Dataset: CO2 × temperature specific early life survival and growth of Menidia menidia assessed by 5 factorial experiments

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

Abstract: In five individual rearing experiments, wild-caught M. menidia adults were spawned to test offspring sensitivity to factorial combinations of pCO2 (nominal: 400, 2200, 4000, and 6000 µatm) and temperature (17, 20, 24, and 28 °C) through measurements of early-life survival and growth. For experiment 1, adults were collected from Poquot Beach (40.947376, -73.10258), and the experiment took place at Stony Brook University's Flax Pond Marine Laboratory. For experiments 2–5, spawning adults were collected from Mumford Cove (41.321526, -72.015247), and experiments were conducted in the Rankin Seawater Facility at University of Connecticut's Avery Point campus. The experiments quantified two survival and two growth traits for each replicate and CO2 × temperature treatment; embryo survival (fertilization to 1 dph), larval survival (1 dph to experiment termination), size (SL) at hatch (1 dph), and growth rate ((SL at end of experiment – SL 1dph)/number days reared post hatch). These data are published in: Murray, C.S., and Baumann, H. (2018) You Better Repeat It: Complex CO2 × Temperature Effects in Atlantic Silverside Offspring Revealed by Serial experimentation. Diversity. doi:10.3390/d10030069. 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/732818

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These data are published in: Murray, C.S. & Baumann, H. 2018 You better repeat it: complex temperature × CO2 effects in Atlantic silverside offspring revealed by serial experimentation. Diversity 10, 1-19. doi:10.3390/d10030069

Acquisition Description: **CO2 × temperature manipulations and measurements:**

For 2 × 2 and 3 × 2 factorial designs, replicate rearing containers (20 L) were placed into large temperature-controlled water baths. Elevated CO_2 levels were achieved via gas proportioners (ColeParmer®) mixing air with 100% CO_2 (bone dry grade) that was delivered continuously to the bottom of each replicate rearing container via airstone. To counteract metabolic CO_2 accumulation, control CO_2 conditions were achieved by forcing compressed laboratory air through a series of CO_2 stripping units containing granular soda lime (AirGas®), a particle filter (1 µm),

and then to each replicate via airstone. Target pH levels were monitored daily using a handheld pH probes (Orion Ross Ultra pH/ATC Triode with Orion Star A121 pH Portable Meter; Intellical PHC281 pH Electrode with Hach® HQ11D Handheld pH/ORP Meter) calibrated bi-weekly with 2-point pH_{NBS} references. Continuous bubbling maintained dissolved oxygen saturation (>8 mg/ DO) in rearing vessels. Target treatment temperatures were controlled by thermostats (Aqualogic®) which powered chillers (DeltaStar®) or glass submersible heaters to maintain water bath temperatures. For 3 × 3 factorial experiments, we developed an automated acidification system composed of nine discrete recirculation units designed for larval fish rearing. We designed a LabView (National Instruments®) based program to fully automate the control of seawater chemistry. The software interfaces with the recirculating units via a data-acquisition module (NI cDAQ-9184, National Instruments®), which controls nine sampling pumps (one per tank) and a series of gas and water solenoid valves, while receiving input from a central pH electrode (Hach pHD® digital electrode calibrated weekly using 2-point pH_{NBS} references) and dissolved oxygen probe (Hach LDO® Model 2). The software sequentially assesses the pH conditions in each rearing unit (each tank once per hour) by pumping water for ~450 seconds through the housing of the central pH probe, comparing measured pH levels to set-points and then adjusting levels by bubbling standardized amounts 100% CO_2 (bone dry grade, AirGas®) or CO_2-stripped air into the sump of each tank. The software also maintains DO saturation (>8 mg/l) by bubbling in CO_2-stripped air. LabView logs current pH, temperature, and DO conditions before cycling to the next unit. Temperatures were controlled by thermostats (Aqualogic®) that powered submersible heaters or in-line chillers (DeltaStar®).

Actual treatment CO_2 levels were determined based on measurements of pH, temperature, salinity, and total alkalinity (A_T). Treatment tanks were sampled three times per experiment for measurements of A_T (μmol kg^{-1}). Seawater was siphoned and filtered (to 10 μm) into 300 ml borosilicate bottles. Salinity was measured at the time of sampling using a refractometer. Bottles were stored at 3˚C and measured for A_T within two weeks of sampling using an endpoint titration (Mettler Toledo® G20 Potentiometric Titrator). Methodological accuracy (within ±1%) of alkalinity titrations were verified and calibrated using Dr. Andrew Dickson's (University of California San Diego, Scripps Institution of Oceanography) certified reference material for A_T in seawater. The partial pressure of CO_2 (pCO_2,; μatm) was calculated in CO2SYS (V2.1, http://cdiac.ornl.gov/ftp/co2sys) based on measured A_T, 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

$KHSO_4$.

Field sampling and experimental designs:
Collections of wild, spawning ripe Atlantic silversides were made during high tide 1-3 days prior to full or new moons during the species spawning season. Adults were caught with a 30 m × 2 m beach seine from local salt marshes and transported live to our laboratory facilities. Ripe adults were held overnight at 20° C in well aerated tanks at low densities with no food and strip spawned the next day.

For each experiment, eggs from 20+ running-ripe females were gently mixed into shallow plastic dishes lined with 1 mm plastic window screening. 20+ males were stripped-spawned together into 500 ml glass beakers, mixed with seawater, stirred, then gently poured into spawning dishes and mixed with eggs for ~15 minutes. Screens were rinsed with seawater to remove unfertilized eggs and then soaked in a 100 ppm buffered iodine (Ovadine®) solution for 15 minutes to prevent fungal infection. Experiments were initiated within two hours of fertilization when replicate rearing vessels received precisely 100 embryos. Vessels were filled with clean seawater (filtered to 1 µm and UV sterilized). Optimal salinity (27-31) and light conditions (15 h light:9 h dark) for rearing *M. menidia* were maintained across experiments. Upon hatching larvae were immediately provided *ad libitum* rations of newly hatched brine shrimp nauplii (*Artemia salina,* San Francisco strain, brineshrimpdirect.com) and equal rations of powdered weaning diet (Otohime Marine Fish Diet, size A1, Reed Mariculture®). To quantify hatching survival, one day post first hatch larvae were counted by gently scooping small groups into replacement rearing vessels. For initial hatch measurements, random sub-samples (N = 10) from each replicate were preserved in 5% formaldehyde/freshwater solution buffered with saturated sodium tetraborate. All experiments were terminated when larvae reached ~10 mm standard length (SL). At termination, all survivors were counted and measured for standard length (SL, nearest 0.01 mm) via calibrated digital images (Image Pro Premier® V9.0).

Processing Description:

BCO-DMO Processing:
- modified parameter names to conform with BCO-DMO naming conventions (replaced spaces with underscores);
- changed date format from mm/dd/yyyy to yyyy/mm/dd;
- replaced "n/a" with "nd";
- replaced spaces with underscores in columns: species, adult_collection_site;
- removed commas from adult_collection_site field;
- replaced original lat/lon values with decimal degree values provided by PI.

Project Information

Collaborative research: 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 Mumford_Cove

Shallow, coastal embayment in outer Long Island Sound, US Atlantic coast.

Instrument Information

Instrument	handheld pH probe
Description	Target pH levels were monitored daily using a handheld pH probes (Orion Ross Ultra pH/ATC Triode with Orion Star A121 pH Portable Meter; Intellical PHC281 pH Electrode with Hach HQ11D Handheld pH/ORP Meter) calibrated bi-weekly with 2-point pHNBS references.
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	refractometer
Description	Salinity was measured at the time of sampling using a refractometer.
Generic Instrument Name	Refractometer
Generic Instrument Description	A refractometer is a laboratory or field device for the measurement of an index of refraction (refractometry). The index of refraction is calculated from Snell's law and can be calculated from the composition of the material using the Gladstone-Dale relation. In optics the refractive index (or index of refraction) n of a substance (optical medium) is a dimensionless number that describes how light, or any other radiation, propagates through that medium.

Instrument	Mettler Toledo G20 Potentiometric Titrator
Description	Bottles were stored at 3˚C and measured for AT within two weeks of sampling using an endpoint titration (Mettler Toledo G20 Potentiometric Titrator).
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.

Instrument	dissolved oxygen probe
Description	*local description not specified*
Generic Instrument Name	Dissolved Oxygen Sensor
Generic Instrument Description	An electronic device that measures the proportion of oxygen (O2) in the gas or liquid being analyzed

Instrument	airstone

Description	Elevated CO2 levels were achieved via gas proportioners (ColeParmer) mixing air with 100% CO2 (bone dry grade) that was delivered continuously to the bottom of each replicate rearing container via airstone.
Generic Instrument Name	Airstone
Generic Instrument Description	Airstone - Also called an aquarium bubbler, is a piece of aquarium furniture, traditionally a piece of limewood or porous stone, whose purpose is to gradually diffuse air into the tank, eliminating the noise and large bubbles of conventional air filtration systems

Instrument	glass submersible heaters
Description	Target treatment temperatures were controlled by thermostats (Aqualogic) which powered chillers (DeltaStar) or glass submersible heaters to maintain water bath temperatures.
Generic Instrument Name	Immersion heater
Generic Instrument Description	Submersible heating element for water tanks and aquaria.

Instrument	DeltaStar chiller
Description	Target treatment temperatures were controlled by thermostats (Aqualogic) which powered chillers (DeltaStar) or glass submersible heaters to maintain water bath temperatures.
Generic Instrument Name	Aquarium chiller
Generic Instrument Description	Immersible or in-line liquid cooling device, usually with temperature control.

Instrument	beach seine
Description	Adults were caught with a 30 m × 2 m beach seine from local salt marshes

	and transported live to our laboratory facilities.
Generic Instrument Name	Seine Net
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)