

Data from the spawning trial in a study of CO₂ and temperature-specific reproductive traits in *Menidia menidia*

Website: <https://www.bco-dmo.org/dataset/845633>

Data Type: experimental

Version: 1

Version Date: 2021-04-23

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 results from the spawning trial.

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Coverage

Spatial Extent: Lat:41.32 Lon:-72.02

Temporal Extent: 2015-09-25 - 2016-05-03

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 results from the spawning trial. See Related Datasets for additional results from this study.

Methods & Sampling

Experimental CO₂ and temperature treatments: The spawning trial reared wild-caught juveniles to maturity at 20°C under duplicate control (~600 µatm CO₂) and high CO₂ conditions (~2,000 µatm CO₂), whereas the fecundity trial reared newly fertilized embryos to maturity under similar control vs. high CO₂ 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 CO₂ controlled

by gas proportioners (ColeParmer®). In control CO₂ treatments, metabolic acidification by the fish was offset by bubbling CO₂-stripped air (~50 µatm CO₂) 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 CO₂ 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 CO₂ 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 CO₂ 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 ($GSI = 100 \cdot wW_g / wW_{ng}$). 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 Health) 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 ($F_{Rel} = F_{Pot} \cdot wW^{-1}$, 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 CO₂ treatments ($n_{17^\circ C} = 6$, $n_{24^\circ C} = 11$) and 20 females at high CO₂ treatments ($n_{17^\circ C} = 8$, $n_{24^\circ C} = 12$) 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 Ganas 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 CO₂ level (single temperature), while the fecundity trial had single replicates for each CO₂ × temperature combination. Although the 24°C treatments ended with two equal density groups per CO₂ 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 CO₂ 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 pCO₂ levels were calculated via CO2SYS (v2.1) from total alkalinity (A_T) measurements in samples of tank and lab source water ($N_{\text{spawn}} = 12$, $N_{\text{fecund}} = 17$) measured via endpoint titration (G20 Potentiometric Titrator, Mettler Toledo®) with an accuracy of $\pm 1\%$ (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 CO₂ partial pressure (pCO₂, μatm), dissolved inorganic carbon (C_T , $\mu\text{mol kg}^{-1}$), and carbonate ion concentrations (CO_3^{2-} , $\mu\text{mol kg}^{-1}$).

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 |
|--|
| spawning_trial.csv (Comma Separated Values (.csv), 9.18 KB) MD5:8713463f03d31c165d452268bad590b9 |
| Primary data file for dataset ID 845633 |

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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](https://doi.org/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](https://doi.org/10.1093/icesjms/fsab217)
Results

Dickson, A. G. (1990). Standard potential of the reaction: $\text{AgCl(s)} + 1/2 \text{H}_2(\text{g}) = \text{Ag(s)} + \text{HCl(aq)}$ and the standard acidity constant of the ion HSO_4^- 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](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](https://doi.org/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](https://doi.org/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](https://doi.org/10.1080/00028487.2013.862178)
Methods

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicz, 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/lm.1973.18.6.0897](https://doi.org/10.4319/lm.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](https://doi.org/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](https://doi.org/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](https://doi.org/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 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](#)]

Baumann, H., Nye, J. (2021) **Egg measurements 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.845921.1 [[view at BCO-DMO](#)]

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Parameters

| Parameter | Description | Units |
|------------------------------|---|-------------------------------|
| Species | Atlantic silverside, <i>Menidia menidia</i> , sampled in Mumford Cove, CT | unitless |
| Sample_date_field | Date (EST/EDT) when juvenile founders of the spawning trial were sampled in the field; format: YYYY-MM-DD | unitless |
| Sample_TL_field | Mean total length of juvenile founders from 9-25-15 | millimeters (mm) |
| Spawning_date_spawning_trial | Date (EST/EDT) when the spawning trial was concluded; format: YYYY-MM-DD | unitless |
| Rearing_temp | static temperature used for rearing juveniles during the spawning 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 (μ atm) |
| Ind_nr | Individual identifier number | unitless |
| Replicate | each CO2 levels had two replicates | unitless |
| TL | Total length of the individual at the end of the experiment | millimeters (mm) |
| wW | wet weight of the individual at the end of the experiment | grams (g) |
| Sex | Sex; only females are given (f) | unitless |

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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](https://doi.org/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](https://doi.org/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](https://doi.org/10.3354/meps11142)

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Funding

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

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