Size and porosity of squid statolith from laboratory study from June to August 2011 (OA Squid Rearing project)

Website: https://www.bco-dmo.org/dataset/3983
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Project
» Examining Impacts on Squid Paralarval Development, Behavior, and Survival (OA Squid Rearing)

Program
» Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Contributors	Affiliation	Role
Mooney, T. Aran	Woods Hole Oceanographic Institution (WHOI)	Principal Investigator
Kaplan, Maxwell B.	University of St. Andrews	Student

Table of Contents

Dataset Description

Surface area and porosity of hatchling squid statoliths under two pH levels.

Longfin squid, Doryteuthis pealeii, were reared under two pH levels in a laboratory setting to study the effect of ocean acidification on hatching and paralarval mantle growth and statolith condition.

This dataset is the result of M. Kaplan's WHOI summer student fellow project.

This work is also associated with project NSF OCE-1041106, "An Investigation of the Role of Nutrition in the Coral Calcification Response to Ocean Acidification".

Methods & Sampling

Experiments were conducted at the Woods Hole Oceanographic Institution between June-August 2011. The use and care of the animals was performed with approval from the Woods Hole Oceanographic Institution's Animal Care and Utilization Committee (IACUC). All necessary permits were obtained for the described studies including the collection of adult squid. These were gathered by the Marine Biological Laboratory, which has a research permit issued by the Massachusetts Division of Marine Fisheries to collect invertebrates in various life stages for research and education (permit number 152087). No specific permissions were required for these locations/activities because they fall under the permit provisions. The collecting location was not privately owned or protected in any way and the field studies did not involve endangered or protected species.

Collection and husbandry
Squid were captured by trawl in Vineyard Sound on two occasions in 10-30 meters of water. Temperature in the area at the time of capture was 16.7 °C and 17.9 °C, and salinity was 30.7 and 30.8, respectively (data

from the 12 meter node at the Martha's Vineyard Coastal Observatory). These values are typical for longfin squid recruits, which are primarily found in water ranging from 6-35 m depth, 4-28 °C, and with salinity of 30-37 between the spring and fall [29]. Similar to some other inshore squid species, D. pealeii is able to tolerate temperature and salinity variations [39]. Adults in healthy condition (free of cuts and scrapes) were hand-selected from the group, gently placed in individual buckets, and transported from the Marine Biological Laboratory to a holding tank in the Environmental Systems Laboratory (Woods Hole Oceanographic Institution, Woods Hole, MA) within 1 hour of being caught. To encourage mating, more females than males were selected from the trawl (f:m ratio; 6:2 and 16:3). The holding tank (120 cm diameter; 70 cm depth) contained a layer of fine-grained sand at the bottom (~2 cm thick). The sand was collected from a nearby beach and was rinsed thoroughly with sand-filtered seawater prior to being added to the holding tank. The holding tank was set up in a flow-through system with sand-filtered seawater that was temperature-controlled to ~20 C using aquarium heaters and chillers. In accordance with our animal care protocols, squid were fed twice daily with live Fundulus heteroclitus, which were gathered from a local bay.

Experimental set-up

Individual aquaria (1-litre Solo PET food service containers) were set up in a flow-through system at the beginning of each trial and were equilibrated with different CO_2 concentrations. The PET containers (16 in total) were placed in a water bath in which temperature was maintained at ~20 °C (monitored using an Onset data logger (pendant model UA-002-64), which recorded ambient light intensity and water temperature every 15 minutes). The containers were covered tightly with lids in which a single hole had been cut (0.5 cm diameter), which allowed the water and gas tubing to fit snugly inside. Vineyard Sound seawater, temperature-controlled to 20 °C and 5 m-filtered, was fed into a header tank from which it flowed through 2 'H'-shaped equilibration chambers. Seawater in chamber 1 was continuously equilibrated with air pumped from an indoor air compressor, while seawater in chamber 2 was equilibrated with the air from the same source enriched with CO_2 using Aalborg Mass Flow Controllers (model GFC17 and GFC37). Both gas mixtures passed through air stones in the equilibration chambers. The concentrations of CO_2 in the gases bubbled in the two sets of aquaria were set to 390 µatm (control) and 2200 µatm (treatment), targeting pH levels of 8.0 and 7.3 for the control and elevated pCO_2 levels respectively. CO_2 concentrations of the gases were analyzed weekly using a Qubit Systems CO_2 Analyzer (model s151) with reference to 3 known commercially prepared standards (1036, 362 and 0 ppm). Gas concentrations in both treatments remained stable for the duration of the experiment (mean ± SE; control: 394 ± 6 ppm; treatment: 2267 ± 10 ppm.

Water from the equilibration chambers entered a PVC manifold from which it was supplied individually to the containers to ensure that the egg capsules were well oxygenated and in order for metabolic byproducts to be expediently removed from the containers (as in [48,49]). Flow to each container was approximately 21 liters d^{-1}. Each container was also bubbled individually with the same air or air + CO_2 mixture to ensure continued equilibration. Bubbling rates and water flow rates in the "H"-shaped equilibration columns (upstream), and in the experimental cups (downstream) were adjusted so that pH was on a plateau and not sensitive to small fluctuations in water flow, water chemistry, or gas flow. Outgoing water dripped out of the container through a hole in the side of the container covered in 500 m mesh to prevent loss of larvae. Water was circulated through the system for several days prior to introducing the eggs, during which the pH in each treatment was tested every other day using a pH meter (Orion 3 Star Plus model 1212001, ThermoElectron Corporation) to ensure that target pH levels had been reached and remained stable. The experiment was carried out in a windowless room that was maintained on a 12:12 light:dark photoperiod by 4 ceiling-mounted fluorescent bulbs.

Multiple females laid several egg capsules in one large egg cluster after 1-2 days in the holding tank prior to the start of both experimental trials. Each egg capsule may contain between 100-200 fertilized eggs, with considerable inter-capsule variability in the number of eggs per capsule both within and between females [50]. Furthermore, female D. pealeii are able to store sperm from multiple males [51], and their egg capsules are known to contain eggs fertilized by multiple males [52]. Thus, it was likely these egg capsules contained fertilized embryos from multiple males.

The morning after eggs were laid, 2 randomly selected egg capsules from the egg cluster were added to each of 6 containers per trial (3 containers per CO_2 concentration per trial). This balanced the need for numerous embryos with the feasibility of measuring paralarvae immediately after hatching. An additional 4 containers were included in the water bath as blanks (2 per CO_2 concentration) and contained no eggs throughout the experiment for comparative purposes so that seawater chemistry measurements could be taken independent of potential biotic effects on chemistry parameters.

Seawater Chemistry

Seawater pH was measured using a pH meter every other day and by spectrophotometer weekly throughout the experiment in a method adapted from Clayton & Byrne [53] and Dickson et al. [54]. Samples for

spectrophotometric pH analysis were first taken on 6 July 2011 (day 5 of trial 1) and were taken weekly thereafter from a subset of aquaria. Electrode-based pH measurements were converted from the NBS scale to the total scale and were used for monitoring purposes only; spectrophotometric pH measurements, also expressed on the total scale, were used for seawater chemistry calculations.

Salinity samples were collected in 120 mL glass bottles weekly concurrently with the samples for spectrophotometric analysis, but were analyzed at a later date. Total alkalinity (AT) samples were also taken weekly in plastic acid-washed 20 mL scintillation vials and poisoned with 11 l of saturated mercuric chloride ($HgCl_2$). These samples were analyzed using automated Gran titrations of 1 mL samples, run in duplicate and standardized using certified reference materials (from the laboratory of Andrew Dickson, SCRIPPS Institution of Oceanography) (method adapted from Holcomb et al. [49]). If there was a discrepancy of more than 4 µequiv/kg, duplicate samples were run again. Using CO2sys software, temperature, pH, salinity, and AT values were used to calculate aragonite saturation state values (Arag) in each treatment [55,56] using dissociation constants from Mehrbach et al. [57] refit by Dickson & Millero [58], sulphate constants from Dickson [59], and carbonate mineral solubilities from Mucci [60].

Measurement Protocol

Containers were checked for hatching daily, and time to first hatching was recorded. Containers continued to be inspected every 24 hours until all eggs had hatched (18 days in trial 1; 19 days in trial 2 from day of egg laying to last hatching). On the day they were observed, all hatchlings were removed from their container and counted in order to calculate the number of animals hatched per day. Larvae were then placed in new containers (one container per treatment containing all of the hatched larvae from that day) to separate the eggs and new hatchlings. Thus, cup densities varied. Paralarvae were not fed, because they hatch with a yolk sac, which fuels their initial (post-hatching) metabolic requirements [61]. Animals were not reared beyond this yolk stage because of the high mortality rate of squid raised in captivity [39].

A subset of the hatchlings (10 paralarvae d-1) from each of the 3 containers per treatment and trial (i.e., n = 30 animals per treatment per trial per day) was subjected to morphological analyses. Randomly selected individuals were gently lifted via pipette and placed lengthwise and dorsal-side up on a glass slide in a small drop of water. Multiple photographs of each individual were taken using a Dino-Lite Pro2 AD-413TL USB-microscope (calibrated twice daily prior to taking the first measurement of each treatment by placing a standard in the field of view) and the DinoXcope software. Only photographs of the dorsal side, of undamaged individuals, and of animals that were not moving when the image was captured, were retained for mantle length analysis. In order to facilitate rapid assessments of the hatchings, dorsal mantle length (ML) was determined from the image later using the measurement tools in the DinoXcope software.

Every day another random subset of paralarvae (n=10 per treatment, pooled across containers) were fixed in 97% ethanol. Statoliths were dissected out of a further subset of these ethanol-fixed paralarvae (pooled across days but separated by CO_2 treatment). Only one statolith per dissected individual was retained in order to maintain sample independence. All statoliths were soaked briefly (~15 minutes) in a dilute bleach solution in order to dissolve any remaining tissue. This is a standard method to remove tissue from otoliths and coral skeletons before detailed mass and visual morphological measurements [62-64]; visual assessments of the statoliths indicated that this method did not impact morphology. We obtained 36 statoliths from control and 22 from treatment CO_2-reared individuals available for comparison; unequal numbers resulted from the difficult nature of statolith extraction and a limited number of preserved larvae from which samples could be taken. The statoliths were mounted on stubs in a uniform orientation (anterior view) for scanning electron microscopy (SEM) using a fine paintbrush and were sputter-coated with platinum or gold. Images were collected using both a Zeiss NTS Supra 40VP with a field emissions source for the electrons and a JEOL JSM 35CF. Surface area measures were made using the programs Axiovision (Carl Zeiss, USA) and SemAfore (JEOL, Germany) with outline and ROI tools, which captured 2-dimensional surface area. These tools were calibrated individually for each SEM image using the scale bar present.

Statoliths were graded according to their porosity [e.g., 65] and shape from the SEM images using a categorical grading system (Fig. 1, Kaplan et al, 2013). This method was based on a system of categorizing morphological abnormalities in developing squid by Rosa et al. [66] and was defined as: (1) standard statolith shape and normal/minimal porosity, (2) standard shape with some abnormalities in the surface structure, slightly porous, and (3) porous and/or abnormal shape. Standard statolith shape is well described elsewhere [44,67-69]. Briefly, statoliths are oriented in the long axis approximately in line with the dorso-ventral plane of the animal [69]. Adult squid statoliths are composed of four parts: dorsal dome, lateral dome, rostrum wing, with variation between species [69]; however, paralarval statoliths are typically droplet-shaped [68]. Abnormalities were defined by varying degrees of pitting in the statolith surface and morphological deformations (i.e., deviations from the droplet shape).

Data Processing Description

Statistical analysis was carried out in PASW 18.0 and SPSS 19.0 for Windows (IBM Corporation, NY, USA). Values reported are means ± SE unless otherwise noted. One-way ANOVAs, General Linear Models (GLMs) and Chi-squared tests were used to compare between treatments and trials. Where ANOVAs were used, data were normally distributed. The hatching data represent counts in a J-by-K table where J is the number of treatments and K is the number of days. We tested the null hypothesis that the distribution of hatching across the days was the same for all treatments using a Chi-squared test. Because egg capsules contained variable numbers of embryos, Fig. 2, Kaplan et al, 2013, shows the proportion hatching per day to better reflect relative differences between treatments and trials. All results were adjusted using the Bonferroni correction, which reduces the critical p value based on the number of parameters tested.

Related Reference:

Kaplan MB, Mooney TA, McCorkle DC, Cohen AL (2013) Adverse Effects of Ocean Acidification on Early Development of Squid (Doryteuthis pealeii). PLoS ONE 8(5): e63714. doi:10.1371/journal.pone.0063714

[table of contents | back to top]

Data Files

File
statolith.csv (Comma Separated Values (.csv), 1.34 KB) MD5:a5662ca6089688ca36ff50c0371ed5cb Primary data file for dataset ID 3983

[table of contents | back to top]

Parameters

Parameter	Description	Units
treatment	treatment code	unitless
trial	trial number	unitless
sample	sample code	unitless
len_statolith	length of the statolith along its longest dimension	microns
surf_area	surface area of statolith	microns^2
grade	grade for shape and porosity	unitless

[table of contents | back to top]

Instruments

Dataset-specific Instrument Name	Microscope-Electron
Generic Instrument Name	Electron Microscope
Dataset-specific Description	Zeiss NTS Supra 40VP with a field emissions source for the electrons and a JEOL JSM 35C
Generic Instrument Description	Instruments that generate enlarged images of samples using the phenomena of reflection and absorption of electrons behaving as waves.

[table of contents | back to top]

Deployments

lab_Mooney

Website	https://www.bco-dmo.org/deployment/59047
Platform	WHOI
Start Date	2011-06-01
End Date	2011-08-31
Description	squid rearing studies performed in laboratory setting

[table of contents | back to top]

Project Information

Examining Impacts on Squid Paralarval Development, Behavior, and Survival (OA Squid Rearing)

Coverage: Woods Hole, MA

Longfin squid, *Doryteuthis pealeii*, were reared under two pH levels in a laboratory setting to study the effect of ocean acidification on hatching and paralarval mantle growth and statolith condition.

Proposal Abstract

Squid are often referred to as keystone species because of their central role in ocean ecosystems. They play vital roles in marine food webs both as prey for many of top predators such as seabirds, dolphins, sharks, and tunas, and as voracious predators of smaller, often deep-water (mesopelagic) fishes and invertebrates. Squid comprise a substantial fisheries resource, both directly as commercial and recreational fisheries, and indirectly as an important food source for many of the fishes consumed by a growing human population. Changes in squid abundances can dramatically impact the ecology of the ocean and fisheries yields.

Despite the importance of squid to both ecosystems and economies, there has been little investigation of the effects of ocean acidification (OA) on these taxa. Impacts upon juvenile squid are a primary concern because: (i) early developmental stage animals are highly sensitive to environmental conditions and (ii) their successful early life history growth, behavior and survival are critical to founding future cohorts that support ecosystem food webs and global fisheries. The goal of this work is to quantify how OA conditions impact squid embryo and juvenile development, behavior, and survival. The studies will provide a mechanistic foundation for understanding potential impacts of OA on squid populations. Squid are dynamic organisms, which may now encounter high carbon dioxide and lower pH in areas of ocean upwelling, and measures of these environmental variables will be collected in this study. This research takes an extensive whole-organism approach to understanding OA impacts on squid by examining anatomy, physiology, and behavior of these animals at levels of environmental carbon dioxide and pH encompassing present-day and predicted, future values.

These are the first tests to comprehensively examine OA impacts on squid. The experiments are timely in the context of changing ocean conditions, increased pressure on squid fisheries, and preliminary data showing potential OA impacts on squid. Understanding these effects will aid fisheries managers in estimating ecosystem and economic impacts in this and other cephalopod species. Information from this research will be used to engage and educate the general public, who are fascinated by squid biology, about ocean acidification through a summer seminar series at Woods Hole Oceanographic Institution. The research team and the Woods Hole Oceanographic Communications Department will communicate findings from this study to journalists; interact with teachers about this work through existing fellowship programs; construct and convey education materials for grade-school children at a local science school; and develop a squid dissection module for high school teachers entitled "Squid have ears too!"

This study was supported by a WHOI Student Summer Fellowship and WHOI-MIT Joint Program, the Penzance Endowed Fund, the John E. and Anne W.Sawyer Endowed Fund and NSF Research Grant No. EF-1220034. Additional support came from NSF OCE 1041106 to ALC and DCM, and NOAA Sea Grant award #NA10OAR4170083 to ALC and DCM.

[table of contents | back to top]

Program Information

Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES): Ocean Acidification (formerly CRI-OA) (SEES-OA)

Website: https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=503477

Coverage: global

NSF Climate Research Investment (CRI) activities that were initiated in 2010 are now included under Science, Engineering and Education for Sustainability NSF-Wide Investment (SEES). SEES is a portfolio of activities that highlights NSF's unique role in helping society address the challenge(s) of achieving sustainability. Detailed information about the SEES program is available from NSF (https://www.nsf.gov/funding/pgm_summ.jsp?pims_id=504707).

In recognition of the need for basic research concerning the nature, extent and impact of ocean acidification on oceanic environments in the past, present and future, the goal of the SEES: OA program is to understand (a) the chemistry and physical chemistry of ocean acidification; (b) how ocean acidification interacts with processes at the organismal level; and (c) how the earth system history informs our understanding of the effects of ocean acidification on the present day and future ocean.

Solicitations issued under this program:
NSF 10-530, FY 2010-FY2011
NSF 12-500, FY 2012
NSF 12-600, FY 2013
NSF 13-586, FY 2014
NSF 13-586 was the final solicitation that will be released for this program.

PI Meetings:
1st U.S. Ocean Acidification PI Meeting(March 22-24, 2011, Woods Hole, MA)
2nd U.S. Ocean Acidification PI Meeting(Sept. 18-20, 2013, Washington, DC)
3rd U.S. Ocean Acidification PI Meeting (June 9-11, 2015, Woods Hole, MA – Tentative)

NSF media releases for the Ocean Acidification Program:

Press Release 10-186 NSF Awards Grants to Study Effects of Ocean Acidification

Discovery Blue Mussels "Hang On" Along Rocky Shores: For How Long?

Discovery nsf.gov - National Science Foundation (NSF) Discoveries - Trouble in Paradise: Ocean Acidification This Way Comes - US National Science Foundation (NSF)

Press Release 12-179 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: Finding New Answers Through National Science Foundation Research Grants - US National Science Foundation (NSF)

Press Release 13-102 World Oceans Month Brings Mixed News for Oysters

Press Release 13-108 nsf.gov - National Science Foundation (NSF) News - Natural Underwater Springs Show How Coral Reefs Respond to Ocean Acidification - US National Science Foundation (NSF)

Press Release 13-148 Ocean acidification: Making new discoveries through National Science Foundation research grants

Press Release 13-148 - Video nsf.gov - News - Video - NSF Ocean Sciences Division Director David Conover answers questions about ocean acidification. - US National Science Foundation (NSF)

Press Release 14-010 nsf.gov - National Science Foundation (NSF) News - Palau's coral reefs surprisingly resistant to ocean acidification - US National Science Foundation (NSF)

Press Release 14-116 nsf.gov - National Science Foundation (NSF) News - Ocean Acidification: NSF awards $11.4 million in new grants to study effects on marine ecosystems - US National Science Foundation (NSF)

[table of contents | back to top]

Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1041106
Woods Hole Sea Grant (WH Sea Grant)	NA10OAR4170083, R/O-42
NSF Emerging Frontiers Division (NSF EF)	EF-1220034

[table of contents | back to top]