

Morphometrics of Atlantic silversides (*Menidia menidia*) reared at contrasting pCO₂ conditions in laboratory experiments conducted with embryos from adults collected in Long Island Sound in 2021 and 2022

Website: <https://www.bco-dmo.org/dataset/1009596>

Version: 1

Version Date: 2026-10-09

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

We experimentally examined the ontogeny of schooling and cranial neuromast development under contrasting CO₂ conditions in Atlantic silverside larvae (*Menidia menidia*), an ecologically important forage fish in the Northwest Atlantic. Larvae were produced from wild spawners and reared them until 25 days post hatch (dph; Schooling Exp) or 34 dph (Neuromast Exp) at three contrasting pCO₂ conditions (~400, ~2200, ~4200 µatm). We then measured larval morphometrics throughout and at the conclusion of two experiments.

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Coverage

Location: Northwest Atlantic

Spatial Extent: Lat:41.323611 Lon:-71.9059

Temporal Extent: 2021-05 - 2022-06

Methods & Sampling

Spawning Atlantic silversides (*Menidia menidia*) were caught on May 24th, 2021, and May 15th, 2022 (for lateral line development and schooling ontogeny experiments, respectively, see below) in Mumford Cove (41°19'25"N 72°01'07"W). Fish were captured using a beach seine (30 × 2m) and were transported to the University of Connecticut's Rankin Seawater Laboratory, separated by sex, and held at 20°C in 80-l aerated tanks. The following day, fish were strip-spawned (following Murray et al., 2014, 2017; Malvezzi et al., 2015), and within one hour embryos were randomly distributed into 20-l replicate rearing containers.

Experimental Design:

Each container was equipped with 4 mesh-covered holes (7 cm diameter, 150 μm mesh) to allow water exchange with the individual surrounding 600-l tanks in the Automatic Larval Fish Rearing System (ALFiRiS). This custom-built system consists of nine such recirculation units that are independently controlled for pH and temperature via a customized LabView program (Baumann et al., 2022; Zavell and Baumann, 2024). ALFiRiS measures conditions once per hour via sampling pumps connected to a central pH sensor (Hach pH/D digital electrode) that is calibrated weekly using NIST 2-point pH references. As needed, the system automatically adjusts pH / $p\text{CO}_2$ levels by triggering solenoid valves to inject standardized amounts of 100% bone dry CO_2 (to decrease pH) or CO_2 -stripped air (to increase pH), while maintaining desired temperatures via computer-controlled submersible heaters (Finnex Deluxe Titanium, 800W) or in-line chillers (DeltaStar $\frac{1}{3}$ horsepower). All trials experiments were conducted at 22°C to resemble late spring conditions at Mumford Cove normally experienced by spawning silversides and their larvae (Murray and Baumann, 2018).

Two experiments were done to quantify schooling and cranial neuromast development. In our **schooling ontogeny experiment** we reared silversides from fertilization to 25 days post hatch (dph) at three CO_2 levels, while repeatedly conducting standardized burst photography to identify larval positions – and subsequently larval distance distributions – in each rearing container. This simple but robust, quantitative, and observer-unbiased metric showed ontogenetic changes that clearly differed between CO_2 treatments. A separate **lateral line development experiment** then specifically tested our hypothesis that high CO_2 environments affect schooling due to morphological changes and/or the development of the LL system. In both experiments three CO_2 treatments were used, referred to hereafter as control (8.15 pH units, $\sim 400 \mu\text{atm CO}_2$), elevated (7.45 pH units, $\sim 2200 \mu\text{atm CO}_2$), or extreme (7.20 pH units, $\sim 4200 \mu\text{atm CO}_2$) CO_2 conditions. The control CO_2 treatment is characteristic of the average local surface ocean and of coastal systems at the start of the silverside spawning season in spring (Murray et al., 2014). The elevated CO_2 level represents the maximum predicted open ocean CO_2 for the next 300 years (Caldeira and Wickett, 2005), and it is both a common benchmark in ocean acidification studies as well as a level already observed in productive coastal systems during summer (Baumann et al., 2015). The extreme CO_2 level is rarely reached yet in contemporary systems (Baumann et al., 2015), but may become more common in productive nearshore habitats under future climate and eutrophication scenarios (Wallace et al., 2014).

Actual CO_2 levels were determined biweekly by measuring total alkalinity (A_T ; Murray and Baumann, 2018) in 300 mL samples of filtered seawater (10 μm) taken from each tank. Salinity was measured concurrently using a handheld refractometer. Endpoint titrations (G20 Potentiometric Titrator, Mettler Toledo) were calibrated with Dr. Andrew Dickson's certified reference material for A_T in seawater (UCSD Scripps Institution of Marine Science; Dickson et al., 2003) and titration accuracy was ($\pm 1\%$). Measured temperature, salinity, pH, and A_T were then used to calculate CO_2 (CO2SYS V2.1; https://cdiac.ess-dive.lbl.gov/ftp/co2sys/CO2SYS_calc_XLS_v2.1/) using K_1 , K_2 constants from Mehrbach et al. (1973), refitted by Dickson & Millero (1987), and Dickson (1990) for potassium bisulfate (KHSO_4).

Upon hatch, larvae were fed both a powdered weaning diet (Otohime Marine Fish Diet, size A1, Reed Mariculture, Campbell, CA, USA) and newly hatched brine shrimp nauplii ad libitum (Artemia salina, San Francisco Strain, brineshrimpdirect.com, Ogdon, UT, USA). During the remainder of each experiment larvae were fed daily ad libitum rations of newly hatched nauplii. Ammonia was measured daily (API Saltwater Test Kit) to assure that levels remained < 0.25 ppm. Rearing containers were siphoned daily to remove uneaten food and detritus, and 25% partial water changes were conducted weekly. Salinity (27 – 31 psu) and light conditions (15 h light: 9 h dark) reflected optimal levels for growth for this species (Middaugh et al., 1987). Throughout both experiments, sampled larvae were euthanized and fixed in cold (2–4°C) 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). 48 hours following fixation, larvae were transferred to 70% ethanol in deionized water, which was changed twice to remove excess PFA.

Schooling Ontogeny Experiment:

To address the effects of ocean acidification on schooling, a schooling experiment quantified larval behavior under the three different CO₂ conditions. Approximately 500 embryos were reared to hatch in one 20-l rearing container for each of 3 ALFiRiS units tanks per CO₂ treatment (except for the extreme CO₂ treatment, where a fungal outbreak rendered one unit unusable). Hatching occurred 8 days post fertilization (May 23rd, 2022). At 1 day post hatch (dph), 50 larvae were randomly distributed into each of two 'schooling' containers per ALFiRiS unit. Schooling containers were the same as all other containers, but had one additional, mesh-covered hole located 2 cm above the container bottom to drain excess water during schooling trials. Therefore, the control and elevated CO₂ treatments each had 6 schooling containers (2 per ALFiRiS unit) and 3 rearing containers (1 per ALFiRiS unit). In the extreme treatment, we added a third schooling container per ALFiRiS unit (total of 6 schooling and 2 rearing containers). Larvae were reared until 25 dph and surviving larvae were euthanized and fixed. After being euthanized and fixed, larvae were photographed using calibrated images (Nikon SMZ1000; Image-Pro Premier V.9.3.3, Media Cybernetics, Rockville, MD) and measured for standard length (SL, in mm), eye diameter (ED; horizontal diameter of the orbit, in mm), and lens diameter (LD, in mm), and the ratio of ED:SL was calculated as a proxy for relative visual capacity (Caves et al. 2017, Lisney et al 2020).

Lateral Line Development Experiment:

The lateral line development experiment quantified the rate of development of the cranial lateral line system including the number of neuromasts under contrasting CO₂ conditions (control and extreme, as defined above). The cranial lateral line system (as opposed to the trunk LL system) was studied given its role in maintaining spatial positioning within a school of fish (Mekdara et al., 2021). Approximately 500 embryos were randomly allocated to each of two 20-l rearing containers (one control and one extreme CO₂ treatment). Hatching occurred 9 days post fertilization (dpf; June 1st, 2021), and 15 larvae were sampled daily from 0 to 7 dph, and then 3× per week until 34 dph (to ~14 mm SL). Each day 5 larvae were used for vital fluorescent staining of the neuromasts using 4-Di-2-ASP 4-(4-Diethylaminostyryl)-1-methylpyridinium iodide (4-Di-2-ASP; Nakae et al., 2012; Nickles et al., 2020) and the other 10 sampled larvae were euthanized and fixed as previously described for future imaging of neuromasts using SEM (scanning electron microscopy). Fluorescent imaging of stained larvae was done on a Nikon SMZ 1500 dissecting microscope with epifluorescence (GFP filter set) and captured with a SPOT® digital camera and SPOT software (v 5.2.21, Diagnostic Instruments Inc., Sterling Heights, MI). Then larvae were fixed as previously described and subsequently measured (notochordal length, NL, for pre-flexion larvae; or SL, for post-flexion larvae; and total length, TL). Adobe Photoshop® CS3 was used to generate panoramas from multiple images to visualize complete distribution of neuromasts. Adobe Illustrator® CS3 was then used to make diagrams of neuromast distributions from panoramas. Neuromast numbers were determined for each larva by two independent analysts blinded to the treatments.

Organism Identifier (LSID, Life Sciences Identifier):

Menidia menidia, urn:lsid:marinespecies.org:taxname:159228

BCO-DMO Curation Notes

This section documents curation actions performed prior to publication review with the submitter, and additional information relevant to understanding and reusing this dataset. It distinguishes changes made to the submitted (meta)data from unresolved issues and/or enhancements that improve future reuse and interoperability.

CURATION ACTIONS PERFORMED ON DATA

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CURATION ACTIONS PERFORMED ON METADATA

- BCO-DMO's standard metadata entry and text formatting steps were performed. See: <https://www.bco-dmo.org/how-to/standard-curation-edits>

ISSUES POTENTIALLY IMPACTING REUSE

Problem Description

Larval morphometrics were pooled by DPH, Tank, and rearing container ("Bucket.ID"). There is no individual fish ID.

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Parameters

Parameters for this dataset have not yet been identified

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Instruments

Dataset-specific Instrument Name	Automated Larval Fish Rearing System (ALFiRiS)
Generic Instrument Name	Automated Larval Fish Rearing System
Dataset-specific Description	Automated Larval Fish Rearing System (ALFiRiS) - UConn Avery Point Rankin Lab - self-designed and assembled
Generic Instrument Description	The Automated Larval Fish Rearing System (ALFiRiS) was self-designed and assembled in the Rankin Lab at the University of Connecticut Avery Point. It consists of a 3 x 3 array of recirculating units (600 liters/150 gallons) that have independent computer-control over their temperature, oxygen, and pH conditions. The system was designed to sequentially monitor tank conditions via industrial-grade oxygen and pH sensors (Hach) and then control gas solenoids (air, N2, CO2) to maintain and modulate environmental conditions. The system can apply static as well as fluctuating conditions on diel and tidal scales. Computerized temperature control allows simulating heatwaves and other non-static thermal regimes. More information can be found at https://befel.marinesciences.uconn.edu/alfiris/

Dataset-specific Instrument Name	Infinity2-1R, 1.4 Megapixel USB 2.0 Microscopy Camera CCD
Generic Instrument Name	Camera
Dataset-specific Description	Infinity2-1R, 1.4 Megapixel USB 2.0 Microscopy Camera CCD (Teledyne Lumenera, Ottawa, Canada)
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	SPOT digital microscope camera
Generic Instrument Name	Camera
Dataset-specific Description	SPOT digital microscope camera (SPOT Imaging, Sterling Heights, Michigan)
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	
Generic Instrument Name	digital thermometer
Dataset-specific Description	As needed, the system automatically adjusts pH /pCO ₂ levels by triggering solenoid valves to inject standardized amounts of 100% bone dry CO ₂ (to decrease pH) or CO ₂ -stripped air (to increase pH), while maintaining desired temperatures via computer-controlled submersible heaters (Finnex Deluxe Titanium, 800W) or in-line chillers (DeltaStar ⅓ horsepower).
Generic Instrument Description	An instrument that measures temperature digitally.

Dataset-specific Instrument Name	Nikon SMZ1000 Zoom Stereo Microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Nikon SMZ1000 Zoom Stereo Microscope (Nikon Instruments Inc., Melville, New York)
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	Nikon SMZ1500 Stereo Microscope
Generic Instrument Name	Microscope - Optical
Dataset-specific Description	Nikon SMZ1500 Stereo Microscope (Nikon Instruments Inc., Melville, New York)
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	Hach pHD digital electrode
Generic Instrument Name	pH Sensor
Dataset-specific Description	ALFiRiS measures conditions once per hour via sampling pumps connected to a central pH sensor (Hach pHD digital electrode) that is calibrated weekly using NIST 2-point pH references.
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	handheld refractometer
Generic Instrument Name	Refractometer
Dataset-specific Description	Actual CO ₂ levels were determined biweekly by measuring total alkalinity (AT; Murray and Baumann, 2018) in 300 mL samples of filtered seawater (10 µm) taken from each tank. Salinity was measured concurrently using a handheld 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) <i>n</i> 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	beach seine (30 × 2m)
Generic Instrument Name	Seine Net
Dataset-specific Description	Fish were captured using a beach seine (30 × 2m) and were transported to the University of Connecticut's Rankin Seawater Laboratory, separated by sex, and held at 20°C in 80-l aerated tanks.
Generic Instrument Description	A seine net is a very long net, with or without a bag in the centre, which is set either from the shore or from a boat for surrounding a certain area and is operated with two (long) ropes fixed to its ends (for hauling and herding the fish). Seine nets are operated both in inland and in marine waters. The surrounded and catching area depends on the length of the seine and of the hauling lines. (definition from: fao.org)

Dataset-specific Instrument Name	G20 Potentiometric Titrator
Generic Instrument Name	Titrator
Dataset-specific Description	G20 Potentiometric Titrator (Mettler Toledo, Columbus Ohio) Endpoint titrations (G20 Potentiometric Titrator, Mettler Toledo) were calibrated with Dr. Andrew Dickson's certified reference material for AT in seawater (UCSD Scripps Institution of Marine Science; Dickson et al., 2003) and titration accuracy was (±1%).
Generic Instrument Description	Titrators are instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

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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-1536336

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