Egg measurements from the fecundity trial in a study of CO2 and temperature-specific reproductive traits in Menidia menidia

Website: https://www.bco-dmo.org/dataset/845921
Data Type: experimental
Version: 1
Version Date: 2021-03-18

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
The study examined the temperature- and CO2-specific size and reproductive traits in female Atlantic silversides (Menidia menidia) after long-term and whole-life rearing. This dataset includes egg measurements from the fecundity trial.

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Coverage

Spatial Extent: **Lat**:41.32 **Lon**:-72.02
Temporal Extent: 2018-06-05 - 2019-05-03

Dataset Description

The study examined the temperature- and CO2-specific size and reproductive traits in female Atlantic silversides (*Menidia menidia*) after long-term and whole-life rearing. This dataset includes egg measurements from the fecundity trial. See Related Datasets for additional results from this study.

Methods & Sampling

Experimental CO_2 and temperature treatments: The spawning trial reared wild-caught juveniles to maturity at 20°C under duplicate control (~600 µatm CO_2) and high CO_2 conditions (~2,000 µatm CO_2), whereas the fecundity trial reared newly fertilized embryos to maturity under similar control vs. high CO_2 conditions (~350 µatm, ~2,200 µatm) crossed with two rearing temperatures (17°C, 24°C).

Experimental treatment tanks were continuously bubbled with mixes of air and 100% bone-dry CO_2 controlled

by gas proportioners (ColeParmer®). In control CO_2 treatments, metabolic acidification by the fish was offset by bubbling CO_2-stripped air (~50 µatm CO_2) via diffuser tubes into the rearing tanks (Murray and Baumann, 2020). All treatments maintained near 100% dissolved oxygen saturations (~8.1, 7.6, and 7.1 mg L^{-1} at 17°C, 20°C, and 24°C, respectively). Salinity (~31 psu) was controlled via refractometer, temperature conditions were maintained by thermostats (Aqualogic®) connected to submersible heaters or in-line chillers (DeltaStar®), and target pH conditions were monitored daily using a handheld pH meter (Hach® Intellical PHC281 pH electrode with HQ11D handheld pH/ORP meter, calibrated bi-weekly using two-point NIST buffers).

Offspring production and laboratory rearing: For the spawning trial, juveniles were caught on 25 September 2015 via beach seine (30 × 2 m) in Mumford Cove; a small seagrass dominated embayment connected to eastern Long Island Sound (41.32°N, 72.02°W). Individuals were transported to the Rankin Seawater Facility at the University of Connecticut, where approximately 100 individuals (mean TL = 4.5 cm) were randomly assigned to each of two 700-L replicate rearing tanks per CO_2 treatment and reared to maturity over the next five months at 20°C.

For the fecundity trial, spawning-ripe silversides were captured in Mumford Cove on 29 May 2018, transported to the laboratory, separated by sex, and held without food in 80-L aerated containers at ambient CO_2 and temperature conditions (17°C). The day after, 20 females and 27 males were strip-spawned together using established protocols (Baumann et al., 2018; Murray and Baumann, 2020). The fecundity trial was terminated before spawning occurred at 317-325 dph, when all fish were euthanized with MS-222 (Tricaine-S).

Trait measurements: For the spawning trial, spawning in each tank began when silversides were provided bundled threads of green yarn suspended mid-tank. Yarns were checked every 48h, deposited embryos counted, and egg diameters measured in a subsample (n = 15) via calibrated pictures (4× magnification, ImagePro Premier, v9.0, Media Cybernetics®). The spawning trial was terminated after 60 days, which is approximately the extent of silverside spawning season in the wild (Pringle and Baumann, 2019). Surviving fish were euthanized, measured for TL (0.1 mm) and wet weight (wW, 0.01g), and their sex determined by gonad inspection under a stereo microscope (Nikon SMZ-1000). The total number of embryos spawned was divided by the cumulative wet weight of females in each duplicate CO_2 treatment to obtain a mass-specific estimate of embryo production (n_{emb} g $female^{-1}$). This necessarily assumed that all females actually participated in spawning.

For the fecundity trial, euthanized fish were measured for TL and wW, followed by dissection for sex identification and gonad removal. Re-weighing yielded the gonad-free body weight (wW_{ng}), which was subtracted from wW to yield gonad weight (wW_g) and the gonado-somatic index ($GSI = 100*wW_g/wW_{ng}$). Male gonads were discarded; female gonads were preserved in 5% buffered formaldehyde/freshwater solution. One ovary lobe per female was saved for histological analyses; the other was used to quantify all secondary growth (i.e., vitellogenic) oocytes. The ovary lobe was divided into anterior, middle, and posterior sections that were each gently teased apart so that oocytes evenly distributed between four rectangular 2 mL wells. Each well was photographed (3× magnification) and analyzed via ImageJ (v1.52a, National Institute of Heath) with the ObjectJ plug-in (v1.04t, University of Amsterdam) and a customized macro to count and measure the diameter of all oocytes. The counted total number of oocytes per lobe was doubled to obtain each female's potential fecundity (F_{Pot}, oocytes $female^{-1}$) and relative fecundity ($F_{Rel} = F_{Pot}*wW^{-1}$, oocytes g $female^{-1}$). In addition, we averaged the size of the 25 largest oocytes per female (~99% percentile) as a measure of maximum oocyte size.

The other ovary lobe from a subset of 17 females at control CO_2 treatments ($n_{17°C} = 6$, $n_{24°C} = 11$) and 20 females at high CO_2 treatments ($n_{17°C} = 8$, $n_{24°C} = 12$) were embedded in paraffin, sectioned, placed on glass slides, and stained with H&E (Horus Scientific, Worcester, MA). Slides were then photographed (4× magnification) and six oocyte developmental stages identified: primary growth (PG), cortical alveolar (CA), early vitellogenesis (V1), late vitellogenesis (V2), nuclear migration (NM), and hydrated (H) oocytes (following Ganias et al., 2004; Hyle et al., 2014; Press et al., 2014). We also checked for any postovulatory follicles that would indicate the beginning of spawning. Up to 56 oocytes per developmental stage and female were measured (mean = 13.5). Histological preparation deformed most oocytes to ellipses; therefore we first calculated the area of each ellipse from its major and minor axis and then inferred the oocyte diameter (=size) from a circle with the same area. For each female, we determined the stage-specific median and maximum oocyte size, which were then compared across treatments.

Sampling and analytical procedures: With the replication unit being each 700-L tank (instead of each female), the spawning trial thus had duplicates for each CO_2 level (single temperature), while the fecundity trial had single replicates for each CO_2 × temperature combination. Although the 24°C treatments ended with two equal density groups per CO_2 level, these were not true replicates, because fish were only separated after 245

dph. Given that both groups were statistically identical with respect to TL and wW (t-tests, $p > 0.05$), they were pooled for all subsequent analyses. Lacking the 3+ replicates that means-based statistical approaches require (e.g., general linear models), we instead compared trait distributions between CO_2 and temperature treatments using non-parametric Kruskal-Wallis tests. We focused on female traits here, because reproductive investment in fish females is more costly and more tightly linked to body size and fitness than in males (for male distributions, see supplementary material). We examined female TL and wW (spawning + fecundity trials), number and size of spawned embryos (spawning trial), GSI, potential and relative fecundity, maximum oocyte size, as well as stage-specific oocyte sizes (fecundity trial). Three females were removed from analysis, because they had either no discernible or only immature oocytes in their gonads. Statistical analyses were performed in R v3.6.1 (R Core Team, 2019) and SPSS (V20, IBM).

Data Processing Description

Data Processing: Achieved pCO_2 levels were calculated via CO2SYS (v2.1) from total alkalinity (A_T) measurements in samples of tank and lab source water ($N_{Spawn} = 12$, $N_{Fecund} = 17$) measured via endpoint titration (G20 Potentiometric Titrator, Mettler Toledo®) with an accuracy of ±1% (Dr. Andrew Dickson's certified A_T reference, batch number 162). Using A_T, pH, temperature, salinity, and K2 constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and Dickson (1990), we derived CO_2 partial pressure (pCO_2, µatm), dissolved inorganic carbon (C_T, µmol kg^{-1}), and carbonate ion concentrations (CO_3^{2-}, µmol kg^{-1}).

BCO-DMO Processing:
- converted all dates to YYYY-MM-DD format;
- renamed fields to comply with BCO-DMO naming conventions.

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Data Files

File
egg_measurements.csv (Comma Separated Values (.csv), 129.13 KB) MD5:83deda220c2dd7bfab301470790ec28d Primary data file for dataset ID 845921

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Related Publications

Baumann, H., Cross, E. L., & Murray, C. S. (2018). Robust quantification of fish early life CO2 sensitivities via serial experimentation. Biology Letters, 14(11), 20180408. doi:10.1098/rsbl.2018.0408
Methods

Concannon, C. A., Cross, E. L., Jones, L. F., Murray, C. S., Matassa, C. M., McBride, R. S., & Baumann, H. (2021). Temperature-dependent effects on fecundity in a serial broadcast spawning fish after whole-life high CO2 exposure. ICES Journal of Marine Science. doi:10.1093/icesjms/fsab217
Results

Dickson, A. G. (1990). Standard potential of the reaction: AgCl(s) + 1/2 H2(g) = Ag(s) + HCl(aq) and the standard acidity constant of the ion HSO4− in synthetic sea water from 273.15 to 318.15 K. The Journal of Chemical Thermodynamics, 22(2), 113–127. doi:10.1016/0021-9614(90)90074-z https://doi.org/10.1016/0021-9614(90)90074-Z
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

Ganias, K., Somarakis, S., Machias, A., & Theodorou, A. (2004). Pattern of oocyte development and batch fecundity in the Mediterranean sardine. Fisheries Research, 67(1), 13–23. doi:10.1016/j.fishres.2003.08.008
Methods

Hyle, A. R., McBride, R. S., & Olney, J. E. (2014). Determinate Versus Indeterminate Fecundity in American Shad, an Anadromous Clupeid. Transactions of the American Fisheries Society, 143(3), 618–633. doi:10.1080/00028487.2013.862178
Methods

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. S., & Baumann, H. (2020). Are long-term growth responses to elevated pCO2 sex-specific in fish? PLOS ONE, 15(7), e0235817. doi:10.1371/journal.pone.0235817
Methods

Press, Y. K., McBride, R. S., & Wuenschel, M. J. (2014). Time course of oocyte development in winter flounder Pseudopleuronectes americanus and spawning seasonality for the Gulf of Maine, Georges Bank and southern New England stocks. Journal of Fish Biology, 85(2), 421–445. doi:10.1111/jfb.12431
Methods

Pringle, J., & Baumann, H. (2019). Otolith-based growth reconstructions in young-of-year Atlantic silversides (Menidia menidia) and their implications for sex-selective survival. Marine Ecology Progress Series. doi:10.3354/meps13174
Methods

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Related Datasets

IsRelatedTo

Baumann, H., Nye, J. (2021) **Data from the fecundity trial in a study of CO2 and temperature-specific reproductive traits in Menidia menidia.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-03-18 doi:10.26008/1912/bco-dmo.845906.1 [view at BCO-DMO]

Baumann, H., Nye, J. (2021) **Data from the spawning trial in a study of CO2 and temperature-specific reproductive traits in Menidia menidia.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-04-23 doi:10.26008/1912/bco-dmo.845633.1 [view at BCO-DMO]

Baumann, H., Nye, J. (2021) **Data on egg production resulting from the spawning trial in a study of CO2 and temperature-specific reproductive traits in Menidia menidia.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2021-03-18 doi:10.26008/1912/bco-dmo.845804.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Species	Atlantic silverside, Menidia menidia, sampled in Mumford Cove, CT	unitless
Temp	static temperature used for whole life cycle rearing during the fecundity trial	degrees Celsius
CO2_T	CO2 rearing treatment, control vs. high	unitless
pH	Average pH during the experimental period	NIST
pCO2	Average pCO2 partial pressure during the rearing period	microatmospheres (µatm)
FishID	Individual female identifier number	unitless
Stage	Oocyte developmental stage based on histology samples. PG=primary growth, CA=cortical alveolar, V1=early vitellogenesis, V2=late vitellogenesis, NM=nuclear migration, H=hydrated	unitless
Dia	Oocyte diameter	micrometers (µm)

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Instruments

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

Dataset-specific Instrument Name	G20 Potentiometric Titrator (Mettler Toledo)
Generic Instrument Name	Automatic titrator
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	stereo microscope (Nikon SMZ-1000)
Generic Instrument Name	Microscope - Optical
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	handheld pH meter
Generic Instrument Name	pH Sensor
Dataset-specific Description	handheld pH meter (Hach® Intellical PHC281 pH electrode with HQ11D handheld pH/ORP meter, calibrated bi-weekly using two-point NIST buffers)
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	refractometer
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.

Dataset-specific Instrument Name	thermostats (Aqualogic®)
Generic Instrument Name	thermostat
Generic Instrument Description	A device designed to regulate temperature by controlling the starting and stopping of a heating/cooling system.

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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-1536165

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