



Critical swimming speed of juvenile rockfishes (*Sebastes*) following long- and short-term exposures to acidification and deoxygenation

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ABSTRACT

Reef fishes in the California Current Ecosystem have evolved in habitats affected by seasonally variable, episodic upwelling of high $p\text{CO}_2$ (acidified, low pH) and low dissolved oxygen (deoxygenated) water, which suggests that these fishes might exhibit resilience to ocean acidification (OA) and deoxygenation. Yet, how the fitness of these fish are affected by natural variability in pH and DO over short time scales remains poorly understood, as do the effects of longer-term trends in pH and DO driven by climate change. We conducted a complementary suite of experiments to study the effects of acidification and deoxygenation on the critical swimming speed (U_{crit}) of juvenile copper (*Sebastes caurinus*) and black (*S. melanops*) rockfish collected from nearshore habitats in an ocean acidification “hotspot” off Northern California. We consistently observed that U_{crit} declined more strongly in response to deoxygenation than to acidification, at least under ranges of these stressors consistent with current conditions and plausible future scenarios, and that reduction in swimming performance reflected additive rather than synergistic responses to concurrent exposure. Reductions in swimming performance manifested quickly—on the scale of hours—in response to exposure to elevated $p\text{CO}_2$ /reduced DO, yet are reversible: swimming performance of juvenile rockfish recovers within a matter of days, and perhaps much more quickly, after acidified/deoxygenated conditions have subsided. Insights from this study address potential effects of variability in upwelling intensity at event and seasonal scales for nearshore rockfishes and contribute to our understanding of fish responses to future ocean conditions driven by ongoing climate change.

1. Introduction

In the California Current, as in other eastern boundary current systems, coastal upwelling exposes coastal environments to acidified and deoxygenated waters in at least two ways: the upwelling of deep waters, which are naturally more acidic and depleted in oxygen relative to the surface (Hauri et al., 2013; Booth et al., 2012; García-Reyes and Largier, 2010), and through biogenic hypoxia-acidification events driven by decay following intense, nutrient-fueled plankton blooms (Grantham et al., 2004; Chan et al., 2008; Bakun et al., 2010). The extent and intensity of these events are increasingly exacerbated by ongoing ocean acidification (Doney et al., 2009; Hauri et al., 2009; Feely et al., 2008), concurrent warming of surface waters that has reduced oxygen solubility and increased stratification (thereby reducing ventilation of deeper layers) (Keeling et al., 2010; Shaffer et al., 2009; Helm et al., 2011; Breitburg et al., 2018), and changes in atmospheric and oceanic circulation patterns that affect the source and characteristics of deep waters

that upwell along the coast (Bakun et al., 2015; Rykaczewski and Dunne, 2010). Thus, acidification and deoxygenation tend to be strongly correlated over a broad range of temporal scales, ranging from day- to week-long upwelling events, through seasonal fluctuations, to interannual and longer-term trends (Booth et al., 2012; Carstensen and Duarte, 2019).

Sensitivity to acidification and deoxygenation varies substantially across marine taxa (Vaquer-Sunyer and Duarte, 2008; Kroeker et al., 2010; Kroeker et al., 2013a; Steckbauer et al., 2020; Gobler and Baumann, 2016; Leung et al., 2022; Hu et al., 2022). Relative to calcifying organisms, fishes are expected to be less sensitive to ocean acidification due to well-developed acid-base regulation (Heuer and Grosell, 2014; Kroeker et al., 2010; Kroeker et al., 2013a). Studies of fishes from temperate systems, which are subject to strong seasonal fluctuations in pH (Feely et al., 2008; Hofmann et al., 2011), have documented resilience to acidification in some species (Melzner et al., 2009; Hurst et al., 2013), but have also indicated responses to acidification similar to those

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reported for fishes from less variable coral reef ecosystems (Hamilton et al., 2017; Jutfelt et al., 2013). More recent work indicates that variable conditions (oscillating treatments) at temporal scales matching natural systems can moderate the impacts of acidification relative to sustained exposures at levels approaching extrema of natural distributions (Toy et al., 2022).

Likewise, while many benthic or demersal fishes that inhabit nearshore habitats are capable of physiological, behavioral, and molecular responses that facilitate tolerance of low-oxygen (Mandic et al., 2009; Domenici et al., 2007), most fishes are broadly sensitive to changes in dissolved oxygen (Pollock et al., 2007; Farell and Richards, 2009; Domenici et al., 2013; Chabot and Dutil, 1999; Pichavant et al., 2001; Via et al., 1998; Lefrançois et al., 2005; Mattiasen et al., 2020). Sensitivity to changes in pH and dissolved oxygen can be especially acute during early life history stages (Brauner, 2008; DePasquale et al., 2015). Given the coupled nature of acidification and deoxygenation, it is critical to understand the physiological responses of fish to concurrent reductions in pH and DO (Booth et al., 2012). Available evidence indicates that these stressors predominantly have additive (non-interactive) effects on most taxa, including fishes (Steckbauer et al., 2020). Yet, it remains poorly understood how fish that evolved in habitats exposed to dynamic pH and DO conditions are affected by natural variability in pH and DO over short time scales (e.g., upwelling events), in addition to longer-term trends in pH and DO driven by climate change.

In this study, we examine the sensitivity of recently settled juvenile rockfish to acidification and deoxygenation, using as our metric critical swimming speed (U_{crit}), a standardized measure of maximal swimming performance that combines swimming speed (scaled to body length) and endurance (Brett, 1964; Tierney, 2011; Cano-Barbacid et al., 2020). Standard protocols for estimating U_{crit} have been widely used to assess the effects of environmental conditions on fish performance and provide insight as one reasonable proxy for ecological performance and fitness (Plaut, 2001; but see Leis, 2020). Negative effects of oxygen depletion on swimming performance have been well documented using the U_{crit} protocol for a broad range of freshwater and marine fishes (Domenici et al., 2013; Dahlberg et al., 1968; Jones, 1971; Jourdan-Pineau et al., 2010; Fu et al., 2011; Herbert and Steffensen, 2005; Brady et al., 2009). To a lesser degree, the U_{crit} protocol has also been used to study the effects of ocean acidification on larval and juvenile fish swimming performance, with responses ranging from limited or no sensitivity to acidification (Cominassi et al., 2019) to reduced swimming performance under acidification stress (Bignami et al., 2014; Hamilton et al., 2017).

For this study, the effects of exposure to acidification and deoxygenation on swimming performance were tested on juveniles of two species of rockfish: black rockfish (*Sebastes melanops*) and copper rockfish (*S. caurinus*). Both species occupy nearshore rocky reef and kelp forest habitats but differ in how they use these habitats and the timing of production and settlement of early life stages (Echeverria, 1987; Lenarz et al., 1995; Love et al., 2002; Carr, 1983). Black rockfish spend substantial time in the water column as adults, release larvae into the plankton in late winter, and settle into nearshore habitats in early summer. Copper rockfish are closely associated with benthic habitats, release their larvae during the early spring upwelling season, and settle into nearshore habitats in late summer. These differences in reproductive phenology and adult habits raise the possibility that these species also differ with respect to exposure to acidic, deoxygenated conditions arising from natural seasonal patterns, especially during their early life history (i.e., as larvae and pelagic juveniles, and during settlement to nearshore habitats). Even minor differences in a species depth distribution as they settle to nearshore habitats (e.g., to kelp canopies or to benthic reefs) can have important implications for exposure to low pH and low DO conditions (Frieder et al., 2012; Takeshita et al., 2015). Having evolved in dynamic upwelling systems, rockfishes are likely to have some resilience or ability to acclimate to acidified, deoxygenated conditions, even as juveniles (Davis et al., 2018). However, exposure to these drivers has been shown to induce changes in swimming

performance and behaviors that potentially increase predation risk for juvenile rockfishes as they transition from pelagic to benthic habitats (Hamilton et al., 2014; Hamilton et al., 2017; Mattiasen et al., 2020). Natural levels of variability in exposure (e.g., variability on temporal scales comparable to upwelling-relaxation events) have been observed to drive moderate responses relative to those arising under continuous, chronic exposure (Toy et al., 2022).

In this paper, we present results from a suite of experiments that (1) resolve independent and interactive effects of sustained exposure to acidification and deoxygenation on swimming performance of juvenile rockfish, (2) compare changes in swimming performance arising from sustained exposures (as a proxy for seasonal to longer-term trends) to those manifesting over short-term exposures at the scale of upwelling events, (3) assess response and resilience to short-term variability analogous to upwelling-relaxation events, and (4) where possible, assess differences between species in their sensitivity to acidification and deoxygenation. Long-term experiments are intended to assess responses to chronic exposure, while allowing sufficient time for acclimation responses to develop and ideally stabilize, whereas short-term or variable exposure experiments are designed to provide insight to responses on more immediate time scales. By examining effects on swimming performance in recently-settled juvenile rockfishes, we focus on a trait that is likely to have fitness consequences related to tradeoffs between foraging success and predator avoidance during the critical life history transition from pelagic habitats to nearshore reefs (Almany and Webster, 2006; Stier et al., 2017), and so represents a potential link between acidification and deoxygenation trends and population dynamics (recruitment) of a diverse suite of ecologically and economically important fishes in coastal ecosystems of the California Current.

2. Materials and methods

2.1. Ethics statement

All collections and experiments conducted for this study conformed to the ethical guidelines of California State Polytechnic University, Humboldt (Cal Poly Humboldt) Institutional Animal Care and Use Committee (permit #13114.M.43-A) and the California Department of Fish and Wildlife (Scientific Collecting Permit #13205).

2.2. Fish collections and husbandry

Recently settled juvenile rockfish were collected from shallow, rocky reef habitats in Trinidad Bay, CA (41.0593° N, 124.1431° W) during summer to early fall (June–September) of 2015–17. Most individuals were collected using SMURFs (Standard Monitoring Unit for the Recruitment of Fishes, Ammann, 2004) deployed 1 m below the surface on moored buoys to mimic kelp canopy habitat. SMURFs were recovered at intervals ranging from several days to two weeks by free-divers using hand-held BINCKE nets (Benthic Ichthyofauna Net for Coral/Kelp Environments, Anderson and Carr, 1998) to envelop the SMURF and associated fishes. SMURFs were subsequently brought aboard a small boat, where fish were collected in buckets of seawater and quickly brought to land for preliminary identification and measurement. Additional fish were captured by directly sweeping BINCKE nets through subsurface kelp canopy adjacent to SMURF sites (maximum depth of 3 m). All fish retained for laboratory trials were transported immediately to the Marine Lab at Cal Poly Humboldt, where they were carefully segregated by size and species into holding tanks supplied with well-aerated, chilled seawater (~12 °C, pH ~7.95, ~8.5 mg O₂ L⁻¹) from the recirculating laboratory seawater system.

Juvenile rockfish were acclimated to ambient laboratory conditions for at least two weeks prior to introduction to experimental conditions. Early in this acclimation period, each fish selected to be in the experimental pool was given a unique mark based on a combination of colors and locations of subcutaneous elastomer tags (Northwest Technologies).

Fish were fed commercially available krill every day in 2015 and every other day in 2016, and were fed nutritionally enriched pellets every other day in 2017. Tanks were cleaned regularly using handheld nets or siphons to remove uneaten food and debris. Timer-controlled white LEDs affixed directly to the top of each tank were used to maintain a consistent 12 h:12 h light:dark photoperiod throughout the experimental period. Tanks were shrouded in black plastic to minimize visual disturbance of fishes and to reduce disruption of photoperiod due to lab lighting, and were equipped with artificial habitat (folded plastic fencing affixed to weights) to approximate natural structure and allow individuals to seek refuge at will. All elements of aquarium infrastructure were conditioned in lab seawater for several weeks prior to introduction of fish.

2.3. Estimation of critical swimming speed (U_{crit})

Swimming trials were conducted in a 10 L swim tunnel (Loligo Model 10) designed to generate laminar flow in the test chamber (dimensions: 38x10x10 cm), immersed in a larger (30L) water bath to maintain target temperatures. A calibration curve relating motor speed (Hz) to flow speed ($m\ s^{-1}$) in the measurement chamber was developed from measurements of flow speed with a hand-held digital flow meter equipped with a vane wheel probe (Höentzsch).

In all cases, fish were starved for approximately 24 (range 20–28) hours prior to swimming trials, and velocities were scaled to the total body length (BL, in mm) of each fish. At the start of a swimming trial, the fish was measured, weighed, and evaluated with a rapid assessment of general condition and health (Depestele et al., 2014), then gently introduced to the test chamber facing into a slow ($1/2\ BL\ s^{-1}$) current and allowed to acclimate for 15 min. Environmental conditions in the swim tunnel were matched to the appropriate experimental treatment conditions by recreating conditions in a recirculating sump connected to the swim tunnel. Following acclimation, flow speed was increased to $1\ BL\ s^{-1}$ for two minutes, then increased by $1\ BL\ s^{-1}$ every two minutes until the fish became fatigued (could no longer maintain swimming

position for the entire two minutes). If the fish rested against the back grate, the observer first used pulses of bright light to motivate the fish to continue swimming, or, if that failed, used a brief current reversal and resumption of flow to push the fish away from the rear grate to continue the trial. After a fish rested against the back grate three times, it was considered fatigued, the trial was ended, and the fish was returned to its treatment tank for recovery. Any substantial (but brief) pauses were excluded from the measure of time swum so that the data reflect the time a fish spent actively swimming into the current. Each fish was tested once.

Following protocols used previously to test swimming performance of juvenile rockfish (Fisher et al., 2007; Kashef et al., 2014), U_{crit} was calculated as

$$U_{crit} = \left(U_i + U \times \left(\frac{t}{t_i} \right) \right)$$

where U_i is the highest velocity maintained for a full-time interval ($t_i = 120\ s$), U is the velocity increment ($1\ BL\ sec^{-1}$), and t is time elapsed at fatigue velocity (in seconds). Although U_{crit} is scaled to body length, fish were selected to span similar size distributions across all treatments within a species (see Table 1). To account for the effects of variability in flume temperature among trials (Table 1), U_{crit} estimates were standardized to $12\ ^\circ C$ using a temperature coefficient $Q_{10} = 1.9$, which is consistent with Q_{10} values estimated in respiration studies of scorpaenid fishes reported in the literature (Boehlert and Yoklavich, 1983; Kita et al., 1996; Vetter and Lynn, 1997). Swimming trials resulted in no acute or delayed mortalities.

2.4. Experimental infrastructure

Experimental conditions were generated and maintained in 270 L cylindrical reservoirs (~2 m height, 60 cm diameter), each of which supplied a set of rectangular treatment tanks (80 L; 40 cm × 40 cm × 60 cm). Specific reservoir-treatment tank configurations are described below for each experiment. The issue of limited replication inherent in

Table 1

Summary of realized treatment conditions (pH, DO, temperature, pCO₂) and fish length for a series of experiments (Experiments 1, 2a, 2b, and 3) assessing the effect of long-term exposure to acidified or deoxygenated conditions on critical swimming speed (U_{crit}) of juvenile copper and black rockfish. Standard deviations for length, pH, DO, and temperature, and the upper and lower limits of measured pCO₂ are shown parenthetically.

Year, Species	Treatment	Length (mm)	pH	DO (mg O ₂ L ⁻¹)	Temp (°C)	pCO ₂ (ppm)
Experiment 1: Independent effects of long-term exposure to acidification or deoxygenation stress						
2015, Copper	Control	63.3 (3.4)	7.95 (0.03)	8.38 (0.09)	12.36 (0.16)	492 (479,505)
2015, Copper	pH 7.8	64.9 (3.4)	7.80 (0.05)	8.33 (0.17)	12.50 (0.14)	699 (681,718)
2015, Copper	pH 7.5	64.8 (2.4)	7.53 (0.04)	8.28 (0.21)	12.65 (0.17)	1387 (1350,1424)
2015, Copper	pH 7.3	62.9 (3.2)	7.28 (0.04)	8.34 (0.17)	12.61 (0.15)	2602 (2533,2672)
2015, Copper	DO 6.0	61.8 (4.2)	7.94 (0.02)	5.99 (0.11)	12.74 (0.18)	503 (490,517)
2015, Copper	DO 4.0	64.1 (2.4)	7.94 (0.02)	4.01 (0.13)	12.71 (0.14)	505 (491,518)
2015, Copper	DO 2.0	65.2 (2.8)	7.96 (0.01)	2.20 (0.11)	12.73 (0.11)	476 (464,489)
Experiments 2a & 2b: Independent and concurrent effects of long-term exposure to acidification/deoxygenation stress						
Year, Species	Treatment	Length (mm)	pH	DO (mg O ₂ L ⁻¹)	Temp (°C)	pCO ₂ (ppm)
2016, Black	Control	75.6 (3.2)	7.95 (0.02)	8.59 (0.21)	11.79 (0.56)	485 (473,499)
2016, Black	pH 7.5	76.0 (3.0)	7.47 (0.06)	8.55 (0.22)	12.02 (0.62)	1592 (1550,1636)
2016, Black	DO 4.0	78.4 (2.7)	7.95 (0.02)	4.09 (0.23)	12.14 (0.61)	489 (476,502)
2016, Black	pH 7.5*DO 4.0	76.8 (1.8)	7.51 (0.05)	4.30 (0.21)	12.07 (0.69)	1470 (1431,1510)
2016, Copper	Control	60.6 (2.5)	7.95 (0.02)	8.59 (0.21)	11.79 (0.56)	485 (473,499)
2016, Copper	pH 7.5	60.8 (1.2)	7.47 (0.06)	8.55 (0.22)	12.02 (0.62)	1592 (1550,1636)
2016, Copper	DO 4.0	59.6 (1.2)	7.95 (0.02)	4.05 (0.23)	12.14 (0.61)	489 (476,502)
2016, Copper	pH 7.5*DO 4.0	58.9 (1.5)	7.51 (0.05)	4.30 (0.31)	12.07 (0.69)	1470 (1431,1510)
Experiment 3: Chronic vs. cyclic exposure to concurrent acidification/deoxygenation stress						
Year, Species	Treatment	Length (mm)	pH	DO (mg O ₂ L ⁻¹)	Temp (°C)	pCO ₂ (ppm)
2017, Black	Control	83.0 (6.5)	7.84 (0.04)	8.24 (0.20)	12.28 (0.14)	638 (621,655)
2017, Black	Static moderate	79.5 (8.1)	7.50 (0.06)	4.25 (0.82)	12.09 (0.24)	1505 (1465,1545)
2017, Black	Static extreme	78.7 (6.7)	7.32 (0.09)	2.69 (0.97)	12.54 (0.32)	2318 (2256,2380)
2017, Black	Upwelling	81.5 (5.8)	7.35 (0.01)	2.26 (0.13)	12.13 (0.09)	2169 (2112,2228)
2017, Black	Relaxation	79.5 (5.9)	7.79 (0.01)	7.95 (0.13)	12.35 (0.09)	723 (704,743)

these designs (per Cornwall and Hurd, 2016) are recognized; we address relevant concerns in detail in the Discussion and through synthesis of repeated experiments sharing common design elements.

Seawater from the reservoirs was delivered at 10 mL s^{-1} . Turnover time for water in the treatment tanks was approximately 2.2 h. Treatment tanks were fitted with lids and drain ports were installed to minimize head space and the potential for gas exchange with the atmosphere. Water temperature in each reservoir was maintained at approximately $12 \text{ }^\circ\text{C}$ by partially submerging all reservoirs in a 1200 L temperature-controlled water bath.

Treatment conditions were established and maintained in reservoirs as follows. Acidified conditions were generated by delivery of CO_2 (industrial grade, Eureka Oxygen Supply) at low pressure through an air stone, controlled by Loligo Systems CapCTRL software (v 1.0) in response to measurements taken at 1 s intervals with integrated temperature-pH probes (WTW pH 3310, Loligo Systems). pH sensors were calibrated prior to installation, recalibrated intermittently throughout the course of experiments, and were replaced as needed. Several rounds of alkalinity assays were conducted over the course of the experiments reported here, and combined with concurrent measurements of salinity, temperature, and pH to estimate $p\text{CO}_2$ levels corresponding to our acidification treatments (Table 1). Estimated total alkalinity was approximately $2108 \pm 65 \text{ } \mu\text{mol kg}^{-1}$ across all assays. $p\text{CO}_2$ in our treatment tanks was $\sim 490 \text{ } \mu\text{atm}$ at pH 7.95 (ambient laboratory water), $\sim 710 \text{ } \mu\text{atm}$ at pH 7.8, $\sim 1490 \text{ } \mu\text{atm}$ at pH 7.5 and $\sim 3140 \text{ } \mu\text{atm}$ at pH 7.3.

Deoxygenated conditions were generated by bubbling nearly pure ($>97\%$) nitrogen gas (N_2 ; industrial grade, Eureka Oxygen Supply) through a micro-diffuser (Pentair Aquatic Ecosystems, 0.4 Micro Bubble Diffuser, $30 \times 3 \text{ cm}$) suspended approximately 1 m depth. The supply of nitrogen gas was controlled via Loligo Systems WitroxCTRL software for Windows in response to measurements of oxygen saturation taken at 1 s intervals using fiber optic mini sensors connected to a Loligo System Witrox 4. Adjustments to dissolved oxygen measurements were based on temperature measured in a central reservoir that served as a proxy for all reservoirs, salinity measurements taken from daily water quality assessments of the laboratory water supply, and fixed barometric pressure (1013 hPa). Oxygen sensors were calibrated prior to installment and intermittently throughout the course of experiments using a two-point calibration (0% and 100% air-saturated distilled water).

Temperature, DO, and pH in reservoirs and treatment tanks were checked regularly (typically daily) with a Hach HQ40d multi-parameter meter fitted with pH (pHC101) and DO (LDO101) probes. Assessments based on concurrent, high-frequency monitoring of conditions in treatment tanks and reservoirs confirmed that input flows to treatment tanks were sufficient to maintain consistent temperature regimes across all tanks and to prevent fish from affecting water chemistry, and thus to maintain stable conditions in treatment tanks.

2.5. Experiments

The following sections outline the experimental design, species tested, and statistical analysis for our set of long- and short-exposure experiments. Differences in species used across experiments reflect natural variability in the availability of recently settled juveniles of our two target species. All statistical analyses were conducted in the statistical programming language R (R version 4.2.2, 2022), taking advantage of capacities for data manipulation and visualization provided under the *tidyverse* family of packages (Wickham et al., 2019), and methods for model fitting and analysis implemented in several R packages indicated below.

Table 1 and Table 2 summarize realized conditions and experiment design for a set of three long-term exposure experiments. For each long-term exposure experiment, juvenile rockfish were introduced to treatment tanks and allowed to acclimate further for 2–3 days under ambient laboratory conditions ($\sim 12 \text{ }^\circ\text{C}$, pH ~ 7.95 , $\sim 8.5 \text{ mg O}_2 \text{ L}^{-1}$).

Table 2

Summary of experimental design (year, species, treatment, target pH, target DO, range of exposure duration, and sample size per treatment tank) for a series of experiments (Experiments 1, 2a, 2b, and 3) assessing the effect of long-term exposure to acidified or deoxygenated conditions on critical swimming speed (U_{crit}) of juvenile copper and black rockfish.

Year	Species	Treatment	Target pH	Target DO (mg $\text{O}_2 \text{ L}^{-1}$)	Exposure duration (d)	N/tank
Experiment 1: Independent effects of long-term exposure to acidification or deoxygenation stress						
2015	Copper rockfish	Control	7.95	8.5	45–46	4, 4
2015	Copper rockfish	pH 7.8	7.8	8.5	60–62	5, 5
2015	Copper rockfish	pH 7.5	7.5	8.5	56	4, 4
2015	Copper rockfish	pH 7.3	7.3	8.5	55–57	4, 4
2015	Copper rockfish	DO 6.0	7.95	6	44–52	4, 5
2015	Copper rockfish	DO 4.0	7.95	4	43–61	4, 4
2015	Copper rockfish	DO 2.0	7.95	2	48–54	6
Experiments 2a & 2b: Independent and concurrent effects of long-term exposure to acidification/deoxygenation stress						
2016	Black rockfish	Control	7.95	8.5	66–69	4, 4
2016	Black rockfish	pH 7.5	7.5	8.5	66–68	4, 4
2016	Black rockfish	DO 4.0	7.95	4	68–91	4, 4
2016	Black rockfish	pH 7.5*DO 4.0	7.5	4	67–100	4, 4
2016	Copper rockfish	Control	7.95	8.5	66–71	4, 4
2016	Copper rockfish	pH 7.5	7.5	8.5	66–67	4, 4
2016	Copper rockfish	DO 4.0	7.95	4	68–73	4, 4
2016	Copper rockfish	pH 7.5* DO 4.0	7.5	4	67–68	4, 4
Experiment 3: Chronic vs. cyclic exposure to concurrent acidification/deoxygenation stress						
2017	Black rockfish	Control	7.95	8.5	58–65	7, 8
2017	Black rockfish	Static moderate	7.5	4	56–63	6, 7
2017	Black rockfish	Static extreme	7.3	2	57–64	5, 8
2017	Black rockfish	Upwelling (pulsed)	7.3, 7.95	2, 8.5	59–60	7, 8
2017	Black rockfish	Relaxation (pulsed)	7.95, 7.3	8.5, 2	61–62	8, 8

Experimental conditions were then gradually established over the course of 24–48 h. Swimming performance experiments were conducted after 44–100 days of exposure to treatment conditions (Table 2). Prior to the experiments reported here, individual fish had been tested in two low-stress behavioral trials (escape response and brain lateralization as reported in Flannery, 2018 following protocols adapted from Domenici et al., 2013, Jutfelt et al., 2013), but had been allowed to recuperate in their treatment tanks for at least 10 days prior to swimming trials.

We account for possible correlation among individuals in the same tank by including “tank” as a random effect (on intercept) nested within treatment (reservoir) in a linear mixed effects model (LME). Treatment and fish length (measured at the time of U_{crit} assessment) were included as fixed effects. LME models were fit using functions in R package *lmerTest* (Kuznetsova et al., 2017). Models that also included random tank effects on the slope of responses were explored, but almost

universally failed to converge given the available data and were not considered further. Statistical significance of random tank effects was evaluated by a likelihood-ratio test (function *lmertest::rand*; Kuznetsova et al., 2017). Measures of goodness-of-fit for models that included (r_{full}^2) or excluded (r_{fix}^2) random effects were estimated with function *r.squaredGLMM* in package *MuMIn* (Bartoń, 2019) to characterize the relative contribution of random effects in explaining variability in the data. Regardless of significance, random tank effects were retained in all models to faithfully reflect experimental design and as a source of information for future experiments (Faraway, 2016). Given limitations on sample size, we made no effort to exclude outliers. Statistical significance was evaluated at $\alpha = 0.05$. Where relevant, model selection was based on AICc (Akaike information criterion, with correction for small sample sizes) calculated with function *MuMIn::AICc* (Bartoń, 2019).

2.5.1. Experiment 1: independent effects of long-term exposure to acidification or deoxygenation stress (2015; copper rockfish)

Juvenile copper rockfish were reared under seven different treatment conditions: three reduced pH treatments (target pH of 7.8, 7.5, 7.3; ambient DO ~ 8.5 mg O₂ L⁻¹), three reduced DO treatments (target concentrations of 6.0, 4.0, 2.0 mg O₂ L⁻¹; ambient pH ~ 7.95), and ambient (control) conditions (DO ~ 8.5 mg O₂ L⁻¹, pH ~ 7.95). Each treatment condition (including control conditions) was maintained in a single reservoir. Each reservoir supplied two treatment tanks, except in the case of the DO = 2.0 mg O₂ L⁻¹ treatment, for which challenges in producing sufficiently deoxygenated water without elevating pN₂ to the point of inducing nitrogen-bubble disease precluded adequate supply for a second treatment tank. Ten juvenile copper rockfish were introduced to each treatment tank. U_{crit} was measured for only a subset of individuals in each treatment ($n = 4$ –6/tank; 6–10/treatment). U_{crit} experiments were conducted 43–62 days post treatment exposure. Separate LME models were fit to describe U_{crit} as a linear response to either acidification or deoxygenation using the same set of control fish for both analyses.

2.5.2. Experiments 2a & 2b: independent and concurrent effects of long-term exposure to acidification/deoxygenation stress (2016; copper and black rockfish)

Juvenile copper and black rockfish were reared under four treatments generated by crossing pH and DO levels: control (\sim pH 7.95, 8.5 mg O₂ L⁻¹), acidified (pH 7.5, 8.5 mg O₂ L⁻¹), deoxygenated (\sim pH 7.95, 4.0 mg O₂ L⁻¹), and combined stressors (pH 7.5, 4.0 mg O₂ L⁻¹). The three treatment conditions were each replicated in two reservoirs, each of which supplied two treatment tanks. Control conditions were maintained in a single reservoir that served four treatment tanks. Ten rockfish were introduced to each tank, and rockfish were segregated by species to avoid potential effects of species interactions on individuals' responses. This resulted in two tanks per treatment for both species (one per species per treatment reservoir, two per species for the control reservoir; ten individuals per tank). U_{crit} was estimated for a subset of copper ($n = 4$ /tank; 8/treatment) and black rockfish ($n = 4$ /tank; 8/treatment) from each treatment. U_{crit} experiments on copper rockfish were conducted after 66–73 days treatment exposure and on black rockfish after 66–100 days treatment exposure.

Species responses were modeled separately. For each species, we used AICc to assess relative support for an LME model describing the independent effects of acidification or deoxygenation on U_{crit} relative to a similar model that included an interaction between acidification and deoxygenation effects.

2.5.3. Experiment 3: chronic vs. cyclic exposure to concurrent acidification/deoxygenation stress (2017; black rockfish)

Juvenile black rockfish were exposed to four different treatments: three static treatments spanning a gradient of intensity in concurrent acidification and deoxygenation conditions: control/ambient (\sim pH

7.95, 8.5 mg O₂ L⁻¹), intermediate (pH 7.5, 4.0 mg O₂ L⁻¹), and extreme (pH 7.3, 2.0 mg O₂ L⁻¹) and one oscillating treatment in which conditions shifted between extreme (pH 7.3, 2.0 mg O₂ L⁻¹) and ambient (\sim pH 7.95, 8.5 mg O₂ L⁻¹) conditions every 8 days in order to mimic transitions between periods of strong upwelling and relaxation. Transitions were implemented over the course of 8 h. Static treatments represent constant exposure to one of these two extremes or to the long-term average between upwelling and relaxation conditions.

Each static treatment was maintained in a single reservoir that supplied two treatment tanks. The oscillating treatment was implemented in two reservoirs, set to be out of phase with one another, each of which supplied two treatment tanks. Maintaining complementary, out-of-phase oscillating treatments allowed assessment of swimming performance under both “upwelling” and “relaxation” conditions for fish with similar histories of captivity. Ten juvenile black rockfish were introduced into each treatment tank. U_{crit} was estimated for a subset of black rockfish ($n = 8$ /tank; 16/treatment). U_{crit} trials were conducted 56–65 days post treatment exposure. For fish exposed to fluctuating treatments, U_{crit} trials were conducted on day 6 of exposure to “upwelling” or “relaxation” conditions.

Because acidification and deoxygenation were explicitly linked in this experiment, we analyzed the data using LME models in which treatment (i.e., one of the static treatments, or the phase of the oscillating treatment) was included as a fixed factor in the analysis, and “tank” was treated as a random factor nested within treatment. Post-hoc comparisons among treatments were evaluated with Tukey tests as implemented in package *multcomp* (Hothorn et al., 2017).

2.5.4. Experiment 4: independent and concurrent effects of short-term exposure to deoxygenation/acidification stress (2016 and 2017; copper rockfish)

We assessed the effect of short-term (1–24 h) exposure to acidification (pH 7.5, 8.5 mg O₂ L⁻¹), deoxygenation (pH \sim 7.95, 4.0 mg O₂ L⁻¹), and combined stressors (pH 7.5, 4.0 mg O₂ L⁻¹) on the swimming performance of juvenile copper rockfish captured in 2016 and 2017. These experiments were structured to complement the long-term interactive effects experiment conducted in 2016 and were repeated in 2017 to include a control group that more appropriately accounted for any handling effect (see below).

Prior to exposure, fish were maintained in holding tanks with well-aerated seawater (pH \sim 7.95, 8.5 mg L⁻¹ DO). To implement short-term exposures, individuals were placed in 1 L jars perforated with 1 cm holes and floated in designated treatment tanks for a set period (1, 2, 4, 8, 24 h in 2016; 2, 4, 8, and 24 h in 2017). In 2017, fish were also held in jars for similar periods under ambient (pH \sim 7.95, 8.5 mg O₂ L⁻¹) conditions as a control for any ‘handling effect’ on swimming ability. (Measurements of U_{crit} collected in 2016 for fish taken directly from the acclimation tanks under ambient conditions are excluded from the analysis as they represent an inadequate control for the effect of jar enclosure on swimming performance.) Fewer fish were tested at each treatment-exposure combination in 2017 ($n = 2$ –5) than in 2016 ($n = 4$ –10) due to the limited number of copper rockfish available in 2017.

Data from the two short-term exposure experiments were combined to accommodate the lack of an appropriate control group in 2016. Generalized linear models (GLMs) were fit to model U_{crit} as a function of swim flume pH, swim flume DO, and exposure time. ‘Year’ was included as a fixed factor in this analysis to account for potential differences among cohorts. Model selection (AICc) was used to discriminate among models that differed with respect to inclusion of interactions among the three treatment effects (acidification, deoxygenation, and duration of exposure).

3. Results

For all long-term exposure experiments, we were able to fit mixed-models that included random effects of tank nested within treatment

on the intercept of linear models relating critical swimming speed (U_{crit}) of juvenile rockfish to the severity of acidification (unit pH) or deoxygenation ($\text{mg O}_2 \text{L}^{-1}$) conditions. Models that included an effect of fish length on swimming performance were universally rejected on the basis of parsimony ($\Delta\text{AICc} > 2$ relative to comparable models that lack a term for fish length) and are not considered further. Effects are expressed as (linear) slopes describing a response in terms of the change in U_{crit} per unit change in the stressor, i.e., $\text{BL s}^{-1} (\text{unit pH})^{-1}$ for response to acidification and $\text{BL s}^{-1} (\text{mg O}_2 \text{L}^{-1})^{-1}$ for deoxygenation. To place these metrics in more relevant terms, we also apply modeled relationships to calculate relative effects (percent reductions in U_{crit}) associated with moderate experimental (and ecologically relevant) ranges for each stressor (i.e., $\text{pH} = 7.5$ or $\text{DO} = 4 \text{ mg O}_2 \text{L}^{-1}$).

3.1. Experiment 1: independent effects of long-term exposure to acidification or deoxygenation stress (2015; copper rockfish)

Exposure to acidification stress had a negative but non-significant effect on swimming performance (Fig. 1, $p = 0.217$, slope = 1.27 (s.e. = 0.92); $\sim 7\%$ reduction in U_{crit} at $\text{pH} 7.5$). In this analysis, we detected significant random effects of tank-within-treatment ($p < 0.001$) that accounted for a substantial fraction of the variability in the data ($r_{fix}^2 = 0.12$, $r_{full}^2 = 0.60$).

Swimming performance declined significantly in response to decreased dissolved oxygen (Fig. 1, $p < 0.001$, slope = 0.49 (s.e. = 0.07); $\sim 26\%$ reduction in U_{crit} at $4 \text{ mg O}_2 \text{L}^{-1}$). For these trials, random effects of tank-within-treatment were not significant ($p > 0.5$) and explained only a small fraction of the variability in the data ($r_{fix}^2 = 0.71$ v. $r_{full}^2 = 0.76$).

3.2. Experiments 2a & 2b: independent and concurrent effects of long-term exposure to acidification/deoxygenation stress (2016; copper and black rockfish)

Our observations most strongly indicate that acidification and deoxygenation have independent (additive) effects on swimming performance for both copper rockfish and black rockfish (Fig. 2). For both

species, deoxygenation (exposure to $\text{DO} \sim 4 \text{ mg O}_2 \text{L}^{-1}$) degraded swimming performance (Fig. 2, copper rockfish: $p = 0.02$; slope = 0.180 (s.e. = 0.053), $\sim 11\%$ reduction at $4 \text{ mg O}_2 \text{L}^{-1}$; black rockfish: $p = 0.03$; slope = 0.216 (s.e. = 0.073), $\sim 12\%$ reduction at $4 \text{ mg O}_2 \text{L}^{-1}$). The effect of acidification (exposure to $\text{pH} 7.5$) on swimming performance was not significant, though there was a negative trend for both species (Fig. 2, copper: $p = 0.103$, slope = 1.074 (s.e. = 0.539), $\sim 8\%$ reduction at $\text{pH} 7.5$; black: $p = 0.157$, slope = 1.211 (s.e. = 0.728), $\sim 9\%$ reduction at $\text{pH} 7.5$). Joint exposure reduced U_{crit} by 19% and 21% for copper and black rockfish, respectively. In both cases, our analysis identified significant random effects of tank nested within treatment ($p < 0.03$) that accounted for approximately one-third of the variation in the data explained by the full model (copper rockfish: $r_{fix}^2 = 0.54$ v. $r_{full}^2 = 0.74$; black rockfish: $r_{fix}^2 = 0.46$ v. $r_{full}^2 = 0.68$).

3.3. Experiment 3: chronic vs. cyclic exposure to concurrent acidification/deoxygenation stress (2017; black rockfish)

Swimming performance in black rockfish was reduced by chronic exposure to concurrent acidification and deoxygenation stress, and the magnitude of this decline increased with the intensity of the combined stressors (Fig. 3). The response to cyclical (concurrent) stressors depended on the phase of the exposure cycle during which U_{crit} was measured. Swimming performance for fish on day six of “relaxation” conditions did not differ from that of fish maintained at ambient conditions. Likewise, U_{crit} measured on day six of “upwelling” conditions was indistinguishable from that of fish in the “static extreme” ($\text{pH} 7.3$, $2 \text{ mg O}_2 \text{L}^{-1}$). Statistically significant reductions in U_{crit} ($\sim 22\text{--}24\%$ reduction) were observed under both “upwelling” ($p = 0.033$) and “static extreme” treatments ($p = 0.043$). Continuous exposure to moderate stressors ($\text{pH} 7.5$, $4 \text{ mg O}_2 \text{L}^{-1}$) reduced $U_{crit} \sim 9\%$. Random effects of tank-within-treatment were significant ($p < 0.001$) and accounted for about 40% of the overall fraction of variation explained by the model ($r_{fix}^2 = 0.467$, $r_{full}^2 = 0.754$).

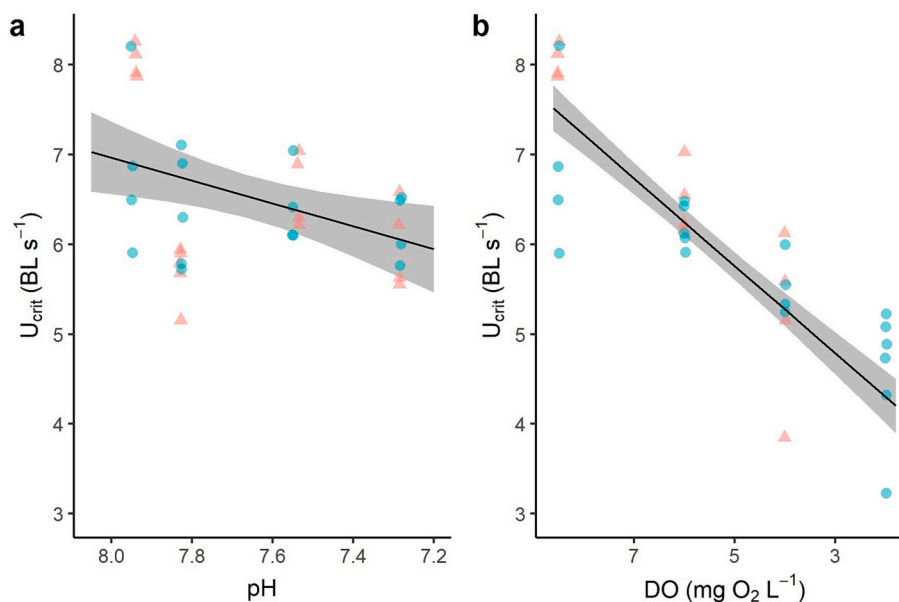


Fig. 1. Results from Experiment 1: Effect of long-term exposure to (a) acidification and (b) deoxygenation on critical swimming speed (U_{crit}) of juvenile copper rockfish. Points are jittered for visualization. Solid line (grey ribbon, standard error) indicates fixed effect of the stressor on swimming performance from linear mixed effects model fit with tank as random effect nested in treatment. Colored symbols (blue circles and red triangles) identify individuals from different tanks within treatments, but do not imply any similarities among tanks across treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

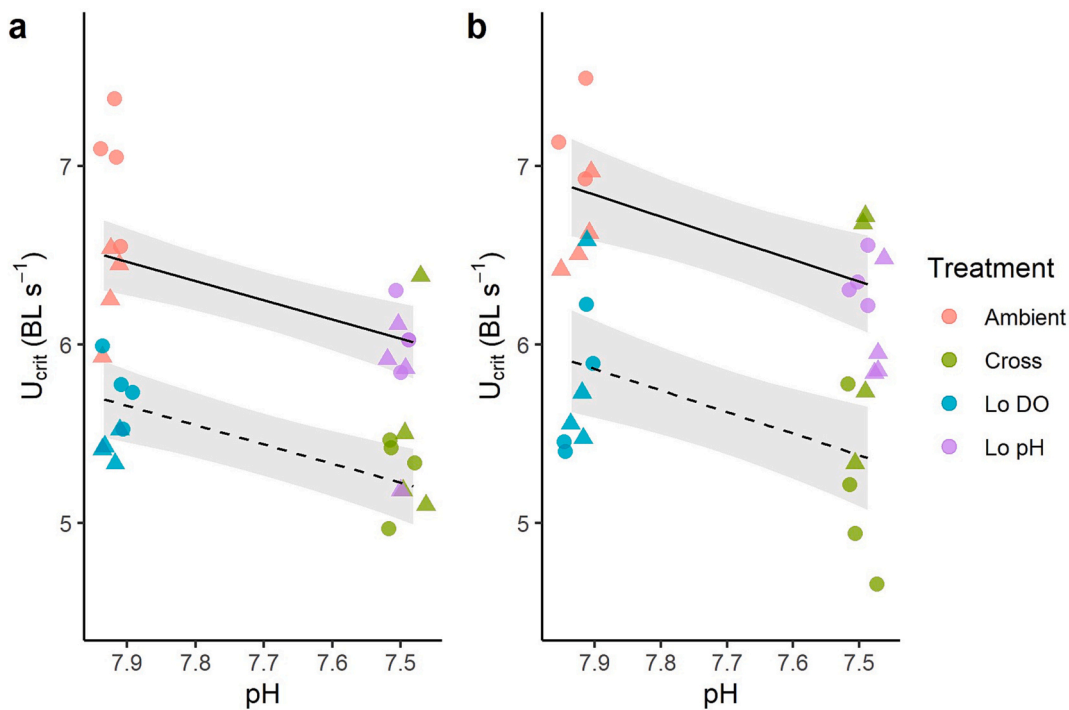


Fig. 2. Results from Experiments 2a & 2b: Responses of critical swimming speed in (a) juvenile copper rockfish (*Sebastes caurinus*) and (b) black rockfish (*S. melanops*) to independent or concurrent exposures to acidification and deoxygenation stressors based on fits of linear mixed effects model with additive effects and random intercepts associated with tank (nested within treatment). Response to acidification is indicated by the slope of the line. Response to deoxygenation is indicated by the offset between lines (upper, solid lines are cases with $\text{DO} \sim 8 \text{ mg O}_2 \text{ L}^{-1}$; lower, dashed lines are for $\text{DO} \sim 4 \text{ mg O}_2 \text{ L}^{-1}$). Each symbol indicates an individual U_{crit} measurement, jittered along the x-axis to improve visualization. Color indicates experimental treatment. Symbols differentiate tanks within treatment but do not imply correlation across treatments.

3.4. Synthesis of responses to long-term exposure

Based on review of results from the long-term experiments, we pursued two post hoc syntheses to leverage information gained from repeated tests and to test for similarity of responses across experiments.

3.4.1. Meta-analysis of long-term exposure experiments (experiments 1, 2a, & 2b)

The magnitude and direction of U_{crit} stressor slopes show remarkable agreement across the three long-term experiments for which independent responses to acidification and deoxygenation were examined (Experiments 1, 2a, and 2b). Estimates of effect sizes from a simple meta-analysis (function *rma* in package *metafor*; Viechtbauer, 2010; Fig. 4) indicate that, collectively, these experiments resolve statistically significant responses of swimming performance to both stressors (deoxygenation: $p = 0.003$; slope = 0.292 (s.e. = 0.097); acidification: $p = 0.002$; slope = 1.150 (s.e. = 0.392)). Using our moderate cases as a baseline, these effects correspond to declines on the order of 1.3 BL s^{-1} for a drop in DO from $8.5 \text{ mg O}_2 \text{ L}^{-1}$ to $4.0 \text{ mg O}_2 \text{ L}^{-1}$ and of 0.5 BL s^{-1} for a drop in pH from 7.95 to 7.5.

3.4.2. Combined analysis of black rockfish response to acidification and deoxygenation stress (experiment 2b [2016] & 3 [2017])

Correlation between acidification and deoxygenation conditions precludes analysis to decompose independent contributions of each stressor to the observed response using only the Experiment 3 (2017) data. Visual comparison, however, suggests that the joint response to acidification and deoxygenation was similar to that observed in the Experiment 2b (2016) cross-factorial experiment. To explore the hypothesis of a consistent response to acidification and deoxygenation, we built on the analysis conducted for Experiment 2b (2016) by (1) augmenting the data set to include observations from the chronic exposure treatments in the Experiment 3 (2017), treating these as additional

observations of responses to concurrent acidification and deoxygenation, and (2) including ‘year’ as a fixed effect (factor) to account for differences among cohorts. This analysis identified a significant effect of cohort (year) ($p < 0.001$); on average, fish swam 1.68 BL s^{-1} faster in 2016 than in 2017, a difference which accounted for approximately 40% of the variability in the combined data set. Random tank effects were statistically significant ($p < 0.001$) but accounted for a modest amount of variation in the data explained by the overall model ($r_{\text{fix}}^2 = 0.789$, $r_{\text{full}}^2 = 0.901$) (Fig. 5).

Deoxygenation stress had a detrimental effect on swimming performance across the combined data set ($p = 0.021$, slope = 0.164 (s.e. = 0.06)). Swimming performance also decreased with increasing acidification, but this relationship was not statistically significant ($p = 0.242$, slope = 0.774 (s.e. = 0.623)). The model that included acidification and deoxygenation was moderately preferred to a model that included only deoxygenation ($\Delta\text{AICc} = 1.61$). A model that included interaction between deoxygenation and acidification had relatively poor support based on the available data ($\Delta\text{AICc} = 2.43$).

3.5. Experiment 4: independent and concurrent effects of short-term exposure to deoxygenation/acidification stress (2016 and 2017; copper rockfish)

All models fit to combined data for the short-term exposure experiments indicate that confinement in holding jars reduced U_{crit} ($p \leq 0.001$, slope = -0.025 (s.e. = 0.007) $\text{BL s}^{-1} \text{ h}^{-1}$ in the best model) and that swimming performance differed significantly between cohorts ($p \leq 0.001$; U_{crit} was approximately 0.87 BL s^{-1} greater in 2016 than in 2017). The best model among those examined identified additive effects of acidification ($p = 0.015$, slope = 0.926 (s.e. = 0.377)) and deoxygenation ($p < 0.001$, slope = 0.207 (s.e. = 0.033)) (Fig. 6). Restricting analysis to the sparser 2017 data returned qualitatively similar results, with the exception that the detrimental effect of exposure time was less

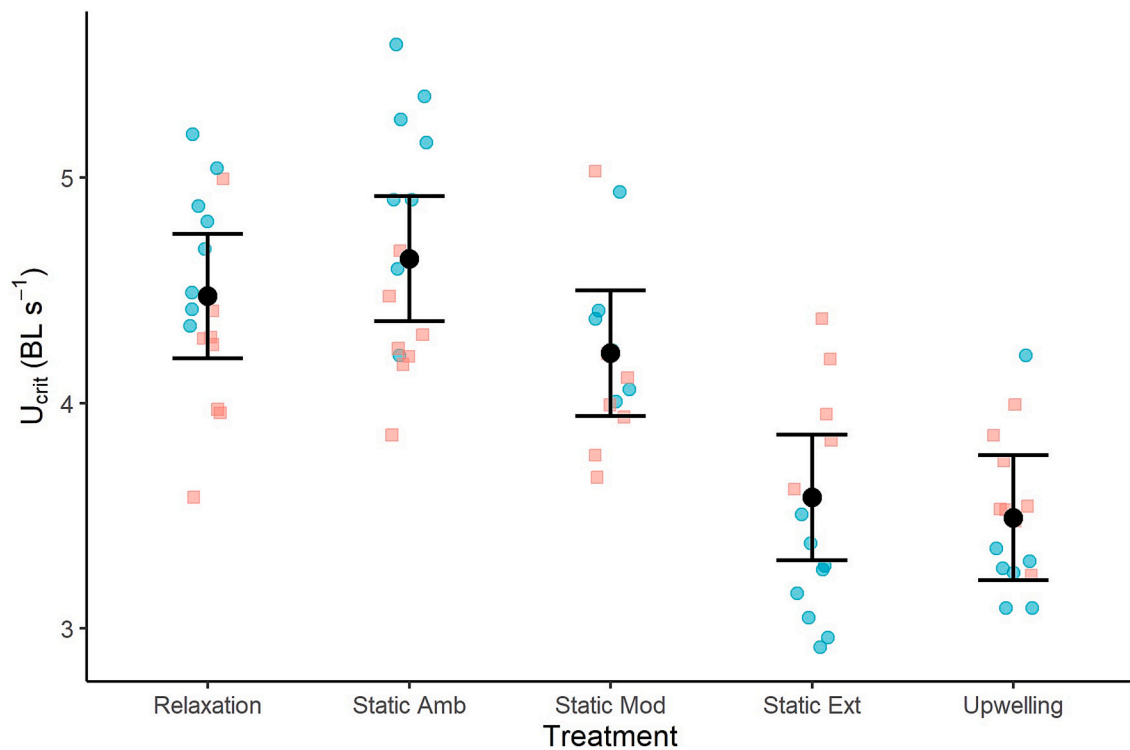


Fig. 3. Results from Experiment 3: Critical swimming speed in juvenile black rockfish (*Sebastes melanops*) following exposure to sustained, concurrent acidification and deoxygenation scenarios (“Static.Amb” = control conditions (~pH 7.95, 8.5 mg O₂ L⁻¹), “Static.Mod” = pH 7.5, 4.0 mg O₂ L⁻¹, and “Static.Ext” = pH 7.3, 2.0 mg O₂ L⁻¹), or to conditions that switched between strong “upwelling” (pH 7.3, 2.0 mg O₂ L⁻¹) and “relaxation” (~pH 7.95, 8.5 mg O₂ L⁻¹) conditions every eight days. Swimming trials for fish were conducted six days into the latest phase of “upwelling” or “relaxation”. Each symbol indicates an individual U_{crit} measurement, jittered along the x-axis to improve visualization. Colored symbols (red squares and blue circles) differentiate tanks within treatment but do not imply correlation across treatments. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clearly resolved (results not shown).

4. Discussion

This study presents data from a suite of complementary experiments designed to assess how swimming performance in two species of juvenile rockfish (copper rockfish, *S. caurinus*, and black rockfish, *S. melanops*) is affected by exposure to acidification and deoxygenation over periods ranging from a few hours to several weeks, and across levels of acidification and deoxygenation ranging from present-day conditions to those anticipated to emerge in the coming decades under continued anthropogenic enrichment of atmospheric (and thus oceanic) carbon dioxide concentrations. Results from these experiments support three general conclusions. First, despite having evolved in a highly dynamic upwelling environment naturally subject to strong fluctuations in pH and dissolved oxygen, juvenile black and copper rockfishes are susceptible to detrimental impacts of deoxygenation and acidification on their swimming ability under both acute and chronic exposures. Second, the effect of reduced oxygen on swimming performance appears to be stronger than that of acidification, at least under realistic ranges for these stressors. Third, juvenile rockfish exhibit the ability to recover following exposure to acidification and deoxygenation conditions, at least on the scale of upwelling events. We revisit these patterns in detail below, following a brief discussion of statistical considerations that weigh on our interpretations.

4.1. Statistical considerations and caveats

With respect to our long-term experiments, the configuration of treatment tanks (‘evaluation units’) associated with reservoirs (‘experimental units’), and the potential for correlated responses by individual

fish housed in a given treatment tank substantially limits the degree of replication realized in each experiment (Cornwall and Hurd, 2016). Such constraints on (true) replication inherent in our study designs mandate cautious interpretation of results from our long-term experiments, especially if they are considered in isolation. Nevertheless, the consistency among responses observed across the suite of experiments provides substantial confidence – bolstered here by application of meta-analytical approaches – that the effects measured in our experiments are real and represent patterns that at the very least provide strong motivation for further investigation through repeated or better replicated experimental studies.

We generally found it to be essential to include random effects in our models to account for coherent, and in some cases surprisingly strong, shifts in mean U_{crit} among tanks within treatments. Indeed, for some experiments, random effects accounted for a substantial fraction of the variability captured in the fitted models. Random effects tended to carry greater weight in analyses of the response of U_{crit} to acidification than in analyses of deoxygenation effects, reflecting the relatively weak effects of changes in pH on swimming performance. By retaining random tank effects in our models, regardless of statistical significance, we ensure that our results faithfully reflect experimental design and can serve as a richer source of information for future experiments (Faraway, 2016).

Some fraction of this variability could reflect our practice of assessing all fish from a tank in sequence, before moving on to assess fish in another tank. Such differences in the date or time of day (e.g., morning versus afternoon) that fish in one tank were assessed relative to fish in another could have contributed to the observed variability. We adopted this protocol over a more random arrangement of trials across treatments to minimize the extent of capture efforts and the need for extended segregation of tested from not-yet-tested individuals before returning them to their treatment tank. We also suspect that other

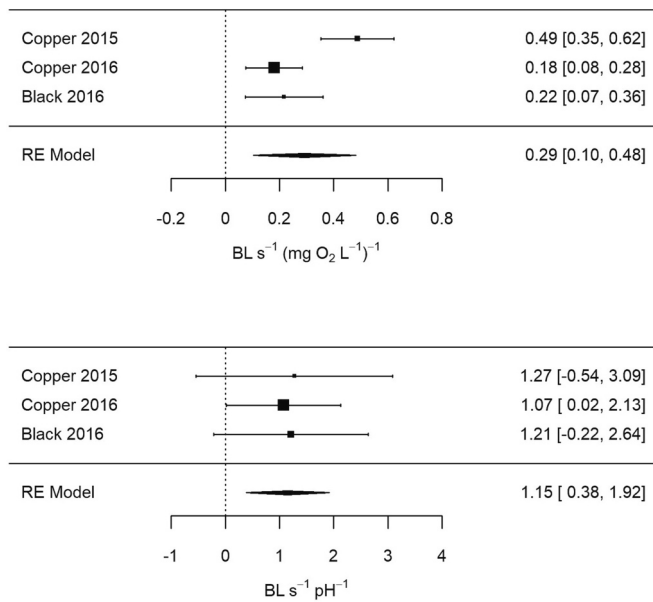


Fig. 4. Forest plot summarizing meta-analytical results from independent random effects (RE) models fit to effect size (slope) of deoxygenation (top panel) and acidification (bottom panel) on swimming speed (U_{crit} in $BL s^{-1}$) of juvenile black and copper rockfish during long-term exposure experiments (Experiments 1, 2a, and 2b). For each experiment, mean effect size is indicated by a square with upper and lower bars representing 95% confidence intervals, and symbol size indicates the relative weight of the experiment estimated during the meta-analysis (a function of sample size and uncertainty in the estimated effect size). The diamond summarizing results from the RE model is centered on the overall effect size and spans the confidence interval around that estimate.

factors might contribute to observed variability among tanks, such as subtle differences in environmental conditions associated with the arrangement of treatment tanks. We attempted to explore these hypotheses through analysis of models that included any of several candidate covariates, e.g., duration of exposure, mean tank conditions, conditions in the swim flume, etc., but found no compelling evidence for an underlying driver of coherent variability associated with individual treatment tanks.

4.2. Concurrent responses to deoxygenation and acidification

Exposure to deoxygenation clearly reduced swimming performance of juvenile copper and black rockfish in our experiments, a pattern that is consistent with a broad literature on fishes' physiological response to reduced oxygen and, more specifically, with results from studies focused on swimming performance (Domenici et al., 2013; Jourdan-Pineau et al., 2010; Fu et al., 2011; Brady et al., 2009; Mattiasen et al., 2020). We strongly suspect that this response arises from constraints on oxygen supply to the slow-twitch red muscles that enable prolonged swimming (Domenici et al., 2013).

Exposure to acidified conditions had a negative, but weaker effect than deoxygenation on swimming performance of juvenile copper and black rockfish, at least under the range of conditions explored in our experiments. This is consistent with previous evidence for reduced swimming performance in response to acidification in juveniles of some rockfish species (Hamilton et al., 2017) and several other taxa (Pimentel et al., 2014; Pimentel et al., 2015), as well as reports of relative insensitivity to acidification in other fishes (Bignami et al., 2014; Melzner et al., 2009), including blue rockfish (*S. mystinus*) (Hamilton et al., 2017). Erosion of swimming performance by exposure to acidified waters has been linked to reduction of oxygen uptake and delivery driven by acidification of the blood (e.g., hypercapnia) and respiratory tissue

pigments (Pörtner et al., 2004) and shifts in energy allocation and depletion of ATP stores driven by costly acid-base regulation (Heuer and Grosell, 2014).

Our results are broadly consonant with patterns reported by Steckbauer et al. (2020) for vertebrate responses to deoxygenation and acidification, in that responses to deoxygenation were stronger than to acidification over ecologically relevant ranges, and that additive rather than synergistic or antagonistic responses to concurrent stressors are most strongly supported by available evidence. Data from both experiments that employed a crossed-treatment design offered mild support ($\Delta AICc \sim 1.1-1.4$) for models that included interaction terms, but in both cases, the interaction term was insignificant, and the model offered a trivial increase in explained variation. Our short-term experiment offered some evidence for a synergistic interaction between acidification and deoxygenation on swimming U_{crit} in which (if real) acidified conditions increase sensitivity to deoxygenation (or deoxygenation increases sensitivity to acidification). Such a synergy would be consistent with patterns of acidification increasing physiological sensitivity of estuarine silversides to hypoxia reported by Miller et al. (2016) and evidence that acidification-driven hypercapnia reduces the efficacy of oxygen uptake and delivery (Pörtner et al., 2004; Heuer and Grosell, 2014). Future studies should continue to assess the potential for synergistic effects in a multi-stressor context (Boyd et al., 2018) and consider the potential for acclimation to mitigate the responses to acidification or deoxygenation during extended exposures (Hamilton et al., 2017; Davis et al., 2018).

4.3. Time scales of response and recovery

Our short-term experiments suggest that reductions in swimming performance manifest quickly upon exposure to deoxygenated or acidified water. We suspect that the decline in swimming performance that were statistically attributed to the duration of exposure (and confinement in the holding jars) might reflect physiological relaxation from immediate, acute "flight" responses to handling (e.g., Fu et al., 2011) or diminishing capacity caused by close confinement in the holding jar prior to the swimming trial. Based on observations of consistent reductions in U_{crit} across treatments over the range of exposure durations, we conclude that exposure to acidified or deoxygenated water has a rapid effect on swimming performance, independent of handling and recovery effects (Fu et al., 2011). Rapid onset of stress impacts on swimming performance is corroborated by our oscillating upwelling-relaxation experiments, which revealed that reductions in swimming performance are substantially developed within days (in contrast to weeks or months) of exposure to concurrent acidification and deoxygenation.

The oscillating experiments also demonstrated the potential for rockfish to recover swimming abilities comparable to unexposed fish within six days after "upwelling conditions" had subsided. We suspect that responses and recovery were achieved much more rapidly than the several-day time scale examined in the oscillating-conditions experiment. Regardless, these observations demonstrate that effects are temporary, at least at scales of upwelling-event variability. Further studies are necessary to test how quickly recovery manifests, whether the intensity or duration of acidification and deoxygenation exposures constrains recovery, and, more generally, how realistic variability affects the magnitude and persistence of responses that emerge across the organism (Hamilton et al., 2017; Toy et al., 2022).

4.4. Temperature/warming

In this study, we isolate responses of swimming performance to acidification and deoxygenation from responses to temperature by maintaining all fish within a narrow range of temperatures ($\sim 11-13^\circ C$) consistent with conditions in coastal waters affected by weak upwelling and by applying Q_{10} relationships to account for the limited variability

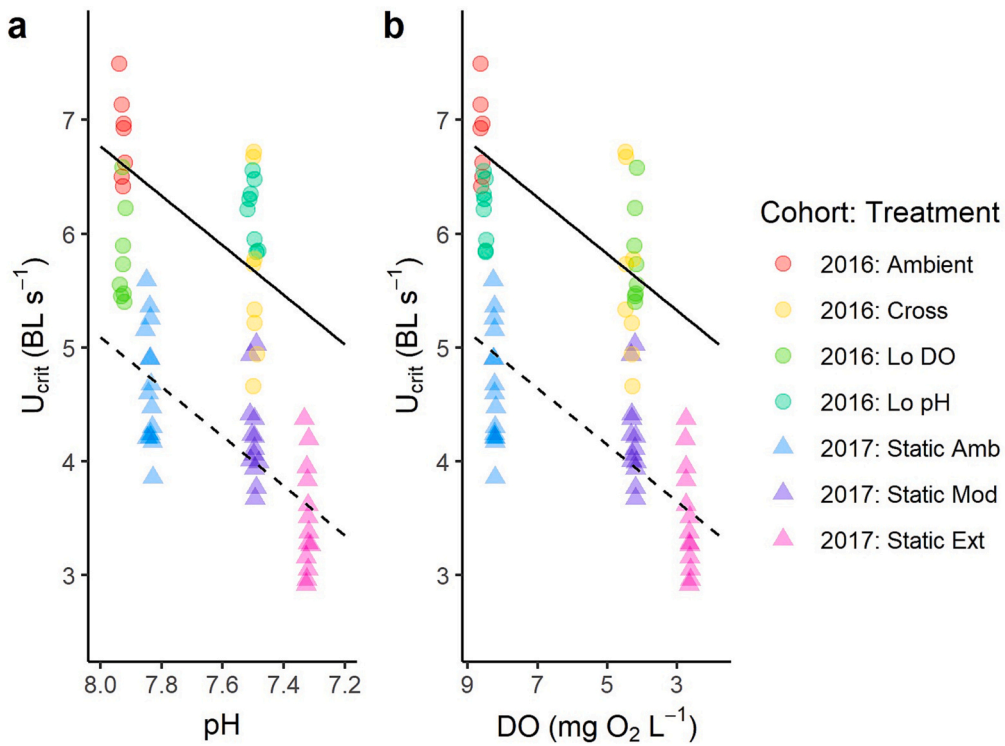


Fig. 5. Additive effects of acidification stress (left panel) and deoxygenation stress (right panel) on U_{crit} of juvenile black rockfish from Experiment 2b (2016) and static, interactive treatments from Experiment 3 (2017). Lines indicate joint effect of acidification and deoxygenation plotted against pH (left) and DO (right); note that the data are the same, and only the scale and spacing along the x-axis changes with the stressor. Note that data points for the independent stressor cases (2016: Lo DO and 2016: Lo pH) are not modeled by these lines (and shift position along the respective x-axes) but are presented as part of the combined data set considered in the analysis. The vertical offset indicates the difference in swimming ability between 2016 (solid line) and 2017 (dashed line) cohorts. Symbol shape and color indicate the cohort and treatment for each set of observations combined in this analysis. Note that observations from un-crossed treatments (“Lo DO” and “Lo pH”) in Experiment 2b (2016) are included for context, but independent effects are not illustrated in this plot.

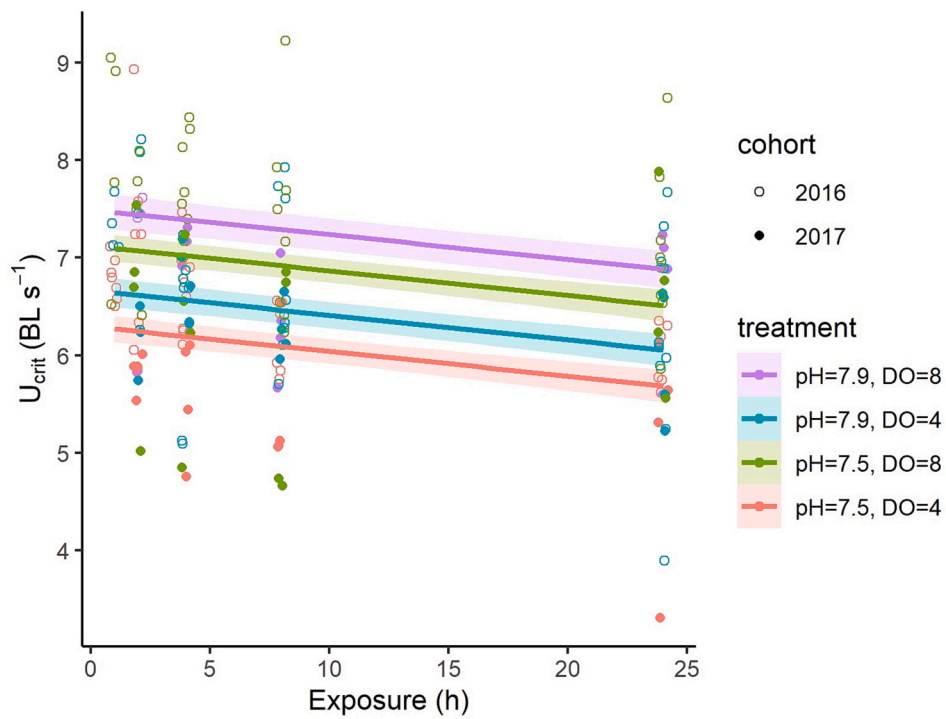


Fig. 6. Results from Experiment 4: U_{crit} of juvenile copper rockfish (*Sebastes caurinus*) following short-term (1–24 h) exposures to independent or concurrent acidification and deoxygenation stressors. Lines indicate fixed effects responses from a linear mixed effects model with additive effects for stressor treatments and exposure duration and random intercepts associated with cohort (year). Data set combines observations from trials conducted on fish collected in 2016 (open circles) and 2017 (filled circles).

in temperature observed during swimming trials. Though we are unaware of any direct assessments of juvenile rockfish swimming performance in response to temperature, it is assumed rockfishes are like other fishes in being able to swim faster under warmer conditions up to some tolerance threshold (e.g., McMahon et al., 2020; McDonnell and Chapman, 2016; Yu et al., 2018). Though it is possible that long-term increases in ocean temperature might offset some of the negative effects of reduced oxygen and acidification on swimming performance, acidification and hypoxia events in upwelling systems typically occur in concert with the delivery of colder waters into nearshore habitats. Thus, it is plausible that reductions in swimming performance under acidified, deoxygenated conditions will be exacerbated by cooler temperatures. Future studies are needed to assess swimming performance in the context of concurrent changes in temperature, pH, and dissolved oxygen, whether driven by upwelling-relaxation dynamics or ongoing climate change. These experiments will require careful design to efficiently assess interactive effects of multiple stressors (Boyd et al., 2018), and, especially in the case of coastal upwelling systems, require careful selection of scenarios likely to represent future conditions.

4.5. Comparisons across species and cohorts

Black and copper rockfish represent two broad classes of rockfishes present in the California Current Ecosystem that differ in the timing of early life history relative to seasonal upwelling (Love et al., 2002) and so span an important axis of diversity within the genus *Sebastes*. Despite these differences, we found that swimming performance of juvenile black and copper rockfish was similarly sensitive to acidified and deoxygenated conditions, a conclusion most robustly supported by similarities in species-specific responses to independent deoxygenation and acidification treatments (2016 experiments).

Observed sensitivities (slopes of responses) were also consistent across cohorts, despite evidence that, for both species, juveniles captured in 2017 did not swim as strongly as those in the 2016 cohort. We suspect that differences in cohorts' swimming capabilities are a consequence of systemic year-to-year changes in the duration in laboratory acclimation and husbandry conditions, especially diet, rather than any unperceived changes in how the swimming trial protocol was implemented from one year to the next. Fish assessed in both long- and short-term experiments in 2016 had been in captivity longer than fish tested in 2017, and at the time of testing, juvenile black rockfish in the long-term experiments were smaller in 2016 (mean TL = 76.7 ± 2.8 mm) than in 2017 (80.5 ± 6.6 mm). Copper rockfish used in the short-term experiments were of similar mean size but spanned a broader range of sizes in 2017 (2016: 55.0 ± 3.1 mm; 2017: 57.6 ± 8.1 mm). Changes in diet (krill in 2016 to pellets in 2017) supported more rapid growth in 2017, suggesting enhanced nutritional benefits of pellets, and a hypothesis that satiation might reduce possibly motivation to swim and forage and subsequent degradation of swimming ability. Additionally, the ambient/control laboratory seawater pH was naturally lower in 2017 compared to 2016 (Table 1), which might have led to differences in swimming performance among cohorts.

We cannot rule out lingering effects on individuals' sensitivity to low-pH, low-DO conditions linked to environmental conditions experienced by juvenile rockfish during early life history (i.e., plasticity) or by their parents during oogenesis (i.e., transgenerational effects) (Miller et al., 2012; Murray et al., 2014; Schunter et al., 2018). Ocean conditions affecting the 2016 cohort were strongly influenced by the 2015–16 El Niño (Jacox et al., 2016), during which conditions were warmer and the scope and intensity of acidification and deoxygenation events in coastal waters was not strongly driven by upwelling. In contrast, the 2017 cohort experienced conditions marked by a moderate return to more typical upwelling conditions and greater potential to expose rockfish early life history stages to cooler temperatures and a greater range of acidified and deoxygenated conditions (Thompson et al., 2018).

We found that the sensitivity of copper rockfish to acidification was

generally similar to that reported by Hamilton et al. (2017) for copper rockfish collected off central California. Our observations for black rockfish, however, contrast sharply with their findings for blue rockfish (*S. mystinus*) from central California, which showed no sensitivity in swimming performance under acidified conditions (Hamilton et al., 2017). We had anticipated the possibility of observing milder responses (indicative of greater tolerance) in copper rockfish collected off northern California, a region noted as an acidification hotspot marked by strong, sustained exposures of nearshore habitats to low pH waters during active upwelling events (Feely et al., 2008; J. Jacobsen, E. Bjorkstedt, J. Abell unpublished results). Our study, however, coincided with unusual climate forcing that generally suppressed strong upwelling and thus reduced the magnitude of naturally occurring acidification and deoxygenation exposures that might have otherwise induced resilience. The qualitative difference in response between black and blue rockfish is somewhat surprising, as these species share many life history characteristics, particularly with respect to the phenology of early life history (Love et al., 2002; Echeverria, 1987), but might reflect slight differences in earlier recruitment and shallower depth distribution of black rockfish compared to blue rockfish during early life history (Lenarz et al., 1991; Carr, 1983). Exposure to acidification and deoxygenation can vary substantially over minor differences in depth distribution (Frieder et al., 2012; Takeshita et al., 2015). Future studies based on common-garden experiments involving fish from different regions under common rearing conditions would provide opportunity to assess latitudinal variation in species' sensitivity, whether arising from local adaptation or plasticity (Vargas et al., 2017), and to take advantage of comparative assays of metabolic activity, gene-regulation, or genetic variability to elucidate drivers of shared or divergent responses (e.g., Hamilton et al., 2017).

4.6. Ecological implications

Juvenile rockfishes used in this study were captured shortly after completing the critical life history transition from pelagic to nearshore habitats, a process that involves reaching and selecting a settlement habitat while navigating a 'predator gauntlet' (Almany and Webster, 2006; Stier et al., 2017). While directly relating laboratory measures of performance, such as sustained swimming speed, to ecologically relevant behaviors or dynamics is not entirely straightforward (Leis, 2020; Leis and Fisher, 2006; Plaut, 2001; Fuiman et al., 2006); swimming ability is fundamental to an individual's ability to escape predation, forage effectively, maintain position in a current, and perhaps, to complete settlement to nearshore habitats in the first place (e.g., Montgomery et al., 2001). The impact of reduced swimming may be exacerbated by concurrent (and possibly correlated, Laubenstein et al., 2019) behavioral or sensory sensitivities to acidification or deoxygenation that affect individuals' ability to identify and orient towards suitable habitats, increase their susceptibility to predation, or reduce their ability to forage efficiently (e.g., Hamilton et al., 2014; Jutfelt et al., 2013; Hamilton et al., 2017; Davis et al., 2018).

Our results indicate juvenile rockfish lack the ability to rapidly acclimate to changes in pH and DO conditions at the scale of discrete upwelling events. The potential for predators of juvenile rockfish also to experience concurrent impacts on swimming performance, olfaction, and behavior (e.g., attack responses) complicates the challenge of elucidating ecological impacts of acidification and deoxygenation (Davis et al., 2018; Kroeker et al., 2013b). If predators and prey differ in their sensitivities to acidification and deoxygenation, the cumulative ecological impact of such exposures on rockfish population dynamics will depend in part on temporal patterns of settlement and subsequent exposure to upwelling conditions in predator and prey (Jones and Mulligan, 2014; Ammann, 2004). Likewise, just as alongshore structure and gradients in upwelling intensity structure settlement over space (Woodson et al., 2012), spatial variability in coastal oceanographic structure will influence exposure to acidification and deoxygenation stressors (e.g., Chan et al., 2017), with possible implications for post-

settlement survival and subsequent population dynamics. At larger spatial and temporal scales, ongoing intensification and poleward shifts in upwelling (Sydeman et al., 2014; Rykaczewski et al., 2015), changes in the characteristics of source waters (Rykaczewski and Dunne, 2010) and novel climate events (e.g., marine heatwaves) can be expected to alter the spatial pattern of acidification and deoxygenation exposures, and how these stressors overlap with temporal and spatial drivers of recruitment in these stocks (Field and Ralston, 2005; Ralston et al., 2013).

5. Conclusions

Insights from this study add to the growing body of evidence that marine organisms are susceptible to acidification and deoxygenation stresses that continue to intensify in marine environments (Breitburg et al., 2018; Doney et al., 2020) and that presently manifest in coastal upwelling regions (Gruber et al., 2012; Bograd et al., 2022). We consistently observed that U_{crit} declined more strongly in response to deoxygenation than to acidification, at least under ranges of these stressors consistent with current conditions and plausible future scenarios, and that reductions in swimming performance reflected additive rather than synergistic responses to concurrent exposure. While reductions in swimming performance manifested quickly (within hours) in response to exposure to reduced pH and DO, the effects are also reversible: swimming performance of juvenile rockfish recovered within a matter of days, and perhaps much more quickly, after acidified/deoxygenated conditions had subsided.

Much of this work yields insight into what might best be characterized as worst-case responses to emerging future conditions. More recently, research has focused on the potential for acclimation to stressful conditions, adaptive phenotypic plasticity, epigenetic responses that buffer offspring against stressors experienced by parents, or local adaptation at generational time scales (Vargas et al., 2017; Ho and Burggren, 2012; Miller et al., 2012; Murray et al., 2014), and has stressed the need for understanding impacts across multiple life history stages (Hurst et al., 2021).

Our ability to forecast future impacts of acidification and deoxygenation on important species and ecosystems will require carefully designed experiments and long-term studies that focus on trophic and competitive interactions (e.g., Davis et al., 2018; Kroeker et al., 2013b) and account for the range of sensitivities across individuals (Fuiman and Cowan Jr, 2003; Laubenstein et al., 2019). Performance measures such as U_{crit} are useful indicators of how individuals are affected by stressful conditions, but these effects ultimately need to be explored in a more holistic context and translated into ecological outcomes. More directly, future experiments should build on results such as ours to better resolve non-linear responses and to identify thresholds beyond which species' abilities to tolerate further stress rapidly degrades (Sokolova, 2013). Identifying such thresholds within individuals (and the cumulative population impact of individual responses) is essential for realistically projecting species responses to long-term shifts in the envelope of acidification and deoxygenation exposures (Andersson et al., 2015; Kroeker et al., 2017). Any such forecasts are, of course, conditional on robust understanding of how ongoing enrichment of atmospheric CO₂ and resultant environmental trends will alter mean conditions in the environment and the range and intensity of short-term variability around these trends.

CRedit authorship contribution statement

Corianna Flannery: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Eric P. Bjorkstedt:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Data availability

Data will be made available on request.

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