# Genotyped parents used in a study of survival of Menidia menidia larvae under high CO2 conditions; conducted at Southampton Marine Station from 2011-2015

**Website**: https://www.bco-dmo.org/dataset/551857
**Version**: 24 Feb 2015
**Version Date**: 2015-02-24

**Project**
» Will rising pCO2 levels in the ocean affect growth and survival of marine fish early life stages? (OA Fish)


| Contributors | Affiliation | Role |
|---|---|---|
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## Dataset Description

The investigators reared offspring, selected from a wild coastal fish population (Atlantic silverside, *Menidia menidia*), at high $CO_2$ conditions (~2300 uatm) from fertilization to 15 days posthatch, which significantly reduced survival compared to controls. Perished and surviving offspring were quantitatively sampled and genotyped along with their parents, using eight polymorphic microsatellite loci, to reconstruct a parent–offspring pedigree and estimate variance components.

Related Dataset: Menidia menidia - Genotyped Offspring

This dataset provides the source data to:
Alex J Malvezzi, Christopher S Murray, Kevin A Feldheim, Joseph D DiBattista, Dany Garant, Christopher J Gobler, Demian D Chapman, Hannes Baumann. 2015. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. Evolutionary Applications. doi: 10.1111/eva.12248

Note: This dataset has also been contributed to Dryad and can be found at http://dx.doi.org/10.5061/dryad.bd6vs

### Methods & Sampling

For more information on the dataset acquistion and processing methods, see Malvezzi et al. (2015) (doi: 10.1111/eva.12248). A summary follows:

**Species origin:**

We collected ripe, adult *M. menidia* at the beginning of the spawning season (25 April 2013) from a tidal salt marsh on the North shore of Long Island (Poquot, 40°58.12'N, 73°5.28'W). Males and females were caught with a 30 × 2 m beach seine, then transported to our laboratory facility (Flax Pond Marine Laboratory) and held overnight in separate temperature-controlled baths (200 L, 21 degrees C). The next morning, 29 females were strip-spawned by gently squeezing their hydrated eggs into a large shallow container containing seawater-activated sperm of 42 males and a large sheet of window screen (1 mm mesh).

**Estimation of 'Days survived at high CO2' (DS):**
To quantify *DS* for each hatched larva, the bottom of every high CO2 rearing container was gently siphoned twice daily, and all dead individuals were removed, recorded, and individually transferred to a tissue lysis solution (100 uL tissue lysis buffer + 12 uL proteinase K) for subsequent genomic DNA extraction. Frequent siphoning was critical, because fish larvae decompose beyond recognition within hours after death (at 24 degrees C). The pattern of daily posthatch mortality was typical for early larval fish, with mortality peaking soon after hatch (1–4 dph), but then declining exponentially over the remaining days of the experiment. After two consecutive days without any mortality (days 14 and 15 posthatch), the experiment was terminated and all surviving larvae (i.e., *DS* = 15) were individually transferred to tissue lysis solution for DNA extraction. To specifically evaluate the resistance of the population to elevated CO2, only hatched larvae of the 10 high CO2 replicates were sampled for genetic analyses.

**DNA extraction and amplification:**
Adult spawner DNA was extracted from tail clips (15–35 mg) following the animal tissue protocol of Qiagen DNeasy kits. Larval DNA (*DS* ranging from 1 to 15 dph) was extracted using a more cost-effective 'salting out' protocol that yielded useable DNA from larval silversides. Ten polymorphic microsatellite loci for *M. menidia* were amplified for all parents and offspring in a 10 uL reaction containing 1× PCR buffer, 10× bovine serum albumin, 1.5–3.5 mm MgCl2, 0.12 mm dNTPs, 0.16 um of the reverse primer and fluorescently labeled M13 primer, 0.04 um of the species-specific forward primer and 1 unit Taq polymerase, and 10–40 ng of genomic DNA. Thermal cycling consisted of 5 min at 94 degrees C followed by 35 cycles of 94 degrees C for 30 s, primer specific annealing temperature (*T*a) for 30 s and 72 degrees C for 60 s with a final extension at 72 degrees C for 10 min. Fluorescently labeled PCR products were electrophoresed on an ABI 3730 DNA analyzer along with an internal fluorescent ladder (LIZ-500; Applied Biosystems). Alleles were scored by a single analyst (AJM) using the software Peakscanner 1.0 (Applied Biosystems, Life Technologies, Grand Island, NY, USA). A subset of approximately 10% of genotypes was verified by a second analyst (KAF) using GENEMAPPER v4.0 (Applied Biosystems). One locus (Mm09) did not amplify and another one did not yield easily scored peaks (Mm119), hence only 8 of 10 loci were eventually used for parentage assignment and genetic analyses.

**Data Processing Description**

**Genetic analyses:**
We used CERVUS 3.0 (www.fieldgenetics.com) to calculate summary statistics (e.g., number of occurrences of each allele at each locus) and assess the suitability of loci for parentage analysis. CERVUS was then used to calculate the following four measures for each of the 10 high CO2 replicates: relative allelic richness (i.e., NAlleles, replicate/NAlleles, total), observed heterozygosity, number of dams, and number of sires. Linear regression was used to relate the four proxies to survival. Subsequently, CERVUS was used to assign individual offspring to candidate mothers and fathers. For each offspring tested, candidate parents were assigned with at least 95% confidence using exclusion probability first and the maximum likelihood score second. Comparing each offspring to all parent genotypes, exclusion of candidate parents followed if mismatches occurred at more than one locus. In total, 704 of 772 individuals (91%) were successfully assigned to parents, a success rate comparable to other studies on fish (75–95%).Unassigned offspring, resulting from poor amplification at some loci, were excluded from further analyses. All females, but only 35 of 42 males, were assigned to offspring, likely because the sperm from seven males was not activated or the fertilized eggs were not selected for in the experiment.

Last, we ran a hierarchical set of univariate 'animal models' for the trait *DS*, using the restricted maximum likelihood (REML) software ASReml v3.0.5 (VSN International Ltd, Wood Lane, Hemel Hempstead, UK). An 'animal model' is a mixed model (i.e., a form of linear regression with 'fixed' and 'random' effects as explanatory variables) that has been found advantageous for estimating phenotypic variance components in wild populations. For fixed effects, we only considered replicate *ID* (1–10), which corresponds to the experimental vessel, given that age (all individuals had the same day of fertilization) and sex (individuals sampled in their entirety) were not relevant factors. Replicate *ID* significantly influenced *DS* (df = 9, $P < 0.001$, univariate general linear model SPSS; IBM) therefore all subsequent analyses included replicate *ID* as a fixed effect to remove its effect prior to the estimation of genetic parameters. We then estimated the heritability of

*DS* by testing three distinct models: (i) the base model including only the fixed effect, (ii) a model including the fixed effect and an additive genetic random effect ($\alpha i$ with variance *V*A), and (iii) a model including the fixed effect, additive genetic random effect, and dam identity (*m*i with variance *V*M) as a random effect. The components of total phenotypic variance in *DS* (*V*DS = *V*A + *V*M + *V*R) were then used to calculate narrow sense heritability *h*2 of *DS* as the ratio of additive genetic variance to total phenotypic variance (*h*2 = *V*A/*V*DS) and maternal effects *m*2 of *DS* as the ratio of maternal variance to total phenotypic variance (*m*2 = *V*M/*V*DS). Statistical significance was assessed with likelihood-ratio tests [LRT = −2 * (likelihood base model–likelihood model with random effects)] tested against the chi-square distribution (df = 1).

**BCO-DMO Processing:**
- Replaced spaces with underscores in species column.
- Replaced blanks with 'nd' to indicate 'no data'.
- Modified parameter names to conform with BCO-DMO naming conventions.
- Changed positive degrees west (long) to negative degrees east.

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## Data Files

| File | |
|---|---|
| **genotyped_parents.csv** | (Comma Separated Values (.csv), 37.22 KB) MD5:95b3809d743fbf7d16053a277ae2cd8d |
| Primary data file for dataset ID 551857 | |

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| species | Name of the species studied. This study was done on the Atlantic Silverside (Menidia menidia). | unitless |
| date_sampled | Date of sampling in mm/dd/yyyy format. All parents used in this study were caught on 4/25/2013 . | unitless |
| lat | Latitude of sample site (Poquot, Long Island, NY). | decimal degrees |
| lon | Longitude of sample site (Poquot, Long Island, NY). | decimal degrees |
| sample_id | Sample ID number (F1-29 and M1-41). F and M stand for female and male, respectively. This study used a total of 29 females and 41 males to produce offspring. | unitless |
| locus | This study used 9 polymorphic loci (Mm02, 108, 119, 202, 204, 240, 248, 251, and 272). | unitless |
| allele_1 | Microsatellite allele 1. | base pairs |
| allele_2 | Microsatellite allele 2. | base pairs |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | Thermal Cycler |
| **Generic Instrument Name** | Thermal Cycler |
| **Generic Instrument Description** | A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html) |

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## Deployments

**lab_Baumann_Gobler_FP**

| | |
|---|---|
| **Website** | https://www.bco-dmo.org/deployment/551842 |
| **Platform** | Flax Pond Marine Lab |
| **Start Date** | 2011-09-01 |
| **End Date** | 2015-02-01 |

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## Project Information

**Will rising pCO2 levels in the ocean affect growth and survival of marine fish early life stages? (OA Fish)**

**Coverage**: Long Island Sound, Shinnecock Bay, Long Island, NY

*Description from NSF award abstract:*
Ocean acidification has the potential to affect a broad spectrum of marine organisms and thereby transform the composition and function of our oceans. In contrast to calcifying marine invertebrates, marine fish are widely believed to be unaffected by the CO2 concentrations projected for the future. While this may be so for juvenile and adult fish stages, the fate of fish embryos and larvae in high CO2 oceans is less certain as CO2-sensitivity data for these stages are largely unavailable. Recognizing this knowledge gap and inspired by the findings of two recent studies on clownfish and sea bass larvae (Munday et al. PNAS 107 (2010); Checkley et al. Science 324 (2009)), the investigators performed a series of experiments exposing eggs and early larvae of inland silversides (*Menidia beryllina*) to elevated CO2 levels while strictly adhering to current "best practice" guidelines for ocean acidification research. At 1,000 ppm CO2, average *M. beryllina* survival ~1wk post-hatch significantly and consistently (five experiments) declined by ~75% compared to current day CO2 levels (390 ppm), while average length of newly hatched larvae decreased by 22%. Together with prior studies, these results suggest a surprisingly high susceptibility of fish early life stages to the CO2 increases that are projected to occur this century. Given that the abundance of many fish stocks, including most commercial species, is often regulated by processes affecting early life history growth and survival, ocean acidification may impact the dynamics of future fish populations and become yet another challenge to sustainable fisheries.

The investigators believe that there is now a pressing need to better understand how CO2 affects the viability of fish embryos and larvae in the ocean. This requires novel approaches involving longer-term, larger-scale experiments across multiple species. The investigators will comprehensively examine the impacts of current and future CO2 levels (400 - 1,000 ppm) during the egg and larval stages of three model fish species: Atlantic silversides (*M. menidia*), inland silversides (*M. beryllina*) and sheepshead minnows (*Cyprinodon variegatus*). They will also investigate populations of the same species (*M. menidia*) from differing latitudes. These species/populations are ecologically important due to their intermediate trophic position, have comparable life histories to commercial marine fish, offer differences in genetic growth capacity and presumed sensitivity, and are highly amenable to laboratory experimentation. Survival and growth (weight- and length-based) will be measured in experiments performed at different CO2, temperature (21, 27°C) and feeding conditions (low, ad libitum), thus permitting the affects of CO2 to be considered in parallel with thermal stress and food limitation. Quantification of feeding rates, gross growth efficiency, and oxygen consumption will characterize the physiological costs of high CO2 environments. Changes in calcification of larval fish otoliths and skeletal elements will be determined from weights and a Ca45 radiotracer approach. Finally, surviving *M. menidia* (or *M. beryllina*) will be reared to maturity and their offspring will be challenged with differing levels of CO2. Repeating this approach over several generations will demonstrate the extent to which CO2 resistance may evolve through natural selection. Collectively, this study will make significant advances toward understanding how ocean acidification may challenge the world's most valuable marine resource, fish.

Note that PI Hannes Baumann has since moved to the University of Connecticut. See his current contact information.

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## Funding


| Funding Source | Award |
| --- | --- |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1129622 |


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