CO2, temperature, and oxygen effects on Atlantic silverside metabolic rates

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Data Type: experimental
Version: 1
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Project
» Collaborative research: Understanding the effects of acidification and hypoxia within and across generations in a coastal marine fish (HYPOA)

Contributors	Affiliation	Role
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Abstract
Metabolic rates of Atlantic silverside (Menidia menidia) embryos and larvae reared in six separate experiments in 2016 and 2017. Four experiments used factorial combinations of CO2 and temperature, and two experiments used combinations of CO2 and oxygen.

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Coverage

Spatial Extent: **Lat**:41.321526 **Lon**:-72.015247
Temporal Extent: 2016-04-22 - 2017-06-18

Dataset Description

This dataset includes metabolic rates of Atlantic silverside (*Menidia menidia*) embryos and larvae reared in six separate experiments in 2016 and 2017. Four experiments used factorial combinations of CO_2 and

temperature, and two experiments used combinations of CO_2 and oxygen.

Acquisition Description

Sampling and analytical procedures:
Detailed experimental methods are provided for experimental design and water chemistry in Murray and Baumann (2018) and Cross et al. (2019), and for respirometry methods in Schwemmer et al. (2020). In summary, spawning-ripe adult *Menidia menidia* were collected from Mumford Cove (41°19'25" N, 72°1'7" W), Groton, CT, in late spring and early summer of 2016 and 2017 and transported to the Rankin seawater laboratory at University of Connecticut's Avery Point Campus. Adults were strip-spawned, and fertilized eggs were randomly distributed into 20-L rearing containers, which were placed into each treatment tank within 2h post-fertilization. All experimental methods were approved and conducted according to University of Connecticut Institutional Animal Care and Use Committee protocol #A14-032. Of six factorial experiments conducted in 2016 and 2017, experiments 1-4 quantified CO_2 × temperature effects and experiments 5-6 quantified CO_2 × oxygen effects. Experiment 1 used 400 and 2200 uatm as target pCO_2 levels, crossed with two temperatures: 17°C and 24°C. Experiments 2 and 3 factorially crossed three pCO_2 levels (400, 2200, and 4200 uatm) with three temperatures (17°C, 20°C, and 24°C). Experiment 4 used the same three target pCO_2 levels crossed with 24°C and 28°C. Experiments 5 and 6 exposed *M. menidia* early life stages to three levels of pCO_2 (400, 2200, and 4200 uatm) crossed factorially with three target levels of oxygen partial pressure (pO_2): normoxic (23 kPa), suboxic (12 kPa) and hypoxic (7.5 or 9 kPa). The pCO_2 levels were calculated based on measured pH, temperature, salinity, and total alkalinity (AT). AT samples were collected three times per experiment and measured using an endpoint titration. Based on these measurements, the pCO_2 (uatm) was calculated in CO2SYS (V2.1). In the CO_2 × temperature experiments, oxygen was maintained at ~100% air saturation (>20 kPa). For experiments 1 and 4 this was achieved with continuous bubbling and validated daily for each tank with a handheld probe. For experiments 2, 3, 5, and 6, dissolved oxygen (DO, mg L^{-1}) measurements were automatically taken twice hourly in each tank by a DO probe connected to a LabView program, which adjusted bubbling of CO_2-stripped air or nitrogen gas to maintain target oxygen levels.

Closed respirometry measurements were conducted on embryos randomly sampled from each treatment 1-3 days prior to hatch and larvae sampled on the day of hatching. Oxygen consumption rates were measured by two 24-channel SensorDish readers (SDR) and glass well plates equipped with an optical oxygen sensor spot in each well. Each 0.5-mL well received a single embryo or larva, and at least one well contained only treatment water to measure background microbial respiration. Well plates were sealed and placed in temperature-controlled water baths and dissolved oxygen (DO, mg L^{-1}) was recorded every fifteen seconds by the SDR software until DO had decreased by 3 mg L^{-1} in at least one of the wells, for 15-60 minutes. In the case of the suboxic and hypoxic treatments from experiments 5 and 6, however, the trials lasted five minutes regardless of the DO differential, given the already low oxygen in the treatment water. At the end of each measurement period, embryos and larvae were checked for injury or death, and any other factors that might have affected oxygen consumption rates were noted.

Known Problems:
Embryos were not sampled in Experiment 2 due to logistical issues. Some fish IDs might be missing because the individual died or escaped, and was therefore not included in the dataset although it had already been assigned a number before respirometry.

Processing Description

Data Processing:
Routine metabolic rates were calculated in R statistical software (v4.0.0; R Core Team, 2020). Because temperature influences oxygen solubility and metabolic rates of fish, we measured temperature simultaneously with DO throughout the measurement period and only used data for periods of time in which the temperature changed by less than ~0.03°C min^{-1}. A linear model was fit to the DO values with respect to time for each well. The slope (mg O_2 L^{-1} s^{-1}) of the linear model was used to calculate oxygen

consumption rate (RO_2; umol O_2 h^{-1}) with the following formula: RO_2=slope/(0.032×3600×0.0005), where 0.032 is the molar mass of O_2 (mg mol^{-1}), 3600 converts seconds to hours, and 0.0005 L is the well volume. The mean RO2 from control wells was subtracted from each fish-containing well of the same treatment to account for microbial respiration and obtain fish RO2. Size differences in embryos were negligible and quantifying embryo mass was impractical, so RO2 was not normalized to mass and is reported as whole-embryo routine metabolic rate (RMR). Larval total length (TL, mm) was measured in images (Image J) taken by digital camera (TrueChrome Metrics, Tucsen Photonics Co., Fuzhou, Fujian, China) connected to a stereo microscope (Nikon Eclipse E200). TL was then converted to dry weight (DW, mg) using the relationship ln(DW)=2.997×ln(TL)-6.703 (H. Baumann, personal communication, June 23, 2017). DW was then used to calculate the larval mass-specific RMR (umol mg^{-1} h^{-1}) as RMR=RO2/DW.

BCO-DMO processing:
- converted dates to format YYYY-MM-DD;
- rounded values of embryo_RMR and larva_RMR to 6 decimal places.

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Related Publications

Cross, E. L., Murray, C. S., & Baumann, H. (2019). Diel and tidal pCO2 × O2 fluctuations provide physiological refuge to early life stages of a coastal forage fish. Scientific Reports, 9(1). doi:10.1038/s41598-019-53930-8
Methods

Murray, C., & Baumann, H. (2018). You Better Repeat It: Complex CO2 × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. Diversity, 10(3), 69. doi:10.3390/d10030069
Methods

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/
Software

Schwemmer, T. G., Baumann, H., Murray, C. S., Molina, A. I., & Nye, J. A. (2020). Acidification and hypoxia interactively affect metabolism in embryos, but not larvae, of the coastal forage fish Menidia menidia. The Journal of Experimental Biology, jeb.228015. doi:10.1242/jeb.228015
Results

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Parameters

Parameter	Description	Units
experiment	experiment number	unitless
exp_type	type of experiment (independent variables)	unitless
species	subject species	unitless
adult_collection_site	site adults used for spawning were collected	unitless
latitude	latitude of collection site	degrees North
longitude	longitude of collection site	degrees East
fertilization_date	date eggs were fertilized; format: YYYY-MM-DD	unitless
fish_id	ID number assigned to fish	unitless
tank	tank fish were reared in	unitless
target_pCO2	target partial pressure of CO2	microatmospheres (uatm)
target_temp	target temperature level	degress Celsius
target_DO	target dissolved oxygen level	milligrams per liter (mg L-1)
target_PO2	target partial pressure of oxygen	kilopascals (kPa)
mean_pCO2	mean partial pressure of CO2	microatmospheres (uatm)
mean_pH	mean water pH	unitless
mean_temp	mean water temperature	degrees Celsius
mean_DO	mean dissolved oxygen	milligrams per liter (mg L-1)
mean_PO2	mean partial pressure of oxygen	kilopascals (kPa)
stage	life stage at sampling	unitless
sample_date	date fish was sampled; format: YYYY-MM-DD	unitless
embryo_RMR	metabolic rate of embryo	micromoles O2 per individual per hour (umol O2 individual-1 h-1)
larva_RMR	mass-specific metabolic rate of larva	micromoles O2 per milligram per hour (umol O2 mg-1 h-1)

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Instruments

Dataset-specific Instrument Name	Digital microscope-mounted camera
Generic Instrument Name	Camera
Dataset-specific Description	TrueChrome Metrics, Tucsen Photonics Co., Fuzhou, Fujian, China.
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	pH sensors
Generic Instrument Name	pH Sensor
Dataset-specific Description	The following pH sensors were used: Orion Ross Ultra pH/ATC Triode with Orion Star A121 pH Portable Meter,Thermo Fisher Scientific®, Waltham, MA, USA. Intellical PHC281 pH Electrode with HQ11D Handheld pH/ORP Meter, Hach®, Loveland, CO, USA. Hach® pHD digital electrode.
Generic Instrument Description	General term for an instrument that measures the pH or how acidic or basic a solution is.

Dataset-specific Instrument Name	Alkalinity titrator
Generic Instrument Name	Automatic titrator
Dataset-specific Description	G20 Potentiometric Titrator, Mettler Toledo®, Columbus, OH, USA.
Generic Instrument Description	Instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

Dataset-specific Instrument Name	Microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Nikon Eclipse E200, Nikon Corporation, Tokyo, Japan.
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of visible light. Includes conventional and inverted instruments. Also called a "light microscope".

Dataset-specific Instrument Name	Chiller
Generic Instrument Name	Aquarium chiller
Dataset-specific Description	DeltaStar®, Lynchburg, VA, USA
Generic Instrument Description	Immersible or in-line liquid cooling device, usually with temperature control.

Dataset-specific Instrument Name	Respirometry oxygen sensor readers
Generic Instrument Name	plate reader
Dataset-specific Description	24-channel SensorDish Readers, Presens Precision Sensing, GmbH, Regensburg, Germany.
Generic Instrument Description	Plate readers (also known as microplate readers) are laboratory instruments designed to detect biological, chemical or physical events of samples in microtiter plates. They are widely used in research, drug discovery, bioassay validation, quality control and manufacturing processes in the pharmaceutical and biotechnological industry and academic organizations. Sample reactions can be assayed in 6-1536 well format microtiter plates. The most common microplate format used in academic research laboratories or clinical diagnostic laboratories is 96-well (8 by 12 matrix) with a typical reaction volume between 100 and 200 uL per well. Higher density microplates (384- or 1536-well microplates) are typically used for screening applications, when throughput (number of samples per day processed) and assay cost per sample become critical parameters, with a typical assay volume between 5 and 50 µL per well. Common detection modes for microplate assays are absorbance, fluorescence intensity, luminescence, time-resolved fluorescence, and fluorescence polarization. From: http://en.wikipedia.org/wiki/Plate_reader, 2014-09-0-23.

Dataset-specific Instrument Name	Thermostat
Generic Instrument Name	thermostat
Dataset-specific Description	Aqualogic®, San Diego, CA, USA.
Generic Instrument Description	A device designed to regulate temperature by controlling the starting and stopping of a heating/cooling system.

Dataset-specific Instrument Name	Respirometry microplates
Generic Instrument Name	microplate
Dataset-specific Description	500-uL 24-chamber glass well plates with optical oxygen sensor spots, Loligo Systems®, Viborg, Denmark.
Generic Instrument Description	A flat dish with multiple individual wells that are arrayed in a standardized number, size, and arrangement.

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Project Information

Collaborative research: Understanding the effects of acidification and hypoxia within and across generations in a coastal marine fish (HYPOA)

Coverage: Eastern Long Island Sound, CT, USA

Description from NSF award abstract: Coastal marine ecosystems provide a number of important services and resources for humans, and at the same time, coastal waters are subject to environmental stressors such as increases in ocean acidification and reductions in dissolved oxygen. The effects of these stressors on coastal marine organisms remain poorly understood because most research to date has examined the sensitivity of species to one factor, but not to more than one in combination. This project will determine how a model fish species, the Atlantic silverside, will respond to observed and predicted levels of dissolved carbon dioxide (CO2) and oxygen (O2). Shorter-term experiments will measure embryo and larval survival, growth, and metabolism, and determine whether parents experiencing stressful conditions produce more robust offspring. Longer-term experiments will study the consequences of ocean acidification over the entire life span by quantifying the effects of high-CO2 conditions on the ratio of males to females, lifetime growth, and reproductive investment. These studies will provide a more comprehensive view of how multiple stressors may impact populations of Atlantic silversides and potentially other important forage fish species. This collaborative project will support and train three graduate students at the University of Connecticut and the Stony Brook University (NY), two institutions that attract students from minority groups. It will also provide a variety of opportunities for undergraduates to participate in research and the public to learn about the study, through summer research projects, incorporation in the "Women in Science and Engineering" program, and interactive displays of environmental data from monitoring buoys. The two early-career investigators are committed to increasing ocean literacy and awareness of NSF-funded research through public talks and presentations. This project responds to the recognized need for multi-stressor assessments of species sensitivities to anthropogenic environmental change. It will combine environmental monitoring with advanced experimental approaches to characterize early and whole life consequences of acidification and hypoxia in the Atlantic silverside (Menidia menidia), a valued model species and important forage fish along most of the US east coast. Experiments will employ a newly constructed, computer-controlled fish rearing system to allow independent and combined manipulation of seawater pCO2 and dissolved oxygen (DO) content and the application of static and fluctuating pCO2 and DO levels that were chosen to represent contemporary and potential future scenarios in productive coastal habitats. First CO2, DO, and CO2 × DO dependent reaction norms will be quantified for fitness-relevant early life history (ELH) traits including pre- and post-hatch survival, time to hatch, post-hatch growth, by rearing offspring collected from wild adults from fertilization to 20 days post hatch (dph) using a full factorial design of 3 CO2 × 3 DO levels. Second, the effects of tidal and diel CO2 × DO fluctuations of different amplitudes on silverside ELH traits will be quantified. To address knowledge gaps regarding the CO2-sensitivity in this species, laboratory manipulations of adult spawner environments and reciprocal offspring exposure experiments will elucidate the role of transgenerational plasticity as a potential short-term mechanism to cope with changing environments. To better understand the mechanisms of fish early life CO2-sensitivity, the effects of temperature × CO2 on pre- and post-hatch metabolism will be robustly quantified. The final objective is to rear silversides from fertilization to maturity under different CO2 levels and assess potential CO2-effects on sex ratio and whole life growth and fecundity. Related references: Gobler, C.J. and Baumann, H. (2016) Hypoxia and acidification in ocean ecosystems: Coupled dynamics and effects on marine life. Biology Letters 12:20150976. doi:10.1098/rsbl.2015.0976 Baumann, H. (2016) Combined effects of ocean acidification, warming, and hypoxia on marine organisms. Limnology and Oceanography e-Lectures 6:1-43. doi:10.1002/loe2.10002 Depasquale, E., Baumann, H., and Gobler, C.J. (2015) Variation in early life stage vulnerability among Northwest Atlantic estuarine forage fish to ocean acidification and low oxygen Marine Ecology Progress Series 523: 145–156.doi:10.3354/meps11142

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536336
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536165

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