# Larval positioning in acrylic tubes describing the behavior of coral larvae in high pCO2 within shallow tropical reefs in Okinawa, Japan from 2016-07 to 2016-08

**Website**: https://www.bco-dmo.org/dataset/751013
**Data Type**: Other Field Results
**Version**: 1
**Version Date**: 2018-12-07

**Project**
» Collaborative Research: Ocean Acidification and Coral Reefs: Scale Dependence and Adaptive Capacity (OA coral adaptation)

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

| Contributors | Affiliation | Role |
|---|---|---|
| Bergman, Jessica | California State University Northridge (CSUN) | Principal Investigator |
| Edmunds, Peter J. | California State University Northridge (CSUN) | Co-Principal Investigator |
| Biddle, Mathew | Woods Hole Oceanographic Institution (WHOI BCO-DMO) | BCO-DMO Data Manager |

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## Coverage

**Spatial Extent**: **Lat**:26.6717 **Lon**:127.8847
**Temporal Extent**: 2016-07 - 2016-08

## Dataset Description

Twelve colonies of Pocillopora damicornis (Linnaeus 1758) were collected in July and August 2016 from ~ 1-m depth on a patch reef on the northwest shore of Okinawa (26°40’18.24” N, 127°53’4.78” E). Colonies were collected prior to expected larval release in Okinawa in July and August (S. Harii, unpublished data on the study site), with peak release occurring ~ 7 days after the new moon.

Following collection, colonies were transferred to Sesoko Station, part of the Tropical Biosphere Research Center University of the Ryukyus, where they were incubated outdoors in individual containers exposed to natural irradiance in flow-through seawater. Ambient seawater was pumped at 3.0 L min-1 (AC Flowmeter, Tokyo Keiso Co., Japan) from 4–5 m depth and stored in two 10 L reservoirs. Air was bubbled constantly in to the reservoirs at 3.0 L min-1 to maintain ambient seawater pCO2 (i.e., the control conditions). Seawater temperature was measured hourly at 1–2-m depth near the collection site prior to, and during, the experiment (HOBO Pro v2, Onset Computer Corporation, USA), and was 29.9 ± 0.2°C (mean ± SE, n = 45 days), with a daily minimum of 28.3°C and daily maximum of 31.8°C (R. Prasetia & S. Harii, unpublished data) that reflects

summertime diurnal warming in this location. Temperature in the containers holding the corals was maintained within this range during the experiment (29.8 ± < 0.2°C, mean ± SE, n = 31) using a chiller (ZR-130E, Zensui, Japan).

Planulae released from P. damicornis during the first quarter moon of July and August were collected at ~ 05:00 hrs following their release at ~ 03:00 hrs, using containers lined with 110 µm plankton mesh. As larvae from P. damicornis are physiologically dissimilar among days of release, larvae were collected from the inferred day of peak release and pooled among colonies releasing larvae on this day. Larvae from July and August were used to test the effects of pCO2 (two levels) and depths (two levels) on larval behavior, and the experiment was conducted in two parts. The first part (July 2016) tested the effects of two pCO2 regimes on larval behavior with the tubes positioned with their upper opening adjacent to the air-water interface of the seawater (hereafter “shallow” tubes), and the second part (August 2016) tested the effects of the same two pCO2 regimes on larval behavior with the tubes positioned with their upper opening ~3–4 m below the surface (hereafter “deep” tubes).

**Methods & Sampling**

Response of larval behavior to high pCO2
To determine the position of P. damicornis larvae in the seawater in response to high pCO2, larvae were transferred from collection containers to UV-transparent acrylic tubes (UV-T, ACRYLITE Colorless 0070 GT, Evonik Industries, New Jersey, USA) and incubated in situ at two depths, which centered the tubes vertically at ~ 0.3 m (July 2016) or ~ 3.3 m (August 2016). The tubes had a wall thickness of 6 mm, were 68-cm long with an inner diameter of 4.5 cm, and were sealed at either end with UV-T acrylic caps. A 6-mm hole was drilled in the top cap so the tubes could be filled with seawater; the hole was later covered with vinyl electrical tape. While the objective was to completely fill the tubes with seawater, in most cases small bubbles were introduced during the filling process and combined when the tubes were positioned vertically to create an air gap of ≤ 1-cm height at the top of the tube. The acrylic in these tubes transmitted ~ 92% of ultraviolet radiation (UV-R) (280–400 nm), ensuring larvae were exposed to ecologically relevant light conditions for shallow seawater. Previous studies of the vertical movement of P. damicornis larvae in acrylic tubes, as well as preliminary observations of larval behavior used in the present study, show that larvae of P. damicornis exposed to ambient seawater generally aggregate either in the top or bottom 0–2 cm of tubes regardless of light conditions (i.e., light versus dark). Therefore, the vertically-oriented tubes were marked into two sections for the purpose of scoring the larvae by position: an upper section (21-cm length) and lower section (47-cm length), since we reasoned it would be unlikely that the larvae would accumulate in the center of the tubes. Of the larvae found in the lower section, 83–88% were located in the bottom 23 cm of the tube, which suggested that the greater size of the lower scoring section in the tubes did not upwardly bias estimates of larvae scored as moving downward.

Eight tubes were used for each experiment, during which four were filled with ambient (~ 400 µatm pCO2) seawater, and four with treatment (~ 1000 µatm pCO2) seawater that simulated the elevated pCO2 conditions predicted under a pessimistic scenario of human activity to occur by 2100 (RCP6.0). Once tubes were filled with seawater, 50 larvae were haphazardly selected from the larval stock obtained by pooling larvae released that morning from maternal colonies, and added using a Pasteur pipette through the 6-mm hole in the cap of the tube. The hole was sealed when the tubes were stocked with larvae. Each tube was carefully inverted to gently mix the 50 larvae, so each trial began with the larvae scattered along the length of the tube. Although the pelagic larval duration (PLD) of P. damicornis can be > 100 d, their larvae commonly settle ≤ 24 h following release, and therefore, the present incubations were designed to last 12 h starting at 08:00 hrs.

After the tubes were stocked with larvae on the shore, they were transported by a snorkeler to an adjacent fringing reef and suspended vertically, with the midpoint of the tubes at a median depth (MD) of either ~ 0.34 m (July) or 3.34 m (August) (hereafter referred to as 0.3 and 3.3-m MD) (Fig. 1). The median depth was recorded at the middle of the vertically oriented tubes, thus the depth range for the 0.3 m MD tubes extended from the surface to 0.68 m, and from 3 – 3.68 m for the 3.3 m MD tubes. To maintain this configuration, the tubes were attached to a weight at 5-m depth, and a float was used to keep them suspended vertically. While it is not possible to exclude an effect of time (July versus August) in confounding the contrast of depth, experimental conditions were kept virtually identical between months to reduce temporal bias. Tubes were stocked with larvae shortly after sunrise, and were installed on the reef within 30 minutes of filling at ~ 07:30 hrs. Thereafter, the vertical position of the larvae was scored every 4 hours starting at 08:00 hrs (initial) and finishing at ~19:30–20:00 hrs (sunset); the tubes were removed from the water at 08:00 hrs the following day and the larvae processed for lipid content (described below). At each census, the position of the larvae in the tubes was scored as the number of larvae in either the top or bottom sections of the tube. Tubes were not

evaluated at night because of logistical constraints associated with nighttime snorkeling. The percentage of the total number of larvae present in the tubes at the time of each sampling was calculated for the top section of the tubes and used as the response variable to evaluate the effects of the treatments (depth and pCO2).

Statistical analysis
Statistical analyses were conducted using SYSTAT Version 11 software (Systat Software, San Jose, CA). A two-factor RM ANOVA was used to compare the effects of depth and pCO2 on larval position in the tubes, with time of day as the RM factor and arcsine-transformed values of the percentage of larvae found in the top of each tube as the dependent variable. Differences in lipid content of larvae were evaluated using a two-sample t-test to compare the effect of pCO2 on total lipid between depths, and a Kruskal-Wallis non-parametric test (due to violations of normality in the data) to compare lipid content after pCO2 incubations regardless of month. Assumptions of normality and homogeneity of variance for the RM-ANOVA were assessed through graphical analyses of the residuals.

**Data Processing Description**

BCO-DMO Processing Notes:

- translated Excel spreadsheet to a comma separated file
- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions

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## Data Files

| File |
|---|
| **larvae_count.csv** (Comma Separated Values (.csv), 1.31 KB) MD5:5e44b327f63cb4d5a9ee2fec8f0b6981 Primary data file for dataset ID 751013 |

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## Related Publications

Bergman, J. L., Harii, S., Kurihara, H., & Edmunds, P. J. (2018). Behavior of Brooded Coral Larvae in Response to Elevated pCO2. Frontiers in Marine Science, 5. doi:10.3389/fmars.2018.00051
*Results*

Harii, S., Yamamoto, M., & Hoegh-Guldberg, O. (2010). The relative contribution of dinoflagellate photosynthesis and stored lipids to the survivorship of symbiotic larvae of the reef-building corals. Marine Biology, 157(6), 1215–1224. doi:10.1007/s00227-010-1401-0
*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*

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| pcnt_at_top | percent of larvae counted in the upper 21 cm of each tube | percent |
| Treatment | Ambient (400 uatm) or high (1000 uatm) pCO2 conditions | unitless |
| Tube_No | Tube number (1-8) | unitless |
| Depth | Depth at which larvae were incubated | meters (m) |
| Time_of_Day | Time point where % of larvae was determined | unitless |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | autoburette titrator |
| **Generic Instrument Name** | Automatic titrator |
| **Dataset-specific Description** | The salinity of the seawater used to fill the larval incubation tubes was measured using a conductivity meter (TetraCon 325, WTW, Germany), and AT was determined using open-cell titrations conducted with an autoburette titrator (Kimoto, ATT-05, Japan). |
| **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** | handheld meter |
| **Generic Instrument Name** | Multi Parameter Portable Meter |
| **Dataset-specific Description** | Seawater pH and temperature in the reservoir were measured daily between 09:00 hrs and 11:00 hrs using a handheld meter (Multi 3410, WTW, Germany) fitted with a combination probe that recorded pH (± 0.001 pH unit) and temperature (± 0.1°C) (SenTix 940, WTW, Germany). |
| **Generic Instrument Description** | An analytical instrument that can measure multiple parameters, such as pH, EC, TDS, DO and temperature with one device and is portable or hand-held. |

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## Project Information

**Collaborative Research: Ocean Acidification and Coral Reefs: Scale Dependence and Adaptive Capacity (OA coral adaptation)**

**Website**: http://mcr.lternet.edu

**Coverage**: Moorea, French Polynesia

*Extracted from the NSF award abstract:*

This project focuses on the most serious threat to marine ecosystems, Ocean Acidification (OA), and addresses the problem in the most diverse and beautiful ecosystem on the planet, coral reefs. The research utilizes Moorea, French Polynesia as a model system, and builds from the NSF investment in the Moorea Coral Reef Long Term Ecological Research Site (LTER) to exploit physical and biological monitoring of coral reefs as a context for a program of studies focused on the ways in which OA will affect corals, calcified algae, and coral reef ecosystems. The project builds on a four-year NSF award with research in five new directions: (1) experiments of year-long duration, (2) studies of coral reefs to 20-m depth, (3) experiments in which carbon dioxide will be administered to plots of coral reef underwater, (4) measurements of the capacity of coral reef organisms to change through evolutionary and induced responses to improve their resistance to OA, and (5) application of emerging theories to couple studies of individual organisms to studies of whole coral reefs. Broader impacts will accrue through a better understanding of the ways in which OA will affect coral reefs that are the poster child for demonstrating climate change effects in the marine environment, and which provide income, food, and coastal protection to millions of people living in coastal areas, including in the United States.

This project focuses on the effects of Ocean Acidification on tropical coral reefs and builds on a program of research results from an existing 4-year award, and closely interfaces with the technical, hardware, and information infrastructure provided through the Moorea Coral Reef (MCR) LTER. The MCR-LTER, provides an unparalleled opportunity to partner with a study of OA effects on a coral reef with a location that arguably is better instrumented and studied in more ecological detail than any other coral reef in the world. Therefore, the results can be both contextualized by a high degree of ecological and physical relevance, and readily integrated into emerging theory seeking to predict the structure and function of coral reefs in warmer and more acidic future oceans. The existing award has involved a program of study in Moorea that has focused mostly on short-term organismic and ecological responses of corals and calcified algae, experiments conducted in mesocosms and flumes, and measurements of reef-scale calcification. This new award involves three new technical advances: for the first time, experiments will be conducted of year-long duration in replicate outdoor flumes; CO2 treatments will be administered to fully intact reef ecosystems in situ using replicated underwater flumes; and replicated common garden cultivation techniques will be used to explore within-species genetic variation in the response to OA conditions. Together, these tools will be used to support research on corals and calcified algae in three thematic areas: (1) tests for long-term (1 year) effects of OA on growth, performance, and fitness, (2) tests for depth-dependent effects of OA on reef communities at 20-m depth where light regimes are attenuated compared to shallow water, and (3) tests for beneficial responses to OA through intrinsic, within-species genetic variability and phenotypic plasticity. Some of the key experiments in these thematic areas will be designed to exploit integral projection models (IPMs) to couple organism with community responses, and to support the use of the metabolic theory of ecology (MTE) to address scale-dependence of OA effects on coral reef organisms and the function of the communities they build.

**The following publications and data resulted from this project:**

Comeau S, Carpenter RC, Lantz CA, Edmunds PJ. (2016) Parameterization of the response of calcification to temperature and pCO2 in the coral Acropora pulchra and the alga Lithophyllum kotschyanum. Coral Reefs 2016. DOI 10.1007/s00338-016-1425-0.
calcification rates (2014)
calcification rates (2010)

Comeau, S., Carpenter, R.C., Edmunds, P.J. (2016) Effects of pCO2 on photosynthesis and respiration of tropical scleractinian corals and calcified algae. ICES Journal of Marine Science doi:10.1093/icesjms/fsv267.
respiration and photosynthesis I
respiration and photosynthesis II

Evensen, N.R. & Edmunds P. J. (2016) Interactive effects of ocean acidification and neighboring corals on the growth of Pocillopora verrucosa. Marine Biology, 163:148. doi: 10.1007/s00227-016-2921-z
coral growth
seawater chemistry
coral colony interactions

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## 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)

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## Funding

| Funding Source | Award |
| --- | --- |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1415268 |


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