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

Website: https://www.bco-dmo.org/dataset/719379
Data Type: experimental
Version: Final
Version Date: 2017-11-14

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

Contributors	Affiliation	Role
Baumann, Hannes	University of Connecticut (UConn)	Principal Investigator, Contact
Nye, Janet	Stony Brook University - SoMAS (SUNY-SB SoMAS)	Co-Principal Investigator
Ake, Hannah	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Coverage

Spatial Extent: **Lat**:41.3213389 **Lon**:-72.0148361

Dataset Description

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 um thick) was used to quantify FAs, and individual FAs were identified by comparison to commercial standards (Supelco, Inc).

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 females exposed to contrasting CO2 environments.

Methods & Sampling

Methodology from **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).**

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 TLmale = 9.14 cm, mean TLfemale = 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-um 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 = (SL16dph – SL1dph)/15).

CO2 regime:

Offspring were reared at ambient (~ 400 uatm, pHNBS = 8.18) and acidified CO2 conditions (~2,300 uatm, pHNBS = 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 umol 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 (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 um 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.

Data Processing Description

BCO-DMO Data Processing Notes:
- 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

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Data Files

File
fatty_acids.csv (Comma Separated Values (.csv), 11.26 KB) MD5:d4867c4c6b0984e78cc60f25d27cc49f Primary data file for dataset ID 719379

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Parameters

Parameter	Description	Units
Species	Atlantic silverside; Menidia menidia	unitless
Collection_site	Mumford Cove Connecticut USA	unitless
Lat	Latitude of field collection site	decimal degrees
Lon	Longitude of field collection site	decimal degrees
Collection_date	Date of field collection for the spawners used in the experiment; YYYY/MM/DD	unitless
Fatty_acid	Individual fatty acid nomenclature	unitless
Female	Five females denoted by letter A B C D E	unitless
Absolute_concentration_spawning_adult	Absolute fatty acid concentration in mg per g of dry weight	milligram per gram
Absolute_concentration_eggs	Absolute fatty acid concentration in mg per g of dry weight	milligram per gram
Relative_concentration_spawning_adult	Relative fatty acid concentration in percent of total fatty concentration	percent
Relative_concentration_eggs	Relative fatty acid concentration in percent of total fatty concentration	percent

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Instruments

Dataset-specific Instrument Name	Mettler Toledo™ G20 Potentiometric Titrator
Generic Instrument Name	Automatic titrator
Dataset-specific Description	Used to measure total alkalinity
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	Shimadzu GC-2014 gas chromatograph set with a Phenomenex ZB-WAX plus capillary column
Generic Instrument Name	Gas Chromatograph
Dataset-specific Description	Used to quantify fatty acids
Generic Instrument Description	Instrument separating gases, volatile substances, or substances dissolved in a volatile solvent by transporting an inert gas through a column packed with a sorbent to a detector for assay. (from SeaDataNet, BODC)

Dataset-specific Instrument Name	Hach® HQ40d portable meter with a PHC201 standard pH-probe
Generic Instrument Name	pH Sensor
Dataset-specific Description	handheld pH probe
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+).

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Deployments

AP_Rankin

Website	https://www.bco-dmo.org/deployment/651907
Platform	Avery_Point
Start Date	2015-05-03
End Date	2015-09-15
Description	This was where the Long-term Menidia menidia growth experiments took place. The samples were collected from offshore in Mumford Cove.

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Project Information

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

Coverage: Eastern Long Island Sound, CT, USA

Description from NSF award abstract:
Coastal marine ecosystems provide a number of important services and resources for humans, and at the same time, coastal waters are subject to environmental stressors such as increases in ocean acidification and reductions in dissolved oxygen. The effects of these stressors on coastal marine organisms remain poorly understood because most research to date has examined the sensitivity of species to one factor, but not to more than one in combination. This project will determine how a model fish species, the Atlantic silverside, will respond to observed and predicted levels of dissolved carbon dioxide (CO2) and oxygen (O2). Shorter-term experiments will measure embryo and larval survival, growth, and metabolism, and determine whether parents experiencing stressful conditions produce more robust offspring. Longer-term experiments will study the consequences of ocean acidification over the entire life span by quantifying the effects of high-CO2 conditions on the ratio of males to females, lifetime growth, and reproductive investment. These studies will provide a more comprehensive view of how multiple stressors may impact populations of Atlantic silversides and potentially other important forage fish species. This collaborative project will support and train three graduate students at the University of Connecticut and the Stony Brook University (NY), two institutions that attract students from minority groups. It will also provide a variety of opportunities for undergraduates to participate in research and the public to learn about the study, through summer research projects, incorporation in the "Women in Science and Engineering" program, and interactive displays of environmental data from monitoring buoys. The two early-career investigators are committed to increasing ocean literacy and awareness of NSF-funded research through public talks and presentations.

This project responds to the recognized need for multi-stressor assessments of species sensitivities to anthropogenic environmental change. It will combine environmental monitoring with advanced experimental approaches to characterize early and whole life consequences of acidification and hypoxia in the Atlantic silverside (Menidia menidia), a valued model species and important forage fish along most of the US east coast. Experiments will employ a newly constructed, computer-controlled fish rearing system to allow independent and combined manipulation of seawater pCO2 and dissolved oxygen (DO) content and the application of static and fluctuating pCO2 and DO levels that were chosen to represent contemporary and potential future scenarios in productive coastal habitats. First CO2, DO, and CO2 × DO dependent reaction norms will be quantified for fitness-relevant early life history (ELH) traits including pre- and post-hatch survival, time to hatch, post-hatch growth, by rearing offspring collected from wild adults from fertilization to 20 days post hatch (dph) using a full factorial design of 3 CO2 × 3 DO levels. Second, the effects of tidal and diel CO2 × DO fluctuations of different amplitudes on silverside ELH traits will be quantified. To address knowledge gaps regarding the CO2-sensitivity in this species, laboratory manipulations of adult spawner environments and reciprocal offspring exposure experiments will elucidate the role of transgenerational plasticity as a potential short-term mechanism to cope with changing environments. To better understand the mechanisms of fish early life CO2-sensitivity, the effects of temperature × CO2 on pre- and post-hatch metabolism will be robustly quantified. The final objective is to rear silversides from fertilization to maturity under different CO2 levels and assess potential CO2-effects on sex ratio and whole life growth and fecundity.

Related references:
Gobler, C.J. and Baumann, H. (2016) Hypoxia and acidification in ocean ecosystems: Coupled dynamics and effects on marine life. Biology Letters 12:20150976. doi:10.1098/rsbl.2015.0976

Baumann, H. (2016) Combined effects of ocean acidification, warming, and hypoxia on marine organisms. Limnology and Oceanography e-Lectures 6:1-43. doi:10.1002/loe2.10002

Depasquale, E., Baumann, H., and Gobler, C.J. (2015) Variation in early life stage vulnerability among Northwest Atlantic estuarine forage fish to ocean acidification and low oxygen Marine Ecology Progress Series 523: 145–156.doi:10.3354/meps11142

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536336

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