Results of modeled growth of Mytilus californianus larvae to pediveliger stage after acute acidification stress

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Project
» A mechanistic understanding of the impacts of ocean acidification on the early life stages of marine bivalves (Mechanisms of bivalve response to acidification)

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

Contributors	Affiliation	Role
Waldbusser, George G.	Oregon State University (OSU-CEOAS)	Principal Investigator
Hales, Burke	Oregon State University (OSU-CEOAS)	Co-Principal Investigator
Haley, Brian	Oregon State University (OSU-CEOAS)	Co-Principal Investigator
Langdon, Christopher	Oregon State University (OSU-HMSC)	Co-Principal Investigator
Rauch, Shannon	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

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Methods & Sampling

Initiation of feeding
Impacts of water treatments on development of larval particle feeding mechanisms were determined by measuring the proportion of mussel larvae from each treatment that ingested fluorescent beads at 44 h post-fertilization (initiation of feeding, IF). Preliminary experiments demonstrated that at 44 h after fertilization >50% of *M. californianus* larvae began feeding when reared at ambient PCO2 (~380 ppm) and 18C.

We expanded upon previous IF findings and determine the length of the delay of the onset of feeding and how this delay affected the modeled growth of larvae to 260 um in shell length, the size at which larvae typically develop into pediveligers. We quantified the delay by first determining the relationship between the proportion of larvae feeding under optimal conditions and time since fertilization. This relationship was best described by the following three parameter logistic equations:

% Feeding= 94.1/((1+Exp (-0.74 × (h-45.1))))

where *h* is the hour post-fertilization. The logistic equation was then rearranged and linearized, enabling us to estimate the functional age of larvae feeding in each ocean acidification (OA) treatment, by comparison with the proportion of larvae feeding under normal conditions.

Particle processing

To assess the effects of OA on particle processing, 48 h old larvae from each treatment were stocked in nine 25 ml VOA vials (10 larvae/ml) containing the same water treatment in which they developed from fertilized eggs. After an acclimation period of one hour, larvae were then exposed to 2 um Fluorescbrite Polychromatic (Polysciences Inc., Warrington, PA) yellow (Y) beads (excitation maxima of 441 nm and emission maxima at 485 nm) at a concentration of 20 beads/ul and allowed to feed on these beads for one hour. A second and equal dose of 2 um red (R) beads (excitation maxima of 491 nm and 512 nm and emission maxima at 554 nm) were added to the vials at a concentration of 20 beads/ul following the hour-long exposure to Y beads. Triplicate vials were assigned to one of three exposure groups (10, 30, and 50 min) after red beads were added to the vials. To terminate feeding activity at the prescribed exposure time and preserve larvae for later analysis, 40 ul (0.2% v/v) of 10% buffered formalin (pH = 8.1-8.2) were added to vials. Later, larvae were crushed under a cover slip to flatten gut contents and allow better enumeration of all ingested beads in larvae under an epifluorescent microscope (objective 20x; Leica DM 1000). Larval sample sizes consisted of greater than or equal to 20 larvae per replicate vial per treatment.

Gut fullness
Gut fullness was defined as the mean total number of ingested beads (Y+R beads) per larva over 10, 30, and 50 min sampling periods.

Ingestion rate
Ingestion rates were estimated by determining the uptake of R beads after the first 10 min of exposure to this bead type. We then doubled the number of ingested beads as larvae were found to consume R and Y beads at equal rates in preliminary experiments.

Standardizing particle processing for shell-length effects
We examined the relationship between larval shell length (SL), gut fullness, and ingestion rate from a subset of treatments spanning the range of experimental omega-aragonite categories (greater than or equal to 10 larvae from 10 different VOA vials). Shell lengths, defined as the longest axis parallel to the shell hinge, were obtained by photographing larvae under a light microscope (50x) and measuring shell lengths using Image-pro (v.7).

After finding a significant relationship between larval shell size and feeding metrics, we applied the following hyperbolic function from Waldbusser et al. (2015), which strongly predicted the shell lengths of these larvae from the omeaga-aragonite for the first 48 h of development, to estimate shell lengths of larvae for all treatments:

$$SL=(884.378 \times OM\_ar)/(1+7.691 \times OM\_ar)$$

Next, we divided gut fullness values and ingestion rates of each treatment by their shell length estimate using the above equation. We then reexamined the effects of carbonate chemistry parameters on these feeding metrics after accounting for shell length.

Modeled effects of initial 48 h OA exposure on subsequent larval energy budgets, growth and development
To estimate the effects of exposure to OA treatments during the first 48 h of larval development on subsequent energy budgets of M. californianus larvae, we first estimated energy contents of larvae in treatments at 44 h post-fertilization by applying the relationship between estimated total body energy content (E_SL; uJoules) and larval shell size reported for Mytilus edulis larvae by Sprung (1984a):

$$E\_SL = 2.28 \times (10^{-7}) \times (SL^{3.12})$$

where SL is the shell length (um). To our knowledge, this is the only known allometric relationship between energy content and shell length for any larval Mytilus species. Additionally, the relationship provided by Sprung (1984a) seemed appropriate to use here as M. edulis and M. californianus are similar with respect to egg size and, presumably, energy content (Strathmann 1987).

The total energy contents of larvae (ET) at 48 h post-fertilization (i.e. after a 4 h period during which larvae could be feeding on algal cells) were then estimated to evaluate the energy budgets of larvae among OA treatments. ET was estimated by accounting for ESL and other potential energy gains and losses using the following equation:

$$ET = ESL + ((I \times AE)/h \times (4-D)) - R$$

where I is the ingestion rate (uJoules/h), AE is the assimilation efficiency (%) that describes the conversion of ingested food to energy available to larvae, R is the energy loss due to respiration (uJoules) over the 4 h period, and D is the energy gain or loss (uJoules) to ET as a result of developmental advancement or delay in feeding activity. Physiological rate processes were converted to energy units under the following assumptions: 1)

growth or change in ESL was negligible between initiation of feeding experiments (44 h) and when the sizes of larvae were measured at 48 h; 2) larval ingestion rates were constant over the period from 44 to 48 h and were equivalent to algal ingestion rates with an estimated algal cell energetic content of 0.61 uJoules/cell (Isochrysis galbana, Sprung 1984a), which was also the food source underpinning the relationship between energy content and shell length; 3) total time for potential gains from ingestion was 4 h +/- any advancement/delay in the initiation of feeding among early larvae; 4) assimilation efficiencies of larvae were 0.38 (Sprung 1982); 5) respiration rates were similar among larvae in all treatments except for those of larvae in the lowest pH treatment (based on Waldbusser et al. 2015); and 6) 1 nl O2 was equivalent to 20.1 uJoule of respired energy (Crisp 1971).

To estimate the impacts of OA during the first 48 h of development on subsequent larval growth, we modeled the developmental time of larvae to reach a shell length of 260 um, the approximate size of pediveliger mussel larvae under normal conditions (Sprung 1984a) and a proxy for larval competency for settlement and metamorphosis; however, we note that larval competency is frequently correlated with but not necessarily dependent on larval size (Coon et al. 1990, Pechenik et al. 1996). To make these extrapolations, we first estimated energy content gains of larvae in 24 h intervals (delta E) using the following equation:

delta E= ESL + (I × AE × NGE)

where ESL is the estimated energy content of the larvae based on their shell length at 48 post-fertilization, I is the ingestion rate (uJoules/h), AE is the assimilation efficiency (%), NGE is the net growth efficiency (%). ESL and I in this study were assumed constant between 48 and 72 h post-fertilization. After this initial 24 h period (i.e. 48-72 h post-fertilization), modeled gains in larval energy content were added to those of larvae of each treatment and larval shell lengths were adjusted by rearranging equation 2. From 72 h post-fertilization onward, we modified larval ingestion rates and larval respiration rates in accordance with allometric equations for M. edulis as described by Sprung (1984b, c). This model explicitly tests how an acute initial 48 h OA exposure could significantly alter the subsequent duration and energy budgets of mussel larvae. Assimilation efficiencies were 0.38, 0.29, and 0.27 for larvae < 200 um, between 200-250 um, and > 250 um, respectively (Bayne 1983). NGE were also adjusted for changes in larval size and estimated at 0.78, 0.67, 0.65 for larva < 200 um, between 200-250 um, and > 250 um (Bayne 1983). The impacts of differences in initiation of feeding, initial feeding rates and shell size after 48 h of development on subsequent larval growth to 260 um were estimated by non-linear multiple regression analysis.

Data Processing Description

BCO-DMO Processing:
- replaced "M. Californianus" with full species name;
- modified parameter names to conform with BCO-DMO naming conventions;
- rounded to 3 decimal places (per dataset contact);
- replaced blanks with "nd".

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Data Files

File
OA_modeled_growth.csv (Comma Separated Values (.csv), 63.58 KB) MD5:5548cfe9321807633ba50e6b7f85cef0 Primary data file for dataset ID 662188

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Parameters

Parameter	Description	Units

species	Species name	unitless
treatment	Treatment identifier	unitless
pCO2	Partial pressure of CO2	microatmospheres (uatm)
aragonite_sat	Aragonite saturation state	dimensionless
pH	pH (total scale)	pH scale units
pH_category	pH category	unitless
omega_category	Aragonite saturation state category	unitless
development_time_post_fert	Development time post-fertilization	hours
time	Time	days
shell_size_est	Shell size estimate	micrometers (um)
energy_est_from_shell_len	Energy estimate from shell length	micro Joules (uJoules)
ingestion_rate_est	Ingestion rate estimate (beads/larva/h)	number of beads per larva per hour
food_energy_content	Food Energy Content (uJ/cell)	micro Joules per cell
assimilation_efficiency	Assimilation effciency (fraction)	unitless
resp_rate	Respiration rate (nl O2/h)	nanoliters O2 per hour
resp_cost	Respiration Cost (uJoules)	micro Joules
energy_est_from_shell_len2	Energy estimate from shell length (uJoules)	micro Joules
daily_ingestion_rate	Daily ingestion rate (beads/larva/day)	number of beads per larva per day
net_growth_efficiency	Net growth efficiency (fraction)	unitless

respiration_costs	Respiration costs (uJoules)	micro Joules
change_in_energy	Change in energy content (uJoules)	micro Joules
scope_for_growth_energy	Scope for Growth Energy (uJoules)	micro Joules
delay_effect	Delay Effect (uJoules)	micro Joules

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Deployments

Waldbusser_HMSC

Website	https://www.bco-dmo.org/deployment/557259
Platform	OSU-HMSC
Start Date	2013-08-19
Description	Laboratory experiments on California mussel larvae (Mytilus californianus) were conducted in the Hatfield Marine Science Center, Newport, OR.

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Project Information

A mechanistic understanding of the impacts of ocean acidification on the early life stages of marine bivalves (Mechanisms of bivalve response to acidification)

Coverage: Coastal and estuarine waters of Oregon, U.S.A.

Extracted from the NSF award abstract:

The shift in the carbonate chemistry of marine waters, as a result of direct anthropogenic CO2 addition and climate-driven changes in circulation, poses a threat to many organisms. A rapidly expanding body of literature has shown that increasing levels of carbonic acid and decreasing carbonate ion levels will have deleterious effects on many marine organisms; however little is known about the mode of action of these changes in water chemistry on marine bivalves. Many marine organisms, particularly bivalves, depend critically on the production of calcium carbonate mineral, and this material becomes thermodynamically unstable under more acidic conditions. The actual mineral precipitation, however, takes place within interstitial volumes intermittently separated from ambient seawater by biological membranes. Therefore, abiotic relationships between solid phase minerals and seawater thermodynamics are oversimplified representations of the complex interplay among seawater chemistry, bivalve physiology, and shell growth processes.

In this integrative, multi-disciplinary project we will develop and apply novel experimental approaches to elucidate fundamental physiological responses to changes in seawater chemistry associated with ocean acidification. The four primary objectives of this project are to: 1) develop a novel experimental approach and system capable of unique combinations of pCO2, pH, and mineral saturation state (Ω), 2) conduct short-term exploratory experiments to determine bivalve responses to different carbonate system variables, 3) conduct longer-term directed studies of the integrated effects of different carbonate system variables over early life history of bivalves, and 4) compare these biological responses among a group of bivalve species that differ in shell mineralogy and nativity to the periodically acidified upwelling region of the Pacific Northwest coast of North America. By isolating the effects of different components of the carbonate system on the early life

stages of marine bivalves, e.g. does an oyster larvae respond more strongly to pCO2 or mineral saturation state?, we can begin to identify the mechanisms behind bivalve responses as well as understand how these organisms survive in transiently corrosive conditions.

Laboratory based experiments on three primary taxa (oyster, mussel, clam) having native and non-native species pairs to Oregon's coastal waters: oysters *Ostrea lurida* and *Crassostrea gigas*; mussels *Mytilus califonianus* and *Mytilus galloprovincialis*; and clams *Macoma nasuta* and *Ruditapes philippinarum*, will allow for species comparisons among different shell mineralogy, microstructure, life-history, and adaptability. High-precision pCO2 and dissolved inorganic carbon (DIC) instruments will be used in experiments to control and properly constrain the carbonate chemistry. A compliment of response variables will be measured across the early life stages of these species that include tissue acid-base balance, shell mineralogy and chemistry, respiration rate, and behavior. Additionally, our emphasis will be placed on observation of development, growth, and shell structure by directly linking observational data with other measured response data. An adaptive strategy using short-term experiments to determine the most salient variables in the carbonate system to manipulate in longer-term studies is being employed. This approach allows us to evaluate acute effects, mimicking diurnal changes to carbonate variables often found in coastal areas, and integrated chronic effects mimicking a more gradual acidification due to the rise in atmospheric CO2.

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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-1041267

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