

Dataset: Fatty acid profiles of *M. menidia* females and their unfertilized eggs.

Project(s): Understanding the effects of acidification and hypoxia within and across generations in a coastal marine fish (HYPOA)

Abstract: Gas chromatography was used to quantify the absolute (mg g dry weight⁻¹) and relative concentrations (% of total) of 27 FAs for each of 5 females (whole individual) and their unfertilized eggs (~ 1 ml). Samples were first dried and then homogenized in a solution of chloroform-methanol (2:1 v/v) and tricosanoic acid (23:0) as an internal standard for quantification of mg g⁻¹ dry mass of fatty acids. Lipids were cold-extracted from approximately 50 mg of dry mass. A Shimadzu GC-2014 gas chromatograph set with a Phenomenex ZB-WAX plus capillary column (30 m long; 0.53 mm ID; 1.0 µm thick) was used to quantify FAs, and individual FAs were identified by comparison to commercial standards (Supelco, Inc). These data are published in "Snyder, J.T.*, Murray, C.S.*, and Baumann, H. (2017) Potential for maternal effects on offspring CO₂-sensitivities in a coastal marine fish. *Journal of Experimental Marine Biology and Ecology*. doi: 10.1016/j.jembe.2017.11.002" and are affiliated with the dataset "Survival, length, and growth responses of *M. menidia* offspring from different females exposed to contrasting CO₂ environments. doi: XXXXX" For a complete list of measurements, refer to the supplemental document 'Field_names.pdf', and a full dataset description is included in the supplemental file 'Dataset_description.pdf'. The most current version of this dataset is available at: <http://www.bco-dmo.org/dataset/719379>

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These data are associated with the corresponding paper:

[Snyder, J.T.*, Murray, C.S.*, and Baumann, H. \(2017\) Potential for maternal effects on offspring CO₂-sensitivities in a coastal marine fish. *Journal of Experimental Marine Biology and Ecology* \(in press\).](#)

Other datasets related to this paper:

[Survival, length, and growth responses of *M. menidia* offspring from different](#)

Acquisition Methodology from **Snyder, J.T.*, Murray, C.S.*, and Baumann, H. (2017)**

Description: Potential for maternal effects on offspring CO2-sensitivities in a coastal marine fish. *Journal of Experimental Marine Biology and Ecology* (in press).

Five randomly selected females were strip-spawned onto cutout sections of window screen (1-mm mesh) that were placed into separate seawater-filled spawning dishes (Murray et al., 2014). To ensure full fertilization success and randomize potential paternal effects, eggs were fertilized with a mixture of milt from 22 males, thus producing full-sib and maternal half-sib embryos from each female. Adults were measured for total length (TL; mean TL_{male} = 9.14 cm, mean TL_{female} = 10.4 cm) and frozen for later analysis of FA. Mesh screens with attached embryos were subsequently cut into smaller sections to allow precise enumeration, and within 2-hr post-fertilization 100 embryos were placed into each of three replicate rearing containers (20 L) per female and CO₂ treatment (i.e., 600 embryos for each of five females, 3 × 100 in ambient and 3 × 100 in acidified treatments). Rearing containers were filled with 1-µm filtered, UV-sterilized seawater (~30 psu) from Long Island Sound and placed in temperature-controlled water baths set to 24 deg C, the known thermal optimum for survival and growth in this species (Middaugh et al., 1987). Offspring were reared for 24 d post fertilization under a 15h light:9h dark lighting regime. After hatch, larvae were fed ad libitum rations of newly hatched brine shrimp nauplii *Artemia salina* (brineshrimpdirect.com), and 50% of water was replaced every 5 d to ensure safe ammonia levels (< 0.25 ppm). Hatched larvae were counted and subsampled (n = 10 per replicate) at 1 d post hatch (dph) by gently scooping them into identical 20 L containers, and final samples were taken at 16 dph. All samples were preserved in 5% buffered formalin for later measurements of larval standard length (SL, 0.01 mm) via calibrated digital images (ImagePro Premier, MediaCybernetics). The experiment thus quantified three related survival and three size traits for each replicate, female, and CO₂ treatment: embryo survival (fertilization to 1 dph), larval survival (1 to 16 dph), overall survival (fertilization to 16 dph), size (SL) at hatch (1 dph), SL at 16 dph, and larval growth rate (GR = (SL_{16dph} – SL_{1dph})/15).

CO₂ regime:

Offspring were reared at ambient (~ 400 uatm, pH_{NBS} = 8.18) and acidified CO₂ conditions (~2,300 uatm, pH_{NBS} = 7.50). The higher value was set to a level commonly used in OA research (consistent with projections of future pCO₂ values for open oceans over in the next 200 yr (IPCC, 2007)) and represents current conditions experienced during seasonal extremes by this species in nature (Murray et al., 2014). Ambient conditions were achieved by bubbling partially

CO₂-stripped air into each rearing container, thereby offsetting metabolic CO₂ accumulation. Acidified conditions were achieved via gas proportioners (Cole Parmer®) that mixed CO₂ stripped air with 100% bone-dry CO₂ delivered to the bottom of each rearing container via air stones. Target pH and temperature were monitored daily via a handheld pH probe (Hach® HQ40d portable meter with a PHC201 standard pH-probe) calibrated regularly via two-point National Bureau of Standards (NBS) pH buffers (electronic supplementary material, Fig.S1). To characterize actual pCO₂ levels and related water chemistry parameters, water was sampled from four randomly chosen rearing containers per treatment three times over the course of the experiment and immediately measured for total alkalinity (AT) via endpoint titration (Mettler Toledo™ G20 Potentiometric Titrator). The instrument has previously been shown to quantify AT in Dr. Andrew Dickson's reference material (batch 147, AT= 2231.39 $\mu\text{mol kg seawater}^{-1}$) with an average error of 0.6%. Actual levels of total dissolved inorganic carbon (CT), partial pressure of CO₂ (pCO₂), fugacity of CO₂ (fCO₂), and carbonate ion concentration were calculated in CO₂SYS (<http://cdiac.ornl.gov/ftp/co2sys>) based on measured AT, pH (NBS), temperature, and salinity using K1 and K2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and Dickson (1990) for KHSO₄ (Table 1).

Fatty acid analysis:

Gas chromatography was used to quantify the absolute ($\text{mg g dry weight}^{-1}$) and relative concentrations (% of total) of 27 FAs for each of the 5 females (whole individual) and their unfertilized eggs (~ 1 ml) following the methods of Faulk and Holt (2005) as recently used for this species in Murray et al. (2016). Briefly, frozen samples were shipped on dry ice to the Fisheries and Mariculture Laboratory (University of Texas, Marine Science Institute), where they were first dried and then homogenized in a solution of chloroform-methanol (2:1 v/v) and tricosanoic acid (23:0) as an internal standard for quantification of mg g^{-1} dry mass of fatty acids. Lipids were cold-extracted from approximately 50 mg of dry mass. Fatty-acid methyl esters were prepared by saponification in potassium hydroxide, followed by transesterification with 14% boron trifluoride in methanol. A Shimadzu GC-2014 gas chromatograph set with a Phenomenex ZB-WAX plus capillary column (30 m long; 0.53 mm ID; 1.0 μm thick) was used to quantify FAs, and individual FAs were identified by comparison to commercial standards (Supelco, Inc). Two FAs, 12:0 and 15:1, were below detection limit or invariant across egg batches and were therefore excluded from subsequent analyses.

Processing BCO-DMO Data Processing Notes:

Description: - added underscores to column headers
- replaced blank cells with nd

- added underscores to site name
- removed commas from data
- changed date format to yyyy/mm/dd

Project Information

Understanding the effects of acidification and hypoxia within and across generations in a coastal marine fish

Description from NSF award abstract: Coastal marine ecosystems provide a number of important services and resources for humans, and at the same time, coastal waters are subject to environmental stressors such as increases in ocean acidification and reductions in dissolved oxygen. The effects of these stressors on coastal marine organisms remain poorly understood because most research to date has examined the sensitivity of species to one factor, but not to more than one in combination. This project will determine how a model fish species, the Atlantic silverside, will respond to observed and predicted levels of dissolved carbon dioxide (CO₂) and oxygen (O₂). Shorter-term experiments will measure embryo and larval survival, growth, and metabolism, and determine whether parents experiencing stressful conditions produce more robust offspring. Longer-term experiments will study the consequences of ocean acidification over the entire life span by quantifying the effects of high-CO₂ conditions on the ratio of males to females, lifetime growth, and reproductive investment. These studies will provide a more comprehensive view of how multiple stressors may impact populations of Atlantic silversides and potentially other important forage fish species. This collaborative project will support and train three graduate students at the University of Connecticut and the Stony Brook University (NY), two institutions that attract students from minority groups. It will also provide a variety of opportunities for undergraduates to participate in research and the public to learn about the study, through summer research projects, incorporation in the "Women in Science and Engineering" program, and interactive displays of environmental data from monitoring buoys. The two early-career investigators are committed to increasing ocean literacy and awareness of NSF-funded research through public talks and presentations. This project responds to the recognized need for multi-stressor assessments of species sensitivities to anthropogenic environmental change. It will combine environmental monitoring with advanced experimental approaches to characterize early and whole life consequences of acidification and hypoxia in the Atlantic silverside (*Menidia menidia*), a valued model species and important forage fish along most of the US east coast. Experiments will employ a newly constructed, computer-controlled fish rearing system to allow independent and combined manipulation of seawater pCO₂ and dissolved oxygen (DO) content and the application of static and fluctuating pCO₂ and DO levels that were chosen to represent contemporary and potential future scenarios in productive coastal habitats. First CO₂, DO, and CO₂ × DO dependent reaction norms will be quantified for fitness-relevant early life history (ELH) traits including pre- and post-hatch

survival, time to hatch, post-hatch growth, by rearing offspring collected from wild adults from fertilization to 20 days post hatch (dph) using a full factorial design of 3 CO₂ × 3 DO levels. Second, the effects of tidal and diel CO₂ × DO fluctuations of different amplitudes on silverside ELH traits will be quantified. To address knowledge gaps regarding the CO₂-sensitivity in this species, laboratory manipulations of adult spawner environments and reciprocal offspring exposure experiments will elucidate the role of transgenerational plasticity as a potential short-term mechanism to cope with changing environments. To better understand the mechanisms of fish early life CO₂-sensitivity, the effects of temperature × CO₂ on pre- and post-hatch metabolism will be robustly quantified. The final objective is to rear silversides from fertilization to maturity under different CO₂ levels and assess potential CO₂-effects on sex ratio and whole life growth and fecundity. Related references: Gobler, C.J. and Baumann, H. (2016) Hypoxia and acidification in ocean ecosystems: Coupled dynamics and effects on marine life. *Biology Letters* 12:20150976. doi:10.1098/rsbl.2015.0976 Baumann, H. (2016) Combined effects of ocean acidification, warming, and hypoxia on marine organisms. *Limnology and Oceanography e-Lectures* 6:1-43. doi:10.1002/loe2.10002 Depasquale, E., Baumann, H., and Gobler, C.J. (2015) Variation in early life stage vulnerability among Northwest Atlantic estuarine forage fish to ocean acidification and low oxygen *Marine Ecology Progress Series* 523: 145–156. doi:10.3354/meps11142

Deployment Information

Deployment description for lab Avery_Point AP_Rankin

This was where the Long-term *Menidia menidia* growth experiments took place. The samples were collected from offshore in Mumford Cove.

Instrument Information

Instrument	Shimadzu GC-2014 gas chromatograph set with a Phenomenex ZB-WAX plus capillary column
Description	Used to quantify fatty acids
Generic Instrument Name	Gas Chromatograph
Generic	Instrument separating gases, volatile substances, or substances dissolved

Instrument Description	in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)
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Instrument	Hach® HQ40d portable meter with a PHC201 standard pH-probe
Description	handheld pH probe
Generic Instrument Name	pH Sensor
Generic Instrument Description	General term for an instrument that measures the pH or how acidic or basic a solution is.

Instrument	Mettler Toledo™ G20 Potentiometric Titrator
Description	Used to measure total alkalinity
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.