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Assessing the Effects of Upwelling-Driven pH and Dissolved Oxygen Variability on Juvenile Rockfishes

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ASSESSING THE EFFECTS OF UPWELLING-DRIVEN PH AND DISSOLVED OXYGEN VARIABILTY ON JUVENILE ROCKFISHES

A Thesis

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by

Melissa Palmisciano

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CALIFORNIA STATE UNIVERSITY MONTEREY BAY

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ASSESSING THE EFFECTS OF UPWELLING-DRIVEN PH AND DISSOLVED OXYGEN VARIABILITY ON JUVENILE ROCKFISHES

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Assessing the Effects of Upwelling-Driven pH and Dissolved Oxygen
Variability on Juvenile Rockfishes
by
Melissa Palmisciano
Masters of Science in Marine Science
California State University Monterey Bay, 2023

Abstract

Global climate change is expected to increase the frequency and severity of upwelling events in the California Current Ecosystem, yielding concurrent reductions in pH and dissolved oxygen (DO) in coastal marine environments. Juvenile copper (*Sebastes caurinus*) and gopher (*Sebastes carnatus*) rockfish may be particularly vulnerable to low pH and DO because they settle nearshore during the upwelling season. Previous studies often use static (i.e., fixed) pH and DO treatments in the laboratory, but ocean chemistry is dynamic and these conditions fluctuate naturally in upwelling zones. To determine how fluctuations in ocean chemistry will impact rockfish behavioral and physiological performance at this critical life history stage, I exposed juveniles of both species to one of the following pH/DO treatments: static control (8.0 pH, 8.3 mg/L DO), static moderate (7.5 pH, 4.0 mg/L DO), static extreme (7.3 pH, 2.0 mg/L DO), and two fluctuating treatments, upwelling and relaxation (recurring cycles of 8 days of 7.3 pH, 2.0 mg/L DO and 8 days of recovery at control conditions). Responses to sublethal stress were evaluated after 1-13 weeks of exposure (or up to six 16-day fluctuating cycles) through behavioral and physiological metrics including lateralization tests, escape time trials, startle response, critical swimming speed (U_{crit}), metabolic performance (standard and maximum metabolic rates, capacity for aerobic activity), hypoxia tolerance (critical oxygen tension [P_{crit}]), growth rates, body condition, and mortality. I did not observe any significant effects of upwelling conditions on the behavioral metrics of lateralization, escape time, or startle response. Rockfish exhibited impairment in aerobic scope (due to a reduced maximum metabolic rate), swimming speed, and increased ventilation rates and hypoxia tolerance, under simulated upwelling conditions, performing equally to fish in the extreme treatment. In the fluctuating upwelling treatment, both species appeared to recover fully for most physiological metrics when returned to control seawater for 6-7 days (i.e., a simulated oceanographic relaxation event). Both species exhibited the lowest growth rate in the extreme low pH/DO treatment and intermediate growth in the two fluctuating treatments, suggesting lasting cumulative negative impacts of exposure to future upwelling conditions. Mortality was highest in the extreme low treatment and body condition tended to decrease as treatments became more extreme. While juvenile rockfish are susceptible to physiological impairment under extreme climate change scenarios, the severity and duration of future hypoxic, acidic events will ultimately set the consequences for survivorship and physiological fitness, influencing the outcome of the population replenishment process and the long-term sustainability of economically and ecologically important nearshore rockfish species.

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I. Introduction

Since pre-industrial times, anthropogenic emissions of fossil fuels have raised atmospheric CO_2 from 280 parts per million (ppm) to over 400 ppm, triggering profound changes in the world's climate and oceans (IPCC 2014). The ability of marine organisms to withstand rapidly changing ocean conditions from climate change has important implications for fisheries sustainability and biodiversity. One major challenge in predicting biological responses to climate change is that rising atmospheric CO_2 is simultaneously altering multiple environmental variables that affect organismal physiology, including temperature, acidity, oxygenation, salinity, currents, and nutrient availability (Gunderson et al. 2016). These factors may act in concert (additive effect), in opposition (antagonistic effect), or may enhance one another (synergistic effect) to affect the behavior, physiology, and survival of marine organisms (Crain et al. 2008). Marine organisms exposed to multiple environmental stressors may face energetic trade-offs between the stress response and maintenance activities and behaviors (e.g., growth and metabolism, predator avoidance, foraging activity), which may ultimately reduce their fitness (Davis et al. 2018).

Increasingly, scientists are recognizing the importance of simulating future oceanic conditions in the laboratory through multiple stressor experiments (DePasquale et al. 2015; Davis et al. 2018; Donham et al. 2022). Most multi-stressor studies to date have examined organismal responses to stable/static stressor parameters representing mean environmental conditions, even for those organisms living in variable coastal environments (Gunderson et al. 2016). However, organismal performance under mean environmental conditions is generally different from mean performance under variable conditions due to the nonlinearity of performance curves, a mathematical principle that is also known as Jensen's inequality (Jensen 1906; Denny 2017; Kroeker et al. 2019). For example, metabolic rate is an accelerating, concave, nonlinear function of temperature, so predicting metabolic rate under variable temperatures using mean temperature could underestimate actual metabolic rate (Denny 2017). Conversely, for convex performance curves, performance under variable conditions might be lower than performance under constant mean conditions due to the shape of the curve.

Coastal habitats undergo significant changes over timescales ranging from hours to seasons (Hofmann et al. 2011; Booth et al. 2012; Frieder et al. 2012; Duarte et al. 2013; Waldbusser & Salisbury 2013) and these spikes or temporary reprieves in physical forcing factors may have disproportionate effects on organismal fitness (Gruber et al. 2012; Gunderson et al. 2016; Small et al. 2016; Mangan et al. 2017). Variability in pH and dissolved oxygen may provide a temporal refuge from stress compared to constant exposure. For example, diel pH fluctuations have been shown to minimize the adverse effects of pH on brain lateralization, predator cue response, and growth in coral reef fishes (Jarrold et al. 2017; Jarrold & Munday 2019). Alternatively, repeatedly acclimating to changing conditions might have a higher energetic cost, increasing overall stress and reducing performance. For example, variable pH increased metabolic rates in the mussel *Mytilus edulis* compared to a static treatment (Mangan et al. 2017). Finally, fluctuations might have no effect on organismal responses to changing ocean chemistry. For example, pH fluctuations did not alter social or individual behavioral responses of juvenile blacksmith (*Chromis punctipinnis*) compared to fish raised in static treatments (Kwan et al. 2017).

Habitats found in Eastern Boundary Current Upwelling Systems, such as the California Current Ecosystem, are highly dynamic and shaped by natural environmental variability. In the California Current, the wind-driven process of upwelling periodically transports CO_2-rich, hypoxic water from depth onto the coastal shelf during the spring. Eastern Boundary Currents have lower pH than other coastal regions due the shoreward advection of deep source waters, which are enriched with CO_2 by the remineralization of organic matter (Gruber et al. 2012). By increasing the land-sea pressure gradient, atmospheric warming over land from climate change is expected to increase the northerly winds that cause upwelling, leading to an increase in the frequency and severity of upwelling events in central and northern CA (Bakun 1990; Wang et al. 2015). Increasingly frequent and more severe upwelling may periodically expose nearshore marine organisms in these regions, such as fishes, to even more extreme acidified, hypoxic conditions (Booth et al. 2012). Intrusion of acidified water with pH below 7.5 and dissolved oxygen (DO) below 3.0 mg/L has been recorded in Stillwater Cove, Carmel (Figure 1) and other areas in the Monterey Bay (Booth et al. 2012). Upwelling in Central California occurs most frequently during the spring and early summer, corresponding with critical settlement stages for ecologically and economically important species, such as nearshore-recruiting rockfishes. Therefore, an emerging concern is that these juveniles, at a vulnerable early life stage, may be particularly impacted by climate change following exposure to upwelling-induced ocean acidification and hypoxia (Cline et al. 2020; Hamilton et al. 2017; Mattiasen et al. 2020).

In addition to the effects of increased advection of hypoxic, acidified water to coastal regions, climate change is causing global changes in baseline pH and oxygenation which are likely to exacerbate the effects on nearshore marine organisms. The world's oceans have absorbed one third of anthropogenic carbon emissions and oceanic pH has fallen from 8.2 to 8.1 since the Industrial Revolution, with up to a 0.3-0.4 drop predicted by 2100 and potentially a 0.7-0.8 unit drop by 2300 (Caldeira & Wickett 2003; Orr et al. 2005; Sabine & Feely 2007; Feely et al. 2008). In Central California, surface waters within 10 km of the coast are expected to fall to a mean pH of 7.82 ± 0.05 by 2050 (Gruber et al. 2012). Additionally, rising atmospheric CO_2 is expected to amplify the amplitude of natural monthly oscillations in oceanic CO_2 concentrations by 2100 (McNeil & Sasse 2016).

Concurrently, global oceanic dissolved oxygen is predicted to decline by 1-7% by 2100 (Keeling et al. 2010). Regions of hypoxia and anoxia – or extreme low DO concentrations, below which many organisms cannot survive – develop naturally in the ocean. A review across multiple fish studies found that the average LC_{50}, or lethal hypoxia concentration for 50% of the individuals, is approximately 2.0 mg/L, but this value can vary widely by species (Vaquer-Sunyer & Duarte 2008). However, climate change is causing large-scale reductions of DO, through a process termed ocean deoxygenation, as well as the expansion of naturally occurring persistent hypoxic strata, known as oxygen minimum zones (OMZs; Keeling et al. 2010; Breitburg et al. 2018). Warmer water is less dense and has a decreased capacity for oxygen storage, increasing stratification and reducing downward transport of oxygen to the ocean interior (Keeling et al. 2010; Helm et al. 2011). The effects of diminishing oxygen on marine ecosystems can range from changes in species composition and reduced biodiversity (Rabalais et al. 2002), compression of habitat both latitudinally (Deutsch et al. 2015) and by depth (Prince & Phillip Goodyear 2007), and mortality and total community decimation in the case of anoxia, even for relatively mobile fishes, including rockfish (Grantham et al. 2004). For example, persistent bouts of extreme hypoxia lasting for multiple months have been observed along the Oregon coast, leading to mortality of benthic invertebrates and an absence of fish from rocky reefs at 50 meters depth during submersible surveys (Chan et al. 2008). Oxygen saturation has declined by 20% in the Southern Californian OMZ since 1984, and its boundaries have shoaled by up to 90 meters (Bograd et al. 2008), causing a loss of aerobic habitat in the California current

(Howard et al. 2020) and compressing depth ranges of multiple rockfish species (Meyer-Gutbrod et al. 2021).

Because of their ability to regulate internal pH through Na^+/H^+ and Cl^-/HCO_3^- ion channels across the gill epithelium, teleost fish were historically presumed to tolerate high oceanic pCO$_2$ (Heuer & Grosell 2014). However, recent studies have revealed significant species-specific behavioral and physiological consequences of low pH exposure in fishes, possibly because of compensatory responses to regulate pH (Munday et al. 2009; Hamilton et al. 2017; Heuer & Grosell 2014). Behavioral changes include differences in anxiety (Hamilton et al. 2014; Ou et al. 2015), learning impairment (Chivers et al. 2014), altered predator-avoidance behavior and olfactory senses (Munday et al. 2010), and changes in brain lateralization (Domenici et al. 2012; Jutfelt et al. 2013), although a recent reproducibility study has questioned the validity of some of these findings (Clark et al. 2020). Physiological deficiencies such as reduced aerobic fitness and swimming performance (Munday et al. 2009; Hamilton et al. 2017), decreased growth and survival (Baumann et al. 2012; DePasquale et al. 2015) and changes in reproductive output (Heuer & Grosell 2014; Nagelkerken et al. 2021) have also been observed. Functional impairment at the organismal scale may result from the high energetic cost of compensatory acid-base regulation and osmoregulation, which require the use of high-energy ion pumps, and changes in oxygen binding and transport (Wittmann & Pörtner 2013; Heuer & Grosell 2014; Lefevre 2016). Behavioral changes are thought to arise from a reversal of the ion gradient at GABA$_A$, an inhibitory neurotransmitter receptor in the brain (Nilsson et al. 2012; Hamilton et al. 2014).

The effects of inadequate oxygenation on teleost fishes are significant and have been extensively studied. Fish respond behaviorally to sublethal hypoxia with compensatory adjustments such as increases in activity level (Petersen & Petersen 1990; Dalla Via et al. 1998) to avoid hypoxic areas (Domenici et al. 2000) or reduced swimming to lower oxygen needs (Metcalfe & Butler 1984). Fish can also compensate for hypoxic exposure by decreasing food intake to reduce energy demands (van Dam & Pauly 1995). Physiologically, fish may increase oxygen uptake through increased ventilation rate (Lomholt & Johansen 1979), adjusting heart rate and stroke volume to improve gas exchange (Randall 1982; Domenici et al. 2007), and by remodeling gill lamellae to increase respiratory surface area (Sollid 2003; Mitrovic et al. 2009). Low oxygen availability reduces the energy available for maintenance activities, requiring trade-offs in energy allocation and ultimately costs in fitness and performance. For example, hypoxia can suppress reproductive capacity (Landry et al. 2007) and result in changes in antipredator behaviors and locomotor performance in fishes (Domenici et al. 2007). Hypoxia may be particularly detrimental to fishes in early life stages, where low DO exposure can cause depression in growth (Pichavant et al. 2000) and increased embryonic deformity (Shang & Wu 2004). Because oxygen is an essential metabolic component required for aerobic ATP production, exposure to extreme hypoxia suppresses metabolic rate in fishes (Richards 2009). Maintenance activities like ventilation or osmoregulation cannot be sustained under extreme hypoxic conditions (Domenici et al. 2007), and it is eventually lethal due to cellular necrosis (Richards 2009).

Both ocean acidification (Hamilton et al. 2017) and hypoxia (Mattiasen et al. 2020) have been shown to negatively impact juvenile rockfishes (genus *Sebastes*). Rockfishes are a commercially and economically important group of groundfish occurring primarily in the North Pacific that were historically overfished (Parker et al. 2000). Rockfishes serve important roles throughout their life cycles as both predators of fishes and crustaceans (Hallacher & Roberts 1985; Murie 1995) and as prey to birds (Elliott et al. 2015), large fish, and mammals (Mills et al. 2007). However, life-history characteristics such as decades-long lifespans and long generation times both make them vulnerable to fishing pressure (Parker et al. 2000) and may limit their ability to adapt to rapid changes (on the order of decades) in ocean conditions caused by climate change. Decreases in survivorship that limit replenishment at the vulnerable juvenile stage could create a population bottleneck (Doherty et al. 2004), with consequences for overall population health.

Because they recruit to the coastal shelf during the spring upwelling season, congeneric juvenile rockfish species such as copper (*Sebastes caurinus*) and gopher (*Sebastes carnatus*) rockfish may be particularly impacted by low pH/low DO upwelling events at the vulnerable juvenile stage (Hamilton et al. 2017; Mattiasen et al. 2020). While species occupying the nearshore zone may already be adapted to fluctuations in pH and dissolved oxygen, large-scale shifts in ocean chemistry in response to climate change may push organisms beyond their physiological limits. In general, early life stages of fishes are more sensitive to stress from changes in ocean chemistry due to the increased energetic demands of rapid growth and development (Wu 2009). Smaller size classes of fishes already face greater susceptibility to predation due to ease of capture and the greater abundance of gape-limited predators that can consume them (Sogard 1997). To avoid predation, many juvenile fish species confine themselves to sheltered microhabitats (Carr 1983), potentially inhibiting their ability to relocate from areas with deleterious ocean chemistry conditions.

Tolerance to acidification and hypoxia can differ significantly between closely related species of fishes with different life history characteristics (Hamilton et al. 2017; Mattiasen et al. 2020). For example, juvenile blue rockfish (*Sebastes mystinus*) demonstrate greater acclimatization potential to low pH (Hamilton et al. 2017) and hypoxia (Mattiasen et al. 2020) than copper rockfish, which may be due to differences in their exposure to low pH/low DO waters in larval and juvenile microhabitats. Blue rockfish develop in deep offshore waters and settle to the benthos (Lenarz et al. 1991; Love et al. 2002), where pH and DO are lower due to benthic respiration and the intrusion of upwelling plumes. In contrast, copper rockfish develop more in surface waters and initially recruit to the kelp canopy (Carr 1991), where photosynthesis and surface mixing buffers $p\text{CO}_2$ and $p\text{O}_2$ (Frieder et al. 2012), before descending to the benthos after a couple of months (Carr 1991; Love et al. 2002). It is currently unknown if more closely related species with similar life histories (e.g., copper and gopher rockfish, *Sebastes caurinus* and *Sebastes carnatus*) will exhibit similar tolerances to low pH and hypoxia. Responses of fishes to combined OA and hypoxia have been highly variable across species in previous studies (DePasquale et al. 2015) and could be due to differences in tolerance across life stages or differences in parental or individual exposure history (Montgomery et al. 2019).

Previous studies demonstrated behavioral and physiological impairment and changes in gene expression in juvenile rockfish after chronic, long-term exposure to both low pH (Hamilton et al. 2017) and low DO (Mattiasen et al. 2020). However, few studies have examined the interactive effects of hypoxia and acidified water (Cline et al. 2020; Gunderson et al. 2016), which are likely to co-occur in the natural environment and could act synergistically to further debilitate juvenile rockfish fitness. Furthermore, few studies have examined the physiological effects of short-term exposure to combined acidification and hypoxia that may become increasingly common during upwelling events, nor fishes' ability to recover once conditions have normalized. To determine how future projected upwelling conditions will impact rockfish fitness, I exposed juvenile copper and gopher rockfish to a range of combined low pH and low DO treatments, both in static conditions and fluctuating conditions simulating a 16-day cycle of upwelling-relaxation that is typical for the California Current region. I then compared behavioral and physiological performance in response to pH/DO exposure history, including: behavioral tests of boldness (escape challenge), brain lateralization, and startle response; and physiological tests of critical swimming speed (U_{crit}), capacity for aerobic activity (aerobic scope), hypoxia tolerance (P_{crit}), and ventilation rate, as well as growth, condition, and mortality. This study will help elucidate the species-specific physiological resilience of rockfishes to ocean acidification and hypoxia through simulated upwelling experiments at a critical early life history stage.

II. Materials and Methods

A. Fish Collections and Tagging

Copper and gopher rockfish recruitment in Central California occurs primarily from March-July, coinciding with the local upwelling season (García-Reyes & Largier 2010). Recently settled young-of-the-year (YOY) copper and gopher rockfish were collected on SCUBA using hand nets from June to July of 2017 (Table 1). Juveniles were obtained from the kelp canopy (~1 m depth) or shallow rocky reef (10-18 m depth) at Stillwater Cove in Carmel Bay, CA (36 ˚34’N, 121˚56’ W). Fish were transported to the NOAA NMFS Southwest Fisheries Science Center aquarium facility in Santa Cruz, CA, and acclimated at 13-14°C and ambient pH and DO (~8.0 pH, ~8.3 mg/L DO) in flow-through seawater tanks for a minimum of 24 hours.

Juveniles were then anesthetized and tagged subdermally with elastomer dye implants (Northwest Marine Technologies, Inc.) that fluoresce under UV-A light. Unique tag color combinations and placements (below the first or second dorsal on the left or right side) distinguished each animal, enabling tracking of individuals across all experimental trials. Following a five-day recovery period, juveniles were removed from tanks, blotted dry and weighed (to the nearest 0.1 mg), measured for standard and total length (to the nearest 1 mm), and sorted into 115 L flow-through treatment tanks, with two replicate tanks per treatment level. Approximately 20 individuals were reared in each treatment tank. Fish were too young to identify to the species level at the beginning of the experiment. Final species designation was performed at the end of the experiment (after 13 weeks), at which point species could be differentiated by examining the coloration surrounding the lateral line (copper rockfish have a white clearing, while gopher rockfish have colored blotches).

B. Rearing Conditions

Source water entered a 2000 L reservoir, which was maintained at 100% pO_2 saturation (~9.0 mg O_2 L^{-1}) by bubbling air through a gas diffuser. Water was pumped from the main reservoir into five 500 L header tanks, where pH and dissolved oxygen were manipulated for each treatment by bubbling in CO_2 and N_2 gas, respectively. Gas flow was regulated by solenoid valves, diffused through Pentair Point Four Micro Bubble Diffusers, and mixed throughout the water column with recirculation pumps. WTW pH 3310 Instruments with SenTix HWD pH probes were used to monitor pH in each header tank and gas flow was controlled by Loligo Systems CapCTRL software. DO was monitored in header tanks using Loligo Systems optical dipping probe oxygen mini sensors, with N_2 gas flow controlled by WitroxCTRL software.

Each 500 L reservoir fed two replicate 115 L treatment tanks, set at constant flow rates of ~30 ml/sec, where juveniles were held. Chiller units connected to the main 2000 L reservoir tank maintained a constant temperature of 14°C. Temperature, pH and DO were checked daily in all tanks with a Hach HQ40d portable multi meter, and setpoints were adjusted if necessary to achieve target levels. Water samples taken every 2 weeks from the treatment tanks were analyzed for total alkalinity (using a Tiamo alkalinity titrator) and pH (using pH spectrophotometer) to enable calculations of the carbonate chemistry parameters in the system using the Excel macro CO2SYS (Lewis & Wallace 1998). Fish were fed every other day with fish pellets and tanks were cleaned after each feeding.

Fish were introduced directly to treatment water in August of 2017 without any ramping down of pH or DO (Table 1). Juveniles were reared in one of five treatments, including three static treatments in which pH and DO were held constant for the duration of the experiment (13 weeks), and two fluctuating treatments that simulated alternating upwelling-relaxation cycles. Static treatments included (1) control pH/DO at 8.0 pH, 8.3 mg/L DO, (2) moderate low pH/DO at 7.5 pH, 4.0 mg/L DO, and (3) extreme low pH/DO at 7.3 pH, 2.0 mg/L DO (Figure 2, Table 2). The two fluctuating treatments ("upwelling" and "relaxation") followed a recurring cycle of 8 days of 7.3 pH, 2.0 mg/L DO, followed by 8 recovery days at control conditions. The two fluctuating treatments had similar cycles of pH/DO, but fish from the upwelling tanks were tested only during days 6-7 of the upwelling phase (7.3 pH, 2.0 mg/L DO) and fish from the relaxation tanks were tested only during days 6-7 of the relaxation phase (8.0 pH, 8.3 mg/L DO). This design assured the independence of the treatments. The mean of the fluctuating treatments was ~7.7 pH and ~5.3 mg/L DO, falling between the control and moderate treatments. The upwelling and relaxation treatment cycles were staggered by two days to allow enough time for trials to be completed. Trials for the static treatments (control, moderate, and extreme) were held in the 2-week period surrounding the fluctuating trials (Table 1). On one occasion during P_{crit} trials, the relaxation/upwelling period was extended to 10 days and trials were spread through days 7-10 due to equipment failure. The moderate treatment reflected the most severe upwelling conditions recorded during 2017 by a SeapHOx instrument deployed at Stillwater Cove (Figure 1). The extreme treatment represents upwelling under projections for additional reductions to pH by 2100 (IPCC 2014) and falls within measured parameters for DO in more severe upwelling regions in the northern California Current System (Chan et al. 2008).

Individual responses to sublethal stress were evaluated through a series of behavioral and physiological trials. The “main experiment” took place in 2017 and involved eight metrics, including the escape challenge, ventilation rate, critical swimming speed, aerobic scope (standard and maximum metabolic rate), hypoxia tolerance, and growth, body condition, and mortality. Efforts were made to measure all eight metrics on the same set of four individual fish per tank at minimum (eight per treatment), but some samples were ultimately excluded from the analyses for some trials due to factors like equipment failure, poor data quality, or mortality before fish could be identified to species level (Table 4). Extra fish were occasionally run through trials as backups in case of mortality. Two short-term experiments were conducted to complement the main experiment, including behavioral lateralization in November 2017 and a startle response test in July of 2018 (see below). Fish for these experiments were reared in separate tanks from those in the main experiment.

Food was withheld for 36 hours before experiments and juveniles were given a minimum 5-day rest period between experiments. All experimental trials were performed in pH and DO conditions matching the assigned treatment conditions (within ± 0.05 units for pH and ± 0.2 mg/L for DO) and in temperatures within ± 0.5°C of treatment tanks. CO_2 and N_2 gas were bubbled into experimental holding tanks and water was mixed and chilled with ice packs or recirculating coil chillers prior to introducing fish. pH and DO levels were confirmed with the

Hach meter immediately before fish were collected from treatment tanks and placed in experimental vessels.

C. Behavioral Trials

Escape Challenge

Juveniles underwent escape challenge trials to evaluate boldness, exploratory behavior, and learning ability (adapted from Jutfelt et al. 2013). Sample sizes ranged from 3-27 fish per treatment, with higher replication for copper rockfish than for gopher rockfish (see Table 4). Although they were introduced to treatment water at the same time as the rest of the fish, a subset of gopher rockfish were not tagged until week six, so they were excluded from this experiment. Fish were given a minimum of a five-day rest period after tagging, and then underwent escape trials at control pH and DO conditions (time point 1, 0 weeks exposure) to establish baseline behavioral patterns for each individual. Escape trials were repeated in treatment water after juveniles had acclimated to their pH/DO treatment conditions for one to two weeks for the static treatments (time point 2), or after one full 16-day cycle for the fluctuating treatments, to identify behavioral disturbances or potential learning responses.

Fish were individually placed inside white PVC cylinders with 5 cm diameter exit holes cut 10 cm above the bottom (Figure 3). A black acrylic divider was inserted into a slit located 8 cm from the top of the cylinder to isolate the fish in the upper half of the chamber and conceal the exit hole below. PVC cylinders were held inside 40 L insulated opaque aquaria held in a water table to maintain constant temperature. Trials were simultaneously performed in eight replicate escape chambers and aquaria. Fish were placed into the upper chamber above the divider. After a 15-minute acclimation, investigators removed the divider while remaining out of view of the fish. The time required for the fish's head and tail to exit through the hole was recorded. Trials were terminated after 30 minutes if the fish did not exit the hole. Only fish that successfully exited from the hole in both time points were included in the analysis.

Behavioral Lateralization

Behavioral lateralization, an expression of brain functional asymmetry, measures an individual's bias for turning left or right. A higher degree of lateralization conveys advantages in cognitive function and predator avoidance in individual fish, improving performance in schooling cohesion (Bisazza & Dadda 2005), escape response (Dadda et al. 2010), spatial orientation (Sovrano et al. 2005), and multitasking (Dadda & Bisazza 2006). However, the degree of lateralization in fishes likely reflects a tradeoff between cognitive advantages in highly lateralized individuals and the ability of poorly lateralized fish to cope with threats from all directions equally (Vallortigara & Rogers 2005). Exposure to elevated $p\mathrm{CO_2}$ has been shown to alter lateralization in both temperate (Jutfelt et al. 2013; Domenici et al. 2014) and tropical marine fishes (Domenici et al. 2012; Nilsson et al. 2012).

Twenty gopher rockfish were held at control conditions until week 10, when they were tagged and divided into two tanks (control and extreme). These fish were assessed for changes in behavioral lateralization through detour tests in a double T-maze (Bisazza et al. 1998), but did not undergo any other behavioral or physiological trials. Due to low availability, copper rockfish were not run through lateralization trials. Fish first underwent lateralization experiments in control water (time point 1; week 11). Lateralization tests were then repeated after a 7-day exposure to the control or a constant extreme treatment (time point 2; week 12). Individual fish were introduced to a 50 × 30 × 25 cm (L × W × H) opaque aquarium containing the two-way T-maze and allowed to acclimate in treatment water for 3 minutes. Fish were gently coaxed down the center channel with a net moving at a constant rate and forced to make a left or right turn decision. Twelve consecutive tests were conducted for each fish, alternating the direction of travel each time to account for possible asymmetry in the experimental set-up. Turn preference was assessed at a population level and compared among treatments using a lateralization index. The absolute lateralization index (L_A) identifies if a directional bias existed at the population level irrespective of direction. Relative lateralization index (L_R) characterizes turn preference for a specific direction, where positive values indicate a right turning bias and negative values indicate a left turning bias.

Absolute lateralization (L_A) = |#right turns - # left turns| / (# right turns + # left turns) x 100
Relative lateralization (L_R) = (#right turns - # left turns) / (# right turns + # left turns) x 100

Startle Response

Fast-start responses, or sudden accelerations to avoid predators, may determine the outcomes of predator-prey interactions and are therefore important for survival (Domenici & Blake 1997). The fast-start response is characterized by a unilateral muscle contraction that bends the body into a C-shape, a return tail flip due to a contra-lateral contraction, and either steady swimming or coasting (Weihs 1973). This startle response is thought to be mediated by Mauthner cells, a pair of neurons in the brain stem that respond to visual and auditory stimulus by creating an action potential and initiating the muscle contractions that create a fast-start (Eaton et al. 2001). The effectiveness of the fast-start response has been shown to be an important predictor of survival for juvenile damselfish (McCormick et al. 2018). Both hypoxia (Lefrançois et al. 2005) and elevated CO_2 (Allan et al. 2014) have been found to negatively affect reactivity to stimuli in fishes.

In June of 2018, YOY copper and gopher rockfish were collected from the kelp forest in Stillwater Cove and acclimated to ambient seawater conditions at the NOAA lab in Santa Cruz for two weeks. Twenty fish were then placed in one of two replicate tanks of three treatment tanks for a period of 6-8 days: control (8.0 pH, 8.3 mg/L DO), moderate (7.5 pH, 4.0 mg/L DO) and extreme (7.3 pH, 2.0 mg/L DO). Fish were too young to differentiate between copper and gopher rockfish species. To compare the effectiveness of predator-avoidance startle response behavior among treatments, fish were placed in a shallow 91.4 cm diameter plexiglass-bottom tank with a 35.6 cm opaque testing arena inside and mirror angled below at 45 degrees (Figure 3). Fish were left to acclimate for 15 minutes prior to the start of the trial. Individuals were then startled from above using a 100-millisecond blast of air from an Arduino-controlled solenoid valve that was deployed next to the fish. To standardize for fish position in relation to the stimulus and prevent the sides of the tank from interfering with movements, fish were only startled when they moved to the center of the opaque divider. A high-speed camera on a tripod was used to record the fish's responses in the mirror at 480 frames per second.

The effectiveness of the Mauthner-initiated startle response (Figure 4) was evaluated in Image J using a manual tracking plug-in to identify and track the individual's center of mass. Response latency was measured as the time between the onset of the stimulus (first visual evidence of a depression in the water surface from the air blast) and the first detectable escape movement of the individual (McCormick et al. 2018). Distance traveled (cm) and duration (s) of the first two axial body bends was recorded and used to calculate fast-start speed (cm/s).

D. Physiological Trials

Ventilation Rate

When exposed to hypoxic conditions, fish compensate by increasing the frequency and volume of their ventilations to raise blood pO_2, also known as the hypoxic ventilatory response (Perry et al. 2009). Oxygen sensing chemoreceptors in the gills detect changes in blood or water pO_2 and trigger a hyperventilatory response (Perry et al. 2009). Ventilation rates were evaluated during weeks three to five, or after two fluctuating cycles. To examine how fish ventilation rates differed among treatments, 9-27 juveniles per treatment and species were placed in a set of ten 5 × 10 × 4 cm chambers within a 25 × 20 × 4 cm (L x W x H) flow-through opaque acrylic tank. Each chamber was fed by a 5 ml/s flow of seawater at treatment pH and DO levels at 13-14°C. A single fish was placed in each chamber atop a 15 × 5 cm piece of waterproof paper that was curved into a U shape to hold the fish in position. Two overlapping GoPro Hero 4 cameras were mounted above the chamber to enable remote recording. The tank was shrouded from view and illuminated from above (Figure 3). Fish were left to acclimate and return to basal ventilation rates for two hours after initial placement. Following the acclimation period, the cameras were remotely activated and juveniles were filmed for 30 minutes. Video files were analyzed to calculate ventilation rates by averaging the number of open/close cycles of the gill operculum (beats per minute, or BPM) from 3 randomly selected 1-minute intervals for each individual.

Critical Swimming Speed (U_{crit})

Critical swimming speed (U_{crit}), a metric for swimming ability and fitness in fishes, is similar to an exercise stress test (Brett 1964) and was investigated using established methods for juvenile rockfish (Kashef et al. 2014; Hamilton et al. 2017). U_{crit} trials took place after six to seven weeks in treatment water or after three16-day fluctuating cycles, with n = 8-12 per treatment and species. Individual fish were measured for total length and placed in treatment water in a Loligo 1.5 L glass swim tunnel with honeycomb inserts that maintained laminar flow (Figure 3). The flume was constantly refreshed with seawater from a water bath using a submersible pump with 5 ml/s flow. Water velocity in the tunnel was calibrated using Loligo's DPTV system. Except for a ~30 × 24 cm viewing window, the flume was shrouded in opaque foam to limit visual cues from the investigator. The end of the tunnel was illuminated to create a light-dark gradient and encourage the fish to swim forward. Fish were left to acclimate for 15 minutes with water flow set to a rate of 0.5 body length (bl) per second. After acclimation, flow was raised to 1 bl/s and increased by increments of 1 bl/s every two minutes until the fish fatigued (could no longer maintain position). Juveniles were discouraged from resting against the rear honeycomb with motion from a flashlight beam. U_{crit} was calculated using the formula:

$$U_{crit} = U_i + U(T/T_i)$$

where U_i is penultimate swimming speed in bl/s, U is the velocity increment (1 bl/s), T is time elapsed at the fatigue velocity, and T_i is the time interval (2 minutes).

Aerobic Scope

Aerobic scope, a measurement of the capacity for aerobic activity and organismal fitness, is equal to the difference between the standard metabolic rate (SMR) and the maximum metabolic rate (MMR). SMR describes the rate of oxygen consumption at a given temperature during the period of lowest activity, while MMR is defined as the highest rate of oxygen consumption that an animal can attain under specified environmental conditions (Fry 1971). The scope, therefore, describes the excess energy available for activities like locomotion, reproduction, and tissue growth (Wang et al. 2009).

SMR for juvenile rockfish was determined using a Loligo Systems intermittent flow respirometer inside a 109 × 49 × 49 cm insulated water bath (Figure 3) during weeks 10-12, after five 16-day fluctuating cycles. Sample sizes ranged from 6-10 fish per species and treatment. Single fish were weighed and placed in four individual 200 mL glass cylindrical chambers. 10 mm OX11220 flow-through oxygen cells recorded dissolved oxygen concentrations in the chambers during repeated 11 minute duration cycles. Loligo AutoResp software controlled temperature and dissolved oxygen using an external recirculating coil chiller and solenoid valve regulating N_2 gas. A Neptune Systems Apex controller with a solenoid valve was used to regulate CO_2 flow. To maintain consistent water quality inside the respirometer, chambers were flushed with water from the water bath (5 mins), allowed to stabilize (1 min), and measured for dissolved oxygen concentration (5 mins) in a repeating loop. To capture SMR during a period of minimum metabolic activity, juveniles were left to rest overnight in the respirometer at treatment pH/DO levels from approximately 7 pm-10 am. SMR was calculated by taking the lowest 20th quantile of oxygen consumption rate scaled to body size (MO_2; mg O_2kg^{-1} hr^{-1}) from the 10 pm-6 am period of intermittent respirometry measurement loops using the fishMO2 package (Chabot 2020) in R. Only loops with $R^2 > 0.95$ were included.

MMR was determined by measuring oxygen consumption rate during exercise during weeks eight and 9 (or at the end of four 16-day fluctuating cycles). Sample sizes ranged from 5-9 individuals per species and treatment. Each fish was placed in the swim flume and encouraged to swim at a velocity of one body length below their previously determined U_{crit} for five minutes, using flashlight cues to discourage resting at the back of the flume, when necessary. MO_2 was measured directly in the 1.5 L swim flume during the 5-minute swimming period.

Hypoxia Tolerance (P_{crit})

Critical oxygen level, or P_{crit}, is a measure of oxygen extraction capacity and hypoxia tolerance in fishes (Chapman et al. 2002; Rogers et al. 2016). Because oxygen is an electron receptor that drives ATP production in aerobic organisms, oxygen consumption rate is considered a proxy measurement for aerobic metabolic rate (Brown et al. 2004). Fishes can typically oxyregulate, or maintain stable oxygen uptake across a wide range of oxygen saturation levels, using physiological coping mechanisms such as increasing ventilation rate and suppressing reproduction, digestion, and growth (Perry et al. 2009; Pörtner & Grieshaber 1993; Wang et al. 2009). When oxygen saturation falls below a critical threshold (P_{crit}), fish transition to anaerobic metabolism and oxyconform, reducing their oxygen uptake with decreasing oxygen tension (Pörtner & Grieshaber 1993). P_{crit} is therefore defined as the pO_2 at which fishes switch from oxyregulating to oxyconforming, signaled by a drop in standard metabolic rate (SMR) as pO_2 decreases (Rogers et al. 2016).

P_{crit} trials were initiated at 8 am on the morning after SMR trials. P_{crit} was determined by decreasing the oxygen saturation by 10% increments from 70% to 40% and recording three flush/wait/measure loops at each increment. To achieve greater precision closer to P_{crit}, oxygen was then lowered by 5% increments until the conclusion of the trial at 15% saturation. P_{crit} was calculated for each individual using the R package fishMO2 (Chabot 2020). The package fits a regression line to a fish's decreasing oxygen conforming MO_2 values and identifies P_{crit} as the intersection between the regression line and a horizontal line at the SMR value. Respirometry loops with $R^2 < 0.95$ and trials with an R^2 value <0.80 for the MO_2 and DO regression were excluded from the analysis. Sample sizes for P_{crit} ranged between 6-12 individuals per treatment and species.

Growth, Body Condition, and Mortality

Standard length (mm), total length (mm), and weight (g) were recorded when fish were first placed in treatment water and at the conclusion of the experiment after 13 weeks in treatment water (six 16-day fluctuating cycles). Specific growth rate (SGR), which represents the percentage of starting body mass gained per day (Wang et al. 2009), was calculated using the formula:

$$SGR = \ln W_t - \ln W_0 / t * 100\%$$

Where W_t = final weight, W_0 = initial weight, and t = days in treatment conditions. A subset (n = 39) of the gopher rockfish were weighed for the first time one month after the start of the experiment, and growth rate for these individuals was calculated using this delayed initial weight. Body condition was calculated at the beginning (or one month later for 39 gopher rockfish) and end of the experiment using the Fulton's K condition metric, where K = weight/length^3. Fish with a higher Fulton's K value are heavier for a given length and presumed to be in better condition. Percent mortality was determined by recording the number of mortalities per day and dividing by the total number of fish (number died / total number of fish x 100%). Because some fish died early in the experiment before they could be definitively identified as a copper or gopher rockfish, both species were grouped together for this analysis. A range of 9-28 fish per treatment and species were evaluated for growth, body condition, and mortality.

III. Statistical Analysis

Behavioral and physiological data from most experiments (escape time, U_{crit}, aerobic scope, P_{crit}, ventilation, growth) were compared among treatments with mixed effects models in R (v4.2.2; R Core Team 2022) using the nlme package (v3.1.160; Pinheiro et al. 2022). Wald chi-square and p-values were obtained using a type-II ANOVA from the car package (v3.1.1; Fox & Weisberg 2019) unless there was a significant interaction term in which case a type-III

ANOVA was used. Treatment and species were included in models as fixed effects. Tank was included as a random intercept to account for the non-independence of fish within a tank in all models except for lateralization, when tank was not replicated. The interaction between species and treatment was retained in the model to determine if species exhibited similar patterns across treatments. For lateralization, escape time, and body condition, where trials were repeated on the same individuals, fish ID was also included as a random intercept and time point was included as a fixed effect. Escape trial data and startle latency were natural log-transformed to achieve normality. Model parameters were estimated using restricted maximum likelihood (REML). Model assumptions including normality of residuals, linearity and homoscedasticity were tested through visual inspection of Q-Q plots and by plotting the residuals vs. the fixed effects. For models with significant effects, additional single-species models were run and Tukey's HSD pairwise comparisons were performed between treatments for each species using the emmeans package (version 1.8.1-1, Lenth 2022) in R. Mortality was analyzed using a generalized linear model with a binomial distribution and no random effect. Residuals for mortality were evaluated using the DHARMa package (version 0.4.6, Harti 2022).

IV. Results

A. Carbonate Chemistry

Experimental treatment conditions were maintained close to intended pH and DO levels. Daily readings from the Hach probe were 0.07 units lower on average than pH results from spectrophotometry of discrete water samples (Table 2). Due to low replication of discrete water samples for some treatments, treatment means are based on daily readings from the Hach meter. Means of fluctuating treatments (upwelling and relaxation) were approximately 7.66 pH and 5.29 mg/L DO, which was higher than the moderate static treatment at 7.5 pH/ 4.26 mg/L DO.

B. Behavior

Escape Time

There was no difference in final escape time among the static pH/DO treatments for either species and no difference between static and fluctuating conditions (Treatment, $\chi^2 = 1.54$, df = 4, p = 0.818; Table 3). There was no difference in escape time between time point 1 in control conditions and time point 2 after two weeks in treatment water for any of the treatments (Time point, $\chi^2 = 1.98$, df = 1, p = 0.160, Figure 5). I also did not detect a difference in escape time between species (Species, $\chi^2 = 0.65$, df = 1, p = 0.42) nor were there significant interactions between any factors. When examining only time point 2, escape time did not differ among treatments (Treatment, $\chi^2 = 4.76$, df = 4, p = 0.31).

Behavioral Lateralization

Gopher rockfish were poorly lateralized (relative lateralization between 20 and -10) and showed no change in relative behavioral lateralization ($\chi^2 = 0.85$, df = 1, p = 0.355, Table 3; Figure 6) or absolute lateralization ($\chi^2 = 1.52$, df = 1, p = 0.22) between control and extreme static treatments. Individuals displayed no changes in relative or absolute lateralization between time point 1 which was performed in control water and time point 2 which was performed in treatment water (Time point, $\chi^2 = 0.51$, df = 1, p = 0.47 for relative; $\chi^2 = 0.01$, df = 1, p = 0.94 for absolute).

Startle Response

All fish, regardless of pH/DO treatment, successfully executed a fast-start in response to the startle stimulus. There was no difference in response latency among treatments (Treatment $\chi^2 = 2.96$, df = 2, p = 0.23, Figure 7). Fast-start velocity did not differ among fish reared in the different static pH/DO treatments (Treatment $\chi^2 = 0.1.19$, df = 2, p = 0.55, Table 3, Figure 7).

C. Physiology

Ventilation Rate

Ventilation rate increased as pH/DO treatments became more extreme (Treatment, $\chi^2 = 151.09$, df = 4, p< 0.0001; Table 3, Figure 8). Ventilation rates increased progressively from the control treatment to the moderate low pH/DO treatment, and were highest in the extreme low pH/DO treatment. Compared to the control, ventilations per minute (VPM) were 71% higher in the moderate treatment and 118% higher in the extreme treatment for copper rockfish. VPM was 80% higher in moderate treatments and 115% higher in extreme treatments compared to the control for gopher rockfish. Interestingly, ventilation rates remained high in the upwelling phase of the fluctuating treatments (similar in magnitude to the extreme treatment fish; with a 119% and 117% increase relative to control for copper and gopher rockfish, respectively), and then appeared to recover in the relaxation phase, with values comparable to the control treatment. Mean VPM was 101% higher during upwelling than relaxation for copper rockfish and 84% higher for gopher rockfish. Ventilation rates did not differ between species (Species, $\chi^2 = 0.04$, df = 1, p = 0.84). However, there was a significant Treatment x Species interaction (Treatment x Species, $\chi^2 = 15.21$, df = 4, $p < 0.01$), due to species differences in the upwelling treatment where copper rockfish had a higher VPM than gopher rockfish.

Critical Swimming Speed (U_{crit})

Critical swimming speed differed as a function of pH/DO treatment according to the main effects of the model (Treatment, $\chi^2 = 27.38$, df = 4, $p < 0.0001$, Table 3; Figure 9). Tukey's HSD test showed that only the upwelling-relaxation and extreme-relaxation pairwise comparisons were significant for copper rockfish, with no significant differences between treatments for gopher rockfish. The high significance of the overall model for gopher rockfish was likely due to the large difference in means between the control and extreme treatment and relaxation and upwelling treatments. Although not always significant in pairwise post-hoc tests, there was a consistent pattern where the slowest swimming speeds occurred in the extreme static pH/DO treatment and the simulated upwelling phase of the fluctuating treatment for both species. For copper rockfish, U_{crit} was 8% lower in the moderate treatment and 17% lower in the extreme treatment when compared to the control. For gophers, U_{crit} dropped by 17% in the moderate and 20% in extreme relative to the control treatment. Juveniles of both species exhibited trends of impairment in swimming capabilities during the upwelling phase compared to control fish (18% reduction for copper rockfish and 24% reduction for gopher rockfish), but appeared to fully recover when returned to control water following 6-7 days of simulated relaxation. Copper rockfish tested during upwelling exhibited a 22% decrease in U_{crit} compared to the relaxation phase and gopher rockfish displayed a 24% decrease. There was no difference in critical swimming speed between species (Species, $\chi^2 = 3.42$, df = 1, p = 0.06) and the relative response patterns were consistent across the treatments (Treatment x Species, $\chi^2 = 3.19$, df = 4, p = 0.53).

Aerobic Scope

Aerobic scope differed significantly across the combined pH/DO treatments (Treatment, $\chi^2 = 62.38$, df = 4, $p < 0.0001$, Table 3; Figure 10) but not between species (Species, $\chi^2 = 1.16$, df = 1, p = 0.28). The relative patterns were consistent across the treatments for each species (Treatment x Species, $\chi^2 = 2.05$, df = 4, p = 0.73). For copper rockfish, aerobic scope fell by 46% for both the moderate and extreme treatments relative to the control. For gopher rockfish, aerobic scope decreased by 28% for the moderate treatment (though not significantly) and 48% for the extreme treatment relative to control. In the two fluctuating treatments, aerobic scope under upwelling conditions matched that in the extreme treatment, while it recovered to values similar to fish in the control treatment at the end of the relaxation period. During upwelling, copper rockfish exhibited a 74% and 72% decrease in aerobic scope values relative to relaxation and control, respectively, and gopher rockfish decreased by 72% and 71%.

Patterns in aerobic scope were not explained by differences in the standard metabolic rate but were driven primarily by changes in maximum metabolic rate. While at rest, juvenile rockfish largely appeared to metabolically compensate for the physiological impacts of exposure to combined pH/DO stressors, and there was no difference in resting metabolic rate among treatments (Treatment, $\chi^2 = 8.10$, df = 4, p = 0.088) or species (Species, $\chi^2 = 2.09$, df = 1, p = 0.15) and no significant interaction (Treatment x Species, $\chi^2 = 9.19$, df = 4, p = 0.06). However, there was a trend towards decreasing resting metabolic rate as treatments became more extreme for gopher rockfish. Maximum metabolic rate decreased significantly as treatments became more extreme (Treatment, $\chi^2 = 62.42$, df = 4, $p < 0.0001$) with no differences between species and no interaction (Species, $\chi^2 = 1.84$, df = 1, p = 0.17; Treatment x Species $\chi^2 = 1.95$, df = 4, p = 0.75), matching the aerobic scope results. Relative to the control, copper rockfish had a 37% and 36% decrease in MMR in the moderate and extreme treatments, respectively, and gopher rockfish experienced a 26% decrease in moderate and 43% decrease in extreme treatments. Similar to aerobic scope, MMR of fish in the upwelling treatment resembled fish in the extreme static treatment. Copper rockfish exhibited a 58% decrease in the upwelling treatment relative to relaxation and a 57% decrease relative to control treatments. Gopher rockfish showed a 57% decrease in the upwelling treatment relative to relaxation and a 56% decrease relative to control.

In addition, fish in the relaxation treatment recovered fully in MMR after 7-8 days in control conditions in both species, mirroring those in the static control treatment.

Hypoxia Tolerance (P_{crit})

In the control treatment, copper rockfish had a mean P_{crit} of 31.9% air saturation (~2.67 mg/L) and gopher rockfish had a mean P_{crit} of 29.3% air saturation (~2.46 mg/L). P_{crit} decreased as the static treatments became more extreme, indicating that exposure to deoxygenated conditions led to an increase in hypoxia tolerance (Treatment, $\chi^2 = 25.11$, df = 4, $p < 0.0001$, Table 3; Figure 11). It was not readily apparent which treatments were responsible for the significant treatment effect from the Tukey's HSD post-hoc tests, but it was likely due to the extreme and control treatments, which exhibited the biggest differences in P_{crit}. For copper rockfish in the moderate and extreme treatments, P_{crit} was reduced to 22.3% (~1.87 mg/L) and 15.0% air saturation (~1.26 mg/L) respectively, dropping by 30% and 53% relative to the control treatment. P_{crit} for gopher rockfish in the moderate and extreme treatments fell to 24.6% (~2.06 mg/L) and 12.2% air saturation (~1.02 mg/L) and was 16% and 58% lower than the control treatment. While the model showed no effect of species (Species, $\chi^2 = 1.01$, df = 1, $p = 0.32$), there was a significant interaction between species and treatment (Treatment x Species, $\chi^2 = 18.97$, df = 4, $p < 0.001$). This was driven by a higher P_{crit} for gopher rockfish compared to copper rockfish in the moderate and relaxation treatments, while gophers had lower P_{crit} for the remaining treatments. For copper rockfish, P_{crit} values showed only a 10% difference in upwelling relative to relaxation (21.2% and 19.3% air saturation or ~1.78 and ~1.62 mg/L). The mean for the upwelling treatment was 34% lower than the control treatment. For gopher rockfish, P_{crit} decreased by 47% in the upwelling treatment relative to relaxation (14.6% vs. 27.4% air saturation, or ~1.22 and 2.30 mg/L) and by 50% relative to control.

Growth, Body Condition, and Mortality

Specific growth rate (after 13 weeks) differed among treatments (Treatment, $\chi^2 = 66.19$, df = 4, $p < 0.0001$, Table 3; Figure 12 and 13). Growth was depressed in the extreme static treatment (59% decline) as well as the upwelling and relaxation treatments (30% and 27% decline, respectively) relative to control treatments for copper rockfish. Gopher rockfish exhibited the same trends, with the lowest growth in the extreme static pH/DO treatment (46%

drop relative to control) and intermediate growth in the upwelling and relaxation treatments (35% and 27% drop). Growth rates in the fluctuating treatment were intermediate between the moderate and extreme low treatments, although pairwise comparisons between these treatments were generally not significant. Growth rate was higher for copper rockfish than for gophers (Species, $\chi^2 = 21.93$, df = 1, $p < 0.0001$) with no significant interaction (Treatment x Species $\chi^2 = 6.25$, df = 4, $p = 0.18$). Growth rate decreased over time in all treatments for both species (Figure 13), with the greatest declines in the extreme and fluctuating upwelling/relaxation treatments.

Body condition differed among treatments (Treatment, $\chi^2 = 11.59$, df = 4, $p = 0.02$) with condition being poorest in the extreme and highest in the control treatment. Condition also differed between the initial and final time points (Time point, $\chi^2 = 50.16$, df = 1, $p < 0.0001$), but not between species (Species, $\chi^2 = 0.15$, df = 1, $p = 0.70$). While all treatments showed an increase in body condition between the start and end of the experiment, the increase was different among treatments and was lowest for the extreme treatment, leading to a significant Time point x Treatment interaction (Treatment x Time Point, $\chi^2 = 11.09$, df = 4, $p = 0.03$). Final body condition differed among treatments (Treatment, $\chi^2 = 12.13$, df = 4, $p = 0.02$) and was lowest in the extreme treatment, but condition did not differ between species (Species, $\chi^2 = 0.06$, df = 1, $p = 0.81$, Figure 12c and 12d). Post-hoc pairwise comparisons were unable to identify which treatments contributed to the significant treatment effect.

Overall, survivorship was high despite chronic exposure to low pH/DO conditions over a 13-week period. Mortality differed significantly among treatments (Treatment, $\chi^2 = 15.69$, df = 4, $p < 0.01$; Figure 14). Cumulatively, three mortalities occurred in the control treatment, nine in the extreme, zero in the moderate treatment, two in relaxation, and three in upwelling.

V. Discussion

Summary

This study presents evidence that fluctuations in pH and dissolved oxygen, simulating future upwelling/relaxation cycles in the California Current, can have significant cumulative detrimental impacts on rockfish physiology (growth) compared to exposure to constant mean conditions. However, fish can recover from impairment of physiological performance that occurs during exposure to extreme low pH/DO conditions (increased ventilation rate, reduced critical swimming speed, reduced aerobic scope), and reacclimate to control conditions within 6-7 days. Trends showing increased hypoxia tolerance under low pH/DO conditions were associated with compensatory increases in ventilation rate. I found no significant effects of combined OA and hypoxia on behavioral metrics, including boldness (escape challenge), behavioral lateralization, and startle response. Copper and gopher rockfish, two closely related congeners that share many life history characteristics, performed similarly across most physiological trials, exhibiting increasing physiological impairment as treatment pH and DO decreased. Because fish had impaired physiological performance during the upwelling stage, followed by full or near-full recovery during the relaxation phase of the fluctuating cycle, it is likely that the duration, frequency, and severity of future hypoxic, acidic events will ultimately determine the impacts of climate change stressors on fitness (Shimps et al. 2005). In addition, despite short-term recovery to conditions in the fluctuating treatment, there may be additional negative cumulative impacts to growth and other energetic costs not measured here that may affect fish fitness.

Behavior

Behaviorally, copper and gopher rockfish demonstrated resilience to both constant and fluctuating OA and hypoxia. I found no difference in escape time, a proxy for boldness or exploratory activity, among combined pH/dissolved oxygen treatments. In contrast, Mattiasen et al. (2020) reported an increase in escape time with decreasing DO levels for copper rockfish, indicating a reduction in boldness under hypoxia. While the results here were inconclusive due to high variance in escape time, the general trend was similar with escape time increasing in the lowest pH/DO treatments. Ocean acidification has been shown to increase anxiety in fishes (Jutfelt et al. 2013), so absence of an effect is contrary to the expected additive or synergistic response under multiple stressors. However, other studies have shown that combinations of low pH and low DO can be antagonistic. For example, exploration during exposure to an alarm cue, another proxy for boldness, decreased as pH and oxygen decreased in single stressor treatments, but did not differ among treatments under exposure to multiple stressors (combined low pH and low DO) in juvenile kelp, gopher, and black & yellow rockfish (Davis et al 2018). One explanation is that increases in CO_2 enhance physiological tolerance to hypoxia in some cases; for example, European sea bass exposed to increases in CO_2 exhibited greater hypoxia tolerance and increased hemoglobin-O_2 affinity than fishes exposed to control CO_2 (Montgomery et al. 2019).

I did not detect a difference in brain lateralization among treatments for gopher rockfish. In contrast, for the closely related copper rockfish, lateralization decreased with decreasing dissolved oxygen (Mattiasen et al. 2020), and fish showed more right turn bias in a detour test as pCO_2 became more extreme (Hamilton et al. 2017). It is possible that the contrasting effects of hypoxia and OA worked in opposition to produce no change in lateralization to combined pH/DO exposure. In addition, behavioral lateralization tests may not be repeatable within individuals (Roche et al. 2020), which might also explain differences between studies and a lack of an effect in this context, where fish were tested before and after exposure to pH/DO stressors. Furthermore, a recent validation study questioned the impact of OA on cognition and behavior in coral reef fishes including lateralization, avoidance of chemical cues, and activity levels (Clark et al. 2020), so these effects may not be as ubiquitous as previously thought.

Gopher and copper rockfish showed no significant difference in startle response latency and velocity with treatment. Similarly, locomotor kinematics and latency for blue rockfish fast-start response are unaffected by short exposures (1 and 10 minutes) to hypoxia only (Leary 2017). However, in contrast to this study where all fish successfully executed a startle response, fewer blue rockfish completed a startle response at lower oxygen concentrations (Leary 2017). This difference could be due to differences between species (gopher and copper vs. blue rockfish); copper rockfish tended to respond more negatively to hypoxia than blue rockfish (Hamilton et al. 2017; Mattiasen et al. 2020), so the lack of an effect for copper rockfish is surprising. Differences could also be due to the duration of exposure to treatment conditions, where Leary (2017) more closely simulated the effect of a fish encountering a patchy hypoxic landscape over a short timescale. Escape responses are fueled with anaerobic metabolism (Domenici & Blake 1997) using fast twitch glycolytic white muscle, and therefore may be unaffected by hypoxia, so long as sensory systems are unimpaired. Unlike the blue rockfish in Leary (2017), juvenile gopher and copper rockfish in this study were given 6-8 days to acclimate to treatment conditions. They were able to lower their P_{crit} below 2.0 mg/L and increase oxygen uptake to maintain a steady SMR in the most extreme treatment. Because their exertion levels and oxygen demands were manageable when the stimulus was initiated, they may not have experienced visual or auditory impairment that would impact responsiveness.

Physiology

Copper and gopher rockfish are closely related congeners that share many life history characteristics. Unsurprisingly, the two species performed similarly across most physiological trials. Fish species that evolved in similarly hypoxic environments tend to converge on similar metabolic response strategies (Mandic & Regan 2018), and both copper and gopher rockfish likely evolved to withstand periodic upwelling and acidified, hypoxic conditions during recruitment. This suggests that information gleaned about one rockfish species can be used to infer responses from closely related species (e.g., kelp, gopher, black and yellow, and copper rockfish, also known as KGB-C complex fishes) with similar evolutionary history (Hyde and Vetter 2007).

For most physiological performance metrics (ventilation rate, critical swimming speed, aerobic scope and MMR), fish exhibited phenotypic plasticity, and were negatively impacted by combined low pH and DO during simulated upwelling, but appeared to recover during simulated relaxation events. This suggests that juvenile rockfish can recover relatively quickly and acclimate back to control environments in less than 6-7 days. The exceptions was hypoxia tolerance in copper rockfish, where the relaxation and fluctuating treatment P_{crits} were similar. It is possible that copper rockfish may acclimate more slowly to variable pH and DO than gopher rockfish and require more than 6-7 days.

Overall, performance tended to decrease as pH and DO decreased, although pairwise tests did not always identify the specific treatments driving the significant response. These findings are consistent with physiological assays and genomics from fishes exposed to low pH and low DO separately. For example, global gene expression in black surfperch, *Embiotoca jacksoni*, increased as acidification increased (Toy et al. 2022). Juvenile copper rockfish showed declines in critical swimming speed and aerobic scope, as well as increased expression of transcription factors and regulatory genes with pH (Hamilton et al. 2017) and showed similar trends in physiological metrics under low oxygen (Mattiasen et al. 2020). Although separate cohorts of fish may not be directly comparable, the similar trends across these studies, rather than a more extreme response under combined stressors, suggests that there is no net synergistic effect of hypoxia and OA on rockfish swimming and aerobic scope. However, for blue rockfish, combined low pH and low oxygen induce a non-additive transcriptomic response, with minimal overlap among genes that respond to single versus combined stressors (Cline et al. 2020).

Juvenile copper and gopher rockfish were able to maintain a steady SMR across treatments, even though the extreme and upwelling treatment had dissolved oxygen concentrations of 2.0 mg/L, which is below the measured P_{crit} for control fish. This is consistent with findings from single-stressor studies which also reported no change in SMR in the lowest DO and pH treatments, respectively (Hamilton et al. 2017; Mattiasen et al. 2020). SMR is less sensitive to hypoxia than MMR (Wang et al. 2009), and a reduction in SMR could indicate suppression of some aspects of maintenance metabolism or that basic cellular metabolic needs are supported by anaerobic metabolism. Fish utilize several methods to maintain their MO_2 during hypoxia such as increases in ventilation volume (driven by increased amplitude/frequency), changes in gill morphology, alterations to Hemoglobin-O_2 affinity, increased hematocrit, and changes to cardiac output (Gamperl and Driedzic 2009; Perry et al. 2009; Silkin et al. 2005; Sollid 2003; Wells 2009). Gopher rockfish showed overall higher SMR than copper rockfish, which may be in part to their smaller size, despite correcting for size in metabolic rate calculations (Sims 1996).

Ventilation rate and aerobic scope appeared to be particularly sensitive to low pH and hypoxia. Dramatic increases in ventilation rate occurred in comparison to fish in control conditions, with significant effects being detected in the moderate treatment. As an important mechanism for increasing oxygen supply while coping with reduced dissolved oxygen levels (Perry et al. 2009), ventilation rate increases linearly as dissolved oxygen levels decrease (Mattiasen et al. 2020). Ventilation rates for copper rockfish exposed to combined 7.3 pH and 2.0 mg/L DO more than doubled compared to control fish (118% increase at 13-14°C), which was greater than the percent change observed when exposed to low oxygen only (56% increase between 8.1 pH and 2.2 mg/L DO at 12°C) in a previous study (Mattiasen et al. 2020). The higher percent increase in ventilation rate suggests that coping with combined stressors could be more energetically costly than a single stressor alone, requiring compensation through increased oxygen uptake at the gills. Ventilation can use up to 10-30% of a fish's energy budget (Soofiani & Hawkins 1985), so the increase in ventilation likely comes at an energetic cost that could otherwise support processes like growth, reproduction, and swimming.

Reductions in growth in the fluctuating treatments compared to the control in both species suggests that short-term exposures to low pH and DO have cumulative impacts that may affect long-term organismal fitness, or carry-over to the adult stage. Changes in growth rate can likely be attributed to reductions in feeding in the moderate and extreme static treatments, as well as during the upwelling phase of the fluctuating cycle (M. Palmisciano, *personal observation*). Because digestion processes including the biochemical conversion of food and protein synthesis is energetically costly (Brown & Cameron 1991a, b; Bureau et al. 2003), suppression of appetite and digestion is a common response to reduce metabolic demands under hypoxic conditions, thereby decreasing oxygen requirements in fishes (van Dam and Pauly 1995; Wang et al. 2009). Despite the ability of juvenile rockfish to recover their swimming performance and aerobic scope during the relaxation phase of the simulated upwelling/relaxation cycle, depressions in growth rate have been shown to reduce survivorship in other species (McCormick & Hoey 2004), and may continue to impact rockfish populations even after acidic and hypoxic conditions abate. For example, the predicted intensification of coastal upwelling (Bakun 1990; Sydeman et al. 2014) could cause growth depression that may lengthen the time to maturation.

While laboratory physiological experiments provide a snapshot of an organism's performance at a specific time, they may fail to capture energetic costs that may endure after an organism appears to have fully recovered due to sub-organismal reallocation of energy and/or an ongoing cellular stress response. Findings from RNA sequencing show that there were high numbers of differentially expressed genes between the static control treatment and the relaxation treatment, suggesting that effects of fluctuations linger at the molecular level (Doerr 2021). Therefore, differential gene expression findings from RNA sequencing may provide a better picture of acclimatory and stress responses, as well as the mechanistic basis of such responses. For example, pH variability from upwelling (decreasing and increasing in pH over a period of 8 days) significantly impacted the number of differentially expressed genes in black surfperch (*E. jacksoni*) at mean pH of 7.7 ± 0.15 and 7.85 ± 0.15 (Toy et al. 2022). Similarly, under diel $p\mathrm{CO}_2$ fluctuations, the brain tissue of coral reef fishes *Acanthochromis polyacanthus* and *Amphiprion percula* exhibited differentially expressed genes involved in circadian rhythm processes and steroid hormone signaling pathways for both species between the fluctuating and stable treatments (Schunter et al. 2021). This suggests that coral reef fishes may utilize the circadian rhythm regulator in the brain to adapt to dynamic pH conditions (Schunter et al. 2021).

Frontloading, or the higher preparatory expression of genes involved in stress response (Barshis et al. 2013), could result in higher differential expression of genes during relaxation and could play a role in acclimation to repeated stressors under environmental variability.

Impacts of fluctuations

This study supports the importance of incorporating natural variability in climate change stressor experiments. The mean pH/DO for the fluctuating treatments (~7.66 pH/5.30 mg/L) was higher than that of the static moderate treatment (7.5 pH/ 4.26 mg/L) and yet growth was reduced in the fluctuating treatment when compared to the static moderate treatment. In this upwelling scenario, fluctuations had an overall negative effect compared to constant mean conditions. Given Jensen's inequality, this suggests that the rockfish performance curve across combined pH and DO levels is convex. However, responses to fluctuations are likely species-specific and depend on the duration and amplitude of the fluctuations (Wang et al. 2009). Diel dissolved oxygen oscillations have been shown to have adverse effects on growth compared to constant moderate treatments in juvenile southern flounder (Taylor & Miller 2001), while in juvenile European sea bass, growth, feeding, and body condition in a diel oscillating DO treatment were not different from a constant normoxic or a constant hypoxic treatment (Thetmeyer et al. 1999). Most studies exposing organisms to fluctuating pH or DO have used cycles that vary on a diel scale, replicating the natural day-night changes in ocean chemistry from photosynthesis and respiration (ex. Dufault et al. 2012; Cornwall et al. 2013; Clark & Gobler 2016; Jarrold et al. 2017; Onitsuka et al. 2018; Enochs et al. 2018; Britton et al. 2019). To my knowledge, fewer studies have simulated cycles with periods that last for multiple days (e.g. Toy et al. 2022), which reflect the California Current's upwelling/relaxation dynamics inhabited by rockfish.

Previous studies have also demonstrated that the effects of fluctuations in pH depend on the species of fish examined. Exposure to fluctuating pH on a diel cycle benefitted three coral reef fish species (*Lutjanus fulviflamma*, *Caesio cuning*, and *Abdudefduf whitleyi*) but not a fourth (*Cheilodipterus quinquelineatus*) compared to static conditions (Hannan et al. 2021). Those species exhibited improvements in swimming performance, MMR, and aerobic scope under fluctuating pH (mean pH = 7.70, fluctuating between 7.60 and 7.80) despite having a similar mean value as the static pH treatment (pH = 7.75). In this case, it seems that variability in pH provides a temporal refuge from stress in cases where fluctuations reduce the amount of time spent in constant undesirable conditions. Conversely, one nocturnal species (*Cheilodipterus quinquelineatus*) was adversely affected with decreased MMR and aerobic scope under fluctuating pH. The negative cumulative growth response to fluctuating pH/DO in our study may be explained by many factors, including variability due to differently shaped performance curves (Jensen's inequality), species differences, the interacting effects of OA and hypoxia, the higher amplitude of pH fluctuation (~± 0.3), or the longer duration of low pH/DO periods (8 days vs. diel cycle in Hannan et al. 2021).

These mixed findings suggest that the amplitude of fluctuations will likely modulate their effects on organismal fitness. The frequency of fluctuations and duration of periods of stress and recovery will also likely play a role in the overall energetic costs of acclimation. Future studies should vary the mean, maximum, minimum, and frequency of fluctuations and compare them to corresponding static treatments with identical means, with both current-day and future projected pH/DO levels, to better evaluate the costs of acclimation. Furthermore, the predictability of fluctuating cycles may influence organismal responses, with higher predictability allowing for more adaptive responses (Kroeker et al. 2019) and lower predictability, combined with higher magnitude fluctuations, correlating with low phenotypic plasticity (Bitter et al. 2021). While this upwelling simulation used a predictable 16-day cycle, true upwelling is stochastic (Figure 1) so this study may overestimate rockfish's acclimatory ability to natural fluctuations. As larger and more customizable control systems are developed, future experiments could use a multi-factorial design where the amplitude, frequency, and predictability of pH/DO fluctuations are altered. This may illuminate any energetic costs of acclimation and identify thresholds where either the mean or variance of climate stressors impact organismal performance. Additionally, sampling at additional points in the cycle, such as during the transition from upwelling to relaxation or on the first day or two would provide information about how acclimation time influences performance.

This study highlights the plasticity of rockfish physiology in response to ocean chemistry as well as the long-term detrimental effects of chronic severe drops in pH and dissolved oxygen on growth. The physiological characteristics measured here (ventilation rate, U_{crit}, aerobic scope, P_{crit}) were dependent on the immediate water chemistry. Impairment was high after a week of exposure to simulated upwelling conditions and fish exhibited acclimatory responses within that time frame (e.g., increased ventilation rate and lowered P_{crit}), but fish quickly recovered or re-acclimated within a week of being returned to control conditions. Growth tended to be greater in the fluctuating treatment than the extreme low, though these pairwise comparisons were not significant, suggesting that temporary reprieves in stressors can have positive long-term effects. My findings demonstrate that acclimatory responses in juvenile rockfish may confer resiliency under extreme projections of future upwelling conditions.

VI. Conclusion

Juvenile copper and gopher rockfish exhibited significant physiological impairment during the upwelling stage, followed by full or near-full recovery during the relaxation phase of the fluctuating cycle. It is likely that the duration, frequency, and severity of future hypoxic, acidic events will ultimately determine the impacts on survivorship and fitness. Future studies should examine how variation in the timing, intensity, and predictability of upwelling pulses across a range of projected climate change scenarios affect fitness in nearshore organisms. More frequent sampling through physiological experiments or RNA sequencing could provide a clearer picture of the time required for full recovery, sub-organismal impacts, as well as any pre-conditioning or frontloading effects (Barshis et al. 2013), where exposure to a stressor increases tolerance to that stressor via acclimatization. Pre-conditioning has been observed in corals exposed to periodic temperature spikes and in fishes exposed to hypoxia (Manchenkov et al. 2015), so it is possible that similar mechanisms could improve a fish's tolerance to combined hypoxia and acidification over time. Additionally, little is known about the avoidance responses (Bell & Eggleston 2005) of nearshore juvenile fishes during upwelling, including vertical and horizontal movements and the use of possible refugia created by kelp and seagrass buffering (Hirsh et al. 2020; Falkenberg et al. 2021).

Rockfishes are long-lived, late to mature, and commercially valuable, so their ability to quickly acclimatize to changing ocean conditions will have far-reaching implications for species diversity and fisheries sustainability (Wittmann & Pörtner 2013). Many species of rockfishes are commercially and recreationally harvested in California. This study shows that concomitant exposure to low pH and low DO significantly impacts the fitness of juvenile rockfish through reductions in aerobic capacity and swimming performance. Reduced fitness in the early life stages due to stress from OA and hypoxia may increase susceptibility to predation and affect the population replenishment process, which will impact fisheries and the ecology of nearshore coastal systems. Understanding the responses of marine organisms to a dynamic ocean environment is becoming progressively more important as climate change intensifies upwelling events along the central and northern California coast (Wang et al. 2015). The ability of organisms to acclimatize and adapt to oceanic variability in pH and dissolved oxygen will have implications for rockfish population health and conservation measures under future environmental forcing.

VII. Tables and Figures

Tables

Table 1. Summary of experiment schedule from May 2017-July 2018.

Experiment or Event	Date	Weeks in Treatment	Number of Fluctuating Cycles (16 days)
YOYs caught on SCUBA	May-June 2017	0	0
Acclimation in control water	May-July 2017	0	0
Tagging	July 10-13 and August 3, 2017	0	0
Control water escapes	July 18-19, 2017	0	0
Introduction to treatment water	August 8, 2017	0	0
Tagging (39 gophers)	August 8 – September 23, 2017	0-6	0-3
Treatment water escapes	August 21-August 25, 2017	1-2	1
Ventilation	September 4-17, 2017	3-5	2
U_{crit}	September 20-October 1, 2017	6-7	3
MMR	October 5-14, 2017	8-9	4
Tagging (20 gophers) for lateralization	October 17, 2017	0	N/A
P_{crit} and SMR	October 19-November 5 2017	10-12	5
Lateralization in control water/time point 1 (gophers only)	October 24 & 26, 2017	0	N/A

Lateralization in treatment water / time point 2 (gophers only)	October 31 & November 2, 2017	1	N/A
Dissections	November 9-12	13	6
Startle response fish collections	June 6-19, 2018	0	N/A
Startle experiment fish introduced to treatment water	July 3, 2018	0	N/A
Startle experiment in treatment water	July 9-13, 2018	1-2	N/A

Table 2. Summary of mean pH, total alkalinity, and pCO_2 from water samples and from daily spot checks taken using the Hach multimeter, averaged by treatment. Values are listed with ± standard error. For the fluctuating treatments, means are also shown when the treatment was on (during upwelling phase when pH and DO fell) and off (relaxation phase where pH and DO returned to control values). N represents the number of samples taken during the experiment.

Treatment	**Discrete water samples**				**Daily Hach multimeter measurements**			
	pH (spec)	**TA**	$\boldsymbol{pCO_2}$	**n**	**pH (Hach)**	**Temperature °C**	**Dissolved Oxygen (mg/L)**	**n**
Control	8.04 ± 0.02	2236.54 ± 3.25	391.25 ± 18.92	8	8.00 ± 0.01	13.86 ± 0.07	8.34 ± 0.03	140
Moderate	7.55 ± 0.05	2241.15 ± 1.97	1405.56 ± 168	5	7.50 ± 0.01	13.95 ± 0.07	4.26 ± 0.06	142
Extreme	7.36 ± 0.04	2248.66 ± 8.48	2237.76 ± 247.36	7	7.32 ± 0.01	13.93 ± 0.07	2.34 ± 0.09	142
Relaxation	7.73	2245.75	1187.56	9	7.64 ± 0.03	13.9 ± 0.08	5.19 ± 0.27	142
(on)	7.40 ± 0.05	2252.74 ± 11.05	2003.71 ± 250.84	5	7.35 ± 0.02	13.93 ± 0.14	2.34 ± 0.14	72
(off)	8.06 ± 0.03	2238.76 ± 0.41	371.4 ± 34.99	4	7.96 ± 0.02	13.85 ± 0.07	8.24 ± 0.13	66
Upwelling	7.77	2257.09	1019.06	9	7.68 ± 0.03	13.93 ± 0.07	5.38 ± 0.26	140
(on)	7.48 ± 0.04	2279.13 ± 40.66	1657.31 ± 166.99	3	7.3 ± 0.00	13.94 ± 0.10	2.06 ± 0.02	64
(off)	8.05 ± 0.02	2235.05 ± 1.92	380.81 ± 15.56	6	8.02 ± 0.01	13.92 ± 0.10	8.30 ± 0.04	72

Table 3. ANOVA results for mixed effects models. Statistically significant p-values (<0.05) are noted in bold text.

Experiment	Factor	χ^2	df	p-value
Escape Time (Initial vs. Final)	Treatment	1.54	4	0.82
	Time point	1.98	1	0.16
	Species	0.65	1	0.42
	Treatment x Time point	5.32	4	0.26
	Treatment x Species	2.51	4	0.64
	Time point x Species	0.01	1	0.94
	Treatment x Time point x Species	2.2	4	0.70
Final Escape Time	Treatment	4.76	4	0.31
	Species	0.19	1	0.66
	Treatment x Species	2.85	4	0.58
Relative Lateralization	Treatment	0.85	1	0.36
	Time point	0.51	1	0.47
	Treatment x Time point	0.15	1	0.70
Absolute Lateralization	Treatment	1.52	1	0.22
	Time point	0.01	1	0.94
	Treatment x Time point	1.28	1	0.26
Startle Latency	Treatment	2.96	2	0.23
Startle Velocity	Treatment	1.19	2	0.55
Ventilation	Treatment	151.09	4	**<0.0001**
	Species	0.04	1	0.84
	Treatment x Species	15.21	4	**<0.01**
U_{crit}	Treatment	27.38	4	**<0.0001**
	Species	3.42	1	0.06
	Treatment x Species	3.19	4	0.53

SMR	Treatment	8.1	4	0.09
	Species	2.09	1	0.15
	Treatment x Species	9.19	4	0.06
MMR	Treatment	62.42	4	**<0.0001**
	Species	1.84	1	0.17
	Treatment x Species	1.95	4	0.75
Absolute Aerobic Scope	Treatment	62.38	4	**<0.0001**
	Species	1.16	1	0.28
	Treatment x Species	2.05	4	0.73
P_{crit}	Treatment	25.11	4	**<0.0001**
	Species	1.01	1	0.32
	Treatment x Species	18.97	4	**<0.001**
Growth	Treatment	66.19	4	**<0.0001**
	Species	21.93	1	**<0.0001**
	Treatment x Species	6.25	4	0.18
Body Condition (Initial vs. Final)	Treatment	11.59	4	**0.02**
	Species	0.15	1	0.70
	Time point	50.16	1	**<0.0001**
	Treatment x Species	3.65	4	0.46
	Treatment x Time point	11.09	4	**0.03**
	Time point x Species	2.18	1	0.14
	Treatment x Time point x Species	4.81	4	0.31
Final Body Condition	Treatment	12.13	4	**0.02**
	Species	0.06	1	0.81
	Treatment x Species	3.76	4	0.44

Table 4. Sample size by treatment and species for each experiment. Additional samples (not shown here) were excluded from analyses in cases where video was blurry or unusable (startle, ventilation) or due to poor data quality (ex. equipment failure, fish failed to swim, etc.).

Treatment	Species	Escape	Lateralization	Startle Latency	Startle Speed	Ventilation Rate	U_{crit}	SMR	MMR	Aerobic Scope	P_{crit}	Growth	Fulton's K	Mortality
Control	Copper	22	N/A	N/A	N/A	18	10	8	8	8	8	21	21	22
Moderate	Copper	27	N/A	N/A	N/A	27	9	9	8	8	9	28	28	22
Extreme	Copper	24	N/A	N/A	N/A	25	9	10	9	9	12	25	25	28
Relaxation	Copper	26	N/A	N/A	N/A	26	12	8	8	8	8	27	27	26
Upwelling	Copper	22	N/A	N/A	N/A	21	10	7	7	7	8	27	26	26
Control	Gopher	8	9	N/A	N/A	17	8	8	8	8	8	17	17	17
Moderate	Gopher	4	N/A	N/A	N/A	11	8	7	7	7	7	11	11	9
Extreme	Gopher	4	10	N/A	N/A	9	8	6	5	5	6	9	9	11
Relaxation	Gopher	3	N/A	N/A	N/A	13	9	7	7	7	8	12	11	13
Upwelling	Gopher	5	N/A	N/A	N/A	11	8	6	5	5	8	13	13	13
Control	Coppers & Gophers	N/A	N/A	15	16	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Moderate	Coppers & Gophers	N/A	N/A	14	17	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Extreme	Coppers & Gophers	N/A	N/A	14	16	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Figures

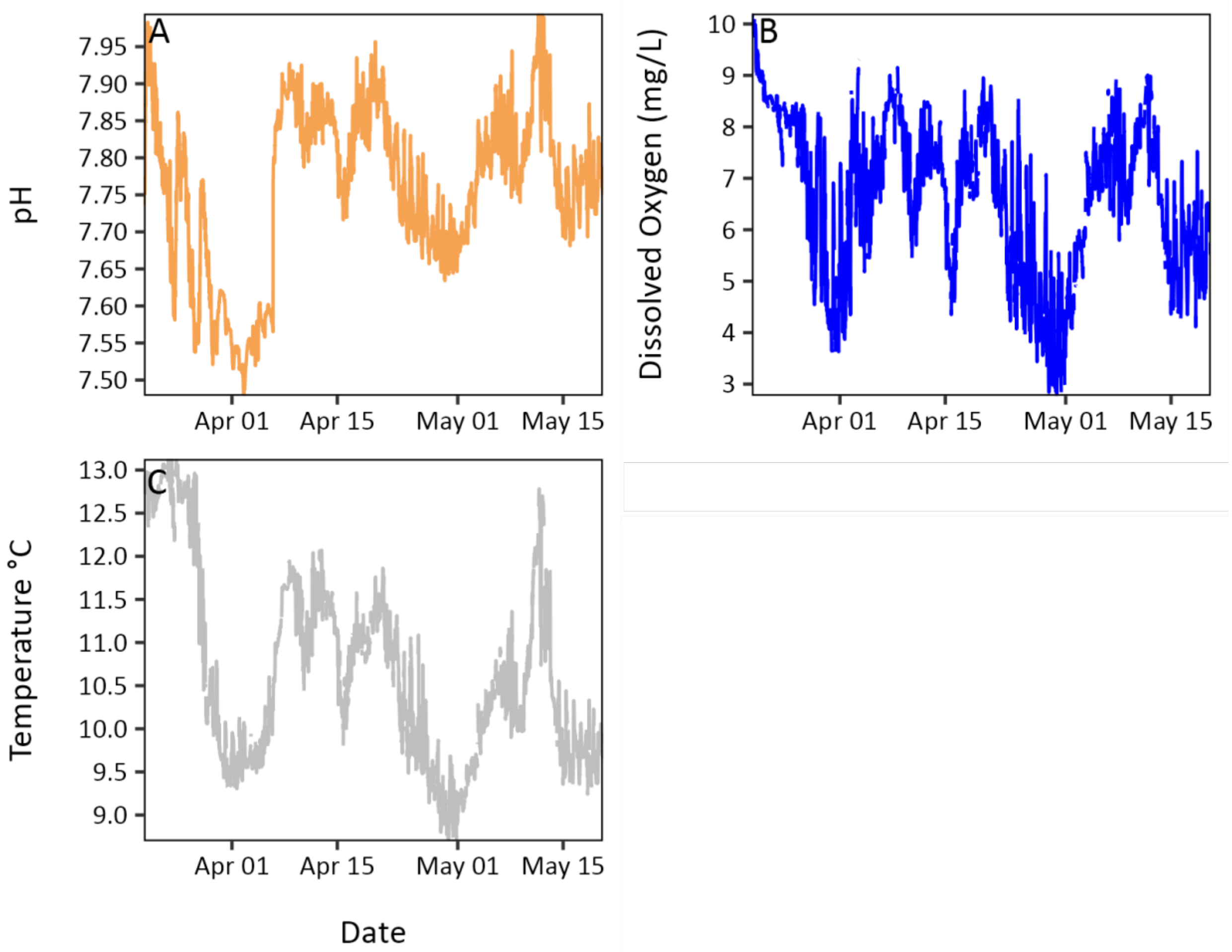

Figure 1. Time series of pH (a, orange), dissolved oxygen (b, blue), and temperature (c, gray) from the SeapHOx sensor moored inside the kelp forest in Stillwater Cove, off the coast of central California, during spring 2017 just prior to fish collections. Data points were collected every 15 minutes. Upwelling events are characterized by simultaneous declines in temperature, pH, and DO. In contrast, relaxation events are characterized by increases in all three environmental parameters.

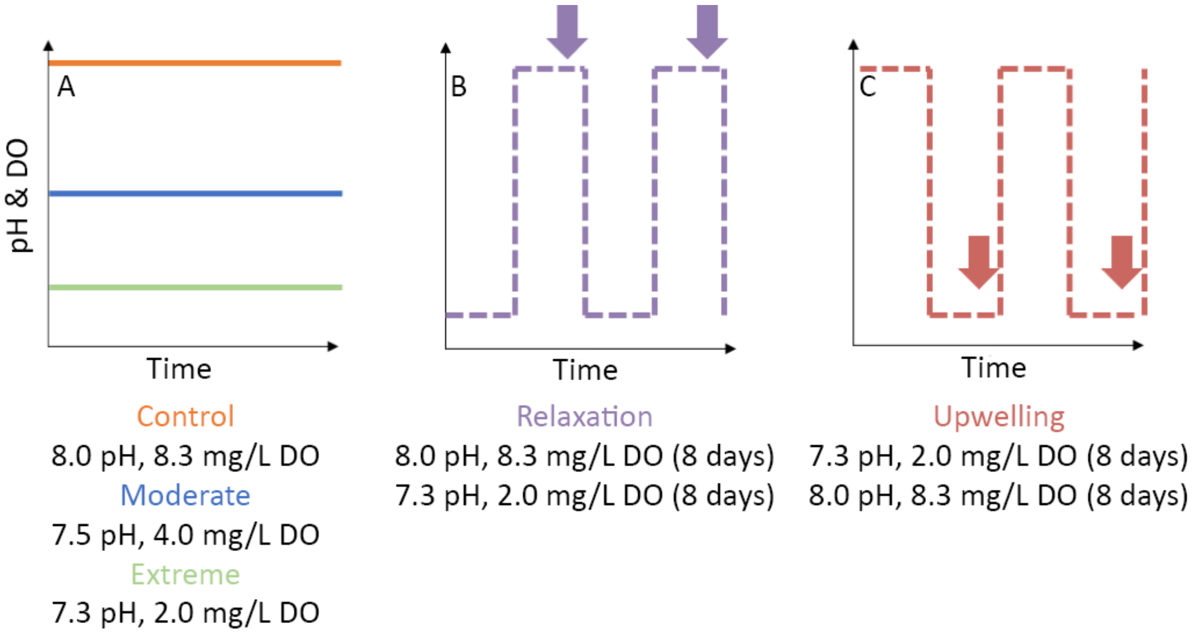

Figure 2. Simplified schematic of treatment conditions. (a) three static treatments were held at a constant pH and DO for the whole experiment. (b) the relaxation treatment on an 8-day cycle. Trials were run on days 6-7 of the relaxation period (indicated by the purple arrows) when treatment conditions were at 8.0 pH / 8.3 mg/L DO. (c) the upwelling treatment fluctuated on an 8-day cycle. Experiments were run on days 6-7 during the upwelling period (indicated by the red arrows) when treatment conditions were at 7.3 pH/2.0 mg/L DO.

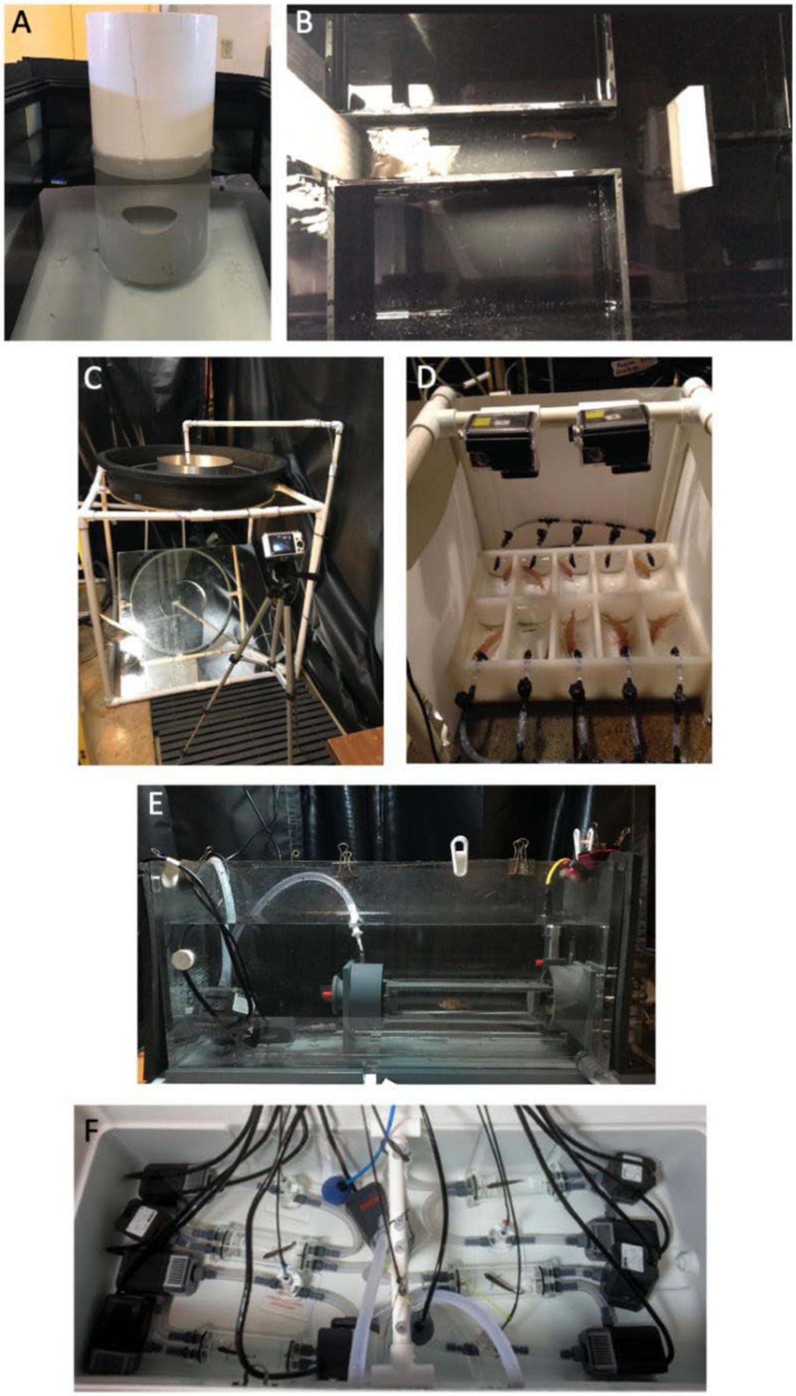

Figure 3. Images of behavioral and physiological experiments. (a) Escape challenge arena (b) Behavioral lateralization double-T maze (c) Startle response arena with camera recording in the mirror (d) Ventilation tank (e) Loligo systems 1.5 L glass swim flume, which was used to calculate critical swimming speed and maximum respiration rate (MMR). (f) Loligo systems intermittent flow respirometer (photo credit Evan Mattiasen) which was used to determine Standard Metabolic Rate (SMR) and P_{crit}.

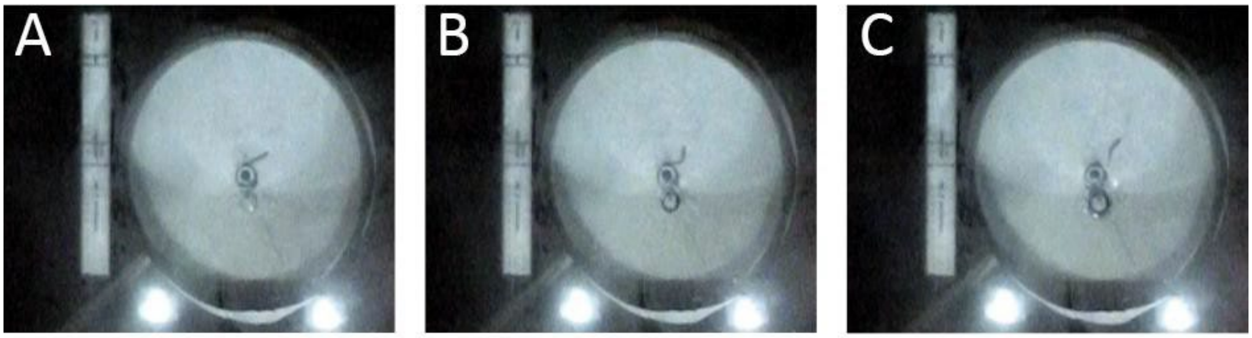

Figure 4. Example of Mauthner-initiated startle response in circular arena. (a) Stimulant (compressed air) is deployed and makes an indent on the water. (b) Stage 1 of C-start, or contralateral bend (c) Stage 2 of C-start, or return flick of the tail.

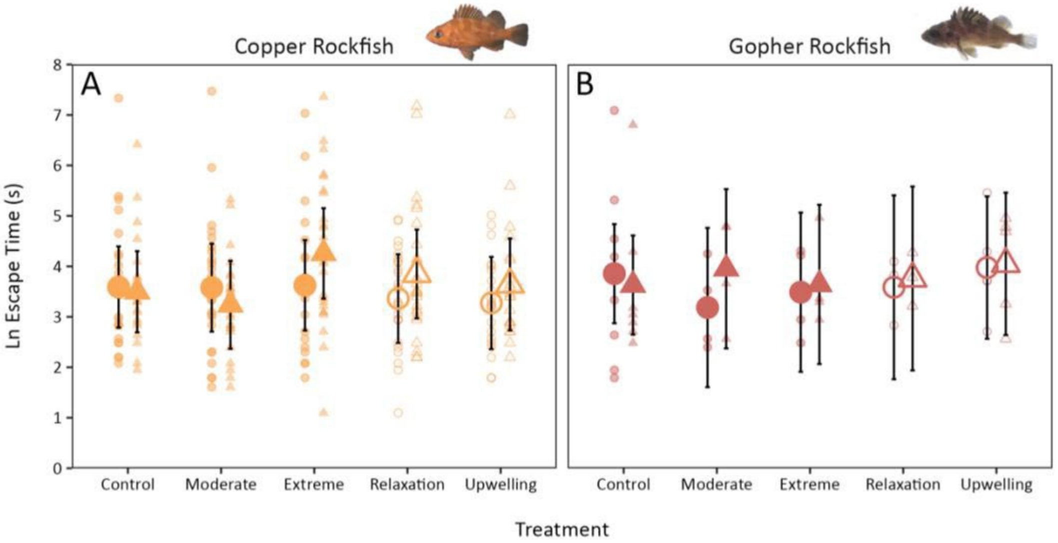

Figure 5. Escape time differences between time points, treatments, and species. Time point 1 (control conditions) is shown as a circle and time point 2 (treatment conditions) is shown as a triangle. Large points represent modeled means and small points show the raw data. Error bars show 95% confidence intervals. Shaded points represent static treatments and open points denote fluctuating treatments. There was no significant difference in escape time among treatments, species, or time points.

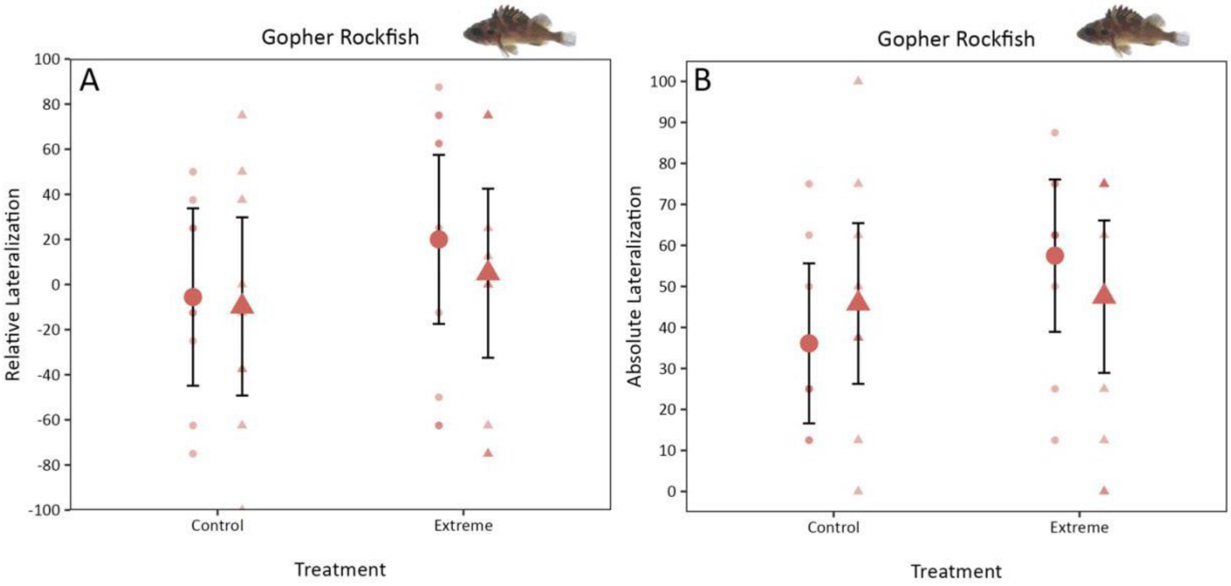

Figure 6. (a) Relative lateralization and (b) absolute lateralization. Large circles/triangles represent modeled means and small circles/triangles show the raw data. Error bars represent 95% confidence intervals. Circles denote time point 1 when fish were in control water and triangles denote time point 2 when fish were in treatment water. For relative lateralization, a positive value indicates a right turn bias and negative value indicates a left-turn bias. There was no significant difference in relative or absolute lateralization among treatments or time points.

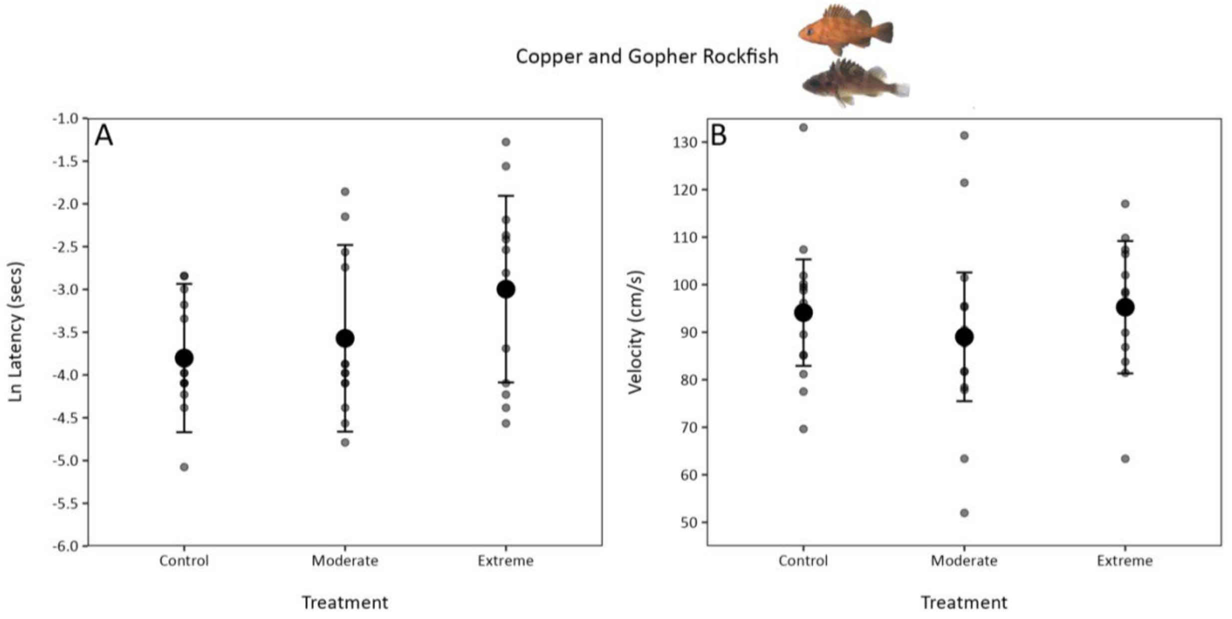

Figure 7. (a) Startle response latency and (b) velocity. Results for copper and gopher rockfish are combined in both graphs. Large circles represent modeled means and small circles show data points. Error bars represent 95% confidence intervals of the model. There was no significant difference in latency or velocity among treatments.

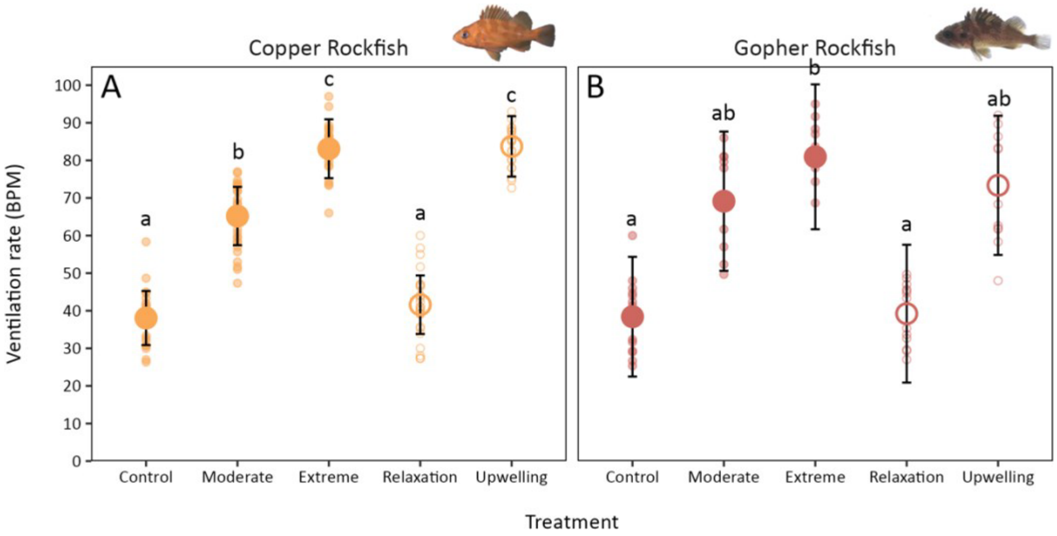

Figure 8. Ventilation Rate for (a) copper and (b) gopher rockfish. Large circles represent modeled means and small circles show data points. Error bars represent 95% confidence intervals. Shaded circles represent static treatments and open circles denote fluctuating treatments. Values not sharing any letters within a plot are significantly different by the Tukey-test at the 5% level of significance.

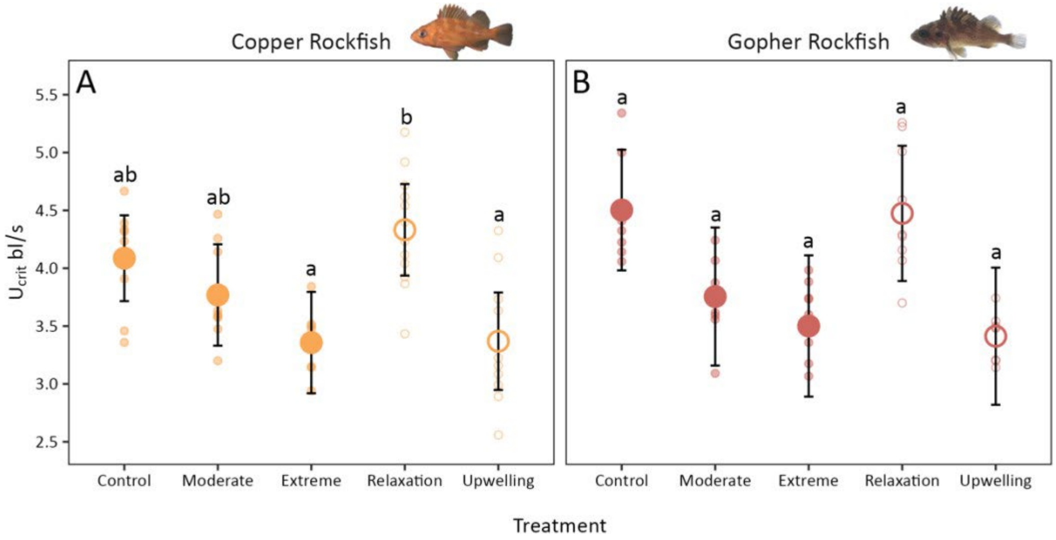

Figure 9. Critical swimming speed for (a) copper and (b) gopher rockfish. Large circles represent modeled means and small circles show data points. Error bars represent 95% confidence intervals. Shaded circles represent static treatments and open circles denote fluctuating treatments. Values not sharing any letters within a plot are significantly different by the Tukey-test at the 5% level of significance.

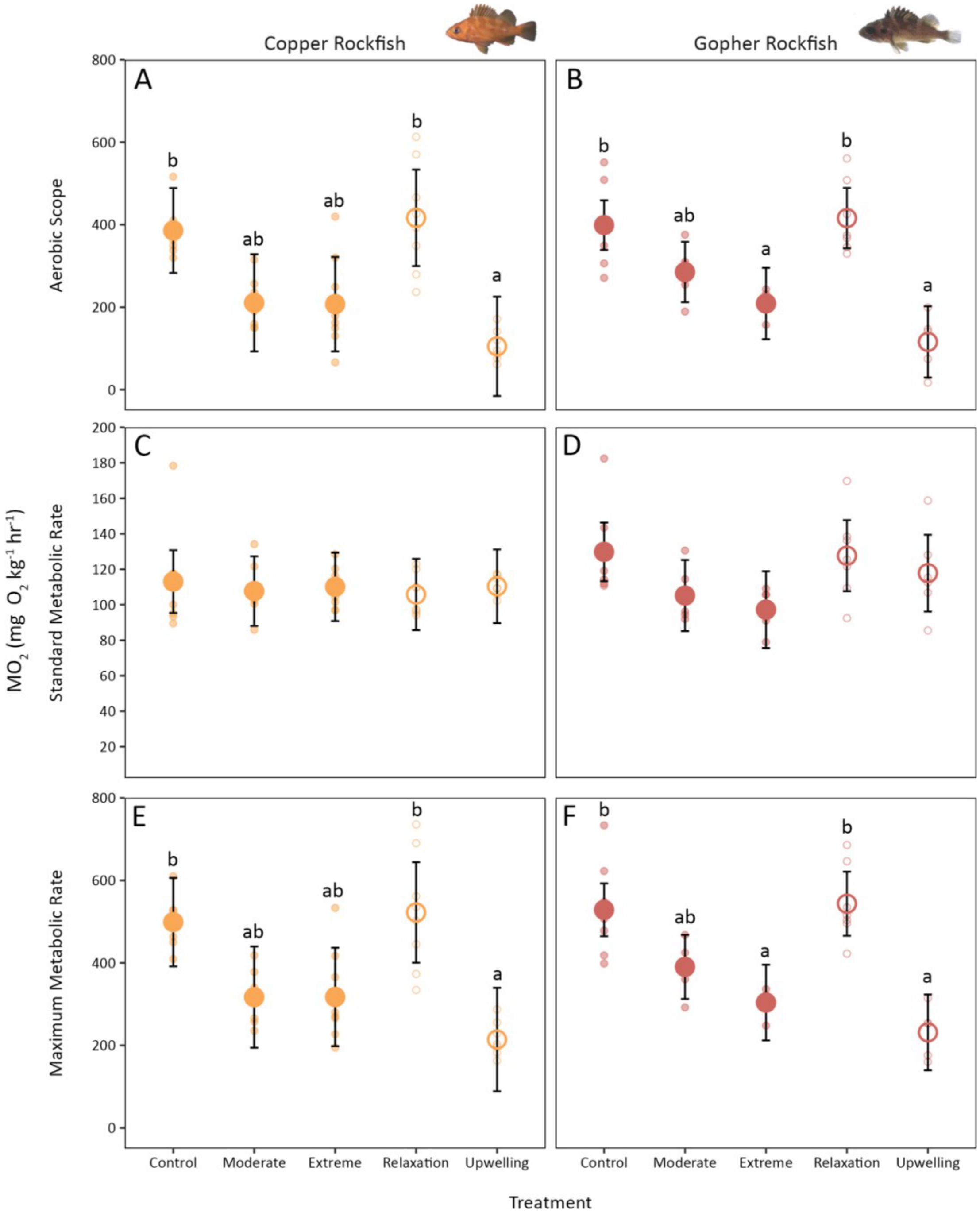

Figure 10. (a, b) Aerobic scope, (c,d) standard metabolic rate, and (e, f) maximum MO2 for copper and gopher rockfish. Large circles represent modeled means and small circles show data points. Error bars represent 95% confidence intervals. Shaded circles represent static treatments and open circles denote fluctuating treatments. Plots with letters had main effects that were significant. Values not sharing any letters within a plot are significantly different by the Tukey-test at the 5% level of significance.

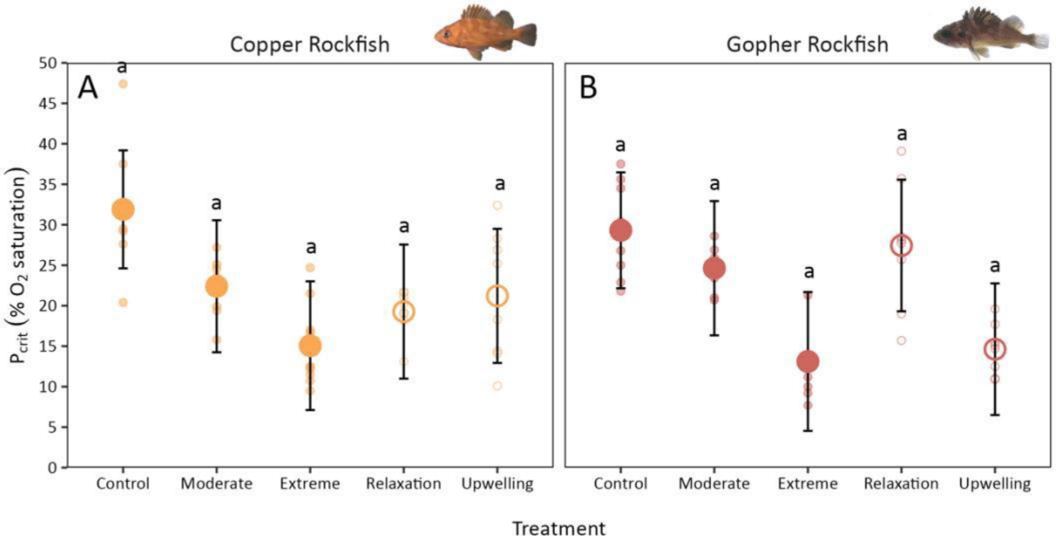

Figure 11. P_{crit}, or hypoxia tolerance for (a) copper and (b) gopher rockfish. A lower P_{crit} indicates a greater hypoxia tolerance. Large circles represent modeled means and small circles show data points. Error bars represent 95% confidence intervals. Shaded circles represent static treatments and open circles denote fluctuating treatments. Values not sharing any letters within a plot are significantly different by the Tukey-test at the 5% level of significance.

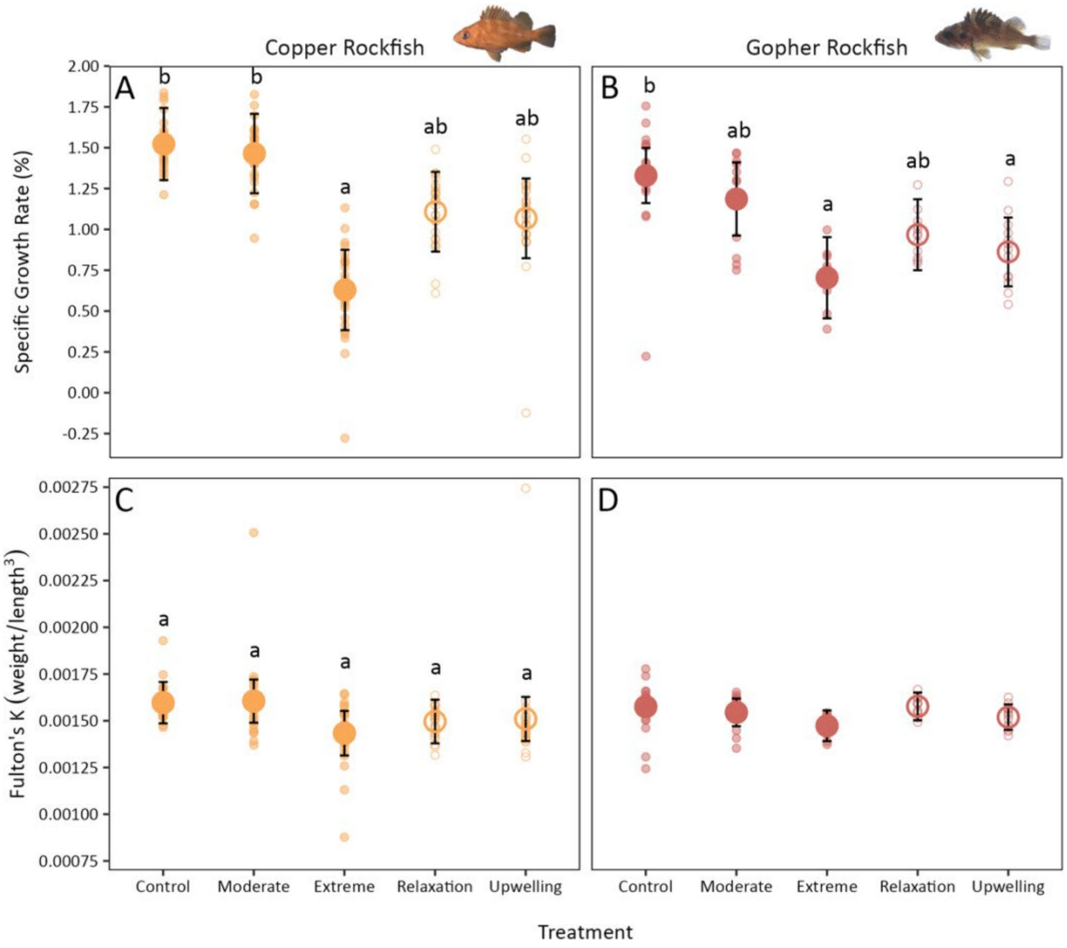

Figure 12. Specific growth rate (% initial mass, a and b) and final Fulton's K condition metric (measured at the end of the experiment, c and d) for copper and gopher rockfish. Large circles represent modeled means and small circles show data points. Error bars represent 95% confidence intervals. Shaded circles represent static treatments and open circles denote fluctuating treatments. Values not sharing any letters within a plot are significantly different by the Tukey-test at the 5% level of significance. Letters are not shown for Fulton's K for gopher rockfish because the single-species model was not significant overall.

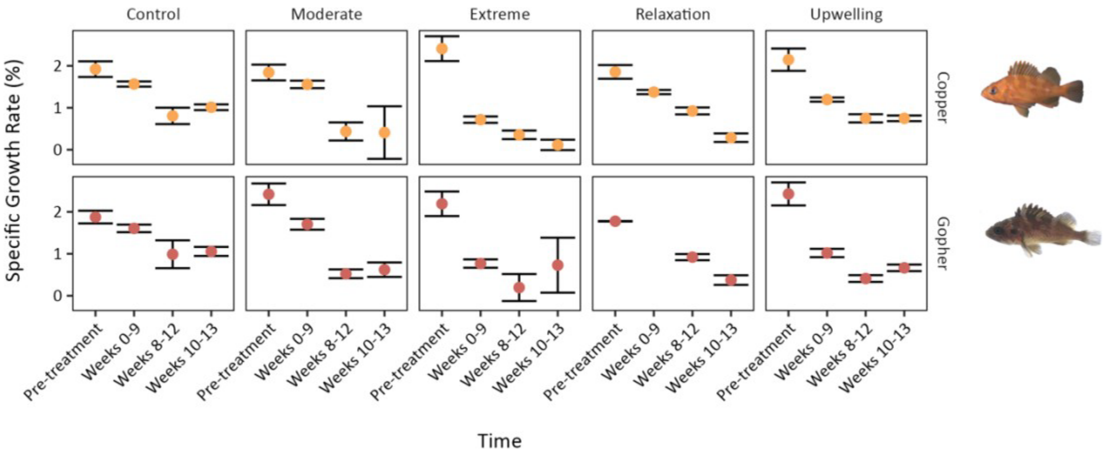

Figure 13. Specific growth rates (% day^{-1}) of copper and gopher rockfish at time points during the experiment. Circles represent means for each period and error bars represent standard error.

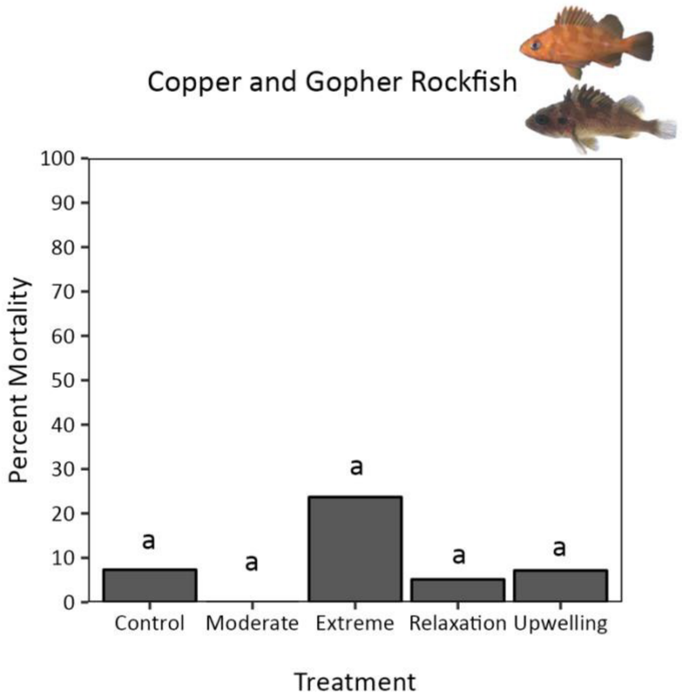

Figure 14. Percent mortality by treatment. Data for both copper and gopher rockfish are combined in this graph. Values not sharing any letters within a plot are significantly different by the Tukey-test at the 5% level of significance.

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