Hatching count and survival statistics of Atlantic silverside (Menida menidia) larvae reared under ambient or hypoxia/acidification treatments during cross-generational laboratory experiments in 2022

Website: https://www.bco-dmo.org/dataset/925081
Data Type: experimental
Version: 1
Version Date: 2024-04-16

Project
» OCE-PRF Detecting signatures of multigenerational plasticity in a marine forage fish (HypOA Cross-generational Plasticity)

Contributors	Affiliation	Role
Murray, Christopher S.	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
York, Amber D.	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This dataset contains daily counts of hatching Atlantic silverside (Menidia menidia) larvae during cross-generational laboratory experiments testing how parental exposure to a combined hypoxia and acidification treatment (hereafter HypOA) affected offspring responses and HypOA tolerance in an important coastal forage fish species. Embryo and larval survival statistics by replicate are provided as a supplemental file in this dataset.

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Coverage

Location: Laboratory-conditioned adults were caught at Mumford Cove, CT
Spatial Extent: **Lat**:41.321694 **Lon**:-72.017444
Temporal Extent: 2022-05-22 - 2022-07-07

Dataset Description

Study description:
*see "Related Datasets" section for access to related datasets from this experiment

These data were generated during a cross-generational laboratory experiment testing how parental exposure to a combined hypoxia and acidification treatment affected offspring responses and HypOA tolerance in an important coastal forage fish species, Atlantic silverside Menidia menidia. The experiment was conducted during June and July 2022 at Woods Hole Oceanographic Institution’s Environmental Systems Laboratory.

Wild adult Atlantic silversides were conditioned to two treatment levels: control (100% dissolved oxygen [DO] / 650 µatm pCO2) or HypOA conditions (40% DO / 2300 µatm pCO2) for 10 days. After artificial fertilization, their offspring were reared in a factorial experimental design to test how parental conditioning to the stressors affected the survival, development, and growth of embryos and larvae when reared under control or HypOA conditions. The larvae were sampled for morphometric measurements at hatch (9 or 10 days post-fertilization) and after 10 days of larval rearing (19 days post-fertilization). We evaluated survival to hatch (fertilization to 10 days post-fertilization), during larval development (hatch to 10 days post hatch), and over the entire rearing interval (fertilization to 19 days post-fertilization). We found that direct offspring exposure to HypOA lowered survival and growth; however, embryos fertilized by HypOA conditioned parents showed better survival under HypOA conditions. By contrast, we found that larvae from HypOA-conditioned parents exhibited, on average, slower post-hatch growth and slightly lower larval survival in offspring groups reared under both treatment conditions. The data demonstrate that parental conditioning does affect offspring responses, with evidence for both positive and negative effects on offspring viability and tolerance to HypOA.

Methods & Sampling

Two experimental treatment levels were used for this experiment: a control treatment [100% DO (8.9 mg L-1) and 650 µatm pCO2 (7.83 pHTotal Scale] and a treatment combining hypoxia and ocean acidification [hereafter referred to as HypOA; 40% DO (3.6 mg L-1) and 2,300 µatm pCO2 (7.33 pHT)]. Following the initial five-day holding period, adult fish were exposed to their respective experimental treatment conditions for 10 days. Parental conditioning to HypOA involved exposing adult fish to a fluctuating DO and pCO2 regime, mimicking the diurnal fluctuations seen in nearshore marine environments. After the conditioning phase, adult silversides were spawned, and their offspring were reared under both conditions in a fully-factorial experimental design. Subsamples for hatch morphometrics (N = 10) were made on the first morning when a growth replicate had at least 20 new hatchlings. The larvae were euthanized by an overdose of MS-222 and were fixed in 4% buffered formalin for 24 h and transferred to 75% ethanol for storage. The remaining hatchlings from growth replicates were moved to a new 20-L container for continued rearing. Larvae were provided daily rations of newly hatched brine shrimp nauplii (San Francisco Bay strain) at 2-3 nauplii ml-1. Replicates were siphoned daily for uneaten food and waste. The continuous seawater flow through ensured that ammonia levels remained near 0 ppm, verified daily via API® Ammonia Test Kit. At 19 dpf (which corresponds to either 9 or 10 days dph based on the treatment and hatch timing) the survival replicates were recounted to determine the final survival rate. Larvae were subsampled for morphometric measurements (N = 12) and fixed using the same protocol as described above.

Dates and Locations:

Wild Atlantic silverside adults were collected from Mumford Cove, CT (41°19'18.1"N 72°01'02.8"W) a shallow embayment adjacent to the eastern end of the Long Island Sound on May 31, 2022. Adults were transported to the Environmental Systems Laboratory at Woods Hole Oceanographic Institution where we have developed at large experimental seawater system to facilitate multistressor research on fish. Parental conditioning of wild adults took place between May 31 to June 17, 2022. Embryo and larval rearing took place between June 17 to July 7, 2022.

Taxnomic Identifier, LifeSciences Identifier (LSID):
Menidia menidia, urn:lsid:marinespecies.org:taxname:159228

Instrument summary:

The experiment was conducted at the Environmental Systems Laboratory at Woods Hole Oceanographic Institution (Falmouth, MA, USA) in a custom designed automated seawater system comprised of eight 900-L circle main tanks (four tanks per treatment level) and one 600-L mixing tank. A schematic of the experimental setup is provided in Fig. S1. Each main tank was outfitted with multiple 20-L rearing containers (white buckets fitted with six 3-cm flowthrough holes covered in 300-µm screening) to accommodate groups of embryos and larvae. Four main tanks were allocated to the control treatment received temperature-controlled seawater sourced from Vineyard Sound. This water was sand-filtered, UV-sterilized, and constantly maintained at the control treatment's DO and pCO2 conditions. The HypOA treatment conditions were automatically maintained within the mixing tank using custom software developed in Arduino IDE and operated by a Teensy® 4.1 Development Board. In short, the mixing tank received a continuous flow of ambient seawater, with DO, pH, and temperature measurements taken every 10 seconds by Pyroscience® Ultra Compact (PICO) DO and pH meters, fitted with fiberoptic DO or pH sensors and PT100 temperature sensors. Calibration of the probes was performed weekly according to the manufacturer's guidelines using oxygen and pHT calibration solutions

provided by Pyroscience®. HypOA levels were regulated through a simple feedback mechanism: when the current DO or pH exceeded the treatment set point, the software opened a solenoid valve to introduce compressed gas (N2 or CO2) into ultrafine gas-diffusers positioned at the bottom of the mixing tank until the setpoint was reached. The software was optimized to implement brief and precisely controlled gas additions, ensuring highly stable DO and pH conditions in the mixing tank. Adjusted HypOA seawater was pumped to the main tanks and individual rearing containers as required.

DO levels in individual rearing units were cross-verified using a handheld Pyroscience Firesting®-GO2 meter fitted with an optical DO sensor. A two-point calibration (100% and 0% DO) was made daily using air-saturated seawater and a concentrated NaSO3 seawater solution. pH conditions were verified via a Hach HQ11D pH meter fitted with a Hach PHC28101 IntelliCal pH probe. The probe was calibrated daily using pHT buffers from PyroScience®. To validate target pCO2 conditions, seawater was sampled every 1 – 2 days from one of the replicate rearing containers per offspring treatment group, resulting in 12 seawater samples per offspring treatment group during the 19-day experiment. Seawater samples were poisoned with mercuric chloride and stored in sealed bottles for later analysis of carbon chemistry parameters. Temperature and salinity (via refractometer) were recorded at the time of sampling. Samples were measured in triplicate for dissolved inorganic carbon (DIC) using an Apollo AS-D1 analyzer connected to a Picarro G-2121i cavity ringdown system. Total alkalinity (TA) was measured using an open-system Gran titration on 5-mL samples in triplicate, using a Metrohm 805 Dosimat and a robotic Titrosampler. Both systems were calibrated against seawater Certified Reference Materials (Andrew Dickson, University of California San Diego, Scripps Institution of Oceanography, https://www.nodc.noaa.gov/ocads/oceans/Dickson_CRM/batches.html). The remaining in situ carbon chemistry parameters of pHT, pCO2, bicarbonate, and carbonate ions were calculated using the R package seacarb (Gattuso et al., 2020) based on direct measurements of DIC and TA and the salinity and temperature at the time of water sampling. Equilibrium constants for the dissociation of carbonic acid in seawater (K1 and K2) followed Mehrbach et al. (1973) and refitted by Dickson and Millero (1987). The constant for KHSO4 followed Dickson (1990).

Data Processing Description

Hatch counts: Larvae were counted from replicate rearing containers and moved to new replicates for continued rearing or sampled for morphometric measurements.

Survival statistics: Larvae were counted at hatch (9 - 10 days post fertilization) and after 9 or 10 days of larval rearing (19 days post fertilization). Survival was summarized over three intervals: 1) embryo survival (fertilization to hatch), 2) larval survival (hatch to 9 or 10 days post hatch), 3) overall survival (fertilization to 9 or 10 days post hatch). Survival is reported by individual rearing replicate (N = 4 per treatment).

BCO-DMO Processing Description

* Sheet "data" of submitted file "Dataset2_Murray et al_HatchingData.xlsx" was imported into the BCO-DMO data system for this dataset.
** Missing data values are displayed differently based on the file format you download from BCO-DMO. They are blank in csv files, "NaN" in MatLab files, etc.
* Dates converted to ISO 8601 format
* Sheet "data" of submitted file "Dataset3_Murray et al_SurvivalStatistics.xlsx" was added as a supplemental file.

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Data Files

File
925081_v1_larval-hatching.csv (Comma Separated Values (.csv), 14.33 KB) MD5:309b7d31d7482976826cfb682ace56ee Primary data file for dataset ID 925081, version 1

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Supplemental Files

File
Survival statistics filename: 925081_v1_survival.csv (Comma Separated Values (.csv), 1.06 KB) MD5:94c1b88fa64bc3a83d29cbae693d5c42 Embryo and larval survival statistics by replicate of Atlantic silverside offspring. Column information: fert_date,fertilization date parental_treatment,parent treatment level (control of hypoxia and acidification (HypOA)) offspring_treatment,offspring treatment level (control of hypoxia and acidification (HypOA)) tank,tank number group,treatment group (first letter is parental treatment and second lettear is offspring treatment) replicate,"replicate ID number, a combination of treatment group and individual replicate number" start_embryos,the number of starting embryos per survival replicate total_hatch,total hatchlings counted per day percent_hatch,ratio of hatchlings counted per day over starting embryo count surv_count_19dpf,total larvae that survived to 19 days post fertilization larval_survival,the ratio of larvae that survived to 19 days post fertilization divided by the total number of hatchlings overall_survival,the ratio of larvae that survived to 19 days post fertilization divided by the number of starting embryos

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Related Publications

Dickson, A. G. (1990). Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K. Deep Sea Research Part A. Oceanographic Research Papers, 37(5), 755–766. doi:10.1016/0198-0149(90)90004-f https://doi.org/10.1016/0198-0149(90)90004-F
Methods

Dickson, A. G., & Millero, F. J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers, 34(10), 1733–1743. doi:10.1016/0198-0149(87)90021-5
Methods

Dickson, A., National Oceanic and Atmospheric Administration (n.d.). CRM Batches: Information on Batches of CO2 in Seawater Reference Material. Ocean Carbon and Acidification Data System. Available from https://www.nodc.noaa.gov/ocads/oceans/Dickson_CRM/batches.html
Methods

Gattuso, JP, Epitalon, JM, Lavigne, H, ...(2023) Seacarb: seawater carbonate chemistry with R, R package version 3.3.2. http://CRAN.R-project.org/package=seacarb
Software

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, R. M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography, 18(6), 897–907. doi:10.4319/lo.1973.18.6.0897
Methods

Murray, C. (2024) Results publication in preparation.
Results

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Related Datasets

IsRelatedTo

Murray, C. S. (2024) **Morphological measurements of Atlantic silverside (Menidia menidia) larvae reared under ambient or hypoxia/acidification treatments during cross-generational laboratory experiments in 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2024-04-16 http://lod.bco-dmo.org/id/dataset/925042 [view at BCO-DMO]
Relationship Description: Data from the same experiments with the same individuals.

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Parameters

Parameter	Description	Units
fert_date	fertilization date	unitless
sample_date	sample date	unitless
dpf	age of sample as days post fertilization	days
tank	tank number	unitless
parental_treatment	parent treatment level (control of hypoxia and acidification (HypOA))	unitless
offspring_treatment	offspring treatment level (control of hypoxia and acidification (HypOA))	unitless
group	treatment group (first letter is parental treatment and second letter is offspring treatment)	unitless
replicate	replicate ID number, a combination of treatment group and individual replicate number	unitless
starting_embryos	the number of starting embryos per survival replicate	unitless
daily_hatch	total hatchlings counted per day	unitless
cum_hatch	cumulative hatch count for all days	unitless
daily_per	ratio of hatchlings counted per day over starting embryo count	unitless
cum_per	ratio of cumulative hatch count over starting embryo count	unitless

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Instruments

Dataset-specific Instrument Name	Apollo AS-D1 analyzer connected to a Picarro G-2121i cavity ringdown system
Generic Instrument Name	Apollo AS-D1 DIC and d13C-DIC Analyzer
Dataset-specific Description	Samples were measured in triplicate for dissolved inorganic carbon (DIC) using an Apollo AS-D1 analyzer connected to a Picarro G-2121i cavity ringdown system.
Generic Instrument Description	The AS-D1 is an instrument designed to prepare natural water samples for Dissolved Inorganic Carbon (DIC) and delta13C analysis and provide the user with the analyses outputs. It has features that are specifically useful for seawater and coastal water samples. The instrument provides the user with DIC values (micromol per kg) and the delta13C content of the DIC (per mille). It consists of a digital syringe pump for delivery of reagent and samples, a mass flow controller to regulate flow rate, a CO2 stripping reactor, and an electronic cooling system to remove moisture. The AS-D1 does not measure the sample but is designed to send the gas to a different analyzer. This second instrument then sends the measurements back to the AS-D1 after analysis. The AS-D1 then calculates the desired DIC and delta13C outputs. This instrument is designed for automatic sampling from multiple bottles. It can be used in laboratories on shore or at sea. The instrument was created to be paired with the Picarro G-2131i Carbon Isotope Analyser, however, other models that measure the isotopic ratio of CO2 may be compatible. The precision is +/- 0.1 % for DIC of seawater and +/- 0.07 % for DIC-delta13C. Sample volume is 1-7 milliliters per analysis, and sample time is under 12 minutes. Additional information from the manufacturer is available at: https://apolloscitech.com/dicdelta.html

Dataset-specific Instrument Name	Pyroscience Firesting®-GO2
Generic Instrument Name	Oxygen Sensor
Dataset-specific Description	DO levels in individual rearing units were cross-verified using a handheld Pyroscience Firesting®-GO2 meter fitted with an optical DO sensor. A two-point calibration (100% and 0% DO) was made daily using air-saturated seawater and a concentrated NaSO3 seawater solution.
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	Pyroscience® Ultra Compact (PICO) DO meter
Generic Instrument Name	Oxygen Sensor
Dataset-specific Description	The mixing tank received a continuous flow of ambient seawater, with DO, pH, and temperature measurements taken every 10 seconds by Pyroscience® Ultra Compact (PICO) DO and pH meters, fitted with fiberoptic DO or pH sensors and PT100 temperature sensors. Calibration of the probes was performed weekly according to the manufacturer's guidelines using oxygen and pHT calibration solutions provided by Pyroscience®. DO levels in individual rearing units were cross-verified using a handheld Pyroscience Firesting®-GO2 meter fitted with an optical DO sensor. A two-point calibration (100% and 0% DO) was made daily using air-saturated seawater and a concentrated NaSO3 seawater solution.
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Dataset-specific Instrument Name	
Generic Instrument Name	pH Sensor
Dataset-specific Description	The mixing tank received a continuous flow of ambient seawater, with DO, pH, and temperature measurements taken every 10 seconds by Pyroscience® Ultra Compact (PICO) DO and pH meters, fitted with fiberoptic DO or pH sensors and PT100 temperature sensors. Calibration of the probes was performed weekly according to the manufacturer's guidelines using oxygen and pHT calibration solutions provided by Pyroscience®.
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

Dataset-specific Instrument Name	
Generic Instrument Name	pH Sensor
Dataset-specific Description	pH conditions were verified via a Hach HQ11D pH meter fitted with a Hach PHC28101 IntelliCal pH probe. The probe was calibrated daily using pHT buffers from PyroScience®.
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

Dataset-specific Instrument Name	
Generic Instrument Name	Refractometer
Generic Instrument Description	A refractometer is a laboratory or field device for the measurement of an index of refraction (refractometry). The index of refraction is calculated from Snell's law and can be calculated from the composition of the material using the Gladstone-Dale relation. In optics the refractive index (or index of refraction) n of a substance (optical medium) is a dimensionless number that describes how light, or any other radiation, propagates through that medium.

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Project Information

OCE-PRF Detecting signatures of multigenerational plasticity in a marine forage fish (HypOA Cross-generational Plasticity)

Coverage: Temperate Coastal Ecosystems

NSF Award Abstract:

This award is funded in whole or in part under the American Rescue Plan Act of 2021 (Public Law 117-2).

Coastal marine ecosystems face multiple anthropogenic stressors including increasingly severe events of co-occurring acidification and hypoxia. These periodic but acute environmental stressors can directly impact the abundance, diversity, and commercial value of coastal fish stocks. Rapid acclimation of key physiological processes can provide short-term protection against extreme conditions. Importantly, these phenotypic modifications can be passed on to subsequent generations thereby priming offspring for increased tolerance. However, for most fish species the scope for phenotypic plasticity and the precise mechanisms of action are poorly understood. This severely limits our ability to anticipate responses in the majority of ecologically and economically important marine species. The overarching objective of this proposal is to investigate the potential for within-generational and multigenerational plasticity in response to co-occurring hypoxia and acidification in the forage fish Atlantic silverside (Menidia menidia). The Atlantic silverside is a foundational species and an essential trophic component of coastal food webs along the North American Atlantic coast and serves as key prey item for many seabirds and commercially important fish. Understanding the long-term bioenergetic impacts and the potential for rapid adaptation in this species will therefore fundamentally advance our understanding of the ecological consequences of rapid environmental change in coastal marine ecosystems.

The project will be centered around a series of laboratory exposure experiments and state-of-the-art metabolic assays utilizing wild-caught Atlantic silversides collected during their spring spawning season. The PI will investigate how parental environments influence offspring phenotype by conditioning mature wild Atlantic silversides (F0) to contrasting fluctuating CO2/O2 treatments. The F1 generation will be then reared in a reciprocal transplant experiment to quantify how parental and offspring treatment levels affect key life-history traits including survival, growth, and aerobic performance. Surviving F1 offspring will be reared until maturity to evaluate how environmental stress experienced during early development affects adult reproductive capacity. Furthermore, the PI will investigate the role of biological memory in multigenerational plasticity by exposing F2 offspring to factorial combinations of grandparental and parental environments. In parallel, the PI will investigate how molecular mechanisms may mediate rapid adaptation to changing environments using transcriptomics (RNAseq) and genome-wide DNA methylation profiling (Methylseq). Linking molecular changes with whole organism phenotypic responses will broaden our understanding of the effects of multiple stressors on an ecologically important species which will provide clues for developing mitigation plans to protect coastal food webs from various climatic factors.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2126533

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