

# Lateral line development in Atlantic silverside (*Menidia menidia*) larvae reared at contrasting pCO<sub>2</sub> conditions in a laboratory experiment conducted with embryos from adults collected in Long Island Sound in 2021

**Website:** <https://www.bco-dmo.org/dataset/1009501>

**Data Type:** Other Field Results, 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

We experimentally examined the ontogeny of schooling and cranial neuromast development under contrasting CO<sub>2</sub> 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 pCO<sub>2</sub> conditions (~400, ~2200, ~4200 µatm). See Related Datasets for resulting data from related experiment and supporting datasets. In this separate trial we then tested whether delayed schooling could result from potential CO<sub>2</sub> effects on the development of the mechanosensory lateral line system. We used vital fluorescent staining and scanning electron microscopy of cranial canal neuromasts but detected no differences in neuromast number, appearance, or in canal enclosure rate between CO<sub>2</sub> treatments.

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## Coverage

**Location:** Northwest Atlantic

**Spatial Extent:** Lat:41.323611 Lon:-71.9059

**Temporal Extent:** 2021-05 - 2021-06

## Methods & Sampling

Spawning Atlantic silversides (*Menidia menidia*) were caught on May 24th, 2021 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, 2016; 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 pHD digital electrode) that is calibrated weekly using NIST 2-point pH references. As needed, the system automatically adjusts pH /pCO<sub>2</sub> levels by triggering solenoid valves to inject standardized amounts of 100% bone dry CO<sub>2</sub> (to decrease pH) or CO<sub>2</sub>-stripped air (to increase pH), while maintaining desired temperatures via computer-controlled submersible heaters (Finnex Deluxe Titanium, 800W) or in-line chillers (DeltaStar 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).

A **lateral line development experiment** specifically tested our hypothesis that high CO<sub>2</sub> environments affect schooling due to morphological changes and/or the development of the LL system. In both experiments three CO<sub>2</sub> treatments were used, referred to hereafter as control (8.15 pH units, ~400 µatm CO<sub>2</sub>), elevated (7.45pH units, ~2200 µatm CO<sub>2</sub>), or extreme (7.20 pH units, ~4200 µatm CO<sub>2</sub>) CO<sub>2</sub> conditions. The control CO<sub>2</sub> 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<sub>2</sub> level represents the maximum predicted open ocean CO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> levels were determined biweekly by measuring total alkalinity (A<sub>T</sub>; 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 AT in seawater (UCSD Scripps Institution of Marine Science; Dickson et al., 2003) and titration accuracy was (±1%). Measured temperature, salinity, pH, and AT were then used to calculate CO<sub>2</sub> (CO<sub>2</sub>SYS V2.1; [https://cdiac.ess-dive.lbl.gov/ftp/co2sys/CO2SYS\\_calc\\_XLS\\_v2.1/](https://cdiac.ess-dive.lbl.gov/ftp/co2sys/CO2SYS_calc_XLS_v2.1/)) using K<sub>1</sub>, K<sub>2</sub> constants from Mehrbach et al. (1973), refitted by Dickson & Millero (1987), and Dickson (1990) for potassium bisulfate (KHSO<sub>4</sub>).

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.

### **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 CO<sub>2</sub> 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 CO<sub>2</sub> 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 Standard length of the sampled larva (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.

Scanning electron microscopy (SEM) was used to determine the degree of canal formation, in larvae in the control (ambient) and extreme groups between 0 and 34 dph (nAmbient = 22; nExtreme = 22). Larvae ranged in size from 5 to 16 mm NL with a mean ( $\pm$  SE) of  $9.3 \pm 0.7$  and  $10.3 \pm 0.5$  mm in the control and extreme CO<sub>2</sub> treatments, respectively. Larvae were dehydrated in an ascending series of ethanol solutions at 1–2-hour intervals (50%, 75% [overnight], 85%, 95%, 100%), critical-point-dried in liquid CO<sub>2</sub> (Leica CPD 300), mounted on aluminum stubs with adhesive carbon discs, and sputter-coated with platinum (Leica MED 020, 15 nm). Imaging was done at 3 kV at a working distance of 10–16 mm on a Zeiss Supra 40VP SEM at the Marine Biological Laboratory (Woods Hole, MA). The degree of canal formation in the vicinity of each neuromast (a canal segment) was assigned to one of four developmental stages (Tarby and Webb, 2003; Webb and Shirey, 2003). Stage 1 - superficial neuromast on epidermis, Stage 2 - neuromast sunken into a groove of unossified or ossified canal walls (could not be distinguished in SEM's), Stage 3 - epithelium fused over the neuromast forming a canal roof, and Stage 4 - bony canal wall fused over the neuromast. Complete ossification of the bony canal (stage 4) could only be quantified using histology, so canal segments that were enclosed with a canal roof were classified as stage 3/4.

#### **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**

- Loaded *Menidia\_Neuromast\_Exp.csv*, using original filename as table name, with headers from row 1; set missing value markers to empty string, "nd", and "N.A" for fields including SL, Count, and NM.ID
- Renamed columns Fish.ID to Fish\_ID and NM.ID to NM\_ID for BCO-DMO naming conventions
- Output file as 1009501\_v1\_menidia\_neuromast.csv

##### **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**

- Data file contains duplicate rows without a clear explanation as to how the observations differ.
- Photos used for analysis are not referenced as an accessible resource or provided here.

#### **Problem Description**

Fish were randomly pooled across tanks for each CO<sub>2</sub> treatment for the "neuromast experiment", hence, there is no associated Tank.ID or Bucket.ID. Further, only SL was measured for these individuals. In the "neuromast experiment" fish that were stained using DiASP were not measured for SL.

## Related Publications

Baumann, H., Wallace, R.B., Tagliaferri, T. et al. Large Natural pH, CO<sub>2</sub> and O<sub>2</sub> Fluctuations in a Temperate Tidal Salt Marsh on Diel, Seasonal, and Interannual Time Scales. *Estuaries and Coasts* 38, 220–231 (2015).

<https://doi.org/10.1007/s12237-014-9800-y>  
*Methods*

Baumann, Jones, L., Murray, C., Siedlecki, S., Alexander, M., & Cross, E. (2022). Impaired hatching exacerbates the high CO<sub>2</sub> sensitivity of embryonic sand lance *Ammodytes dubius*. *Marine Ecology Progress Series*.

<https://doi.org/10.3354/meps14010>  
*Methods*

Caldeira, K., & Wickett, M. E. (2005). Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research: Oceans*, 110(C9). Portico.

<https://doi.org/10.1029/2004jc002671>  
*Methods*

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  $\text{HSO}_4^-$  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*

Dickson, A. G., Afghan, J. D., & Anderson, G. C. (2003). Reference materials for oceanic CO<sub>2</sub> analysis: a method for the certification of total alkalinity. *Marine Chemistry*, 80(2), 185–197.

[https://doi.org/10.1016/S0304-4203\(02\)00133-0](https://doi.org/10.1016/S0304-4203(02)00133-0)  
*Methods*

Image Pro. Image Analysis Software for Microscopy. <https://mediacy.com/>  
*Software*

Malvezzi, A. J., Murray, C. S., Feldheim, K. A., DiBattista, J. D., Garant, D., Gobler, C. J., Chapman, D. D., & Baumann, H. (2015). A quantitative genetic approach to assess the evolutionary potential of a coastal marine fish to ocean acidification. *Evolutionary Applications*, 8(4), 352–362. Portico. <https://doi.org/10.1111/eva.12248>

*Methods*

Max D. Zavell, Jenna N. O'del, Matthew E.P. Mouland, Jacqueline F. Webb, Hannes Baumann (to be submitted). High CO<sub>2</sub> conditions delay the onset of schooling in a common forage fish.

*Results*

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/lb.1973.18.6.0897](https://doi.org/10.4319/lb.1973.18.6.0897)

*Methods*

Mekdara, P. J., Nasimi, F., Schwalbe, M. A. B., & Tytell, E. D. (2021). Tail Beat Synchronization during Schooling Requires a Functional Posterior Lateral Line System in Giant Danios, *Devario aequipinnatus*. *Integrative and Comparative Biology*, 61(2), 427–441. <https://doi.org/10.1093/icb/icab071>

*Methods*

Middaugh, D.P., Hemmer, M.J., & Goodman, L.R. (1987). Methods for Spawning, Culturing and Conducting Toxicity-Tests with Early Life Stages of Four Atherinid Fishes: The inland silverside, *Menidia beryllina*, Atlantic silverside, *M. menidia*, tidewater silverside, *M. peninsulae* and California grunion, *Leuresthes tenuis*. United States Environmental Protection Agency. EPA 600/8-87/004. <https://nepis.epa.gov/Exe/ZyPURL.cgi?Dockey=2000AXXP.txt>

*Methods*

Murray, C. S., Fuiman, L. A., & Baumann, H. (2016). Consequences of elevated CO<sub>2</sub> exposure across multiple life stages in a coastal forage fish. *ICES Journal of Marine Science: Journal Du Conseil*, fsw179.

doi:[10.1093/icesjms/fsw179](https://doi.org/10.1093/icesjms/fsw179)

## Methods

Murray, C., & Baumann, H. (2018). You Better Repeat It: Complex CO<sub>2</sub> × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial Experimentation. *Diversity*, 10(3), 69. doi:[10.3390/d10030069](https://doi.org/10.3390/d10030069)  
*Methods*

## , Methods

Murray, C., Malvezzi, A., Gobler, C., & Baumann, H. (2014). Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Marine Ecology Progress Series*, 504, 1–11. doi:[10.3354/meps10791](https://doi.org/10.3354/meps10791)  
*Methods*

Nakae, M., Asaoka, R., Wada, H., & Sasaki, K. (2012). Fluorescent dye staining of neuromasts in live fishes: an aid to systematic studies. *Ichthyological Research*, 59(3), 286–290. <https://doi.org/10.1007/s10228-012-0274-2>  
*Methods*

Nickles, K. R., Hu, Y., Majoris, J. E., Buston, P. M., & Webb, J. F. (2020). Organization and Ontogeny of a Complex Lateral Line System in a Goby (*Elacatinus lori*), with a Consideration of Function and Ecology. *Copeia*, 108(4). <https://doi.org/10.1643/cg-19-341>  
*Methods*

Tarby, M. L., & Webb, J. F. (2002). Development of the supraorbital and mandibular lateral line canals in the cichlid, *archocentrus nigrofasciatus*. *Journal of Morphology*, 255(1), 44–57. Portico. <https://doi.org/10.1002/jmor.10045>  
*Methods*

Wallace, R. B., Baumann, H., Grear, J. S., Aller, R. C., & Gobler, C. J. (2014). Coastal ocean acidification: The other eutrophication problem. *Estuarine, Coastal and Shelf Science*, 148, 1–13. <https://doi.org/10.1016/j.ecss.2014.05.027>  
*Methods*

Webb, J. F., & Shirey, J. E. (2003). Postembryonic development of the cranial lateral line canals and neuromasts in zebrafish. *Developmental Dynamics*, 228(3), 370–385. Portico. <https://doi.org/10.1002/dvdy.10385>  
*Methods*

Zavell, M. D., & Baumann, H. (2024). Resiliency of black sea bass, *Centropristis striata*, early life stages to future high CO<sub>2</sub> conditions. *Environmental Biology of Fishes*, 107(6), 677–691. <https://doi.org/10.1007/s10641-024-01561-y>  
*Methods*

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## Parameters

| Parameter  | Description                                                                                                                                                                                                                                                                                                                                                 | Units                |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|
| Species    | Atlantic Silverside - <i>Menidia menidia</i>                                                                                                                                                                                                                                                                                                                | unitless             |
| Experiment | Neuromast                                                                                                                                                                                                                                                                                                                                                   | unitless             |
| pH         | Target pH                                                                                                                                                                                                                                                                                                                                                   | unitless             |
| CO2        | Target CO2 Concentration                                                                                                                                                                                                                                                                                                                                    | µatm                 |
| Temp       | Water Temperature                                                                                                                                                                                                                                                                                                                                           | Degrees Celsius (°C) |
| Fish_ID    | Unique identifier for an individual larval fish sampled for lateral line/neuromast analysis                                                                                                                                                                                                                                                                 | unitless             |
| dph        | Age of the sampled larva in days post hatch, used to track the ontogenetic progression of lateral line/neuromast development. Larvae were sampled daily from 0-7 dph and three times per week thereafter, up to 34 dph                                                                                                                                      | days                 |
| SL         | Standard length of the sampled larva (snout to end of vertebral column), measured after fixation in paraformaldehyde/ethanol. Recorded only for larvae examined by SEM; larvae stained with DiASP for neuromast counts were not measured for SL                                                                                                             | millimeters          |
| NM_ID      | Neuromast ID (Cranial Canal and position) from SEM; identifier for a specific neuromast or canal segment, encoding both the cranial lateral line canal (IO = Infraorbital, MD = Mandibular, PO = Preopercular, SO = Supraorbital) and its sequential position within that canal (e.g., IO.1 through IO.6); IO.1 - IO.6; MD.1 - MD.5; PO.1 - PO.7; SO.1-SO.5 | unitless             |
| Canal      | Name of the cranial lateral line canal: Infraorbital, Preopercular, Supraorbital, Mandibular                                                                                                                                                                                                                                                                | unitless             |
| Stage      | Developmental stage of canal enclosure surrounding a neuromast, assessed from SEM images; Stage 1 = neuromast superficial on the epidermis; Stage 2 = neuromast sunk into an unenclosed canal groove; Stage 3 = epithelium fused over the neuromast forming a canal roof; Stage 3/4 = canal roof present                                                    | unitless             |
| Count      | Number of distinct neuromasts counted within a given cranial canal using vital fluorescent staining and epifluorescence microscopy                                                                                                                                                                                                                          | unitless             |
| Method     | Technique used to assess lateral line development for a given larva: SEM or DiASP                                                                                                                                                                                                                                                                           | unitless             |

## Instruments

|                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
|-----------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | Automated Larval Fish Rearing System (ALFiRiS)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Generic Instrument Name</b>          | Automated Larval Fish Rearing System                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <b>Dataset-specific Description</b>     | Automated Larval Fish Rearing System (ALFiRiS) – UConn Avery Point Rankin Lab – self-designed and assembled                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
| <b>Generic Instrument Description</b>   | 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 <a href="https://befel.marinesciences.uconn.edu/alfiris/">https://befel.marinesciences.uconn.edu/alfiris/</a> |

|                                         |                                                                                        |
|-----------------------------------------|----------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | GoPro Hero 4                                                                           |
| <b>Generic Instrument Name</b>          | Camera                                                                                 |
| <b>Dataset-specific Description</b>     | GoPro Hero 4 (GoPro Inc., San Mateo, California)                                       |
| <b>Generic Instrument Description</b>   | All types of photographic equipment including stills, video, film and digital systems. |

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

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

|                                         |                                                                                                                                                                                                                                                                                                                                                                                                           |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> |                                                                                                                                                                                                                                                                                                                                                                                                           |
| <b>Generic Instrument Name</b>          | digital thermometer                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Dataset-specific Description</b>     | As needed, the system automatically adjusts pH /pCO <sub>2</sub> levels by triggering solenoid valves to inject standardized amounts of 100% bone dry CO <sub>2</sub> (to decrease pH) or CO <sub>2</sub> -stripped air (to increase pH), while maintaining desired temperatures via computer-controlled submersible heaters (Finnex Deluxe Titanium, 800W) or in-line chillers (DeltaStar ½ horsepower). |
| <b>Generic Instrument Description</b>   | An instrument that measures temperature digitally.                                                                                                                                                                                                                                                                                                                                                        |

|                                         |                                                      |
|-----------------------------------------|------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | Leica CPD300 Critical Point Dryer                    |
| <b>Generic Instrument Name</b>          | Drying Oven                                          |
| <b>Dataset-specific Description</b>     | Leica CPD300 Critical Point Dryer (Wetzlar, Germany) |
| <b>Generic Instrument Description</b>   | a heated chamber for drying                          |

|                                         |                                                                                                                                       |
|-----------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | Zeiss Supra 40VP Scanning Electron Microscope                                                                                         |
| <b>Generic Instrument Name</b>          | Electron Microscope                                                                                                                   |
| <b>Dataset-specific Description</b>     | Zeiss Supra 40VP Scanning Electron Microscope (Oberkochen, Germany)                                                                   |
| <b>Generic Instrument Description</b>   | Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of electrons behaving as waves. |

|                                         |                                                                                                                                                                                                           |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | Nikon SMZ1000 Zoom Stereo Microscope                                                                                                                                                                      |
| <b>Generic Instrument Name</b>          | Microscope - Optical                                                                                                                                                                                      |
| <b>Dataset-specific Description</b>     | Nikon SMZ1000 Zoom Stereo Microscope (Nikon Instruments Inc., Melville, New York)                                                                                                                         |
| <b>Generic Instrument Description</b>   | 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". |

|                                         |                                                                                                                                                                                                           |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | Nikon SMZ1500 Stereo Microscope                                                                                                                                                                           |
| <b>Generic Instrument Name</b>          | Microscope - Optical                                                                                                                                                                                      |
| <b>Dataset-specific Description</b>     | Nikon SMZ1500 Stereo Microscope (Nikon Instruments Inc., Melville, New York)                                                                                                                              |
| <b>Generic Instrument Description</b>   | 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". |

|                                         |                                                                                                                                                                                                                                            |
|-----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | Hach pHD digital electrode                                                                                                                                                                                                                 |
| <b>Generic Instrument Name</b>          | pH Sensor                                                                                                                                                                                                                                  |
| <b>Dataset-specific Description</b>     | 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.                                                     |
| <b>Generic Instrument Description</b>   | 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+). |

|                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | handheld refractometer                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <b>Generic Instrument Name</b>          | Refractometer                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Dataset-specific Description</b>     | Actual CO <sub>2</sub> 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.                                                                                                                                                                                                                 |
| <b>Generic Instrument Description</b>   | 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. |

|                                         |                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | beach seine                                                                                                                                                                                                                                                                                                                                                                                                                             |
| <b>Generic Instrument Name</b>          | Seine Net                                                                                                                                                                                                                                                                                                                                                                                                                               |
| <b>Dataset-specific Description</b>     | 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.                                                                                                                                                                                                                                          |
| <b>Generic Instrument Description</b>   | 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) |

|                                         |                                                                                                                                                                  |
|-----------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Dataset-specific Instrument Name</b> | G20 Potentiometric Titrator                                                                                                                                      |
| <b>Generic Instrument Name</b>          | Titrator                                                                                                                                                         |
| <b>Dataset-specific Description</b>     | G20 Potentiometric Titrator (Mettler Toledo, Columbus Ohio)                                                                                                      |
| <b>Generic Instrument Description</b>   | Titration is an analytical technique that incrementally adds 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 (CO<sub>2</sub>) and oxygen (O<sub>2</sub>). 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-CO<sub>2</sub> 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 pCO<sub>2</sub> and dissolved oxygen (DO) content and the application of static and fluctuating pCO<sub>2</sub> and DO levels that were chosen to represent contemporary and potential future scenarios in productive coastal habitats. First CO<sub>2</sub>, DO, and CO<sub>2</sub> × 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 CO<sub>2</sub> × 3 DO levels. Second, the effects of tidal and diel CO<sub>2</sub> × DO fluctuations of different amplitudes on silverside ELH traits will be quantified. To address knowledge gaps regarding the CO<sub>2</sub>-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 CO<sub>2</sub>-sensitivity, the effects of temperature × CO<sub>2</sub> on pre- and post-hatch metabolism will be robustly quantified. The final objective is to rear silversides from fertilization to maturity under different CO<sub>2</sub> levels and assess potential CO<sub>2</sub>-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                       |
|----------------------------------------------------------|-----------------------------|
| <a href="#">NSF Division of Ocean Sciences (NSF OCE)</a> | <a href="#">OCE-1536336</a> |

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