Data on chorion thickness from embryos studied in an experiment on CO2 sensitivity of Northern sand lance (Ammodytes dubius) embryos conducted in 2018

Website: https://www.bco-dmo.org/dataset/867837
Data Type: experimental, Other Field Results
Version: 1
Version Date: 2022-01-11

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
Source data of two years of experimentation on Northern sand lance (Ammodytes dubius) embryos at different temperature and pCO2 conditions. Founder adults were sampled at Stellwagen Bank National Marine Sanctuary (SBNMS) (42° 9' 58.26" N, 70° 18' 44.1" W). Two complementary experiments were conducted in late 2018 (E1) and 2020 (E2), each rearing newly fertilized sand lance embryos to hatch over the course of 32-65 days. This dataset includes information on chorion thickness from the first experiment.

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Coverage

Spatial Extent: **Lat**:42.166183 **Lon**:-70.312275
Temporal Extent: 2018-11-15 - 2018-12-20

Methods & Sampling

Experimental setup: Two complementary experiments were conducted in late 2018 (E1) and 2020 (E2), each rearing newly fertilized sand lance embryos to hatch over the course of 32-65 days. Founder adults were sampled at Stellwagen Bank National Marine Sanctuary (SBNMS) (42° 9' 58.26" N, 70° 18' 44.19" W) at the peak of their narrow, local spawning window on November 15th (E1) or 27th (E2), using a 1.3 × 0.7 m beam trawl (6 mm mesh) towed over ground at 3 knots for 15 min. On deck, all flowing-ripe males and females were strip-spawned together (at 10°C, E1: $N_{male/female}$ = 29/13; E2: $N_{male/female}$ = 50/46) and their progeny transported to the University of Connecticut's Rankin Seawater Lab. There, exposure experiments commenced within 8 hours post fertilization by placing a volumetrically measured random sample of 600 (E1) or 1,200 embryos (E2) into each replicate rearing container.

Experimental seawater was drawn from subsurface eastern Long Island Sound (~ 31 psu), filtered to 1 µm, and UV-sterilized before use. Oxygen levels were maintained at ~100% saturation, while the photoperiod was 11L:13D.

Seawater chemistry: Realized pCO_2 conditions and other seawater chemistry parameters (Table 1 of Baumann et al., MEPS (in review)) were estimated in CO2SYS (V2.1, Pierrot et al. 2006) based on samples taken every 10 days and measured for temperature, pHNIST, salinity (refractometer) and total alkalinity (AT, µmol kg-1). Seawater samples were filtered to 10 µm, stored in 300 ml borosilicate bottles at 3°C, and within days measured for AT using endpoint titration (Mettler Toledo® G20 Potentiometric Titrator) with an accuracy of ±1% (Murray et al. 2019; verified and calibrated using Dr. Andrew Dickson's certified reference material for AT in seawater; Scripps Institution of Oceanography, Batch Nr. 162 & 164).

Experimental designs: During E1, we tested factorial combinations of two static temperatures and three target pCO_2 levels, thereby encompassing contemporary thermal conditions on Stellwagen Bank between late fall (10°C) and early winter (6°C), as well as current ambient (400 µatm, pH~8.12), predicted end-of-century (1,000 µatm, pH~7.76), and maximum open ocean pCO_2 benchmarks (2,000 µatm, pH~7.48; Caldeira & Wickett 2003, Salisbury & Jönsson 2018). At 10°C, three additional, pCO_2 levels below 1,000 µatm (570, 690, 890 µatm, Table 1) were included to better describe near future CO_2 sensitivities of sand lance embryos. The replication level for each of the 9 treatments was N = 5. Another 50 embryos per replicate were subsampled 90-190 ddpf and preserved in buffered (sodium tetraborate) 5% formaldehyde-in-freshwater solution. Those embryos sampled just before hatching began (170 ddpf, one random replicate per treatment) were later submitted for sectioning and staining (H&E stain; Horus Scientific, Worcester, MA), and later imaged for analyses of chorionic thickness (Nikon SMZ-1000 with Luminera® Infinity2-2 camera and ImagePro Premier v9.0, Media Cybernetics®).

During E2, we again tested target pCO_2 levels of 400, 1,000, and 2,000 µatm, first at an intermediate static temperature of 7°C and second at a dynamic temperature of 10°C decreasing to 5°C at a rate of 0.2°C d^{-1} (105°C). The latter was chosen to approximate the seasonal decline in bottom temperatures experienced by sand lance embryos on Stellwagen Bank. The two treatments reached thermal equivalence at 32 dpf (224 ddfp) – just after hatching had started. To better describe sand lance upper CO_2-sensitivities (1,000 - 2,000 µatm), we added two intermediate pCO_2 levels at 7°C (~1,300; ~1,700 µatm) and one at 105°C (~1,300 µatm). The initial replication level for each of the 9 treatments was N = 6. However, to disentangle potential pCO_2 effects on embryonic development vs. effects on hatching itself, we switched three random replicates from each extreme pCO_2 treatment per temperature with the opposite pCO_2 treatment (i.e., 3× ~400 --> ~2,000 µatm and 3× ~2,000 --> ~400 µatm). The switch happened at 175 ddpf (25 dpf at 7°C; 22dpf at 105°C) just before hatching started.

Response traits: From 90 ddpf onwards, rearing containers were monitored daily until hatching commenced; then, the number of hatchlings per replicate was recorded daily until hatching ceased. All hatchlings were immediately preserved in buffered 5% formaldehyde/freshwater solution for later morphological measurements. At the conclusion of E1, unhatched remains were imaged at 4× magnification, allowing the later distinction between (a) early arrested embryos (no or only amorphous cell masses visible), (b) partially developed embryos (unpigmented eyes visible, body not fully wrapped around the egg), and (c) fully developed embryos (pigmented eyes, body clearly visible and more than 1× wrapped around; Fig.S2). In E2, we continued daily monitoring for 7 more days after hatching had ceased; then examined the remains microscopically for embryos still alive (i.e., with beating hearts). Absolute hatching numbers were transformed to daily relative frequencies via dividing by the initial number of embryos that was adjusted for subsampling (E1, N = 500 per replicate) or reduced fertilization success (E2, N = 873 per replicate, based on examining independent post-fertilization subsamples). Relative frequencies were then summed to yield cumulative hatching success (HS, %) for each replicate. For E1, we additionally calculated the proportions of (a) fully developed but unhatched embryos and (b) all other arrested embryos combined. The latter also included decayed stages that were no longer detectable at the conclusion of E1.

To characterize hatching phenology, we recorded the day of first hatch (dpf), day of peak hatch (= dpf with the highest relative hatch frequency), and the total hatching period (d) for each replicate. Following Murray et al. (2019), a large number of hatchlings were imaged at 4× magnification (E1: N_{total} = 3,923; E2: N_{total} = 2,659) and then individually measured (ImagePro) for three morphological traits, i.e., standard length (SL, nearest 0.01 mm), yolk sac area (nearest 0.001 mm^2), and the size of the remaining oil globule inside the yolk sac (nearest 0.001 mm^2). The latter two traits are proxies for endogenous energy reserves after hatching, but they were strongly correlated (N = 5,552; R = 0.62, $p < 0.001$). Hence, we used PCA to extract the first principal component (explaining 73% (E1), 81% (E2) of variability) and then used the PC1 scores as the new variable, hereafter referred to as 'Endogenous Energy Reserves' (EER). Histological sections of fully developed, pre-hatch embryos from E1 were imaged at 20× magnification to measure the thickness of the chorion

(ImagePro). Chorion thickness was measured at 10 randomly selected locations around the circumference of the sectioned embryo, with measurements averaged subsequently for each embryo. Unfortunately, fewer than expected embryos were sectioned well enough for quality measurements, ranging from 2-7 per treatment.

Data Processing Description

BCO-DMO Processing:
- changed dates to format YYYY-MM-DD;
- modified parameter names to conform with BCO-DMO naming conventions;
- removed commas from data columns.

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Data Files

File
chorion_thickness.csv (Comma Separated Values (.csv), 36.03 KB) MD5:f80e9301b7dfa14ab6992358d5e0b629 Primary data file for dataset ID 867837

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Related Publications

Baumann, Jones, L., Murray, C., Siedlecki, S., Alexander, M., & Cross, E. (2022). Impaired hatching exacerbates the high CO2 sensitivity of embryonic sand lance Ammodytes dubius. Marine Ecology Progress Series. https://doi.org/10.3354/meps14010
Results

Caldeira, K., & Wickett, M. E. (2003). Anthropogenic carbon and ocean pH. Nature, 425(6956), 365–365. doi:10.1038/425365a
Methods

Murray, C. S., Wiley, D., & Baumann, H. (2019). High sensitivity of a keystone forage fish to elevated CO2 and temperature. Conservation Physiology, 7(1). doi:10.1093/conphys/coz084
Methods

Pierrot, D., Lewis, E., & Wallace, D. 2006. MS Excel program developed for CO2 system calculations. ORNL/CDIAC-105a Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, Book https://cdiac.ess-dive.lbl.gov/ftp/co2sys/CO2SYS_calc_XLS_v2.1/
Methods

Salisbury, J. E., & Jönsson, B. F. (2018). Rapid warming and salinity changes in the Gulf of Maine alter surface ocean carbonate parameters and hide ocean acidification. Biogeochemistry, 141(3), 401–418. doi:10.1007/s10533-018-0505-3
Methods

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Related Datasets

IsRelatedTo

Baumann, H., Nye, J. (2022) **Data on hatching frequency and success from an experiment on CO2 sensitivity of Northern sand lance (Ammodytes dubius) embryos conducted in 2020.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-01-12

doi:10.26008/1912/bco-dmo.867931.1 [view at BCO-DMO]

Baumann, H., Nye, J. (2022) **Data on unhatched embryos from an experiment on CO2 sensitivity of Northern sand lance (Ammodytes dubius) embryos conducted in 2018.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-01-10 doi:10.26008/1912/bco-dmo.867707.1 [view at BCO-DMO]

Baumann, H., Nye, J. (2022) **Hatch data from experiments on CO2 sensitivity of Northern sand lance (Ammodytes dubius) embryos conducted in 2018 and 2020.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-01-03 doi:10.26008/1912/bco-dmo.867401.1 [view at BCO-DMO]

Baumann, H., Nye, J. (2022) **Morphometric data from experiments on CO2 sensitivity of Northern sand lance (Ammodytes dubius) embryos conducted in 2018 and 2020.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2022-01-04 doi:10.26008/1912/bco-dmo.867447.1 [view at BCO-DMO]

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Parameters

Parameter	Description	Units
Species	Species name: Northern sand lance (Ammodytes dubius)	unitless
Source	Area where adult spawners were sampled: Stellwagen Bank, Gulf of Maine	unitless
Year	Year; experiment 1 (E1) = 2018; experiment 2 (E2) = 2020	unitless
Fertilization	Date when embryos were fertilized by strip-spawning adults; format: YYYY-MM-DD	unitless
Tank	Tank number from 1 through 9	unitless
Temp	Rearing temperature	degrees Celsius
Temp_code	Inidcates whether temperatures were static or dynamic	unitless
pCO2	average pCO2 calculated in CO2SYS based on pH, temp, salinity, alkalinity	microatmospheres (uatm)
Replicate	number of rearing container with the same year, temperature and pCO2 conditions	unitless
Sampledate	Date when embryo was sampled; format: YYYY-MM-DD	unitless
Degreedayofsample	Degree-day post fertilization, when embryo was sampled	degree-day post-fertilization (ddpf)
Vial_Numbrt	Vial number where embryo was stored in preservative	unitless
Embryo	Embryo number	unitless
ChorionThickness	Thickness of the egg envelope (chorion)	micrometers (um)

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Instruments

Dataset-specific Instrument Name	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.

Dataset-specific Instrument Name	beam trawl (6 mm mesh)
Generic Instrument Name	Beam Trawl
Generic Instrument Description	A beam trawl consists of a cone-shaped body ending in a bag or codend, which retains the catch. In these trawls the horizontal opening of the net is provided by a beam, made of wood or metal, which is up to 12 m long. The vertical opening is provided by two hoop-like trawl shoes mostly made from steel. No hydrodynamic forces are needed to keep a beam trawl open. The beam trawl is normally towed on outriggers, one trawl on each side. While fishing for flatfish the beam trawl is often equipped with tickler chains to disturb the fish from the seabed. For operations on very rough fishing grounds they can be equipped with chain matrices. Chain matrices are rigged between the beam and the groundrope and prevent boulders/stones from being caught by the trawl. Shrimp beam trawls are not so heavy and have smaller mesh sizes. A bobbin of groundrope with rubber bobbins keeps the shrimp beam trawl in contact with the bottom and gives flatfish the opportunity to escape. Close bottom contact is necessary for successful operation. To avoid bycatch of most juvenile fishes selectivity devices are assembled (sieve nets, sorting grids, escape holes). While targeting flatfish the beam trawls are towed up to seven knots, therefore the gear is very heavy; the largest gears weighs up to 10 ton. The towing speed for shrimp is between 2.5 and 3 knots. (from: http://www.fao.org/fishery/geartype/305/en)

Dataset-specific Instrument Name	Nikon SMZ-1000 stereo microscope
Generic Instrument Name	Microscope - Optical
Generic Instrument Description	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".

Dataset-specific Instrument Name	
Generic Instrument Name	pH Sensor
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	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.

Dataset-specific Instrument Name	
Generic Instrument Name	Water Temperature Sensor
Generic Instrument Description	General term for an instrument that measures the temperature of the water with which it is in contact (thermometer).

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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 CO_2 × 3 DO levels. Second, the effects of tidal and diel CO_2 × DO fluctuations of different amplitudes on silverside ELH traits will be quantified. To address knowledge gaps regarding the CO_2-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_2-sensitivity, the effects of temperature × CO_2 on pre- and post-hatch metabolism will be robustly quantified. The final objective is to rear silversides from fertilization to maturity under different CO_2 levels and assess potential CO_2-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-1536165
Bureau of Ocean Energy Management (BOEM)	M17PG0019

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