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## Effects of Hypoxia on the Behavior and Physiology of Juvenile Stage Temperate Reef Fishes (Genus: Sebastes)

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EFFECTS OF HYPOXIA ON THE BEHAVIOR AND PHYSIOLOGY OF JUVENILE  
STAGE TEMPERATE REEF FISHES (GENUS: *SEBASTES*)

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A Thesis

Presented to the

Faculty of

Moss Landing Marine Laboratories

California State University Monterey Bay

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

in

Marine Science

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by

Evan Gilman Mattiasen

Fall 2018

**CALIFORNIA STATE UNIVERSITY MONTEREY BAY**

The Undersigned Faculty Committee Approves the

Thesis of Evan Mattiasen:

EFFECTS OF HYPOXIA ON THE BEHAVIOR AND PHYSIOLOGY OF  
JUVENILE STAGE TEMPERATE REEF FISHES (GENUS: *SEBASTES*)

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## ABSTRACT

Effects of hypoxia on the behavior and physiology of juvenile stage temperate reef fishes (genus: *Sebastes*)

by

Evan Gilman Mattiasen

Masters of Science in Marine Science

California State University Monterey Bay, 2018

The progression of climate change is predicted to cause large-scale changes to ocean chemistry (i.e., shifts in temperatures, salinity, ocean acidification, etc.) within the California Current. Forecasts from climate models and oceanographic observations indicate an increase in the frequency and duration of hypoxic events in the coastal zone, which have the potential to affect marine biodiversity and fisheries. Many studies have shown that exposure to extreme low dissolved oxygen ( $pO_2$ ) conditions can have deleterious effects on fish in early life stages, such as inhibition to growth and locomotion. Rockfish (*Sebastes* spp.) are a diverse group of species composed of fishes with varying life history characteristics. This study aims to determine how exposure of two species of young-of-the-year (YOY) juvenile rockfishes will perform under chronic exposure to varying dissolved oxygen levels. Copper rockfish (*Sebastes caurinus*) and blue rockfish (*Sebastes mystinus*) are two closely related species that differ in early life history traits. Copper rockfish have a short pelagic duration that begins with parturition in the spring and ends with recruitment to the kelp forest canopy after 1-2 months. Blue rockfish have a longer pelagic duration that begins in the winter and ends with recruitment to the benthic kelp forest habitat after 3-6 months. I compared how behavior and physiology were affected under chronic exposure to low  $pO_2$  at four treatment levels (ambient =  $9.0 \text{ mg O}_2 \text{ L}^{-1}$ , moderate =  $6.0 \text{ mg O}_2 \text{ L}^{-1}$ , low =  $4.0 \text{ mg O}_2 \text{ L}^{-1}$ , hypoxic =  $2.0 \text{ mg O}_2 \text{ L}^{-1}$ ). Behavioral tests were aimed at identifying changes in exploratory behavior (i.e., escape response trial), predator detection through olfactory perception (i.e., olfactory choice test), and changes in turning preference (i.e., behavioral lateralization). Physiological tests focused on determining changes in hypoxia tolerance (critical oxygen tension,  $p_{\text{Crit}}$ ), the capacity for aerobic activity (i.e., aerobic scope), and ventilation rates. Changes in growth rates of both species were also measured. The findings of this study indicate that both species express sensitivity to low  $pO_2$ ; however, the strength of the response differed between species. Copper rockfish exhibited decreased growth rates and reduced absolute lateralization following chronic exposure to the lowest oxygen levels. Behavioral tests did not differ with treatment for blue rockfish. Additionally, growth rates for blue rockfish followed the opposite trend of copper rockfish where individuals in the lowest oxygen treatment grew more than those in the control treatment. Both species exhibited decreases in aerobic scope and increases in ventilation rates with decreasing oxygen levels. Copper rockfish had a lower tolerance of hypoxia and weaker acclimation response compared to blue rockfish as measured by critical oxygen tension threshold ( $p_{\text{Crit}}$ ). A lower  $p_{\text{Crit}}$  for fish exposed to low oxygen conditions indicates the potential for acclimation to those conditions. Despite the physiological changes that occurred for both species in low oxygen conditions, these results

provide evidence of acclimation to chronic hypoxia. Species with a greater capacity for acclimatization are potentially those with life history characteristics where larvae/juveniles have a higher probability of exposure to low oxygen conditions, leading to either acclimatization in the field or pre-adaptation to hypoxia over multiple generations. The differences expressed by each species suggest that acclimatization to changing ocean conditions may vary across closely related species, leading to winners and losers under future ocean conditions. Overall, increases in strength and frequency of coastal hypoxia events may have severe impacts on juvenile stage rockfishes that reside in kelp forests. While this study highlights adaptations to low oxygen, extended exposure to hypoxia decreased fitness of individuals through metabolic and aerobic depression, and changes to behavior. The information gathered in this study is critical for advancing the understanding of how these economically valuable species will fair in the future, and the information presented here will help inform policy makers to protect populations at risk.

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## Introduction

Environmental hypoxia is the phenomenon where the concentration of dissolved oxygen in the water column decreases below  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  (Pihl *et al.* 1991; Diaz & Rosenberg 1995). Hypoxia can occur as a natural phenomenon or it can be stimulated by human activities in the coastal zone. Low oxygen conditions are commonly observed in the spring and summer months in Central California following upwelling events. The driving force behind upwelling along the California coast is strong alongshore winds. These winds initiate Eckman Transport, displacing nearshore surface water with deeper waters that are naturally high in nutrients and low in  $p\text{O}_2$ , because respiration exceeds photosynthesis at depth (Grantham *et al.* 2004; Chan *et al.* 2008; Garcia-Reyes & Largier 2010). Nutrient rich upwelled waters can initiate phytoplankton blooms in the euphotic zone, which can result in fluctuating oxygen concentrations due to high rates of oxygen production from photosynthesis and subsequent consumption of oxygen via the stimulation of microbial respiration (Ryther & Dunstan 1971). Depending on wind and ocean circulation patterns, upwelling-induced hypoxia can extend over large areas of the coastal shelf. These conditions are typically stronger and more frequent during the spring and summer months, which coincides with the critical recruitment season of many temperate rocky reef fishes.

Natural processes governing the dissolved oxygen ( $p\text{O}_2$ ) concentration of the oceans include circulation, ventilation, air-sea exchange, primary production, and respiration (Keeling & Garcia 2002; Bograd *et al.* 2008). These processes are predicted to be altered as climate change progresses, leading to reduced oxygen levels in the midwater oceanic habitats (Sarmiento *et al.* 1998; Keeling & Garcia 2002). Increases in sea surface temperature results in decreases in oxygen solubility and increases in the stratification of surface waters. This causes a decrease in

subsurface mixing, resulting in less atmospheric oxygen in the subsurface water column (Barnett *et al.* 2005). The continuation of this process can result in the shoaling of the oxygen minimum zone (Gilly *et al.* 2013). In addition to increasing sea surface temperatures, emerging evidence suggests that increases in wind speed and wave height will further exacerbate the trend for declining oxygen levels in our global oceans (Young *et al.* 2011; Sydeman *et al.* 2014). The longshore winds that drive the upwelling process are caused by the large-scale gradient in atmospheric pressure across the coastline with a high-pressure system over the ocean and a thermal low-pressure system over the landmass. These pressure systems are sensitive to climate variability and in particular changes in temperature. As land temperatures increase at a faster rate than ocean temperatures, the pressure gradient between land and sea will increase leading to stronger and more frequent upwelling favorable winds (Garcia-Reyes & Largier 2010). Additionally, climate change can lead to a shift in the seasonal timing at which upwelling favorable winds blow most strongly along the coast (Snyder *et al.* 2003). These changes may lead to exacerbated low-oxygen conditions including hypoxia and anoxia in near-shore systems through an increased frequency and strength of coastal upwelling (Bakun 1990; Snyder *et al.* 2003; Diffenbaugh *et al.* 2003; Grantham *et al.* 2004; Bograd *et al.* 2008; Chan *et al.* 2008; Morel *et al.* 2010; Feely *et al.* 2008; Diaz & Rosenberg 2008; Gruber 2011; Sydeman *et al.* 2014). In turn, this may alter species distributions and relative dominance, due to differences in physiological tolerances (Bakun 1990; Perry *et al.* 2005).

In addition to upwelling driven changes in oxygen, coastal waters off Southern California have been exhibiting declines in  $pO_2$  over the past three decades that cannot be directly attributed to climate variability (Collins *et al.* 2003; Booth *et al.* 2014). Other potential culprits include land-based anthropogenic inputs of nutrients, which can further exacerbate hypoxia in coastal

systems. A leading source of eutrophication in the oceans is through runoff of nitrogen- and phosphorus-rich waters from agricultural processes (Bennet *et al.* 2001). Agricultural runoff can cause nutrient over-enrichment of coastal waters in the vicinity of runoff hotspots, which can then initiate phytoplankton blooms and further reduce oxygen through the process of microbial decomposition, as described earlier. Eutrophication and upwelling associated hypoxia have become one of the main environmental constraints affecting coastal marine habitats (Rydberg *et al.* 1990; Druon *et al.* 2004; Hagy *et al.* 2004).

At extreme levels, hypoxia can inhibit growth and cause mortality of marine organisms (Grantham *et al.* 2004). At sub-lethal levels, hypoxia can cause behavioral changes in activity levels and species distributions, and physiological changes to metabolic activity (Kramer 1987; Van Den Thillart *et al.* 1994). Hypoxic conditions have been shown to negatively affect olfactory capabilities of several tropical fish species by causing an altered or reduced response to predator cues (Boutilier *et al.* 1998; Shang & Wu 2004; Landry *et al.* 2007). These affects can lead to indirect mortality events by causing a fish to become less reactive, non-reactive, or attracted to predator odor cues. Hypoxia may increase predation rates by birds, jellyfish, or aquatic marine mammals that are able to take advantage of altered behavior by fishes (Kramer 1987). Respiratory stress and metabolic depression have also been associated with low oxygen conditions and may lead to deviations from normal migratory and predator response behaviors, ultimately resulting in increases in post-settlement mortality (reviewed in Domenici *et al.* 2007). Many studies looking at the individual effects of hypoxia on embryonic, larval, and juvenile coral reef fishes have reported disruptions to physiology and neurological behavior (Farrell & Richards 2009; Richards 2009; Munday *et al.* 2010; Dixon *et al.* 2010; Ferrari *et al.* 2011);



however, it is still largely unknown whether these effects will be seen in similar life stages of temperate rocky reef fishes.

Respiration and metabolic function are key components that determine fitness in fishes, affecting growth and survival to the adult stage. Metabolic activity is the processing of energy and materials by organisms into forms that can be allocated to survival, growth, and reproduction (Brown *et al.* 2004). Metabolism sets powerful constraints on the allocation of resources toward fitness. Conditions affecting metabolic function may have detrimental impacts on population dynamics and mortality rates at juvenile stages when fish are vulnerable. Aerobic metabolic processes of marine organisms rely on the presence of oxygen. Adaptations to changes in oxygen concentration such as increasing the efficiency of acquiring and/ or using oxygen are required for most species to survive under fluctuating or degrading oxygen conditions.

The genus *Sebastes* is comprised of 70 species of rockfish inhabiting a wide range of habitat types from shallow coastal waters to as deep as 1000 m. This wide range of habitat types exposes the *Sebastes* genus to distinct oxygen conditions. Some species of rockfish, such as the longspine thornyhead (*Sebastolobus altivelis*), inhabit depths of 600-1000 m and have adapted to become tolerant of low oxygen conditions (Jacobson & Vetter 1996). Closely related species that inhabit coastal zones are not exposed to the extreme oxygen conditions common to the oxygen minimum zone and thus may not be adapted to low  $pO_2$  conditions. Video surveys on the inner shelf of the Oregon coast, following a 60-day period of hypoxia, reported huge numbers of dead fish and invertebrates, including several species of rockfish (Grantham *et al.* 2004). While extreme, this occurrence shows the dramatic consequences that upwelling-induced hypoxia may have on coastal species that are not adapted to extreme low  $pO_2$  conditions. The results also demonstrate that many mobile species may not be able to simply move away if hypoxic water

masses intrude their habitat. If predictions of an increased frequency, strength, and duration of upwelling events caused by climate change are accurate and those upwelled waters are increasingly hypoxic, many species of shallow water fishes, including economically important species like rockfish, may be in danger of mass mortality.

The effects of increased exposure to low oxygen and hypoxic conditions on fishes in early life stages, such as larvae and juvenile rockfish, are still largely unknown. Fishes at these stages may be more sensitive to low oxygen conditions than adults of the same species. Additionally, it is not known whether some of these near shore species may possess adaptations that make them better suited than other species for low oxygen conditions. This study aims to determine these effects by investigating the behavioral and physiological responses in juvenile rockfish caused by chronic exposure to varying oxygen conditions, ranging from hypoxic conditions of  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  to oxygen saturated water of approximately  $9.0 \text{ mg O}_2 \text{ L}^{-1}$ .

Fishes in the genus *Sebastes* exhibit matrotrophic viviparity (Macfarlane & Bowers 1995). The embryos develop within egg envelopes during most of the gestation period (~1 month) before hatching several days before parturition at the free feeding stage (Wourms 1991). Larvae are then transported offshore where they will metamorphose to pelagic juveniles after 1 to several months. After metamorphosis, juveniles are then transported back to coastal habitats. This thesis focuses on investigating the effects of hypoxia of two closely related rockfish species that differ in their early life history characteristics and exposure to low oxygen conditions during development. Prior to their arrival in the kelp forest, copper rockfish (*Sebastes caurinus*) are generally thought to stay in the surface waters during their offshore larval duration (Lenarz *et al.* 1991). These surface waters are typically higher in  $p\text{O}_2$  than deeper water masses that do not mix with surface water. During the larval and pelagic juvenile periods, blue rockfish (*S. mystinus*) are

often encountered in deeper offshore water masses (Lenarz *et al.* 1991) that are typically lower in oxygen, temperature, and pH. Variability in early life history characteristics of these two species may make them differentially susceptible to environmental conditions, reflecting differences in the timing of parturition and depth in the water column, which may influence their history of exposure to low oxygen conditions during early development.

The settlement period is a critical transition stage where environmental conditions can impact survival and subsequently affect year-class strength. To test hypoxia tolerance among multiple species with different early life history traits, I collected fish at the end of the pelagic larval phase and conducted controlled experiments testing chronic influences of low oxygen levels on behavior and physiology. I hypothesized that exposure to low  $pO_2$  levels will reduce olfactory capabilities (choice flume), disrupt brain functional asymmetry (i.e., lateralization), and increase ventilation rates. Additionally, I hypothesized that low  $pO_2$  will negatively impact aerobic performance (i.e., decreased oxygen consumption rates) and growth for both species. I also predict that blue rockfish will have a stronger acclimation response in the form of a lower  $p_{Crit}$  (i.e., greater hypoxia tolerance) value following exposure to low oxygen exposure. While I predicted that behavior and physiology of both species will be negatively affected, I hypothesized that copper rockfish will be negatively impacted to a greater degree following exposure to low oxygen conditions due to their early life history characteristics of growth and development in surface waters that are typically high in oxygen.

## Methods and Procedures:

### *Study system and study species*

The Monterey Bay is a naturally dynamic system with constantly changing oceanographic conditions, including pH, oxygen, temperature, and wave intensity (Booth *et al.* 2012). For example, the Monterey Bay Aquarium, located at the southern end of the Monterey Bay, has been recording oxygen levels in the bay starting in 2000. Since then, reports have shown recurring events of sub lethal oxygen levels ( $4.5 \text{ mg O}_2 \text{ L}^{-1}$ ) and hypoxic oxygen levels as low as  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  (Booth *et al.* 2012).

Collection of juvenile rockfishes took place in shallow coastal waters of Central California (~10-20 m depth) at Stillwater Cove ( $36^\circ 34' \text{N}$ ,  $121^\circ 56' \text{W}$ ), located within the Carmel Bay State Marine Conservation Area (Fig. 1). This system is a temperate rocky reef and kelp forest habitat, characterized by moderate relief granite, sandstone and conglomerate terraces separated by sand channels. The kelp forest is composed of *Macrocystis pyrifera* with fronds extending from the rocky benthos to the surface, where large canopies are formed. The subcanopy is composed of *Pterygophora californica*, while a diverse assemblage of articulated coralline and fleshy red algae, and sessile invertebrates such as sponges, tunicates, anemones, and cup corals dominate the benthos. This site is located south of Monterey Bay and just north of the Carmel Canyon, and is regularly inundated by upwelling of deep water during the spring and summer months. Due to its south facing orientation, this site is protected from waves during most of the year. Recruitment of rockfishes occurs during the spring and summer months and coincides with the typical upwelling season of March-July (Garcia-Reyes & Largier 2010).

Different rockfish species are characterized by variation in larval duration and the depth occupied by larvae during the pelagic phase, which results in a wide range of settlement times

among species (Love *et al.* 2002). Copper rockfish (*Sebastes caurinus*) are characterized by parturition occurring in the spring, a short pelagic duration of 1-2 months, and settlement to the canopy of coastal kelp forests, typically between the months of April and July (Love *et al.* 2002; Love 2011). Due to this short pelagic phase, recruiting copper rockfish can be as small as 1.2 cm (Love 2011). Blue rockfish are characterized by parturition that occurs in the winter, a longer pelagic duration lasting 3-6 months, and settlement to the benthos of kelp forests and rocky reefs between the months of April and July (Love 2011). Due to the long pelagic phase, blue rockfish typically metamorphose into juveniles while offshore and are at least 3.1 cm in length at the time of recruitment to the kelp forest. Blue rockfish are typically part of the first wave of rockfish species to settle during the spring, followed by copper rockfish during the late spring to summer months. However, in this study, conducted during the 2015 recruitment season, copper rockfish were the first species to recruit and were thus collected first, followed by the recruitment and collection of blue rockfish.

### *Specimen collection & tagging*

Newly recruited rockfish were collected weekly during the settlement period (April-July) by using large mesh hand nets while SCUBA diving. New recruits were identified by a total body length between 2-4 cm for coppers and 3-6 cm for blues. Target species were identified by distinctive color patterns and the timing of arrival in kelp canopy for copper rockfish and benthic substrate for blue rockfish. Collected individuals were then transported to the Moss Landing Marine Laboratories (MLML) aquarium facility in a 40 L cooler equipped with an aerator. The fish were held at MLML until the appropriate sample size of  $n = 140$  copper rockfish (20 individuals per treatment level) and  $n = 140$  blue rockfish was met. Fish were measured for

length and weight and tagged using Visual Implant Elastomer Tags (*Northwest Marine Technology, Inc.*). A unique combination of tag color and body position was used to individually mark each specimen. Fish were given a 2-week recovery period after tagging.

Lingcod (*Ophiodon elongatus*), used as the predator species to obtain odor cues for rockfish olfaction experiments, were collected using hook-and-line fishing. Odor cues that allow prey species to detect the presence of a predator are composed of a mix of naturally occurring excretions (e.g., mucus) and metabolic waste products. Diet studies of lingcod show that rockfish are a major component (Beaudreau & Essington 2007). Lingcod were held in 500 L tanks at the NOAA laboratory and fed a diet of adult rockfish to most closely represent the process of odor cue formation in the wild. All species collected were handled and cared for according to San Jose State University approved IACUC protocol #1007.

#### *Experimental treatment seawater chemistry*

Experiments subjecting juvenile rockfish to simulated future  $pO_2$  levels were conducted at the seawater aquarium facility at the NOAA Southwest Fisheries Science Center laboratory in Santa Cruz, CA. Control water was held in a 2000 L reservoir tank maintained at a constant temperature ( $\sim 12^\circ\text{C}$ ) at approximately 100%  $pO_2$  saturation ( $\sim 9.0 \text{ mg O}_2 \text{ L}^{-1}$ ) using air stones. Control water was fed into four 500 L treatment reservoirs. The 500 L treatment reservoirs were used to create desired  $pO_2$  concentrations by bubbling of  $N_2$  gas (which acts to strip oxygen from the water). The  $N_2$  gas was controlled by a solenoid valve coupled to Loligo Systems optical oxygen probes in the treatment reservoirs to maintain desired  $pO_2$  concentrations. All optical oxygen probes were calibrated at the start of the experiment and re-calibrated every two months

thereafter for the duration of the experiment. The computer software WitroxView was used to monitor and control oxygen levels at desired setpoints.

Using a flow-through system, water was delivered from the 500 L treatment reservoirs into smaller 80 L experimental tanks that held  $n = 10$  randomly assigned juvenile rockfish of each species. Water was fed into the 80 L tanks at a rate of  $20 \text{ mL sec}^{-1}$ . There were 2 replicate tanks per treatment ( $n = 20$  fish per treatment per species). All tanks were covered to eliminate visual cues from investigators. Sections of plastic construction fencing material measuring  $90 \times 120$  cm were bunched together using cable ties and placed in each tank to simulate kelp habitat structure and provide enrichment (Fig. 2A). Tanks were divided into two groups (A and B, Fig. 2B), with each group fed on alternating days and used in experiments on non-feeding days, thereby ensuring a 36-48 hour fasting period prior to behavioral or physiological trials. Fish were fed to satiation with frozen, high protein krill on feeding days, with ration levels increasing as fish grew. Tanks were checked daily for fish health and mortalities.

Four sub-lethal  $p\text{O}_2$  saturation levels, corresponding to those observed in near-shore hypoxia events (Chan *et al.* 2008; Booth *et al.* 2012), were used: 100% saturation ( $\sim 9.0 \text{ mg O}_2 \text{ L}^{-1}$ ), 75% saturation ( $\sim 6.0 \text{ mg O}_2 \text{ L}^{-1}$ ), 45% saturation ( $\sim 4 \text{ mg O}_2 \text{ L}^{-1}$ ), and 20% saturation ( $\sim 2 \text{ mg O}_2 \text{ L}^{-1}$ ). These levels were maintained for the duration of the experiment, exposing fish to chronic levels of oxygen saturation from ambient to hypoxic conditions over several months (Fig. 3). All treatments were held at a constant water temperature ( $\sim 12^\circ\text{C}$ ). Seawater chillers were used to maintain the water temperature at the desired level. Oxygen and temperature levels in all the treatment tanks were measured daily using HACH dissolved oxygen and temperature probes. All experimental trials were conducted in water of  $12^\circ\text{C}$  with the same  $p\text{O}_2$  levels as the rearing tank of the fish being tested. Prior to conducting any behavioral or physiological

measurements, fish were allowed a minimum of 5 days to recover in their treatment tanks from any previous trial.

### *Somatic growth*

Exposure to low  $pO_2$  is known to alter growth rate, feeding, and metabolic function in a variety of marine fishes and invertebrates (Fry 1971; Brett 1979; Diaz & Rosenberg 1995; Chabot & Dutil 1999; Pichavant *et al.* 2000). Decreased food intake and depressed metabolic function can lead to reduced growth and activity levels (Wu 2002). In order to test whether low dissolved oxygen levels affect growth of copper and blue rockfish, measurements of standard length (SL), total length (TL), and weight were taken for all individuals at the time of tagging and again before dissection at the end of the experiment. The change in length or weight was divided by the number of elapsed days to calculate growth rate per day.

### *Escape response*

The escape challenge tests the time required to find the exit of an enclosed chamber and reflects differences in exploratory behavior and perception, serving as a proxy for problem solving ability by reflecting neurological changes in response to hypoxia (Jutfelt *et al.* 2013). To test behavioral responses to chronic low oxygen conditions, copper rockfish were exposed to experimental treatments for 4-6 weeks and blue rockfish for 3-5.5 weeks before being run in the escape challenge experiment (Table 1). Escape chambers were composed of schedule 40 PVC tube measuring 28 cm tall x 9 cm diameter with a 5 cm diameter hole cut in the side (Fig. 2C). A slit located 8 cm from the top of the chamber allowed a black plexiglass divider to be inserted, retaining fish in the top half of the chamber during the acclimation period. The removal of the



divider released the fish into the lower portion of the chamber at the start of the timed trial. The escape chambers were placed in 40 L insulated aquaria on a water table to control temperatures. A total of six replicated escape chambers and aquaria were used, allowing for six simultaneous trials. Experimental seawater conditions were set up in a flow-through configuration to maintain temperature and oxygen conditions (levels were confirmed using HACH water quality O<sub>2</sub> and temperature probes) experienced by each specimen for the duration of the trial. Individual fish were quickly and delicately netted and transferred into the top of the chamber in the same manner for all individuals and allowed a 15 min acclimation period. At the end of the acclimation period the divider was removed without visual interference by the investigator. The response variable recorded was the time taken for the fish to find the exit of the chamber. A fish was confirmed to have found the exit of the chamber when its head moved past the edge of the opening (assessed in real time). A maximum time of 10 min was allowed for fish to exit the chamber before the trial was ended.

### *Behavioral lateralization*

Brain functional asymmetry and behavioral lateralization reflect the bias for left vs. right turning decisions in a detour test and are another behavioral response in fishes that may be sensitive to hypoxia (Domenici *et al.* 2007). The degree of lateralization has been shown to correlate with performance in cognitive tasks, schooling behavior, predator avoidance, and spatial orientation (Munday *et al.* 2009). To measure lateralization in response to *p*O<sub>2</sub> treatment conditions, we employed a detour test with a double T-Maze (Fig. 2D). Copper rockfish were acclimated in seawater treatment conditions for 6-8 weeks and blue rockfish for 6-9 weeks prior to running the behavioral lateralization experiment (Table 1). Individual fish were quickly and

carefully netted and transferred into one end of the two-way T-Maze (50 x 30 x 25 cm L x W x H aquaria), and allowed to acclimate for 3 minutes. The starting side that a fish was placed in was alternated for every trial to minimize the potential for side bias. After the acclimation period, the fish was gently coaxed down the center channel (without touching the fish) using a T-bar, and when it reached a barrier at the end of the channel, the fish was forced to turn right or left. The turn direction was recorded and the experiment was repeated 10 times, 5 times in each direction. Each trial took approximately 10 minutes to complete. I calculated the absolute lateralization index ( $L_A$ ) to compare the turn bias between treatment groups:

$$\text{Absolute lateralization index} = \frac{|\# \text{ right turns} - \# \text{ left turns}|}{\# \text{ right turns} + \# \text{ left turns}} \times 100 \quad (1)$$

This index reflects whether turn bias exists at the population level, irrespective of direction. I also calculated the relative lateralization index ( $L_R$ ) to determine whether the fish in a particular treatment exhibited turning bias for a particular direction (i.e., left or right preference):

$$\text{Relative lateralization index} = \frac{\# \text{ right turns} - \# \text{ left turns}}{\# \text{ right turns} + \# \text{ left turns}} \times 100 \quad (2)$$

Positive values indicate a right turning bias, while negative values indicate a left turning bias.

### *Hypoxia tolerance*

The first response of aquatic animals to hypoxia is to try to maintain oxygen delivery and continue deriving oxygen to support aerobic metabolism. With further oxygen declines, organisms reduce energy expenditure and energy turnover, and finally, if oxygen levels get low enough for a long enough period, the organism will switch to anaerobic metabolism to derive the required levels of energy (Holton & Randall 1967; Van den Thillart & Smit 1984; Wu & Woo 1985; Boutilier *et al.* 1988). The hypoxia tolerance experiment tracked the aerobic metabolic function of individuals as oxygen levels were decreased. Typically, fish can maintain a relatively

constant resting or standard metabolic rate (SMR) across a range of oxygen levels. The critical oxygen tension threshold (pCrit) reflects the point at which the SMR can no longer be maintained by the external oxygen supply, and it begins to decrease in response to further decreases in oxygen, often linearly. This inflection point, pCrit, reflects a switch from oxyregulation to oxyconformation (Chapman *et al.* 2002).

A subset of 16 individuals from each treatment were tested for hypoxia tolerance by estimating pCrit, or the critical oxygen tension threshold after 9-17 weeks of acclimation for copper rockfish, and 11-22 weeks of acclimation for blue rockfish (Table 1). pCrit was measured using the Loligo intermittent flow respirometry system (IFR) (Fig. 2G). This system has four oxygen chambers that house one individual fish per chamber. The animal's oxygen consumption rate is measured using optical oxygen probes that are in line with the chambers. Each chamber set has two attached pumps rated at 300L/h. One pump is for flushing water inside the chamber with the water from the larger reservoir tank that surrounds the chamber. The second pump is used to recirculate the water within the closed system when the oxygen measurement cycle is taking place.

Fish were placed into the respirometry chambers at dusk and the program AutoResp was set to take continuous measurements throughout the night to record the SMR while simultaneously acclimating the fish to the chambers. O<sub>2</sub> levels in the chambers were set to the treatment concentration that the fish were held in. Each measurement loop is composed of three cycles. The first cycle is a five-minute flush cycle where oxygen from the inside of the chamber is replaced with oxygen from the surrounding reservoir. At the end of this cycle the flush pump is turned off and the recirculation pump is turned on, creating a closed system. This initiates a ten-minute wait cycle, which allows time for the oxygen level inside the chamber to become

homogenous and gives the oxygen probes time to stabilize before beginning the measurement cycle. The last cycle is a five-minute measurement period where the oxygen levels decline linearly in relation to the oxygen consumption rate of the fish in the chamber. The entire loop begins again after the end of the measurement cycle. During the nighttime acclimation period, the reservoir water was maintained at 100% O<sub>2</sub> saturation for all treatments.

The hypoxia tolerance experiments were started at 0800 hours by initiating the AutoResp program to begin reducing the oxygen saturation of the reservoir water in a step-wise fashion. This is achieved by pumping nitrogen gas via a solenoid valve and an air stone placed in the reservoir. Three measurement loops were recorded at each oxygen level. The oxygen level was reduced by 10% oxygen saturation after each triplicate of loops until reaching 40% pO<sub>2</sub>. The level was then dropped by 5% pO<sub>2</sub> for the remainder of the trial to obtain a more precise measurement of pCrit. Oxygen consumption rate (MO<sub>2</sub>) is the metric used to measure respiration rate and estimate metabolic function in units of mg O<sub>2</sub> kg<sup>-1</sup> hr<sup>-1</sup> consumed for each individual. MO<sub>2</sub> values were calculated using the following equation:

$$MO_2 = \Delta PO_2 V \alpha M^{-1} \Delta t^{-1} \quad (3)$$

Where  $\Delta PO_2$  is the change in water partial pressure of O<sub>2</sub> (mmHg),  $\Delta t$  is the elapsed time (h),  $V$  is the volume of the respirometer chamber minus the volume of the fish (cm<sup>3</sup>),  $M$  is the total mass of the animal (kg), and  $\alpha$  is the O<sub>2</sub> solubility coefficient at the experimental temperature (Boutilier *et al.* 1984). MO<sub>2</sub> values were monitored as the oxygen level was reduced, and the experiment was ended after approximately six cycles from when it appeared that the fish had reached their pCrit. The IFR system was cleaned using dilute bleach after each trial to eliminate the influence of microbial respiration from subsequent trials.

### *Aerobic scope*

Aerobic scope, or the capacity for aerobic activity, is the measure reflecting the difference between the SMR and the maximum metabolic rate (MMR). Aerobic scope has typically been used to measure the change in fitness-related performance traits such as growth rate, locomotion and reproductive success for fish under thermal stress (Fry 1947; Pörtner & Knust 2007; Munday *et al.* 2009). This study aims to test the hypothesis that aerobic scope declines under oxygen limitation stress. The aerobic scope of a subset of 16 individuals from each treatment was measured using the Loligo Systems swim flume #SW10100 (Fig. 2E) and the Loligo Systems intermittent flow respirometer (Fig. 2G).

SMR was measured after 9-17 weeks of exposure for copper rockfish and 8-17 weeks for blue rockfish at the start of the pCrit trial (Table 1). SMR measures  $MO_2$  of an individual at its resting state. Four individuals were weighed and placed into separate respirometer chambers, with  $MO_2$  measurements taken over a 12 hr period overnight, as described above. SMR was measured during nighttime hours to capture the  $MO_2$  at the time where the fish are at the lowest metabolic activity levels. The lowest 10% of  $MO_2$  measurements per cycle, excluding outliers, were used to calculate the SMR of each individual fish (Clark *et al.* 2012).

MMR was measured using both the swim flume and the IFR. A subset of four individuals were selected from a single treatment tank and weighed. The weights were input to the AutoResp software. The fish were then swum in the flume to reach their maximum energy expenditure (exhaustion). Exhaustion was achieved by swimming the four fish for 5 minutes at a velocity one body length per second below the estimated average critical swimming speed of the group (Kashef *unpublished data*). The fish were then quickly moved from the swim flume to the IFR chambers and run for one measurement cycle to acquire MMR. The SMR was then subtracted

from the MMR for each fish to calculate the aerobic scope (Nilsson and Ostlung-Nilsson 2004; Munday *et al.* 2009). MMR can occur minutes to hours after being chased or swam to exhaustion (Clark *et al.* 2012). Preliminary trials were performed to determine when MMR occurred for the species in this study. These trials concluded that the highest  $MO_2$  values occurred directly following swimming to exhaustion.

### *Ventilation rate*

A fish's ventilation rate (VR) is analogous to the breathing rate of mammals, and represents a parameter that reflects oxygen demands (Kramer 1987). The VR typically increases when individuals are exerting a lot of energy or when oxygen is limited (Holeton 1980). A measurement of VR is a proxy of environmental stress, where increased VR under low oxygen conditions indicate that fish must work harder to acquire the necessary amount of oxygen from the water they are exposed to. Fish experience a high energetic cost to breathing, compared to air-breathers, due to the inherently low oxygen concentrations in water and the process of moving a viscous liquid over the respiratory surface (Kramer 1987). An increase in the amount of water passed over the gills and the associated increase in ion exchange may result in physiological stress associated with maintaining osmoregulatory balance (Evans *et al.* 2005).

Copper and blue rockfish were exposed to treatment conditions for 23-24 weeks before measuring VR (Table 1). To test whether the VR changes as a function of chronic oxygen exposure history, an array containing 10 experimental chambers was constructed using 1.5 cm thick opaque polypropylene sheet (Fig. 2F). The array was constructed so that all chambers are incubated in seawater reflecting the temperature and  $pO_2$  each fish had been acclimated to in its rearing environment. All chambers had separate water flow sources, with continual flushing of

the assigned treatment water. A single array allowed for 5 fish each from two separate seawater treatment conditions to be run at one time, for a total of 10 fish run during each trial. Seawater conditions were maintained at 12°C (+/-) 0.2 °C and desired  $pO_2$  levels for the duration of the trial.  $N = 5$  individuals each were haphazardly chosen from two treatments and placed within the array with one fish per chamber. Each trial had a two-hour acclimation period to ensure behavioral changes caused by handling stress did not influence results. Each trial was recorded for 30 minutes following acclimation using two GoPro hero 4 video cameras mounted side-by-side above the experimental array. The ventilation rate was determined by counting the number of open/closing cycles of the gill operculum within a minute (i.e., ventilations per minute [VPM]). The VPM was calculated by averaging the VPM for 3 one-minute measurements following the acclimation period for each individual per treatment.

### *Olfactory choice*

Olfactory capabilities in fish play a crucial role in predator detection and avoidance, particularly at the juvenile stage (Lima & Dill 1990). Fish are at high risk of predation at this stage when settling to habitats after the pelagic larval phase (Caley *et al.* 1996). Predator recognition and avoidance in aquatic ecosystems often relies on the detection of olfactory cues excreted by predators. Recent studies have shown that exposure to low pH conditions can impair olfactory capabilities in juvenile stage fishes (Dixson *et al.* 2009). It is unknown whether exposure to low oxygen conditions can impact olfactory capabilities of temperate reef fishes.

Juvenile copper rockfishes were exposed to experimental treatments for 16-23 weeks and blues for 16-22 weeks before being used in odor choice assays (Table 1). Olfactory choice trials were conducted using a Loligo Systems 2-channel Choice Tank #AB10250 (Fig. 2H). Water was

continually delivered at the separate head of each channel and drained from the common bottom channel. Predator odor cues were established by turning off the water flow to the 500 L aquaria containing one adult lingcod for 2 hrs before commencing a trial (Dixson *et al.* 2009). This water was delivered to the appropriate channel during the predator exposure period of a trial. Each trial consisted of 3 five-minute measurement intervals, with spot checks every five seconds using tally counters to record the fish's location. Single fish were placed into the experimental chamber of the olfaction choice tank in the same manner for each trial. Control seawater was used in both channels for the first five-minutes to obtain a tank bias baseline, followed by 2 five-minute intervals of predator-odor vs. no-odor water, with the channel water sources swapped at the half way mark. Flow rate was set at 250 L hr<sup>-1</sup>. A sample size of  $n = 8$  juvenile fish was used for each treatment. The percent of time each fish spent in the predator odor cue water source was the metric used for subsequent statistical analyses.

### *Statistical analysis*

A one-way factorial analysis of variance (ANOVA) was used to analyze response variables across different  $pO_2$  treatment levels for olfactory choice trials, behavioral lateralization, escape challenge,  $O_2$  consumption, and ventilation rates. A Levene test was run to determine if the variance was equal and residuals were tested for normality. Data sets that did not meet the assumptions of homoscedasticity and normally distributed residuals were run using a Wilcoxon/Kruskal-Wallis non-parametric test. A Tukey HSD post-hoc test was used to identify significant pairwise treatments. A chi-squared analysis was used to determine statistical significance of mortality data.



pCrit was determined for each fish using the broken stick regression method (Toms & Lesperance 2003). A modified data set with resting measurements removed was loaded into the AutoResp software and pCrit values were calculated using the integrated calculations of the program. pCrit estimates were based on the last 6 measurement cycles for all trials, 3 measurements each for the lowest two oxygen levels. These last 6 measurements represent the point at which  $\text{MO}_2$  began to show marked decreases with decreasing  $p\text{O}_2$ .

## Results

### *Somatic growth*

Copper rockfish exhibited a decrease in growth as a function of decreasing oxygen treatment conditions (Fig. 4A, C). Fish from the  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  treatment grew significantly slower in mass than fish from control treatments (ANOVA,  $F_{3,69} = 3.46$   $P = 0.021$ ). I did not detect a significant change in total length over the course of the experiment for copper rockfish (ANOVA,  $F_{3,69} = 1.84$   $P = 0.15$ ); however, the trends were in the same direction, with fish growing less in length in the lowest  $p\text{O}_2$  treatments. Blue rockfish showed the opposite pattern, where the growth rate increased as a function of decreasing oxygen (Fig. 4B, D). Blue rockfish in the  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  treatment exhibited the largest weight increase compared to the control treatment (ANOVA,  $F_{3,37} = 4.11$   $P = 0.014$ ). The growth rate of blue rockfish measured in total length was also highest in the  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  treatment compared to the control treatment (ANOVA,  $F_{3,45} = 5.53$   $P = 0.0027$ ).

### *Escape response*

Exposure to varying oxygen treatment conditions appeared to have a weak but consistent effect on the escape time of copper rockfish and no effect on blue rockfish. For copper rockfish, fishes exhibited a trend of increasing escape times as a function of decreasing dissolved oxygen levels. Copper rockfish escape times increased by 28%, 10%, and 96% for fish exposed to the 6.0, 4.0, and 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatments relative to the control fish (Fig. 5). These results were not significant (Wilcoxon/Kruskal-Wallis,  $\chi^2_3 = 3.43$ ,  $P = 0.58$ ) due to a high variance in escape response time within a treatment; however, the rank ordering of the treatments followed the predicted response to declining oxygen levels. The escape time for blue rockfish also did not vary significantly as a function of oxygen exposure history (Wilcoxon/Kruskal-Wallis,  $\chi^2_3 = 1.29$ ,  $P = 0.73$ ).

### *Behavioral lateralization*

Lateralization for copper rockfish tended to decrease with decreasing oxygen levels. Absolute lateralization for copper rockfish was significantly reduced for fish exposed to 4.0 mg O<sub>2</sub> L<sup>-1</sup> compared to the control (ANOVA,  $F_{3,71} = 4.63$ ,  $P = 0.0052$ , Fig. 6A). Fish in the 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatment were also less lateralized than the control fish, but the results were not significant. The turning bias that occurred in the control treatments indicated that individuals have an inherent turning bias in one direction or the other, but there is not a specific directional pattern for the population as a whole. This turning bias is reduced for individual copper rockfish with declining oxygen levels. Blue rockfish did not appear to display any change in absolute lateralization turning bias (ANOVA,  $F_{3,61} = 0.86$ ,  $P = 0.47$ , Fig. 6B). There were no changes in

relative lateralization among treatments for either copper rockfish (ANOVA,  $F_{3,71} = 0.22$   $P = 0.88$ , Fig. 6C) or blue rockfish (ANOVA,  $F_{3,61} = 0.83$   $P = 0.48$ , Fig. 6D).

### *Hypoxia tolerance*

Both copper and blue rockfish displayed significant changes in the critical oxygen tension threshold (pCrit) as a function of oxygen treatment. Fish exposed to chronic low oxygen conditions became more tolerant to hypoxia and could withstand lower  $pO_2$  conditions, reflected in lower pCrit values (Fig. 7). The pCrit for copper rockfish differed significantly among the treatment conditions (ANOVA,  $F_{3,26} = 7.28$   $P = 0.0013$ ) with fish in the 4.0 mg  $O_2$   $L^{-1}$  treatment conditions having a pCrit 21% lower than the control treatments, while fish in the 2.0 mg  $O_2$   $L^{-1}$  treatment has a pCrit 16% lower than the control. Blue rockfish showed a similar result with the lowest pCrit (i.e., highest hypoxia tolerance) occurring in the lowest oxygen rearing treatment (ANOVA,  $F_{3,18} = 10.51$ ,  $P = 0.0006$ ). Blue rockfish exposed to 2.0 mg  $O_2$   $L^{-1}$  exhibited a 33% lower pCrit level than the control.

### *Aerobic scope*

For copper rockfish, aerobic scope decreased with decreasing  $pO_2$  treatments (ANOVA,  $F_{3,19} = 24.69$   $P < 0.0001$ ; Fig. 8A). Relative to the control treatment, aerobic scope declined by 19%, 29%, and 79% at levels of 6.0, 4.0, and 2.0 mg  $O_2$   $L^{-1}$ , respectively. Blue rockfish also exhibited a decreased in aerobic scope with decreasing oxygen levels (ANOVA,  $F_{3,23} = 4.31$   $P = 0.017$ ; Fig. 8B), and the fish exposed to 2.0 mg  $O_2$   $L^{-1}$  were significantly lower in aerobic scope than the fish in the control treatment. Relative to the control, aerobic scope was reduced by 52%, 35%, and 64% for fish from the 6.0, 4.0, and 2.0 mg  $O_2$   $L^{-1}$  treatments, respectively. I found no

significant differences in SMR across treatments for either species (ANOVA  $F_{3,21} = 2.36$   $P = 0.10$ ; Fig. 8E, F). However, the MMR differed significantly among treatments for both blue rockfish (ANOVA,  $F_{3,31} = 21.56$ ,  $P < 0.0001$ ) and copper rockfish (ANOVA,  $F_{3,31} = 4.41$ ,  $P = 0.012$ ). The depression of aerobic scope for both species at low oxygen conditions was best explained by a corresponding decline in the MMR and no change in SMR.

#### *Ventilation rate*

The average ventilation rates (VR) for copper rockfish differed significantly as a function of O<sub>2</sub> exposure history (ANOVA,  $F_{3,38} = 19.57$ ,  $P < 0.0001$ , Fig. 9), and VR increased with declining  $pO_2$ . Coppers exposed to 2.0 mg O<sub>2</sub> L<sup>-1</sup> conditions had the highest VR with a mean of 64 VPM, which was significantly different than all other treatments. Coppers exposed to 6.0 mg O<sub>2</sub> L<sup>-1</sup> had an elevated VR of 49 VPM, which was higher than the control of 41 VPM but lower than the hypoxic treatment. Blue rockfish exhibited a similar pattern, with the average VR of fish exposed to 2.0 mg O<sub>2</sub> L<sup>-1</sup> conditions being significantly elevated compared to all other treatments with a mean of 87 VPM (ANOVA,  $F_{3,31} = 12.56$ ,  $P < 0.0001$ ).

#### *Olfactory choice*

The ability to detect olfactory cues produced by predators was retained for fish exposed to all levels of oxygen saturation. Both copper and blue rockfish could detect and avoid predator cues. When presented with two water sources, one containing a predator cue and one containing no cue, fish from all treatment levels avoided the predator cue approximately 80% or more of the time (copper rockfish,  $t_{60} = 16.31$ ,  $P < 0.0001$ ; blue rockfish  $t_{65} = 13.78$ ,  $P < 0.0001$ ; Fig. 10).

There was no significant difference in the avoidance behavior between copper and blue rockfish across all oxygen exposure treatments.

### *Mortality*

Mortality and morbidity were used as proxies for overall well-being of fish health throughout the experiment. As the project progressed and exposure time increased, a pattern was observed of unhealthy fish. This was most apparent for blue rockfish where multiple mortalities were recorded. The highest mortality rates occurred in the 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatment water for blue rockfish ( $\chi^2 = 0.19$ ,  $df = 3$ ,  $P = 0.0003$ ; Fig. 11). The mortality rate of blue rockfish in this treatment was nearly 3 times higher than mortality in any other tanks. Copper rockfish did not experience any mortality in response to exposure to treatment levels alone. The only mortalities for copper rockfish were due to an error in the respirometry automated program during a single trial.

### **Discussion**

The results of this study demonstrate that juvenile rockfish of two species exhibit significant adverse effects of hypoxia, which can impair behavioral and physiological functions. In general, copper rockfish responded more negatively to chronic low oxygen levels than blue rockfish, exhibiting changes in behavioral lateralization and increases in the escape response time. Copper rockfish also experienced reduced growth, reduced aerobic scope, decreased MMR, increased ventilation rate, and only minor increases in hypoxia tolerance. Blue rockfish also experienced reduced aerobic scope and increased ventilation rates in the lowest oxygen conditions, but were additionally characterized by a stronger increase in hypoxia tolerance.

Despite generally responding less dramatically to hypoxia than copper rockfish, the blue rockfish did experience a much higher mortality rate in the lowest oxygen treatment.

### *Behavioral effects*

Lateralization is typically assumed to be advantageous for fish because it results in more efficient reaction times and aids in tasks that require directional responses (Vallortigara & Rogers 2005; Dadda *et al.* 2010). The level of lateralization of an individual is associated with cognitive ability including escape reactivity, spatial orientation, and schooling behavior (Bisazza & Dadda 2005; Sovrano *et al.* 2005; Dadda & Basazza 2006; Munday *et al.* 2009; Dadda *et al.* 2010). Extreme hypoxia has been shown to alter patterns of behavioral lateralization (left vs. right turning bias) in teleost fish, causing individuals to become less lateralized after exposure to low oxygen conditions. Recent studies have shown that exposure to hypoxic conditions can affect behavioral lateralization on the population level (Lucon-Xiccato *et al.* 2014).

A pattern of decreasing absolute lateralization with reduced  $pO_2$  was observed for copper rockfish. Copper rockfish exposed to low oxygen treatments experienced a 60% reduction of absolute lateralization in the  $4.0 \text{ mg O}_2 \text{ L}^{-1}$  treatment. This change is irrespective of a specific direction. As a population, copper rockfish did not show a directional turning preference at ambient oxygen conditions, but rather displayed lateralization at the individual level, and became less lateralized when exposed to reduced oxygen. Changes in behavioral lateralization indicate changes in brain function (Domenici *et al.* 2011). Changed turning preference under exposure to high  $pCO_2$  has been attributed to altered neurotransmitter function responsible for behavioral impairment (Nilsson 2012). Extended periods of reduced oxygen exposure may also alter the neurotransmitter function through reduction of neuronal ion permeability. Nilsson and Renshaw

(2004) showed that hypoxia tolerant carp can control GABA release, the major inhibitory neurotransmitter in the brain. Altered neurotransmitter function leading to reduced lateralization in copper rockfish may be attributed to a change in GABA control and deserves further investigation. Species differing in life history characteristics may be differentially susceptible to changes in regulation of this inhibitory transmitter under low  $pO_2$  exposure.

Differences in the lateralization response between copper and blue rockfish are consistent with Hamilton *et al.* (2017), who similarly found copper rockfish to be more sensitive than blue rockfish to altered water chemistry conditions. Their study found that copper rockfish became more lateralized after exposure to high  $pCO_2$  water, while blue rockfish were unaffected (Hamilton *et al.* 2017). While the directional response for copper rockfish exposed to low  $pO_2$  is opposite of that when exposed to high  $pCO_2$ , these results indicate that copper rockfish appear to have a more plastic response of lateralization to changing ocean chemistry. When exposed to high  $pCO_2$  and low  $pO_2$  in combination, the effects may offset one another. The impairment/disruption of these behaviors may make copper rockfish and similarly affected species more vulnerable to predation.

The escape challenge trial is designed to test exploratory behavior and boldness (Jutfelt *et al.* 2013). A quick escape time relays that a fish is active and exploring its surrounding environment. Although results were not statistically significant due to high variance in escape times within a treatment, the clear trend for increases in escape time with decreasing  $pO_2$  in both copper and blue rockfish may be indicative of a potential impairment of brain function, problem solving abilities, and boldness. A study using similar methods but testing for effect of high  $pCO_2$  exposure on sticklebacks found a similar trend. After 40 days in treatment conditions, fish not exposed to high  $pCO_2$  escaped from the escape chamber in half the time as fish exposed to low

$p\text{CO}_2$  (Jutfelt *et al.* 2013). A potential explanation for the increase in escape time exhibited by juvenile rockfish in reduced oxygen conditions may be that individuals are less willing to explore their habitat, potentially in an effort to conserve energy. An increase in energy use would cause an increase in metabolic rate that would require more oxygen, which is already in short supply. This is particularly true for chronic exposures, where an individual has been acclimated to the oxygen saturation conditions for weeks to months. Results may be different for short-term exposure where species have been shown to actively seek out areas where oxygen conditions are more suitable (Kramer 1987; Pihl *et al.* 1991). Species acutely exposed to low  $p\text{O}_2$  conditions experience a trade-off of increasing swimming activity to increase the probability of finding more oxygenated water and decreasing swimming activity to reduce oxygen requirements (Bushnell *et al.* 1984; Schurmann & Steffensen 1994; Domenici *et al.* 2000). As an artificial test in a laboratory setting, the relevance of these results in an ecological sense are not entirely known.

Juvenile rockfish have an innate ability to detect predators using olfactory cues. A study by Munday *et al.* (2010) showed that this ability can be disrupted in juvenile Orange clownfish (*Amphiprion percula*) that develop under high  $p\text{CO}_2$  conditions. This study tested if olfactory capabilities were also affected by exposure to low  $p\text{O}_2$ . Results indicate that juvenile copper and blue rockfish maintain their olfactory capabilities and consistently avoid water sources containing predator odor cues, suggesting that chronic exposure to reduced oxygen saturation does not impair their olfactory capabilities. For  $\text{CO}_2$  exposed fish, changes in bicarbonate and chloride concentrations can interfere with neuroreceptor function (Nilsson *et al.* 2012). The mechanism for the reversal in the GABA-A receptor function involves shifts in  $\text{Cl}^-$  and/or  $\text{HCO}_3^-$  gradients over neuronal membranes, resulting from acid-base regulation following  $\text{CO}_2$



exposure (Nilsson *et al.* 2012). For low  $pO_2$  exposed fish, the GABA-A receptor is likely maintaining proper function. Exposure to low oxygen conditions does not require the same acid-base regulation changes in fish that could lead to a change in neurotransmitter function. Further investigation is required to determine if juvenile rockfish maintain their predator detection through olfactory senses when given the choice between seawater containing a non-predator cue, or a conspecific cue vs. seawater containing a predator cue. Additionally, these species may be more sensitive to effects of reduced oxygen saturation as larvae, and should thus be tested at this stage as well.

### *Physiological effects*

Several physiological changes in response to hypoxia were detected in both species of rockfish. Chronic exposure to reduced  $pO_2$  negatively impacted the growth rate of copper rockfish. Average daily growth rate for juvenile copper rockfish exposed to  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  was reduced by 37% compared to growth rates in normoxic treatments. Reductions in growth rate for the lowest treatment may be caused by changes in feeding intake. All fish were fed the same quantity of food throughout the duration of the experiment. Although feeding intake was not measured in this study, a reduction in feeding could be one possible approach for individuals to reduce energetic demand, thereby decreasing oxygen requirements (Van Dam & Pauly 1995). Oxygen has been shown to act as a limiting factor for growth in other fish species, including juvenile European sea bass (Thetmeyera *et al.* 1999), and juvenile largemouth bass (Stewart *et al.* 1967), which both showed a reduction in feeding under hypoxia stress. Exposure to low  $pO_2$  did not appear to inhibit growth rates of blue rockfish and in fact fish in the lowest  $pO_2$  condition had the largest growth rate. This contradictory result was likely influenced by a high number of

mortalities in the 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatment. The occurrence of 9 mortalities of blue rockfish in the 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatment tanks may have alleviated competition among the remaining blue rockfish for food. In addition, I observed the copper rockfish to be competitively dominant over the blue rockfish in the shared treatment tanks. Copper rockfish in the lowest oxygen conditions experienced reductions in behavior, feeding, and aggression and the depressed activity levels may have allowed the more oxygen tolerant blue rockfish to increase their food intake in the lowest oxygen treatments. Another possible explanation is selective mortality of the lower performing rockfish in the 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatment, resulting in the survivors exhibiting a higher average growth rate compared to other treatments.

Decreased  $pO_2$  consistently affected overall aerobic performance. Both species examined showed depressed aerobic scope under moderate and extreme oxygen limitation. These results are similar to the results from a study by van den Thillart *et al.* (1994) who showed that the flatfish *Solea solea* showed significant changes to aerobic scope with reduced oxygen levels. Fishes occupying environments with sufficient oxygen will have low SMRs and high MMRs. In the context of limited oxygen conditions, changes in aerobic scope are typically driven by a decrease in the MMR of fish with little to no detectable change in SMR (Hughes 1973). This study supports that claim in that no changes to SMR were observed for either copper or blue rockfish. van den Thillart *et al.* (1994), however, observed no change in SMR between 20-80% oxygen saturation, but decreasing SMR between 6 and 12% saturation. It is possible that rockfish may show decreases in SMR at levels this low as well. I did not reduce the oxygen level as far as 6-12% due to potential for mortality at these levels. MMR can be reduced when the limited capacity of the circulatory and ventilatory systems cannot keep pace with O<sub>2</sub> demands of the fish when available O<sub>2</sub> is limited (Fry 1971; Pörtner & Grieshaber 1993). Lack of oxygen during high

activity can trigger anaerobic metabolism, causing a large decrease in ATP production as the Krebs cycle and electron transport chain, two major ATP-producing pathways, shut down. The reduction observed in aerobic scope likely will lead to an inability to meet oxygen demands of locomotion, growth, behavior, and reproduction (Pörtner & Knust 2007; Wang and Overgaard 2007).

While few studies have looked at the impacts of hypoxia exposure on aerobic scope, similar results have been seen for fish exposed to elevated  $p\text{CO}_2$  conditions (Munday *et al.* 2009; Lefevre 2016; Hamilton *et al.* 2017). A study by Pörtner *et al.* (2004) attributed decreased MMR to reduced oxygen uptake from the environment in response to acidification of blood and respiratory pigments. While this explanation is in the context of elevated  $p\text{CO}_2$ , the underlying mechanism of reduced oxygen uptake is a probable cause for reduced aerobic scope observed in this study. Aerobic scope represents the overall capacity of energy that can be devoted to different activities at any given point (Lefevre 2016). The reduced aerobic scope observed in this study likely represents a decrease in energetics, performance, and fitness. Both species showed a similar pattern of a reduced aerobic scope, but copper rockfish displayed a stronger reduction in aerobic capacity under exposure to low oxygen conditions (79% reduction from control) compared to blue rockfish (64% reduction from control). This may be due in part to blue rockfish having a higher baseline activity level in the kelp forest (i.e. YOY blue rockfish are typically more active in comparison to the kelp associated copper rockfish). A study by Schurmann *et al.* (1998) showed that free-swimming European sea bass spontaneously avoided water layers that were less than 45% oxygen saturation. These results were similarly seen in a study of Atlantic cod (Claireaux *et al.* 2000). Claireaux's study suggested that when an individual's metabolic scope is below a 50% threshold, energy allocation to secondary functions

(locomotion, digestion, reproduction) gradually lessen to fit the animal's metabolic scope while vital activities are sustained. With the application of this concept, it can be inferred that copper rockfish will begin to allocate energy away from these secondary functions at a higher  $pO_2$  level than blue rockfish. The increased sensitivity displayed by copper rockfish can potentially impact population dynamics under longer term exposure to low oxygen.

Further evidence of oxygen deprivation stress on respiration and metabolic function for copper rockfish was observed in the form of higher ventilation rates for fish exposed to the lowest oxygen conditions. An increase in ventilation rate for fish exposed to reduced oxygen levels has also been observed in other species, including juvenile flounder (Tallqvist *et al.* 1999), snake blenny (Pihl *et al.* 1991), striped bass, and naked goby (Breitburg *et al.* 1994). Ventilation requires 10-30% of a fish's energy budget to pass water across the gill membranes (Soofiani & Hawkins 1985). In this study, no significant difference in ventilation rate was recorded above 4.0 mg O<sub>2</sub> L<sup>-1</sup>. With less available  $pO_2$  in the water and increased energy requirements to maintain oxygen supply via higher ventilation rate in the 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatment, the overall energy budget under hypoxia must have shifted given that SMR did not change. Thus, less energy would have been available for non-essential activities, such as growth and locomotion. Low oxygen conditions stimulate a physiological response for individuals to increase their amplitude and rate of ventilation to move more water across the gill epithelium and secondary lamella (Randall & Shelton 1963). An increased flow rate across the gills increases the volume of available O<sub>2</sub> that can be absorbed by the secondary lamellae (Hughes 1973). A study by Marvin and Heath (1968) showed that an increase in ventilation volume is associated with an increase in O<sub>2</sub> consumption. However, even with increased ventilation rates, the tissue O<sub>2</sub> demands may be greater than circulatory O<sub>2</sub> supply at low environmental oxygen conditions. Individuals cannot maintain high

activity levels under these conditions. Although I did not measure ventilation rates under high rates of activity, one could assume that increased ventilation was unable to compensate for oxygen supply when activity levels, and therefore oxygen demand, was high.

For blue rockfish, ventilation rate remained independent of  $pO_2$  level for all treatment conditions except the extreme oxygen condition ( $2.0 \text{ mg O}_2 \text{ L}^{-1}$ ). Blue rockfish in the extreme treatment had a larger increase in ventilation rate (88% increase) compared to copper rockfish of the same treatment (56% increase) when compared to the controls. In this case, blue rockfish were working harder to extract the required oxygen to meet their metabolic demands and still able to maintain growth rates. This is reflected in the increase in hypoxia tolerance at the low oxygen treatment for blue rockfish. Though blue rockfish are required to work harder to extract oxygen in the form of increased ventilation, the payoff is an ability to withstand exposure to a lower oxygen concentration for a longer period.

A marked reduction was observed for the critical oxygen tension threshold ( $p_{\text{Crit}}$ ) with declining oxygen levels for copper rockfish.  $p_{\text{Crit}}$  represents the lowest level of oxygen at which aerobic metabolism is independent of  $pO_2$  (Hochachka & Somero 2002). At  $pO_2$  levels below  $p_{\text{Crit}}$ , survival is dependent upon the capacity to reduce metabolic demands (Farrell & Richards 2009). As ambient oxygen conditions decrease, a level is reached where metabolic rate can no longer be maintained. When pushed past this threshold, fish that are effective oxy-regulators become oxy-conformers and may shift towards reliance on anaerobic metabolic pathways (Schurmann & Steffensen 1997; Hochachka & Somero 2002). If rockfish fully utilize anaerobic metabolism at  $pO_2$  levels below this threshold for long periods they will not be able to meet  $O_2$  requirements and will eventually expire.

Measurements for copper rockfish revealed a lower oxygen threshold for the lowest two oxygen saturation levels. Copper rockfish had a 21% and 16% lower pCrit for 4.0 and 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatments respectively, indicating that fish from these treatments had greater hypoxia tolerance compared to copper rockfish at ambient conditions. Similarly, blue rockfish displayed a 33% reduction in pCrit for the 2.0 mg O<sub>2</sub> L<sup>-1</sup> treatment compared to the control. These results are in line with findings from Mandic *et al.* (2009) showing that environmental acclimation can lead to greater hypoxia tolerance. Additionally, studies such as those done by Saint-Paul (1984) and Hopkins & Powell (2001) show an adaptive ability through modifications leading to enhanced O<sub>2</sub> uptake potential. Sculpins with greater hypoxia tolerance inhabit tidepools with more variable O<sub>2</sub> levels, while species with low tolerance tend to inhabit tidepools characterized by more stable O<sub>2</sub> levels (Mandic *et al.* 2009, Richards 2011). Additionally, hypoxia-induced physiological modifications have been shown for gill surface area (Nilsson 2007), respiration (Saint-Paul 1984), tissue O<sub>2</sub> demand (Hopkins & Powell 2001), and hemoglobin binding characteristics (Jensen & Weber 1982; Brix *et al.* 1999). A low pCrit indicates a higher capacity for oxygen extraction and tissue delivery when *p*O<sub>2</sub> is limited and is thus generally associated with greater hypoxia tolerance (Mandic *et al.* 2009). Greater hypoxia tolerance is advantageous because it allows fish to function aerobically at lower oxygen levels, allowing a more efficient production of ATP (Richards 2009). In an ecological context, pCrit may have major implications for fitness of fishes living in environments prone to hypoxia (Claireaux & Chabot 2016). Species that are better acclimated to hypoxic conditions (lower pCrit) will have a better chance of survival under extreme low *p*O<sub>2</sub> conditions. Evidence for deleterious effects of hypoxia on fish physiology are corroborated in a study by Gracey *et al.* (2001) who found that patterns of gene expression of

*Gillichthys mirabilis* were dramatically altered among individuals raised under hypoxic conditions when compared to those raised under normoxic conditions.

Both copper and blue rockfish became more tolerant to hypoxia exposure when chronically exposed to low oxygen conditions. The 33% reduction in pCrit for blue rockfish exposed to  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  indicate that that blue rockfish have a stronger capacity for acclimation at the most extreme  $p\text{O}_2$  exposure condition compared to copper rockfish. Though not as strong, the response from copper rockfish seemed to scale with pCrit decreasing as a function of decreasing  $p\text{O}_2$ . Increased tolerance to hypoxia may be a fundamental ability required by fish to survive in a dynamic and increasingly hostile environment.

Greater hypoxia tolerance may be associated with enhanced  $\text{O}_2$  extraction capacity. The structure and surface area of the gills is dictated by the oxygen demands of the organism and ion exchange necessary to maintain osmoregulation (Sollid *et al.* 2003; Nilsson 2007). Changes in physical activity and oxygen levels require fish to adjust their behavior and/or physiology to compensate for higher  $\text{O}_2$  demands/uptake. This is most commonly achieved by altering the water flow over the gills, displayed in this study by the increases observed in ventilation rates for fish exposed to low oxygen conditions. This response is likely paired with increasing blood flow within the gills for more effective oxygen extraction (Saunders 1962; Holeyton & Randall 1967). Recent studies have concluded that some fish may possess additional processes for coping with altered environmental conditions, such as adaptive remodeling of the secondary lamella (Nilsson 2007), which acts to increase the surface area of respiratory gas exchange. Adaptive remodeling has been shown to occur in crucian carp (Sollid *et al.* 2003) and mosquito fish (Douglas 2013) when exposed to hypoxic conditions, but it is still unknown whether juvenile rockfish have the capacity to modify their gill surface area. It is well known that exposure to low oxygen

conditions can increase hemoglobin (Hb) content of the blood and the erythrocyte count in vertebrates, including fish (Scott & Rogers 1981, Saint-Paul 1984). In addition, Hb-O<sub>2</sub> binding affinity has been shown to increase in response to hypoxia (Jensen & Weber 1982). These modifications would effectively allow individuals to withstand exposure to lower oxygen conditions, however they may also carry drawbacks as well. Increases in hematocrit can also lead to increased viscosity of the blood, which can lead to stress on the heart (Martini *et al.* 2005). Future studies are required to determine the potential occurrence of these responses in rockfish.

### *Conclusions*

The resilience of a species is often tested during periods of dramatic or sudden environmental change. With current rates of climate change, many species must adapt to maintain healthy populations. As our oceans become more hypoxic through increased sea temperatures, expansion of oxygen minimum zones, and the progression of climate influenced nearshore hypoxia events (i.e. upwelling, eutrophication), it is important to understand how species will be affected by exposure to low oxygen conditions.

Rockfish comprise a valuable component of commercial and recreational groundfish fisheries along the California coast. It is crucial that fisheries managers and policy makers are informed of possible sources of mortality to properly manage susceptible species. Life history characteristics of being long lived, late to mature, and having long generation times already make many rockfish species vulnerable to overfishing (Harvey *et al.* 2006). These traits may also prevent rockfishes from adapting to the rapid changes in ocean chemistry that are predicted to occur within the next century. Additionally, the results of this study show that even closely



related species, like copper and blue rockfish, can have different responses to low oxygen conditions. Determining effects of hypoxia on behavior and physiology of coastal rockfishes can provide scientists with the proper information to predict the potential impacts of climate change on fish populations and fisheries yields. This information will be critical for policy makers to properly protect economically and ecologically important species in the California Current Ecosystem.

For marine species found along the Central California coast, seasonal upwelling and associated variability in ocean chemistry is a natural factor of the environment. Exposure to bouts of low oxygen and hypoxic conditions have potentially made local species more tolerant of changing climates. The rockfish species in this study were tolerant of chronic exposure to varying levels of oxygen depletion. Though metabolically depressed, all copper rockfish survived chronic exposure to  $2.0 \text{ mg O}_2 \text{ L}^{-1}$  conditions for 24 weeks. Both species of rockfish did well under oxygen deprivation for extended periods of time. The degree of resilience displayed by rockfish may be due in part to the fact that they are constantly exposed to dynamic fluctuations in oxygen saturation on a regular basis. The constant exposure to these conditions may allow them to be better adapted to low oxygen conditions than previously thought. In addition, changes in pCrit show the potential for acclimatization to low oxygen conditions in the field. The ability for these species to adapt to changing environments is critical as we progress into an ever-changing world as human impacts alter the natural state of our global oceans.

Although juvenile rockfish displayed some capacity for tolerance to low oxygen conditions, both species had physiological responses that could lead to decreased fitness. Adaptations to low oxygen observed in this study may not be strong enough for increased level of exposure to hypoxic conditions. For example, both species had reductions in maximum

metabolic activity that led to decreased aerobic scope, a proxy for aerobic fitness. Changes to fitness of juvenile rockfish can have significant effects on adult populations if patterns of juvenile survival or growth are altered. Predation is a dominant source of mortality for juvenile stage rockfishes (Adams & Howard 1996; Hobson *et al.* 2001). Decreases in swimming capabilities, limited by reduced metabolic activity, can lead to direct mortality through predation events for both species studied. Juvenile rockfish whose swimming capabilities are impaired can be more easily captured by predators. Extended exposure to low oxygen can reduce growth and locomotion as energy for these processes is reallocated to essential processes such as maintaining metabolic needs due to the loss of ATP production. Large increases in predation at early life stages can potentially affect recruitment success of adult stages leading to population level changes.

It is important to note that hypoxic events typically occur in combination with decreases in pH of the water mass affected (Booth *et al.* 2012). The co-occurrence of hypoxic and acidic water can potentially exacerbate the impacts observed in these fishes (Miller *et al.* 2016). Further studies of the effects of hypoxia, as well as the combined effects of hypoxia and ocean acidification are required to understand the impacts that changing ocean conditions will have on rockfishes.

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### Tables

**Table 1.** Time table of when copper and blue rockfish were run through each experiment. Both species of fish from all treatment conditions were run through each experiment. Species were run through experiments based on a feeding schedule and recovery period to assure that any given individual had been fasted for at least 36 hours, and had not been used in a previous trial for 120 hours. The order of species run through trials was alternated within each experimental time period.

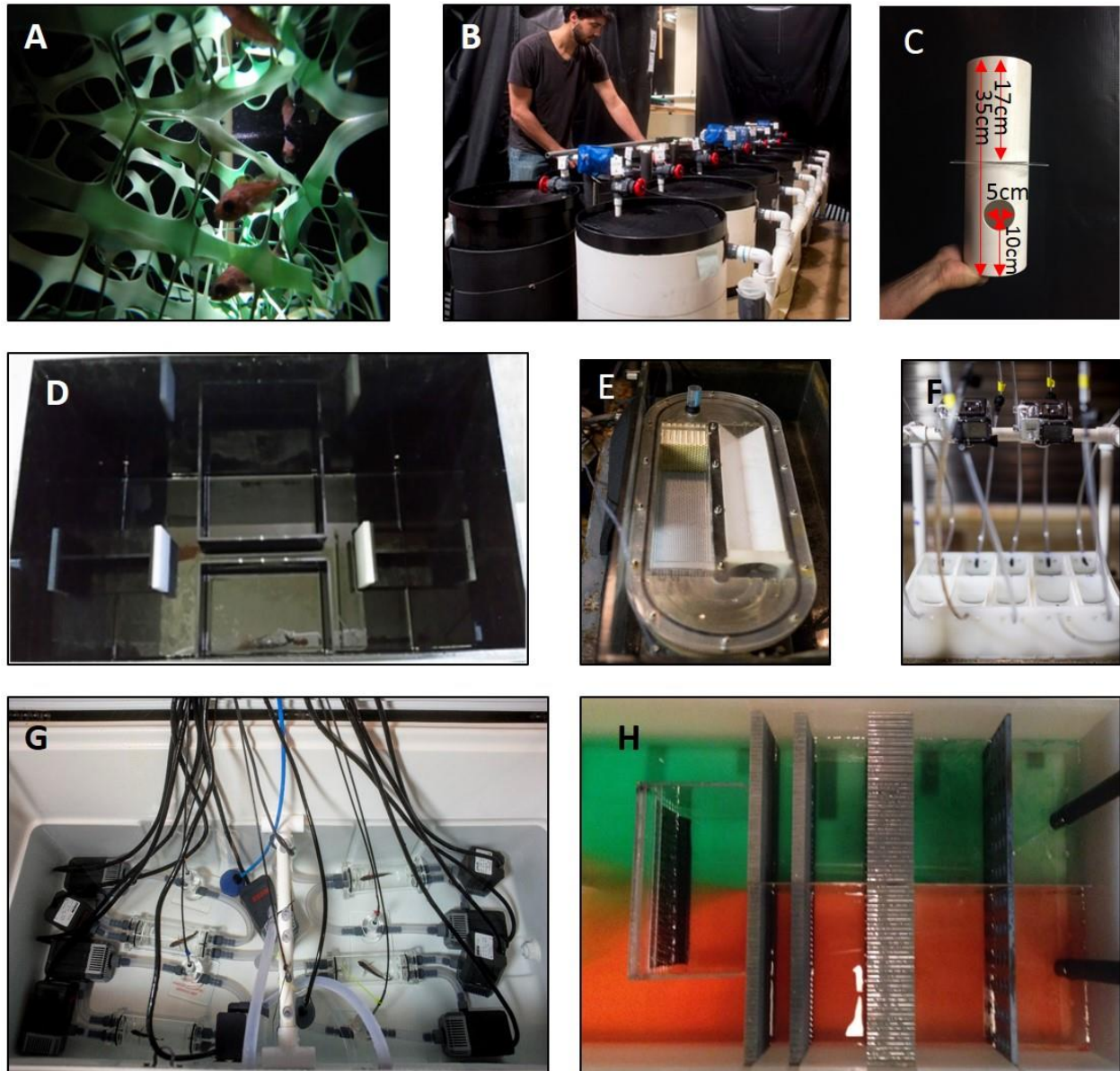
<b>Experiment</b>	<b>Copper Rockfish</b>	<b>Blue Rockfish</b>
Escape Response	4-6 weeks	3-5.5 weeks
Behavioral Lateralization	6-8 weeks	6-9 weeks
Aerobic Scope	9-17 weeks	8-17 weeks
Hypoxia Tolerance	9-17 weeks	11-22 weeks
Olfactory Choice	16-23 weeks	16-22 weeks
Ventilation Rate	23-24 weeks	23-24 weeks



## Figures

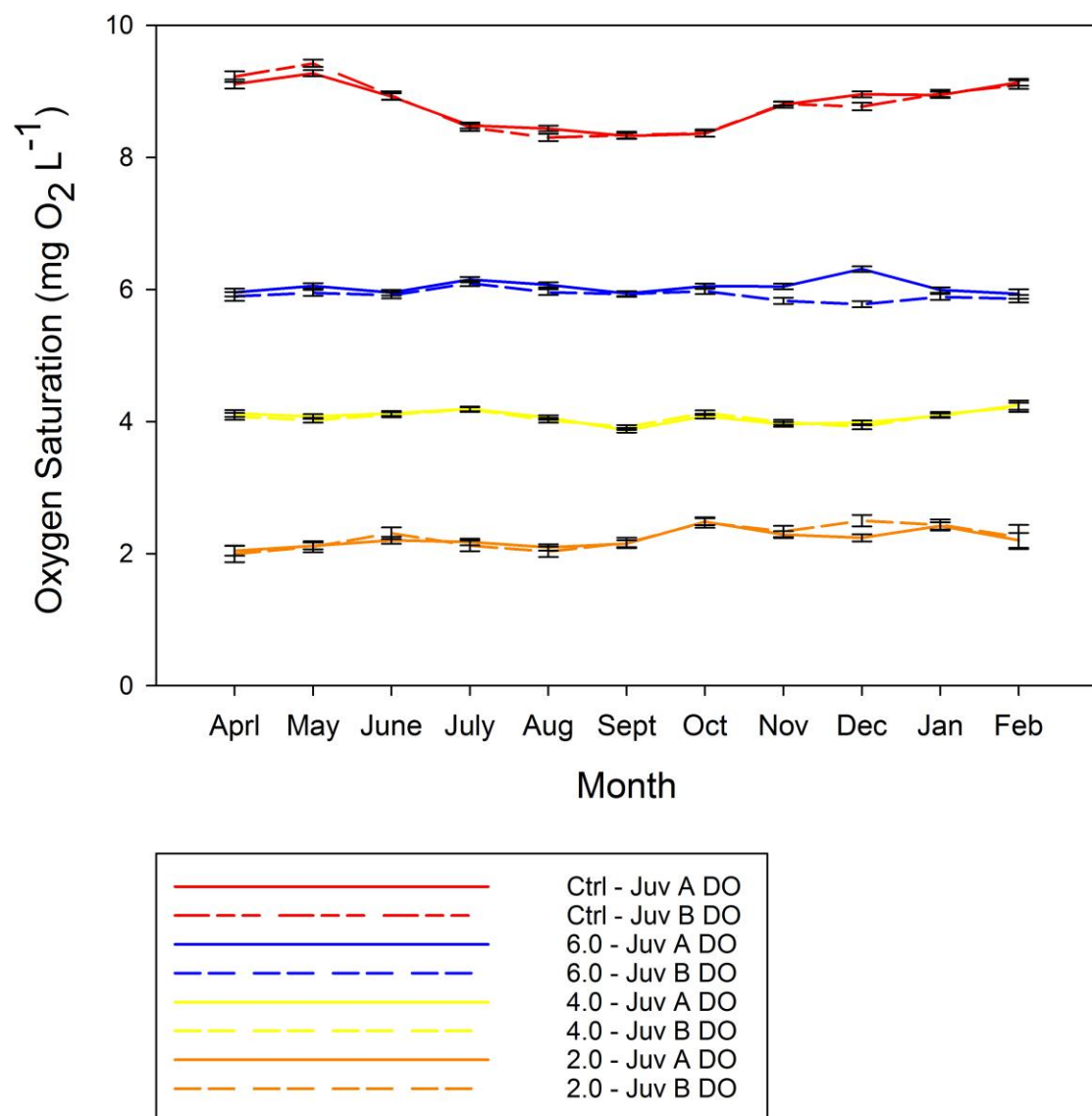


**Figure 1.** Primary collection site in Stillwater Cove, Central California ( $36.5605^{\circ}$  N,  $121.9458^{\circ}$  W), Depth (10-20 m). Stillwater Cove is a temperate rocky reef and kelp forest habitat.

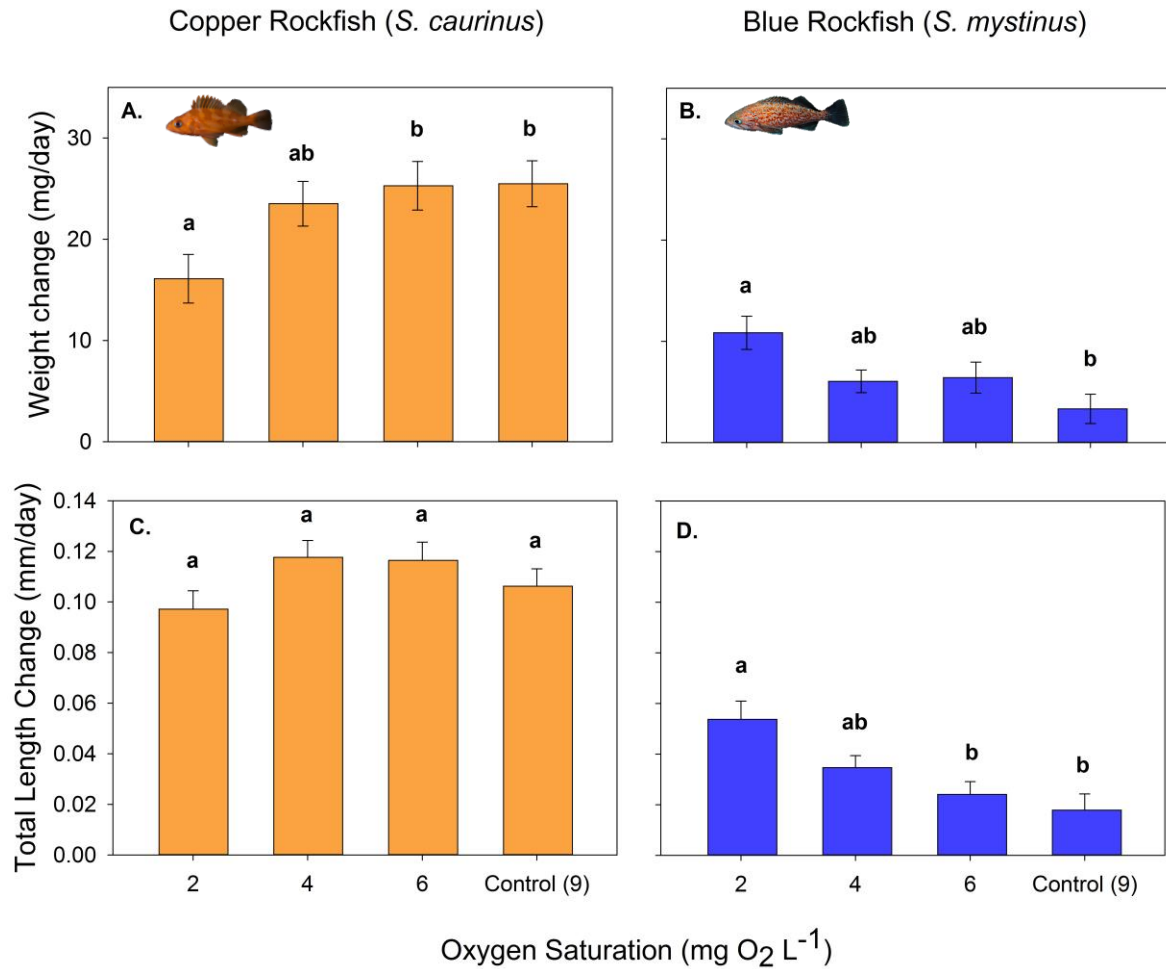


**Figure 2.** (A) Artificial habitat used in fish holding tanks. Plastic construction fencing was made to mimic a kelp canopy structure. (B) Experimental tank set up. Two rows of 7 tanks each were used with independent water sources. Each treatment condition had two replications. (C) Escape challenge experimental chamber composed of an acclimation chamber and escape response chamber. (D) Behavioral lateralization double-T maze. The chamber is made of black plexiglass and is composed of a center channel that leads to a two-way 90° turn at each end. (E) Loligo

swim flume #SW10100 used for swimming fish to maximum activity level before being transferred to the intermittent flow respirometer where MMR was measured. **(F)** Ventilation rate experimental array and GoPro Hero 4 attachment setup. Up to 2 independent water sources provide flow through treatment conditions to 5 chambers for each water source. **(G)** Intermittent flow respirometer measured oxygen consumption with optical oxygen probes. Composed of four separate cylindrical glass chambers that alternate between an open flushing period, a closed waiting period, and a closed measurement period. **(H)** Loligo Systems 2-channel Choice Tank #AB10250. Two independent water sources (colored green and red for visual test only) are kept separate through an experimental chamber where an animal can freely move between the two water sources.

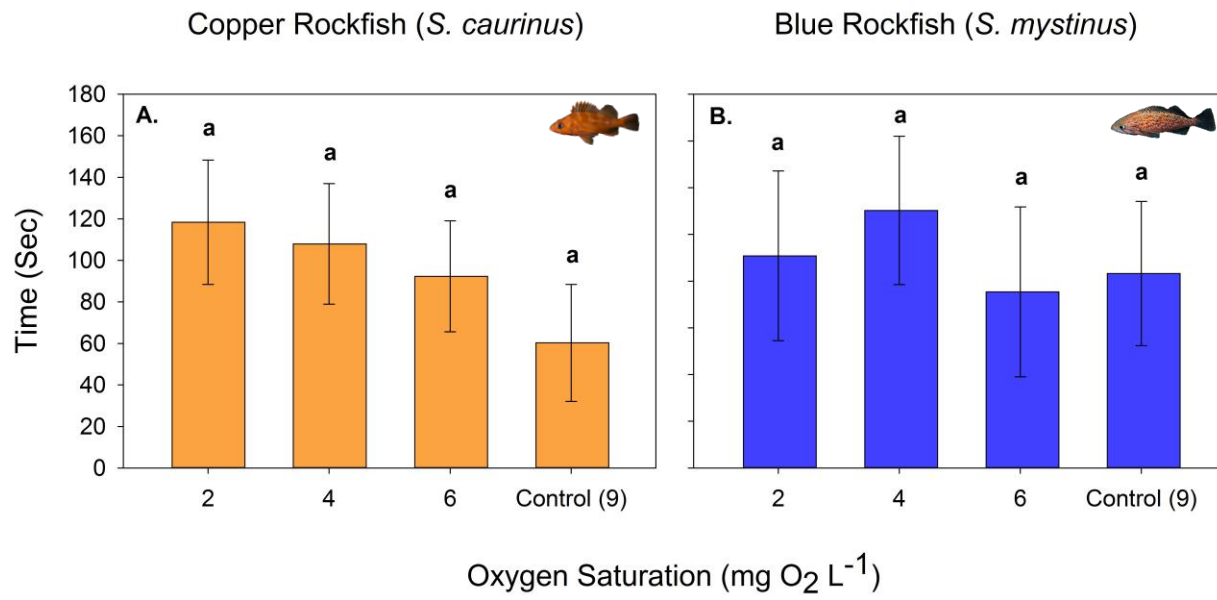


**Figure 3.** Monthly average dissolved oxygen content of experimental fish holding tanks over the duration of the experiment. Conditions were measured using a HACH handheld oxygen meter every morning. The X-axis is time representing the duration of the experiment in months and the Y-axis is dissolved oxygen measured in mg O<sub>2</sub> L<sup>-1</sup>. Row A tanks are represented by solid lines and Row B tanks are represented by dashed lines. Error bars represent calculations of standard error for each monthly average.

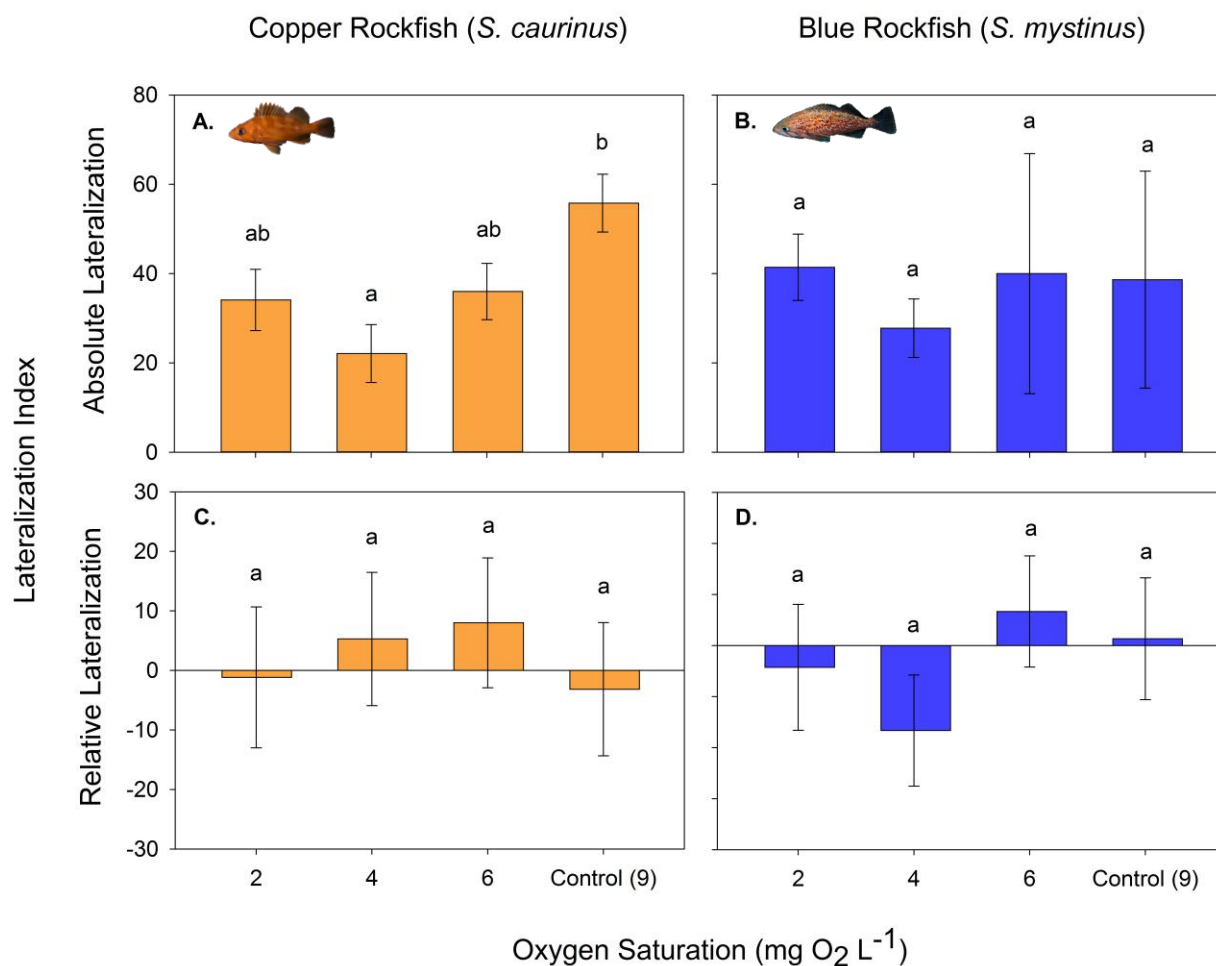


**Figure 4.** Somatic growth measurements of weight change per day for copper rockfish (**A**) and blue rockfish (**B**), and length change per day for copper rockfish (**C**) and blue rockfish (**D**). Bars represent the mean value ( $\pm 1$  SE). Letters above the bars indicate significantly different means following Tukey HSD post-hoc comparisons.

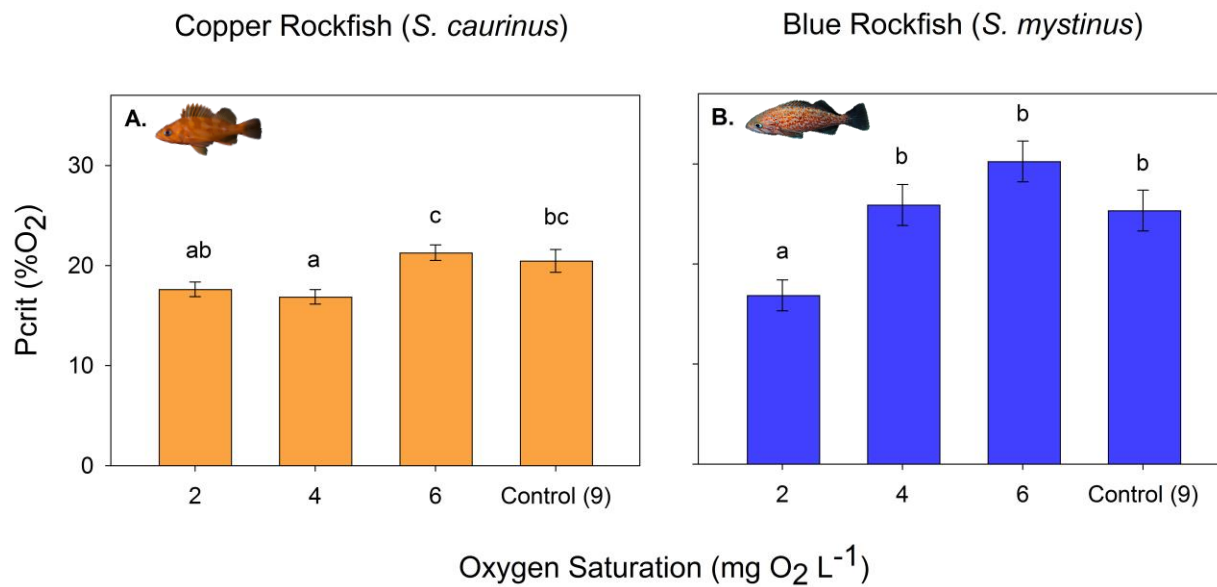




**Figure 5.** Changes in escape time for copper rockfish (**A**) and blue rockfish (**B**). Escape time is measure in seconds from the end of the acclimation period to when the head of the fish exits the chamber. Letters above the bars indicate significantly different means following Tukey HSD post-hoc comparisons.

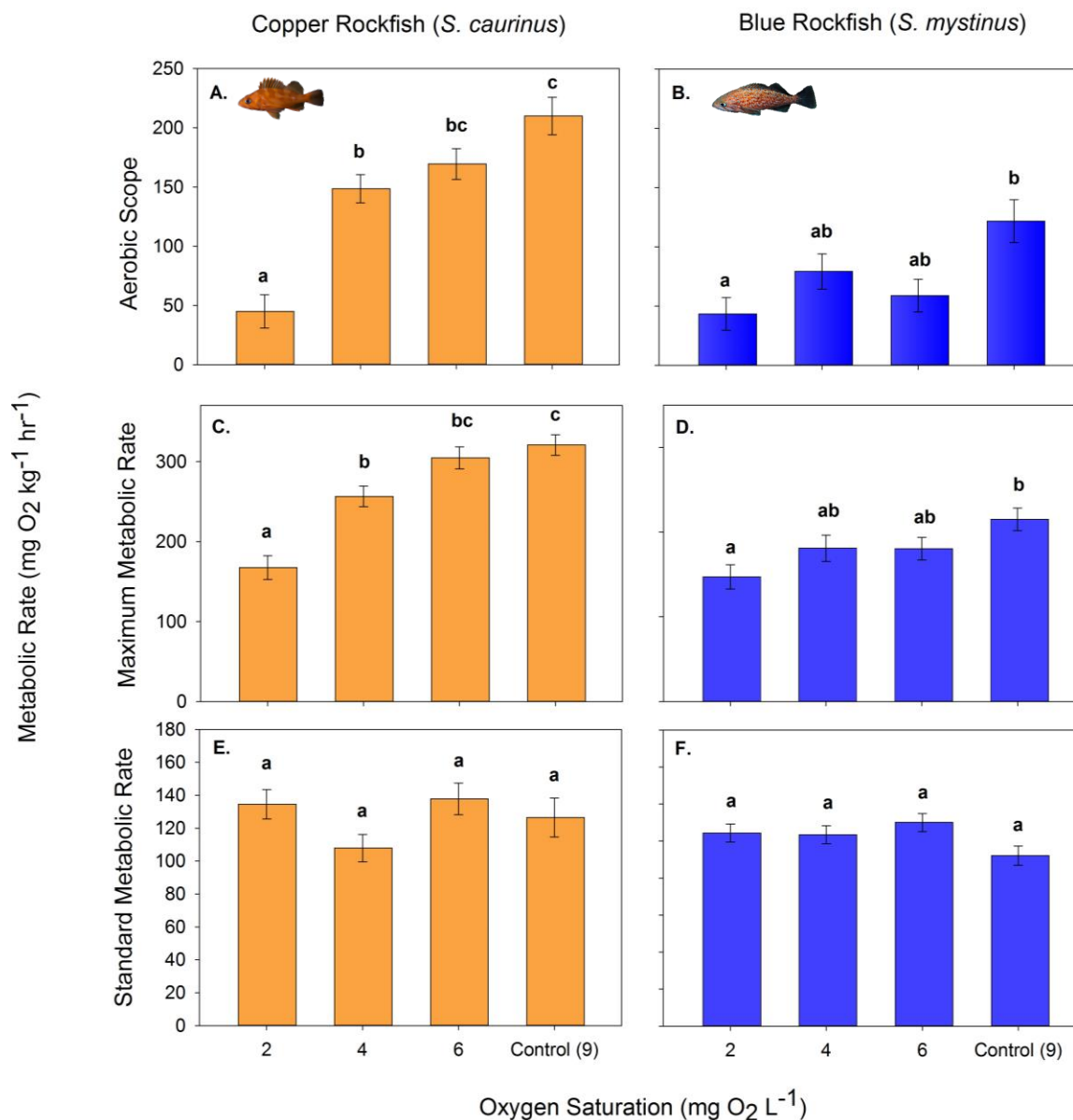


**Figure 6.** Change in absolute lateralization of copper rockfish (**A**) and blue rockfish (**B**), and relative lateralization of copper rockfish (**C**) and blue rockfish (**D**). Absolute lateralization is the turning bias irrespective of direction (a value of 0 indicates equal left to right turning bias, a value of 100 indicates a fish turned one direction exclusively). Relative lateralization is the turning bias specified by either left or right turning preference (a value of 0 indicates a 50:50 turning preference, positive values indicate a right turning bias, and negative values indicate a left turning bias). Bars represent the mean value ( $\pm 1$  SE). Letters above the bars indicate significantly different means following Tukey HSD post-hoc comparisons.

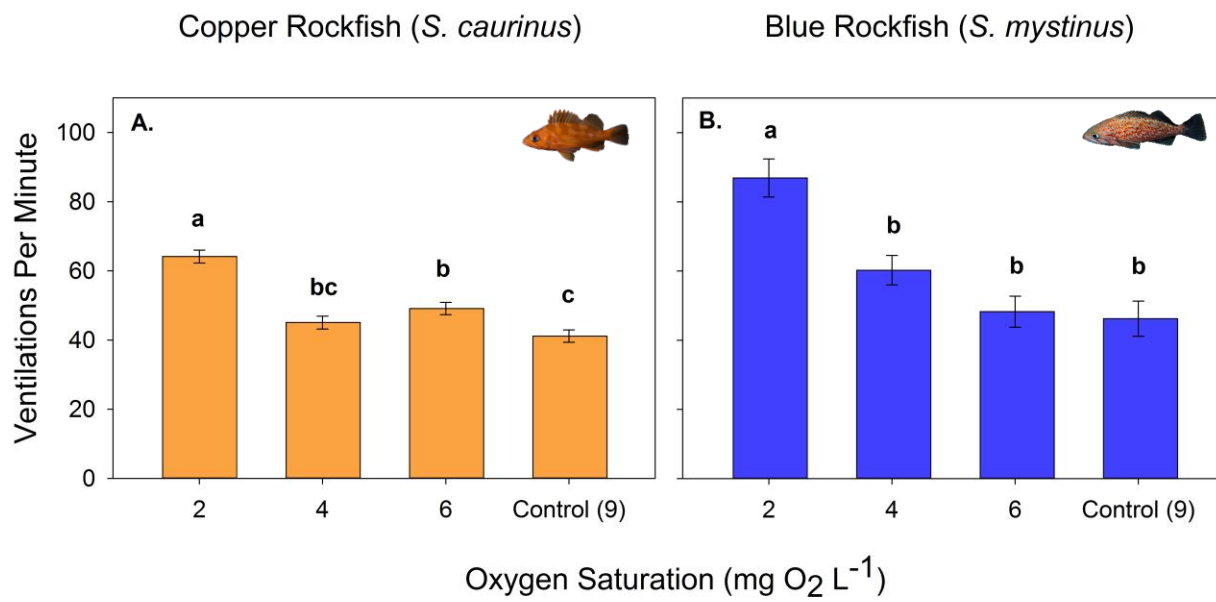


**Figure 7.** Average hypoxia tolerance/ critical oxygen tension (pCrit) for copper rockfish and blue rockfish across all treatment conditions. Bars represent the mean value ( $\pm 1$  SE). Letters above the bars indicate significantly different means following Tukey HSD post-hoc comparisons.

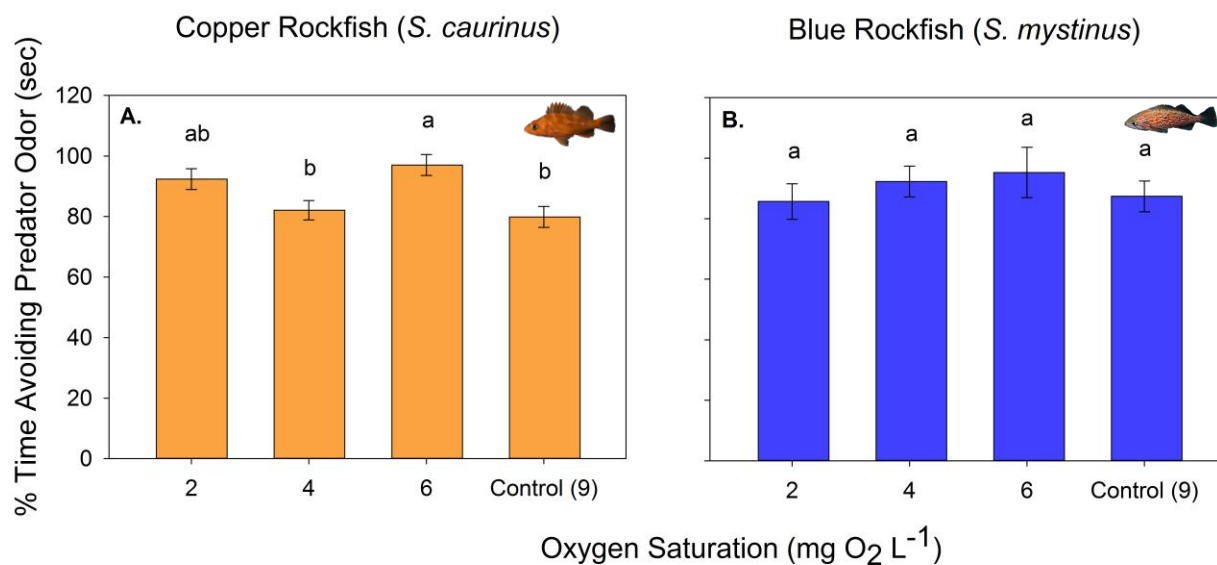




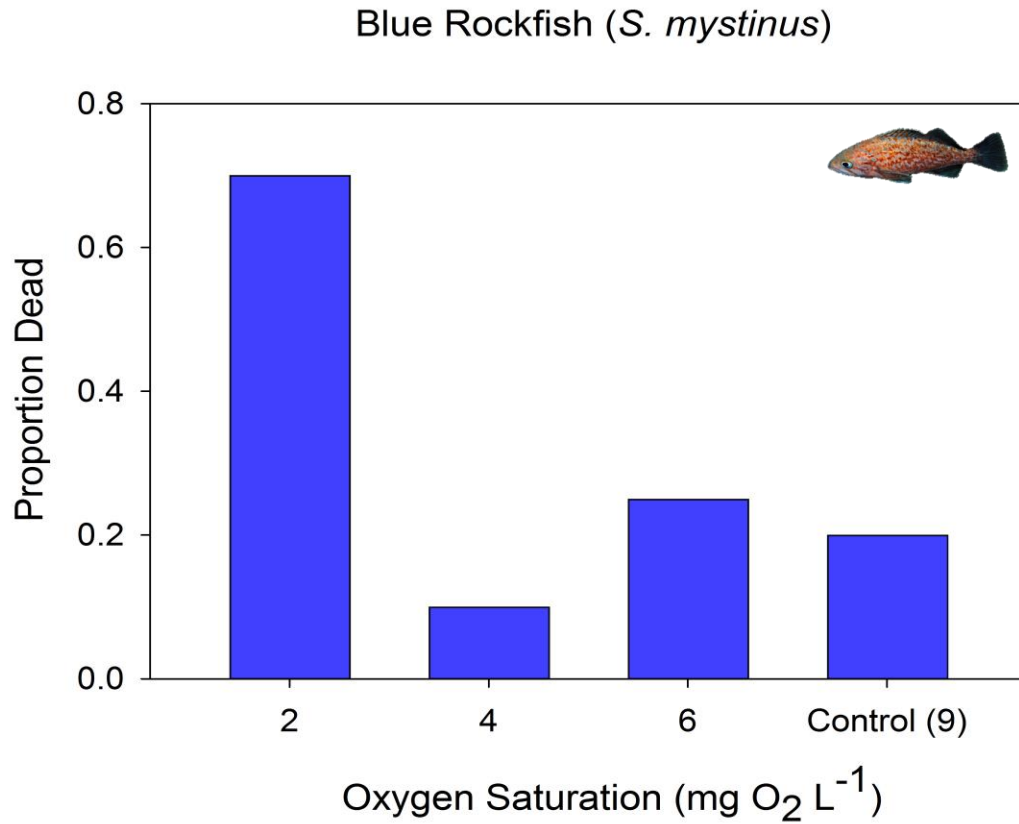
**Figure 8.** Changes in metabolic rate and aerobic capacity as a function of oxygen exposure history. Average aerobic scope (i.e., difference between MMR and SMR) of copper rockfish (A) and blue rockfish (B). Maximum metabolic rate (MMR) of copper rockfish (C) and blue rockfish (D). Standard metabolic rates (SMR) for copper rockfish (E) and blue rockfish (F). Bars represent the mean value ( $\pm 1$  SE). Letters above the bars indicate significantly different means following Tukey HSD post-hoc comparisons.



**Figure 9.** Ventilation rate as a function of oxygen exposure history measured in ventilations per minute for copper rockfish (A) and blue rockfish (B). A single ventilation is defined as the opening and closing of the gill operculum. Bars represent the mean value ( $\pm 1$  SE). Letters above the bars indicate significantly different means following Tukey HSD post-hoc comparisons.



**Figure 10.** Differences in olfactory preference as a function of changing oxygen exposure history using a two-channel water source swim flume. The bars represent the percent time copper rockfish (**A**) and blue rockfish (**B**) spent in the water source void of predator odor. Bars represent the mean value ( $\pm 1$  SE). Letters above the bars indicate significantly different means following Tukey HSD post-hoc comparisons.



**Figure 11.** Differences in mortality rates of blue rockfish as a function of oxygen exposure history. There were no mortalities for copper rockfish treatments.