Laboratory study of long-term growth in juvenile Menidia menidia (Atlantic silverside) at contrasting CO2 levels for 16 to 122 days in 2015

Website: https://www.bco-dmo.org/dataset/651461
Data Type: experimental
Version: final
Version Date: 2016-07-07

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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Coverage

Spatial Extent: **Lat**:41.3213389 **Lon**:-72.0148361
Temporal Extent: 2015-06-01 - 2015-09-15

Dataset Description

Total length, wet weight, and condition factor of juvenile *Menidia menidia* reared at contrasting CO2 levels.

These data are associated with the corresponding paper:

Murray, C.S., Fuiman, L., and Baumann, H. Consequences of elevated CO2 exposure across multiple life stages in a coastal forage fish. *ICES Journal of Marine Science* 74:1051-1061(2017).

Methods & Sampling

Field sampling and experimental design: Experiments were performed at University of Connecticut's Avery Point Campus in the Rankin Laboratory, a seawater facility adjacent to eastern Long Island Sound. Ripe adult *M. menidia* were collected on 1 May 2015 from Mumford Cove (41 19.25' N, 72 1.09'W), a shallow embayment dominated by eelgrasses (*Zostera marina)* and open to the Long Island Sound. Adults were sampled with a 30 m × 2 m beach seine, separated by sex, transported live to our laboratory, and held for 48

hours in large aerated tanks (17 degrees Celsius, ambient CO2, no food). On the day of fertilization (3 May 2015), greater than or equal to 20 ripe individuals from each sex were strip-spawned and eggs evenly distributed onto window screens (1 mm fiberglass mesh) submerged in plastic dishes with clear seawater. Strip-spawned adults were measured for standard length (mean SL, lower 0.5 cm; females 9.7, males 8.7). Fertilized embryos quickly attach to the screens via chorionic filaments, which facilitates precise enumeration and even allotment to treatments and replicates. Following established protocols for rearing *M. menidia* offspring (Murray et al., 2014), replicate containers (20 l) were filled with filtered (to 1 um) and UV sterilized seawater (31 psu) from Long Island Sound and placed in water baths (~300 l) controlled for temperature and light conditions (17 degrees Celsius, 15h light:9h dark) throughout the duration of the experiment. Within 2h of fertilization, each of four replicates per treatment received exactly 200 embryos for measure early life survival, while four other replicates per treatment each received ~400 offspring for long-term rearing. Larvae hatched ~14 days post-fertilization (dpf) and were immediately provided with standardized rations of newly hatched brine shrimp nauplii *Artemia salina* (San Francisco strain, Brine Shrimp Direct) and a commercial larval powder food (first four days, Otohime Marine Weaning Diet, size A, Reed Mariculture). At 2 days post-hatch (dph), living larvae from survival replicates were counted by gently scooping small groups into replacement containers. Between 1 to 14 days post-hatch (dph), all containers were cleaned daily with partial (10%) water exchange.

At 16 dph, larvae from the survival replicates were counted and a sub-sample (Ncontrol = 37, Nhigh = 33) was preserved in 10% formaldehyde/seawater solution for later total length (TL) measurements (nearest 0.01 mm) via calibrated, digital images (ImagePro Premier V9.1). All surviving larvae were transferred to larger (50 l) tubs and maintained under the previously described protocol. At 33 dph, larvae from the survival replicates were counted and then all larvae transferred to 50 l tubs fitted with screen-covered holes (1 mm mesh) to promote water exchange from a 300 l seawater bath. Due to space constraints, from 33 to 54 dph larvae from the survival replicates were pooled into a single container per CO2 treatment. Larvae were provided rations of nauplii and supplemented with commercial powder food (Otohime B1, Reed Mariculture). At 54 dph, all juveniles from survival and grow-out replicates were counted and pooled at equal numbers into 300 l circle tanks (two tanks per treatment, ~615 fish per tank). Juveniles were provided equal rations of newly hatched nauplii and B1 commercial powder food. Tanks were siphoned for waste daily and partial water changes completed twice weekly. Additional sub-samples for length measurements (TL, nearest 0.01 mm) were made at 36 dph (Ncontrol = 20, Nhigh = 20), 68 dph (Ncontrol = 20, Nhigh = 20) and 100 dph (Ncontrol = 28, Nhigh = 28).

At 122 dph, the experiment was terminated and all surviving juveniles were euthanized via an overdose of Tricaine-S (MS 222, Western Chemical) for preservation. While some juveniles from each treatment were immediately frozen at -80 degrees Celsius for fatty acid analyses; ~75% of the samples were fixed in 10% buffered formaldehyde/seawater solution for TL (Ncontrol = 1,025; Nhigh = 1,100, nearest 0.01 mm) and weight measurements (Ncontrol = 720; Nhigh = 786, nearest 0.01g).

Data Processing Description

CO2 treatments and measurements: Following best practices and guidelines for OA research (Riebesell et al., 2010) we used gas proportioners (ColeParmer) to mix air with 100% CO2 (bone dry grade) that was delivered to the bottom of each replicate rearing container via airstones. Control conditions were achieved by forcing compressed laboratory air through a series of CO2 stripping units containing granular soda lime (AirGas), a particle filter (1 um) and then to each replicate via airstone. Two standardized treatment levels were administered; control (CO2 stripped air only, ~500 uatm CO2, pHNIST = 8.05) and high CO2 conditions (air:CO2 mix, ~2,150 uatm CO2, pHNIST = 7.45). These treatments represent levels commonly used in OA research and conditions experienced seasonally by *M. menidia* offspring in the wild (Murray et al., 2014). Target pH levels were monitored daily using a handheld pH probe (Orion ROSS Ultra pH/ATC Triode and Orion Star A121 pH Portable Meter, Thermo Scientific) calibrated regularly with 2-point NIST pH references. Two sets of discrete water samples were taken from each treatment (borosilicate bottles) and immediately analyzed for total alkalinity (TA) using an endpoint titration (Mettler Toledo™ G20 Potentiometric Titrator). Methodological accuracy of alkalinity titrations were verified using Dr. Andrew Dickson's (University of California San Diego, Scripps Institution of Oceanography) certified reference material for TA in seawater (Batch 147 = 2,231 umol TA kg seawater-1). Actual levels of CO2 and dissolved inorganic carbon (DIC) were then calculated in CO2SYS (http://cdiac.ornl.gov/ftp/co2sys) based on measured TA, pH (NIST), temperature, and salinity using K1 and K2 constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and Dickson (1990) for KHSO4. An overview of the carbonate chemistry is given in Table 1.

Controlled for correctness, 3 outliers removed from dataset.

DMO Notes:
- added underscores to column headers
- added underscores to species name
- changed lat and lon to decimal format
- moved tank column last
- added underscores to site name

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Data Files

File
Menidia_growth.csv (Comma Separated Values (.csv), 248.19 KB) MD5:5714e2992f8e255efb80c918b439b42a Primary data file for dataset ID 651461

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Related Publications

Dickson, A. G. (1990). Standard potential of the reaction: AgCl(s) + 1/2 H2(g) = Ag(s) + HCl(aq) and the standard acidity constant of the ion HSO4− 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
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
Methods

Mehrbach, C., Culberson, C. H., Hawley, J. E., & Pytkowicx, 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/lo.1973.18.6.0897
Methods

Murray, C. S., Fuiman, L. A., & Baumann, H. (2016). Consequences of elevated CO2exposure across multiple life stages in a coastal forage fish. ICES Journal of Marine Science: Journal Du Conseil, fsw179. doi:10.1093/icesjms/fsw179
Results

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
Methods

Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.P., (2010). Guide to best practices for ocean acidification research and data reporting. Office for Official Publications of the European Communities, Luxembourg, 260 pp. doi: 10.2777/66906.
Methods

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Parameters

Parameter	Description	Units
species	Menidia menidia; the Atlantic silverside	unitless
adult_collection_site	site of the collection of wild adult spawners on 1 May 2015	unitless
lat	latitude of the adult collection site	decimal degrees
lon	longitude of the adult collections site	decimal degrees
date_fertilization	date offspring were fertilized by strip-spawning; over twenty adults of each sex; format mm-dd-YYYY	unitless
date_sample	date offspring were sampled for length and weight measurements; format mm-dd-YYYY	unitless
age	age in days post hatch (dph) on the date of sampling	days
temp	seventeen degrees Celsius; all fish experienced the same temperature	degrees Celsius
pH_treatment	average pH levels experienced by the fish; 7.45 or 8.05	dimensionless
CO2_treatment	calculated pCO2 levels; calculated by CO2SYS; based on alkalinity pH and temperature	microatomosphere
length_total	total length of the fish at sampling	millimeter
wet_weight	wet weight of the fish at sampling	milligram
condition_factor	condition factor calculated as wetWeight/totalLength to the 2.734608 power	dimensionless
tank	tank number; A B C D	unitless

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Instruments

Dataset-specific Instrument Name	Orion ROSS Ultra pH/ATC Triode
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+).

Dataset-specific Instrument Name	Orion Star A121 pH Portable Meter
Generic Instrument Name	pH Sensor
Dataset-specific Description	portable pH meter
Generic Instrument Description	An instrument that measures the hydrogen ion activity in solutions. The overall concentration of hydrogen ions is inversely related to its pH. The pH scale ranges from 0 to 14 and indicates whether acidic (more H+) or basic (less H+).

Dataset-specific Instrument Name	Mettler Toledo G20
Generic Instrument Name	Titrator
Dataset-specific Description	Potentiometric Titrator
Generic Instrument Description	Titrators are instruments that incrementally add quantified aliquots of a reagent to a sample until the end-point of a chemical reaction is reached.

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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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