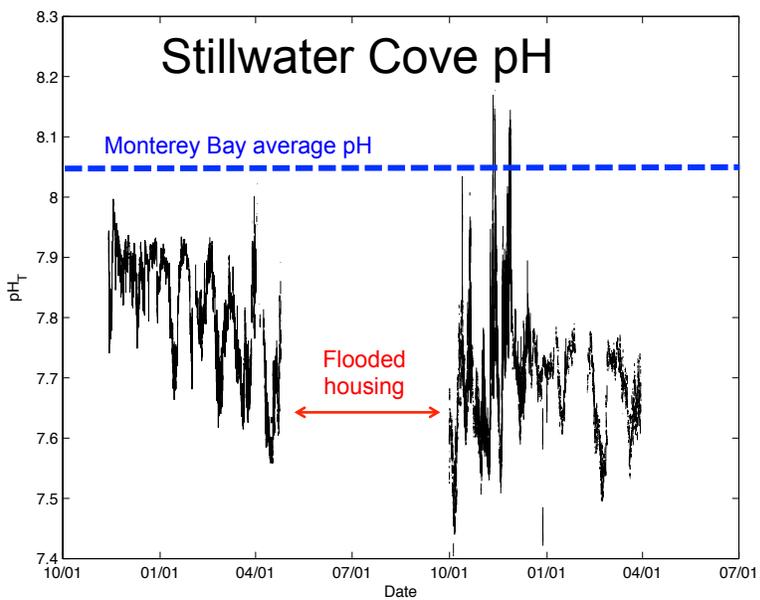
Figures**Figure 1.**

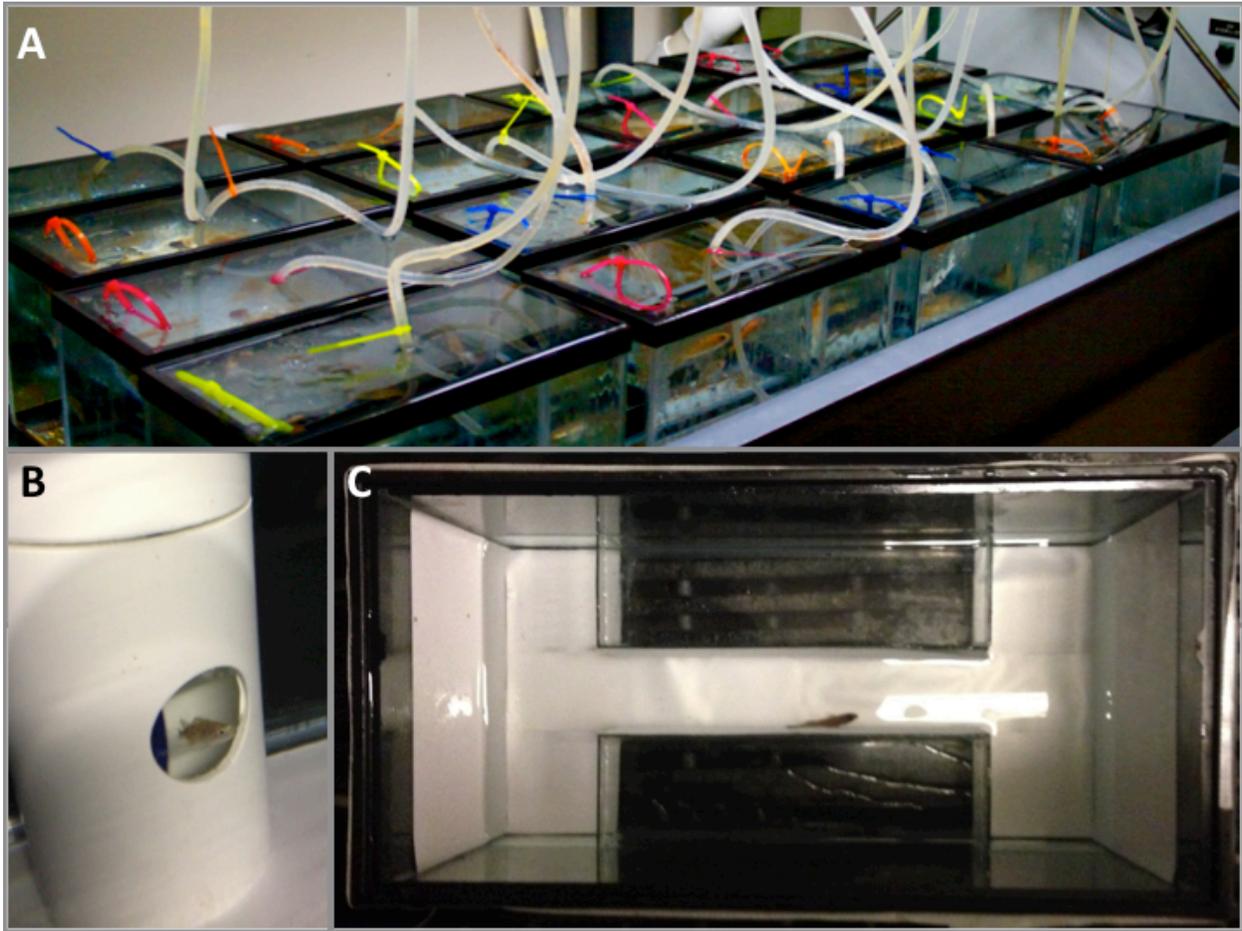
Figure 2

Figure 3.

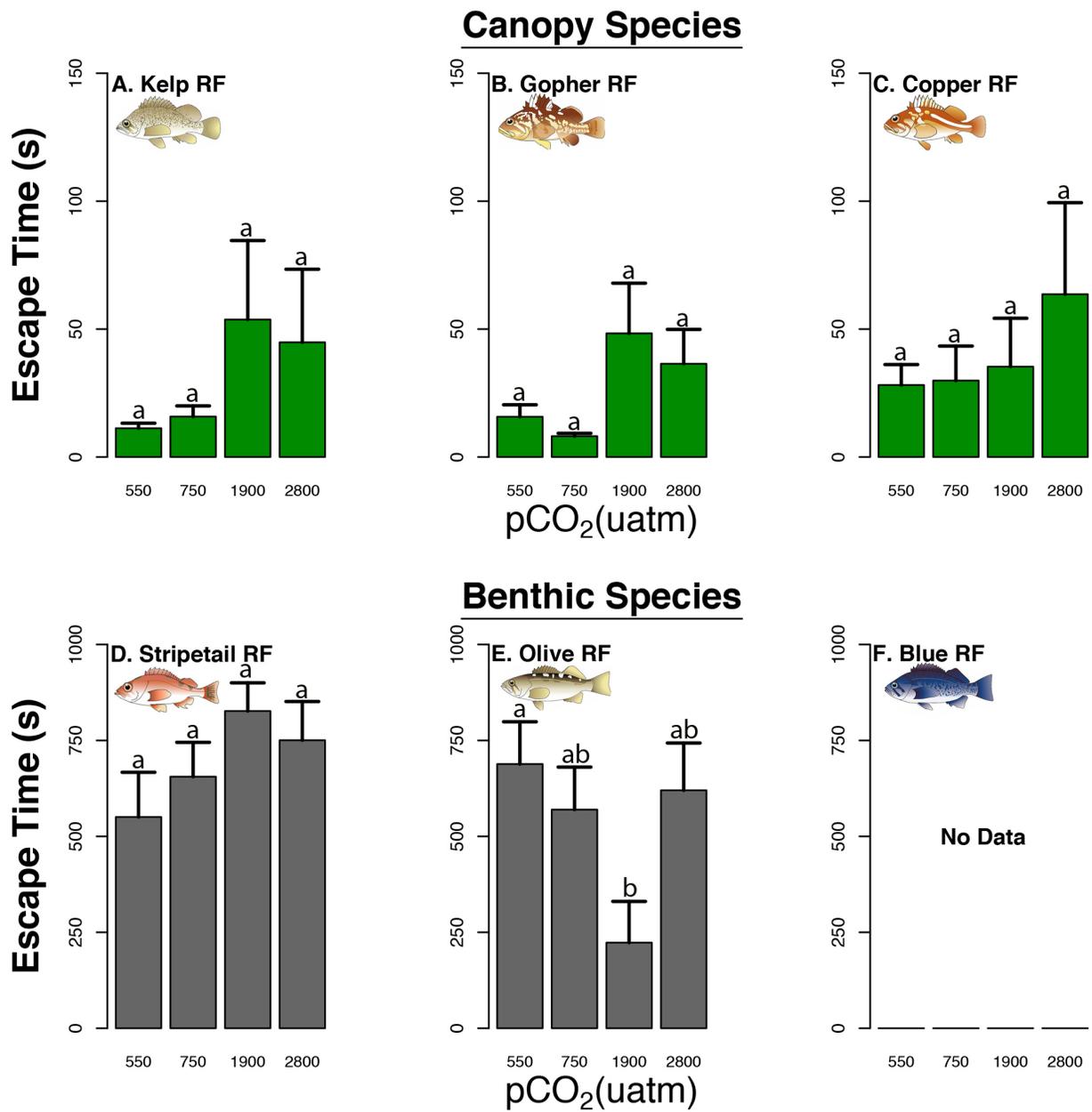


Figure 4.

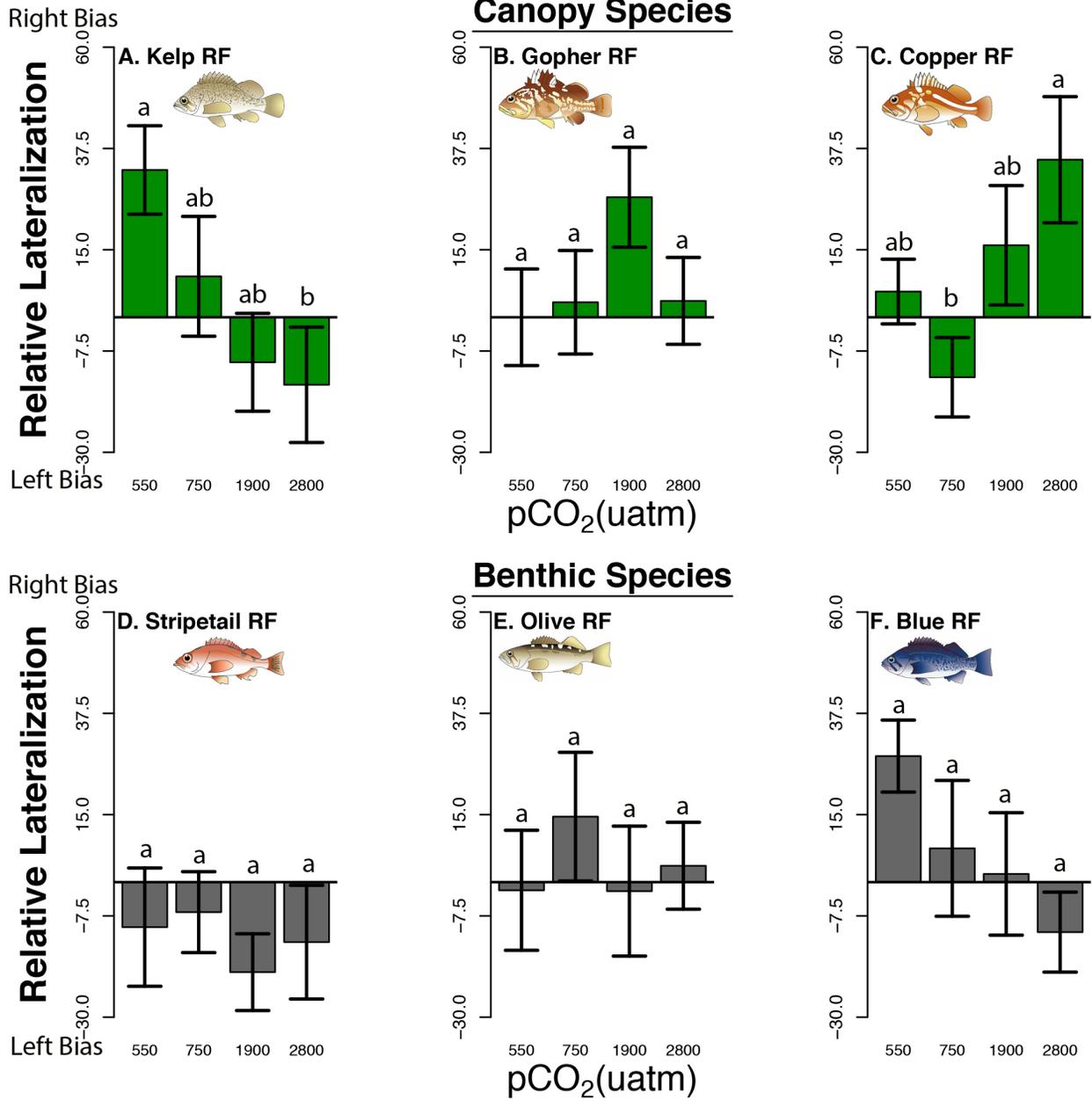


Figure 5.

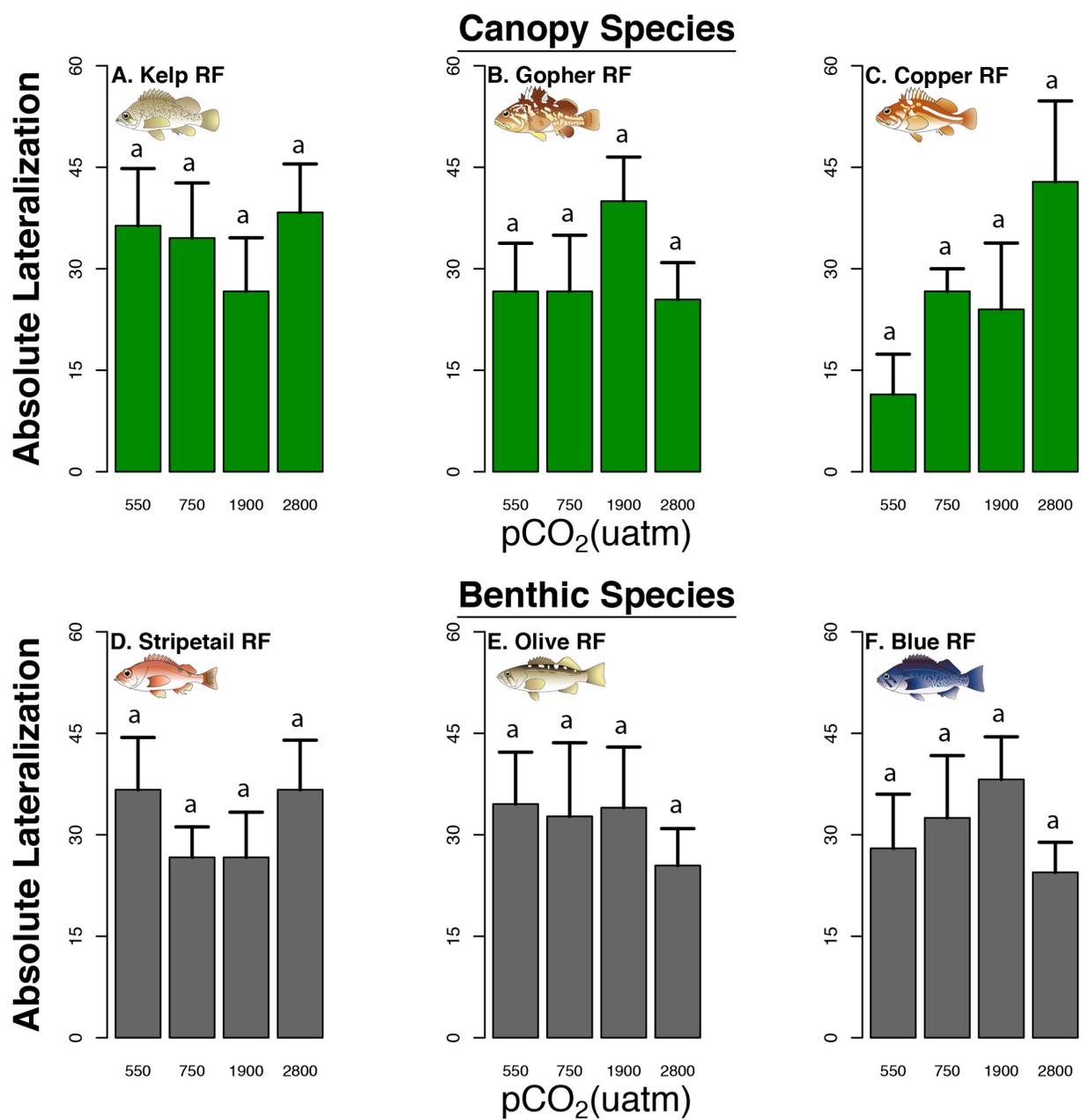


Figure 6.

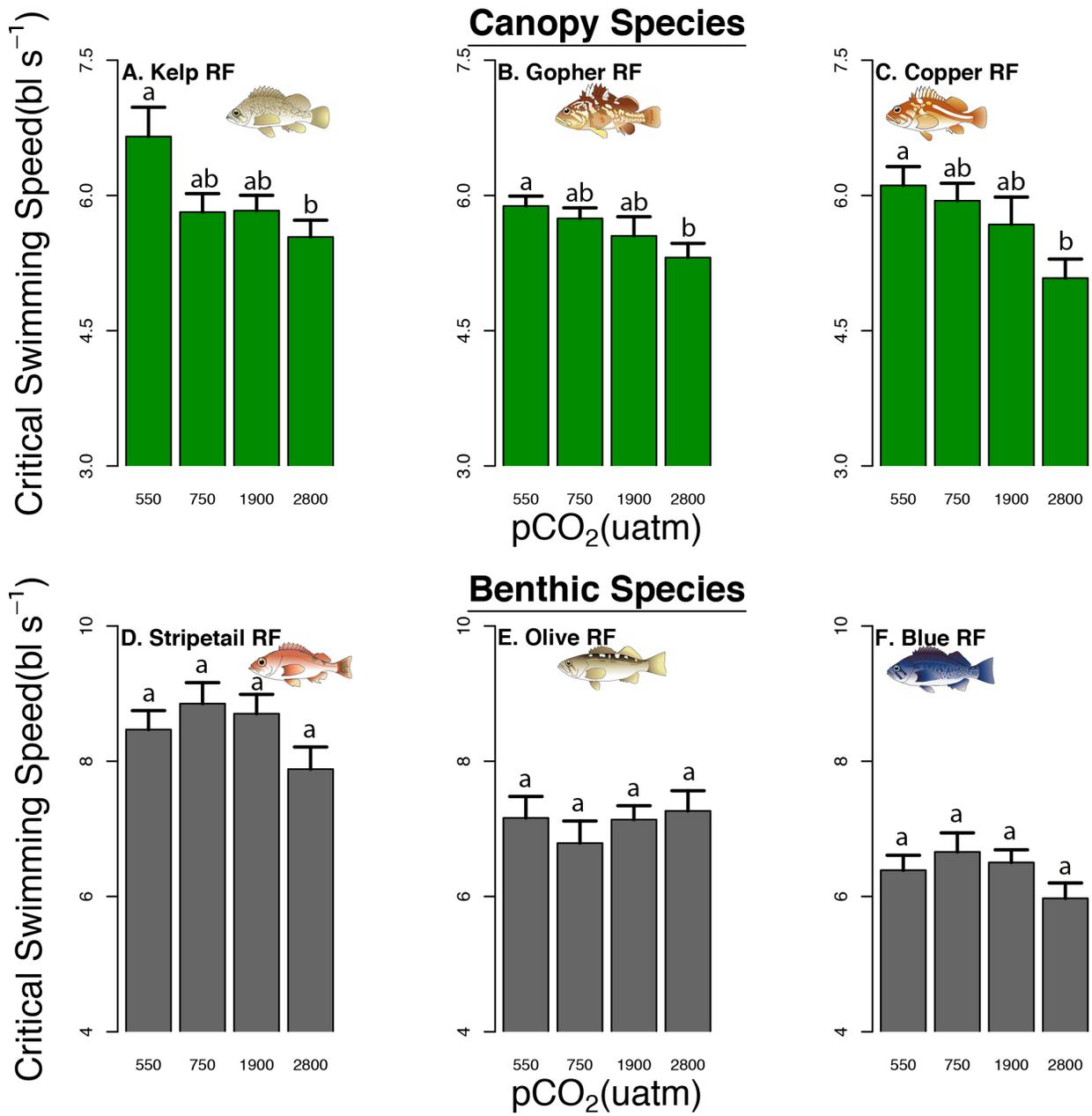


Figure 7.

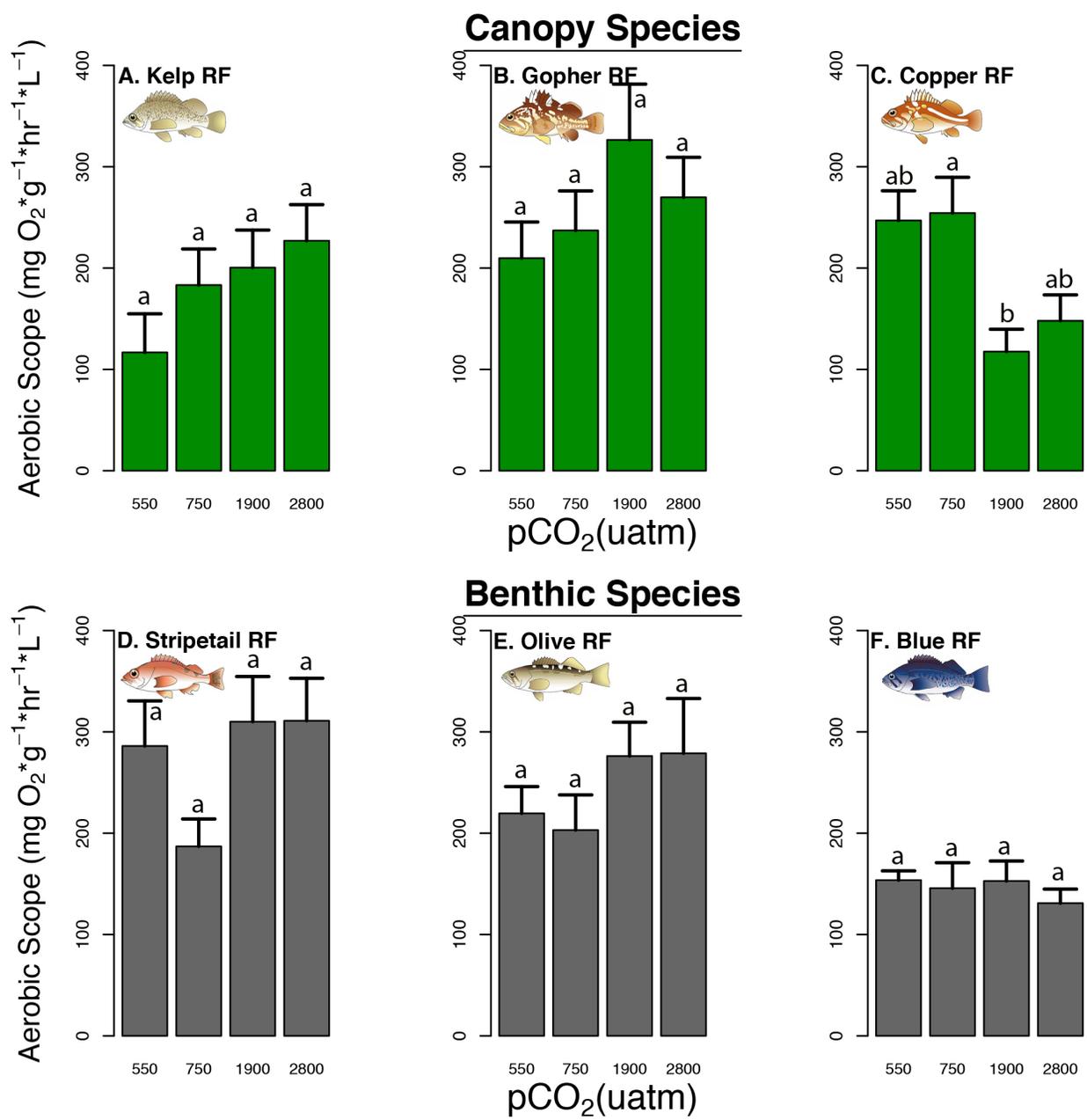


Figure 8.

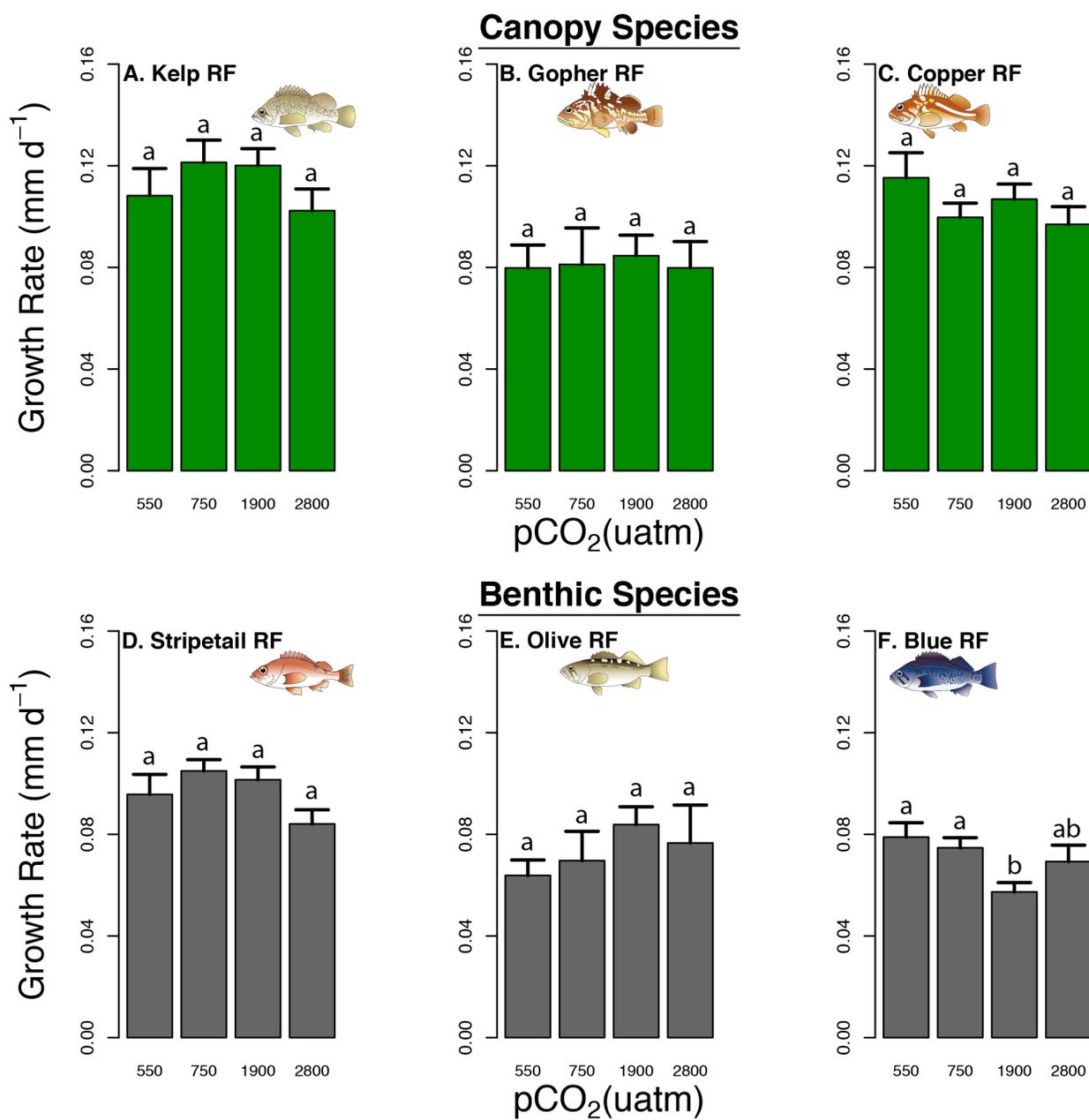


Figure 9.

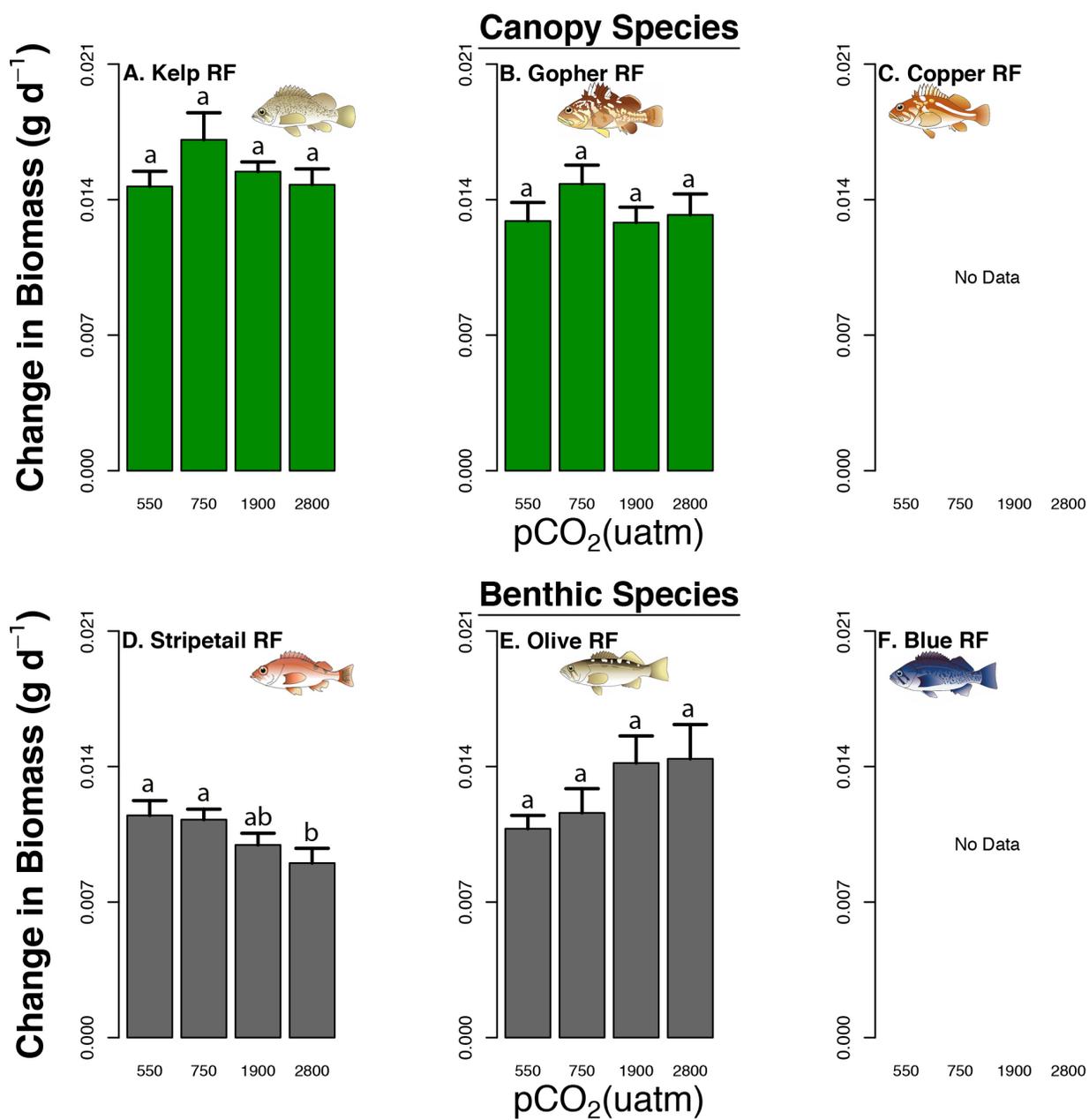


Figure 10.

