Data from experiments testing the effects of hypoxia on behavior and physiology of two species of rockfish from from 2015-2016

Website: https://www.bco-dmo.org/dataset/809321
Data Type: experimental
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Project
» Collaborative Research: Ocean Acidification: RUI: Multiple Stressor Effects of Ocean Acidification and Hypoxia on Behavior, Physiology, and Gene Expression of Temperate Reef Fishes (OA Hypoxia Rockfish)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
Hamilton, Scott	Moss Landing Marine Laboratories (MLML)	Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This study investigated the effects of hypoxia on the behavior and physiology of juvenile rockfishes in a controlled laboratory setting to test how deoxygenation may impact early life stages of common temperate reef fishes. Juvenile rockfishes were collected from shallow rocky reef and kelp forest habitats at Stillwater Cove, central California (36˚ 34' N, 121˚ 56' W) during May-June of 2015. Newly settled copper (Sebastes caurinus) and blue (Sebastes mystinus) rockfish were reared in the laboratory under across a range of oxygen concentrations. Juvenile rockfishes were exposed to one of four dissolved oxygen treatment levels corresponding to conditions that currently occur or are predicted to occur in the future on the central California coast: 100% saturation (8.74 ± 0.03 mg O2 L-1), 68% saturation (6.00 ± 0.04 mg O2 L-1), 46% saturation (4.06 ± 0.04 mg O2 L-1), or 26% saturation (2.25 ± 0.05 mg O2 L-1), with two replicate tanks per treatment level. Behavior and physiological trials were conducted on each individual to test how each species responds to declining oxygen levels, including (1) escape response, (2) behavioral lateralization, (3) standard metabolic rate, (4) maximum metabolic rate, (5) aerobic scope, (6) pCrit (i.e., hypoxia tolerance test), and (7) ventilation rate. These data are published in Mattiasen et al. (2020).

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Coverage

Temporal Extent: 2015-07-29 - 2016-02-22

Dataset Description

Juvenile rockfishes were collected from shallow (~10-20 m depth) rocky reef and kelp forest habitats at

Stillwater Cove, central California (36˚ 34' N, 121˚ 56' W) during May-June of 2015. Newly settled copper (Sebastes caurinus) and blue (Sebastes mystinus) rockfish were collected weekly using large mesh hand nets while SCUBA diving. Fishes were measured for length and weight and tagged using Visual Implant Elastomer Tags. Juvenile rockfishes were exposed to one of four treatment levels corresponding to conditions that currently occur or are predicted to occur in the future on the central California coast (Chan et al., 2008; Booth et al., 2012; Fig. 1): 100% saturation (8.74 ± 0.03 mg O_2 L^{-1}), 68% saturation (6.00 ± 0.04 mg O_2 L^{-1}), 46% saturation (4.06 ± 0.04 mg O_2 L^{-1}), or 26% saturation (2.25 ± 0.05 mg O_2 L^{-1}), with two replicate tanks per treatment level. Behavior and physiological trials were conducted on each individual, including (1) escape response, (2) behavioral lateralization, (3) standard metabolic rate, (4) maximum metabolic rate, (5) aerobic scope, (6) pCrit (i.e., hypoxia tolerance test), and (7) ventilation rate. These data are published in Mattiasen et al. (2020).

Methods & Sampling

Experimental design

Experiments subjecting juvenile rockfishes to simulated future DO levels were conducted at the seawater aquarium facility at the NOAA Southwest Fisheries Science Center laboratory in Santa Cruz, CA. Juvenile rockfishes were exposed to one of four treatment levels corresponding to conditions that currently occur or are predicted to occur in the future on the central California coast: 100% saturation (8.74 ± 0.03 mg O_2 L^{-1}), 68% saturation (6.00 ± 0.04 mg O_2 L^{-1}), 46% saturation (4.06 ± 0.04 mg O_2 L^{-1}), or 26% saturation (2.25 ± 0.05 mg O_2 L^{-1}), with two replicate tanks per treatment level. These levels were maintained for the duration of the experiment to simulate chronic exposure to prolonged hypoxia events and oxygen, pH, and temperature levels in all the treatment tanks were measured daily using a HACH HQ40D multiparameter meter. All experimental trials were conducted under constant temperature (12˚C) and pH (~8.05), with the same DO levels as the rearing tank of the fish being tested.

To manipulate oxygen levels, seawater was first pumped from offshore through a series of settling tanks and sand filters and held in a 2000 L reservoir tank maintained at a constant temperature (12˚C) with aquarium chillers and aerated to approximately 100% air saturation (~9.0 mg O_2 L^{-1}). This source water was fed into four 500 L treatment reservoirs, which were used to create desired DO concentrations by bubbling nitrogen (N_2) gas to strip O_2 from the water. Gas delivery was controlled by the program WitroxView via solenoid valves, and O_2 levels were monitored using Loligo Systems optical oxygen probes. Manipulated (or control) water was delivered at a rate of 20 ml s^{-1} to 80 L experimental tanks in a single-pass, flow-through design.

Ten randomly assigned juvenile rockfish of each species were introduced into each of two replicate tanks for each treatment (20 fish per treatment per species). All tanks were covered to minimize visual disturbance from investigators. Sections of plastic construction fencing material measuring 90 x 120 cm were bunched together and placed in each tank to simulate kelp habitat structure. The two replicate tanks per treatment were fed on alternating days and used in experiments on non-feeding days, thereby ensuring a 36-48 hour fasting period prior to behavioral or physiological trials. Fishes were fed to satiation with frozen, high protein krill on feeding days. Prior to conducting any behavioral or physiological measurements, fish were allowed a minimum of 5 days to recover in their treatment tanks from any previous trial. See Table 1 of Mattiasen et al. (2020) for the schedule of the various experimental trials.

Escape response

Escape response trials tested the time required for a fish to find the exit of an enclosed chamber (Jutfelt et al. 2013). Escape chambers were composed of a PVC tube measuring 28 cm tall x 9 cm diameter with a 5 cm diameter hole cut in the side. A slit located 8 cm from the top of the chamber allowed a black plexiglass divider to be inserted, retaining fish in the top half of the chamber during the acclimation period. Removal of the divider released the fish into the lower portion of the chamber at the start of the timed trial. Escape chambers were placed in 40 L insulated aquaria on a water table to control temperatures. A total of six replicated escape chambers and aquaria were used, allowing for six simultaneous trials. Individual fish were transferred into the top of the chamber and allowed a 15 min acclimation period. At the end of the acclimation period the divider was removed without visual interference by the investigator. Observers watched a mirror above the tanks and recorded the time at which each fish exited the chamber (defined as the time at which the head of the fish exited the chamber). Trials were terminated after 10 min regardless of whether the fish exited.

Behavioral lateralization

Brain functional asymmetry and behavioral lateralization reflect the bias for left vs. right turning decisions in a detour test. To measure lateralization in response to DO treatment conditions, a detour test was employed with a double T-Maze (Domenici et al., 2007). Individual fish were transferred into one end of the two-way T-

Maze (50 x 30 x 25 cm L x W x H aquaria), and allowed to acclimate for 3 minutes. The starting side was alternated for every trial to minimize the potential for side bias. After the acclimation period, the fish was gently coaxed to swim down the center channel (without touching the fish) using a long PVC bar, and when it reached a barrier at the end of the channel, the fish had to decide to turn right or left. The turn direction was recorded and the experiment was repeated 10 times, 5 times in each direction. Each trial took approximately 10 minutes to complete. Absolute lateralization (LA) was calculated as

LA = (|# right turns – # left turns|)/(# right turns + # left turns) x 100

as an index of non-directional turn bias. LA reflects whether turn bias exists at the population level, irrespective of direction. Relative lateralization (LR¬) was also calculated to determine whether the fish in a particular treatment exhibited turning bias for a particular direction (i.e., left or right preference). LR was calculated as

LR = (# right turns – # left turns)/(# right turns + # left turns) x 100

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

Critical oxygen tension (pCrit)

A subset of 8 individuals per species from each treatment was tested for hypoxia tolerance by estimating pCrit using an automated intermittent flow respirometry system (Loligo Systems). Fish were placed in sealed respirometry chambers overnight at their treatment oxygen levels to acclimate to the chambers. Subsequently, pCrit trials were initiated by raising the oxygen saturation of the reservoir to 70% air saturation and three MO2 measurement loops (5 min flush, 10 min wait, 5 min measurement) were recorded at each oxygen level. The DO level was then reduced in a step-wise fashion by 10% air saturation, through the addition of N2 gas until reaching 40% air saturation, below which oxygen saturation was reduced at 5% intervals until reaching 10% air saturation, at which point the trial was terminated. This approach allowed us to obtain a more precise measurement of pCrit and to reduce risk of inadvertent mortality.

Oxygen consumption rate (MO2 in mg O2 kg-1 hr-1) was calculated using the following equation:

MO2 = ΔPO2 V∝M-1 Δt-1

Where ΔPO2 is the change in water partial pressure of O2 (mmHg), Δt is the elapsed time (h), V is the volume of the respirometer chamber minus the volume of the fish (cm3), M is the total mass of the animal (kg), and ∝ is the O2 solubility coefficient at the experimental temperature (Boutilier et al., 1988). The respirometry system was cleaned using dilute bleach after each trial to eliminate the influence of microbial respiration on subsequent trials. pCrit was calculated for each fish using the broken stick regression method (Toms and Lesperance, 2003) by computing the oxygen saturation level at which the metabolic rate began to decrease linearly with decreasing DO.

Aerobic scope

Aerobic scope is the difference between the standard (or resting) metabolic rate (SMR) and the maximum metabolic rate (MMR). We measured the SMR on a subset of 8 fish per species per treatment using the intermittent flow respirometry system. Four individuals at a time were weighed and placed into separate respirometer chambers, with MO2 measurements taken over a 12 hr period overnight. SMR was measured during nighttime hours to capture the MO2 at the time where the fish were at their lowest metabolic activity levels. The lowest 10% of MO2 measurements per cycle, excluding outliers (values > 2 standard deviations), were used to calculate the SMR of each individual fish (Clark et al., 2012). MMR was subsequently measured following swimming to exhaustion using a Loligo Systems 10 L swim flume (model #SW10100). Exhaustion was achieved by swimming the fish for 5 minutes at a velocity one-body length per second below the estimated average critical swimming speed of the group (N. Kashef, unpublished data). The fish were then quickly returned to the respirometry chambers and run for one measurement cycle to acquire MMR. Preliminary trials concluded that the highest MO2 values occurred directly following swimming to exhaustion. Aerobic scope was calculated by subtracting the SMR from the MMR.

Ventilation rate

Ventilation rate was measured on a subset of 10 individuals per species per treatment using a specially designed array of 10 experimental chambers (5 x 15 cm, water depth 4 cm), each holding an individual fish. Each chamber received flow-through seawater of the appropriate rearing DO treatment and constant temperature (12˚C). Following a two-hour acclimation period, two GoPro HERO4 video cameras recorded each fish for 30 minutes. Ventilation rate was determined by counting the number of open/closing cycles of the gill operculum within a minute (i.e., ventilations per minute [VPM]). Average VPM for each fish was calculated for 3 randomly selected one-minute measurements.

Problem report: Some individual fish were used in each of the behavioral and physiological trials, while other fish were only tested in a subset of the possible experimental trials. In addition, if any fish died in the course of the experiments, they were replaced by a new fish.

Data Processing Description

Standard metabolic rate (SMR), maximum metabolic rate (MMR), and pCrit data were processed and summarized using the oxygen time series data from the intermittent flow respirometry system. To calculate SMR, MMR, and pCrit we used the software AutoResp produced by the company Loligo Systems. All other data sources (escape time, lateralization, ventilation rates, etc.) were summarized using spreadsheets of raw values in Microsoft Excel.

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Data Files

File
rockfish_hypoxia_expt.csv (Comma Separated Values (.csv), 19.88 KB) MD5:3fa246d5cb725e9b22761c7b877c74ab Primary data file for dataset ID 809321

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Related Publications

Boutilier, R. G., Dobson, G., Hoeger, U., & Randall, D. J. (1988). Acute exposure to graded levels of hypoxia in rainbow trout (Salmo gairdneri): metabolic and respiratory adaptations. Respiration Physiology, 71(1), 69–82. doi:10.1016/0034-5687(88)90116-8
Methods

Clark, T. D., Donaldson, M. R., Pieperhoff, S., Drenner, S. M., Lotto, A., Cooke, S. J., … Farrell, A. P. (2012). Physiological Benefits of Being Small in a Changing World: Responses of Coho Salmon (Oncorhynchus kisutch) to an Acute Thermal Challenge and a Simulated Capture Event. PLoS ONE, 7(6), e39079. doi:10.1371/journal.pone.0039079
Methods

Domenici, P., Lefrançois, C., & Shingles, A. (2007). Hypoxia and the antipredator behaviours of fishes. Philosophical Transactions of the Royal Society B: Biological Sciences, 362(1487), 2105–2121. doi:10.1098/rstb.2007.2103
Methods

Jutfelt, F., Bresolin de Souza, K., Vuylsteke, A., & Sturve, J. (2013). Behavioural Disturbances in a Temperate Fish Exposed to Sustained High-CO2 Levels. PLoS ONE, 8(6), e65825. doi:10.1371/journal.pone.0065825
Methods

Mattiasen, E. G., Kashef, N. S., Stafford, D. M., Logan, C. A., Sogard, S. M., Bjorkstedt, E. P., & Hamilton, S. L. (2020). Effects of hypoxia on the behavior and physiology of kelp forest fishes. Global Change Biology. doi:10.1111/gcb.15076
Results

Toms, J. D., & Lesperance, M. L. (2003). PIECEWISE REGRESSION: A TOOL FOR IDENTIFYING ECOLOGICAL THRESHOLDS. Ecology, 84(8), 2034–2041. doi:10.1890/02-0472
Methods

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Parameters

Parameter	Description	Units
Fish_ID	Unique identifer	unitless
Species	Species used in the experiments	unitless
Oxygen_treatment_mg_per_L	Experimental treatment condition	milligrams O2 per liter (mg/L)
Tank_Replicate	Two replicate tanks (A and B) per treatment	unitless
Initial_Standard_Length_mm	Initial length measured from tip of mouth to hypural bones	millimeters (mm)
Initial_Total_Length_mm	Initial length measured from tip of mouth to end of caudal fin	millimeters (mm)
Initial_Weight_g	Initial weight	grams (g)
Date_entered_into_experimental_treatments	Date entered into experimental treatments; format: yyyy-mm-dd	unitless
Date_at_end_of_experiment	Date at end of experiment; format: yyyy-mm-dd	unitless
Final_Standard_Length_mm	Final length measured from tip of mouth to hypural bones	millimeters (mm)
Final_Total_Length_mm	Final length measured from tip of mouth to end of caudal fin	millimeters (mm)
Final_Weight_g	Final weight	grams (g)
Escape_Time_seconds	Time required for fish to exit the chamber	seconds
Relative_Lateralization_Score	Percent of time fish turned right (positive score) or left (negative score)	unitless (percent)
Absolute_Lateralization_Score	Percent of time fish showed a turn bias out of 10 trials	unitless (percent)

Ventilation_rate_operculum_beats_per_minute	Average number of breaths per minute	breaths per minute (BPM)
Standard_Metabolic_Rate_mg_O2_per_kg_per_hour	Oxygen consumption of fish at rest	milligrams O2 per kilogram per hour
Maximum_Metabolic_Rate_mg_O2_per_kg_per_hour	Oxygen consumption of fish after exercise	milligrams O2 per kilogram per hour
Aerobic_Scope_mg_O2_per_kg_per_hour	Difference between standard and maximum metabolic rate	milligrams O2 per kilogram per hour
Pcrit_pcnt_air_saturation	Measure of hypoxia tolerance as percent air saturation	unitless (percent)

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Aquarium
Generic Instrument Description	Aquarium - a vivarium consisting of at least one transparent side in which water-dwelling plants or animals are kept

Dataset-specific Instrument Name	
Generic Instrument Name	Aquarium chiller
Generic Instrument Description	Immersible or in-line liquid cooling device, usually with temperature control.

Dataset-specific Instrument Name	GoPro HERO4 video camera
Generic Instrument Name	Camera
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	large mesh hand nets
Generic Instrument Name	Hand Net
Generic Instrument Description	A hand net (also called a scoop net or dip net) is a net or mesh basket held open by a hoop. They are used for scooping fish near the surface of the water.

Dataset-specific Instrument Name	Loligo Systems optical oxygen probes
Generic Instrument Name	Oxygen Sensor
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	SCUBA
Generic Instrument Name	Self-Contained Underwater Breathing Apparatus
Generic Instrument Description	The self-contained underwater breathing apparatus or scuba diving system is the result of technological developments and innovations that began almost 300 years ago. Scuba diving is the most extensively used system for breathing underwater by recreational divers throughout the world and in various forms is also widely used to perform underwater work for military, scientific, and commercial purposes. Reference: https://oceanexplorer.noaa.gov/technology/technical/technical.html

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Project Information

Collaborative Research: Ocean Acidification: RUI: Multiple Stressor Effects of Ocean Acidification and Hypoxia on Behavior, Physiology, and Gene Expression of Temperate Reef Fishes (OA Hypoxia Rockfish)

NSF Award Abstract:
For near shore marine species inhabiting upwelling ecosystems such as the California Current, climate change resulting from the anthropogenic release of CO2 into the atmosphere is likely to induce concurrent conditions of ocean acidification (OA) and hypoxia, which are exacerbated during periods of seasonal upwelling. Although marine fishes have generally been presumed to be tolerant of OA due to their competence in acid-base regulation, recent studies in tropical regions suggest that early life stages may be particularly sensitive to elevated levels of dissolved CO2 (which lowers seawater pH) by impairing respiration, acid-base regulation, and neurotransmitter function. Low levels of dissolved oxygen (DO), which occur during hypoxia, can likewise impact the behavior, physiology and survival of marine fishes. Few studies have addressed the potential interactive effects of a low pH, low DO environment. From molecular tools to whole animal physiology, this research will provide an in-depth examination of an inherently integrative process. The study will use a multiple stressor framework to address the potential threats posed by the independent and combined effects of OA and hypoxia on behavior, physiological capacity, and gene expression in temperate reef fishes. Because mortality in early life stages has important carryover effects, understanding the effects of these stressors is critical for predicting future climate change responses of global fish populations. Such information will lay the groundwork for further studies that address the synergistic effects of multiple stressors and the characteristics of California Current species that influence their ability to tolerate or adapt to changes in ocean chemistry in a rapidly changing climate.

The project goals are to use a combination of laboratory and field studies to examine ecologically and physiologically relevant responses of juvenile rockfish (genus Sebastes) to the independent and interactive effects of ocean acidification and hypoxia. Rockfish will be captured in the field and then reared in the lab at 4 different pCO2 levels and 4 different DO levels to simulate changes in environmental conditions. Response variables include: (1) measures of changes in olfactory capabilities, brain functional asymmetry and problem-solving ability and (2) effects on swimming capabilities, respiration, aerobic performance, and growth. In addition, we will use next generation transcriptome sequencing to examine genome-wide changes in gene expression and enzyme activity for Na+/K+ ATPase (NKA), citrate synthase (CS), and lactate dehydrogenase (LDH), as proxies for acid-base compensation and metabolic shifts between aerobic and anaerobic metabolism. Oceanographic sensors will be deployed in the field to determine the frequency and intensity of hypoxia and low pH events in near shore habitats in Northern and Central California. Adaptive sampling of juvenile rockfish will be used to evaluate gene expression and physiological responses in individuals exposed in situ to low pH and low DO events in the field. The effects of OA and hypoxia will be compared across rockfish species with different life histories (e.g. larval duration, timing of spawning, etc.) and collected from regions differing in exposure to low pH/low DO events to address the potential for local adaptation. The focus of this project is on responses of the early juvenile stage at the time of settlement, because this stage is exposed to near shore changes in ocean chemistry during a critical period where physiological stress and behavioral disruptions may have the strongest demographic effects due to increased risk of predation.

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Program Information

Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

Solicitations issued under this program:
NSF 10-530, FY 2010-FY2011
NSF 12-500, FY 2012
NSF 12-600, FY 2013
NSF 13-586, FY 2014
NSF 13-586 was the final solicitation that will be released for this program.

PI Meetings:
1st U.S. Ocean Acidification PI Meeting(March 22-24, 2011, Woods Hole, MA)
2nd U.S. Ocean Acidification PI Meeting(Sept. 18-20, 2013, Washington, DC)
3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation (NSF)

Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)

Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants

Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation (NSF)

Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation (NSF)

Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards $11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

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Funding

Funding Source	Award
NSF Emerging Frontiers Division (NSF EF)	EF-1416895

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