Phenotypic plasticity in sand dollars in response to predators at the Friday Harbor Laboratories, WA, USA and in sea urchins from Southern Maine, USA from 2018 to 2019

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Data Type: experimental
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Project
» The Ecology of Cloning (Ecology of Cloning)

Contributors	Affiliation	Role
Allen, Jonathan	The College of William & Mary	Principal Investigator, Contact
Barnes, Danielle	Florida State University (FSU)	Student
Heyl, Taylor	Woods Hole Oceanographic Institution (WHOI BCO-DMO)	BCO-DMO Data Manager

Abstract
This dataset describes phenotypic plasticity in sand dollars in response to predators at the Friday Harbor Laboratories, WA, USA and in sea urchins from Southern Maine, USA from 2018 to 2019.

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Coverage

Spatial Extent: **Lat**:48.5456 **Lon**:-123.014107
Temporal Extent: 2018-03-22 - 2019-08-14

Methods & Sampling

This dataset represents data from Danielle Barnes' honors thesis on phenotypic plasticity in juvenile sea urchins in response to predators. Larvae were cultured at the Friday Harbor Laboratories in Washington, USA under standard conditions for metamorphosis when they were measured using standard light microscopy.

For the first experiment, we studied the juvenile phenotypes of *S. droebachiensis* after they were exposed as larvae to waterborne cues from the European green crab (*Carcinus maenas*). Green crabs were selected as a cue source because they are a known predator of juvenile sea urchins (Fagerli et al., 2014). Two trials were conducted in the laboratory in Williamsburg, Virginia in the spring of 2018 and 2019 using two separate male-female pairs to account for differences in genotype and laboratory conditions.

Adult *S. droebachiensis* and green crabs were shipped overnight from the Marine Biological Laboratory at Woods Hole, MA in March of 2018 (Trial 1) and 2019 (Trial 2). Adult urchins were spawned using standard methods by injecting 1 milliliter (mL) of 0.5M KCl into the coelomic cavity (Strathmann, 1987). To collect eggs, female urchins were inverted over glass beakers filled with artificial seawater (ASW; Instant Ocean, Spectrum Brands, Blacksburg, VA) at a temperature of 12 degrees C and salinity of 32. Eggs collected from a single

female were rinsed in ASW and immediately fertilized with dilute sperm collected from a single male. Each trial consisted of offspring from one male-female pair. Eggs were checked to ensure high (>90%) fertilization and then transferred into 1 L glass bowls filled with ASW, kept in aquaria at 12 degrees C, and manually stirred multiple times per day.

The *S. droebachiensis* embryos developed in these conditions until three days post fertilization when they had reached the gastrula stage. Swimming gastrulae were separated into ten, 250 mL glass beakers filled with 200 mL of ASW at a salinity of 32 and a density of 100 embryos per beaker. Beakers were kept in a 12 degrees C water bath and stirred by paddles connected to a 10-rpm motor in order to suspend the larvae and microalgal food (Strathmann, 1987). Beaker locations were rotated every other day in order to minimize potential effects of position in the water bath. Three times per week until settlement, 100 mL of culture water in each beaker was reverse filtered through a 35 micrometer (µm) mesh and replenished with 100 mL of ASW at a salinity of 32. After each water change, larvae were fed a mixed algal diet consisting of *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Rhodomonas lens* at concentrations of 2,500 cells/mL for each species.

Each trial consisted of two treatments: 1) water borne cues from a benthic predator and 2) control with no cue. There were five replicate beakers per treatment (10 beakers total). Adult green crabs were used to generate the benthic predator cue. Upon arrival, crabs were separated into aerated individual 20 L tanks containing ASW at a salinity of 32 in a cold room maintained at 15ºC. Carapace lengths of the crabs ranged from 51 mm to 59 mm (Trial 1) and 46 mm to 59 mm (Trial 2), and the wet weight of the crabs was estimated to range from 30.3 g to 46.3 g (Trial 1) and 22.4 g to 46.3 g (Trial 2; estimated using the following conversion: wet weight (g) = 0.26 (carapace width (cm))$^{2.92}$ R^2= 0.99 from Menge, 1983). Crabs were fed a diet of frozen shrimp every two days except for the window where they were used to create predator cue water. Every two days, one of the crabs was randomly chosen to generate the predator cue water. To generate water with water born cues, an individual crab was housed in an aerated tank filled with 6 L of ASW for 24 hours. The cue water was then filtered through a 5 µm bag filter in order to remove any particulate waste and larger microorganisms. The water for the control treatment was collected from a replicate tank with 6 L of ASW without crabs. The pH, dissolved oxygen, temperature, and salinity of the predator cue and control water were monitored using a YSI Pro 1030 conductivity, pH, and temperature probe (Yellow Springs Instrument, Yellow Springs, OH).

The predator cue water was introduced to each of the beakers beginning when the development of spines as part of the juvenile rudiment were first observed using polarized light under a compound microscope. During water changes, after 100 mL of the original culture water was filtered out, 100 mL of the predator cue water was added in place of the ASW. Predator cues were added to the beakers in the same manner for each subsequent water change (i.e. every other day) until all urchins had settled. Larval settlement was induced by adding a blue mussel shell (*Mytilus edulis*) to each beaker beginning at 28 days post fertilization (Pearce and Scheibling, 1990).

Upon metamorphosis, urchin juveniles were removed from each beaker and morphological measurements were taken at 100x magnification using a compound microscope fitted with an ocular micrometer. Mean spine length was calculated by averaging the length of each juvenile's three longest spines. The total number of spines was also counted. Disk area was determined by measuring the length of the body at the longest axis and then the width of the body at its widest point perpendicular to the length. These two measures were then used to calculate the area of an ellipse as a proxy for sea urchin disk area.

Pacific Sand Dollars

For the second experiment, we studied the juvenile phenotypes of Pacific sand dollars (*Dendraster excentricus*) after they were exposed as larvae to waterborne cues from the red rock crab (*Cancer productus*). *C. productus* are commonly used in experiments as predators of larval *D. excentricus* (Rumrill et al., 1985; Pennington et al., 1986; Allen, 2008), in addition to being frequently associated with sand dollar beds and being observed feeding on adult sand dollars in the field (Merrill and Hobson, 1970). Two trials were conducted at the University of Washington's Friday Harbor Laboratories (FHL) on San Juan Island, Washington in the summer of 2019. For each trial a unique male-female pair was used to account for differences in genotypes and laboratory conditions.

Adult *Dendraster excentricus* were collected from a large sand dollar bed in East Sound, Orcas Island, WA (48^{o} 41' 40" N 122^{o} 53' 45" W) in June, 2019. Sand dollars were transported to FHL where they were housed in tanks connected to a flow-through sea water system. The sand dollars were spawned using standard methods (Strathmann, 1987) by injecting 1 milliliter (mL) of 0.53M KCl into the coelomic cavity. To collect the eggs, female sand dollars were inverted over 50 mL glass beakers filled with 1-micron filtered sea water (FSW) at a salinity of 32. Eggs collected from a single female were rinsed in FSW and immediately fertilized with dilute sperm collected from a single male. Eggs were checked to ensure high (> 90%) fertilization and then

transferred into 1 liter (L) glass bowls filled with FSW and kept in a flow-through sea water table at ambient temperature (range approximately 12ºC – 14ºC), and manually stirred multiple times per day.

Two days post fertilization, when embryos had developed into the prism larval stage, larvae were sorted into twenty replicate 250 milliliter (mL) glass beakers containing 200 milliliter (mL) of FSW at a salinity of 33 with 100 larvae per beaker. Beakers were kept in a flow-through sea table at ambient temperature (range 12ºC – 14ºC). The beakers were stirred by paddles connected to a 10-rpm motor in order to suspend the larvae and microalgal food (Strathmann, 1987). Beaker locations were rotated every other day in order to minimize potential effects of position in the water bath. Every other day until settlement, 100 milliliter (mL) of culture water in each beaker was reverse filtered through a 35 µm mesh and replenished with 100 mL of FSW. After water changes, larvae were fed a mixed algal diet consisting of *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Rhodomonas lens* at concentrations of 2,500 cells/mL for each species.

The first trial consisted of two treatments: benthic predator cue, and a control without cue. There were 10 replicate beakers per treatment (20 beakers total). Adult red rock crabs (*C. productus*) were used to create the benthic predator cue. Red rock crabs were collected intertidally from the intertidal shoreline in front of Friday Harbor Laboratories in July of 2019 and housed in flow-through sea water tanks at ambient salinity and temperature. The crabs' carapace lengths ranged from 53 mm to 61 mm and their masses ranged from 16.8 grams to 23.6 grams. To generate water with a predator cue, a single crab was housed in a closed jar filled with 3 L of FSW for 24 hours. The crab was then removed and the water was filtered through a 5 µm bag filter, re-oxygenated by vigorously shaking for one minute, and diluted with an additional 3 L of FSW. Every other day, a different crab was used to produce the predator cue. The control water came from a jar in the same conditions without a crab. The pH, dissolved oxygen, temperature, and salinity of the predator cue and control water were monitored as done for the green urchin experiment.

The second trial consisted of three treatments: benthic predator cue, planktonic predator cue, and a control. There were 20 total beakers, with seven replicate beakers in the benthic predator cue treatment, seven in the planktonic predator cue treatment, and six in the control. The same population of red rock crabs used in the first trial were used to create the benthic predator cue. To create the planktonic predator cue, *Cancer productus* megalopae were collected from the waters off the dock at Friday Harbor Laboratories. The average mass of an individual megalopa was determined to be 0.019 g, which was used to calculate the number of megalopae needed for a given cue batch, such that the total mass of all megalopae combined was equivalent to the mass of the adult crab used in the corresponding benthic predator cue batch (~150-200 megalopae per 1 L of FSW). The control water came from a jar in the same conditions.

Benthic predator cue water, planktonic predator cue water, or control water was added to the beakers at 14 days post fertilization, when the sand dollar larvae had developed spines on the juvenile rudiment that could be observed with polarized light. 100 mL of predator cue water or control water was added after the culture water was filtered. Predator cues were added to the culture beakers in each subsequent water change until all larvae had settled. Settlement was induced starting at 18 days post fertilization by adding into each beaker approximately 0.2 g of sand from the flow-through sand dollar tanks at Friday Harbor Laboratories that had been conditioned by adult sand dollars for over a year (Highsmith, 1982).

On the day each juvenile metamorphosed, they were removed from the beaker and morphological measurements were taken at 100x magnification using a compound microscope. Mean spine length was calculated by averaging each juvenile's three longest spines. The total number of spines was also counted. Disk area was determined by measuring the length of the body at the longest axis and then the width of the body at its widest point perpendicular to the length under a compound microscope at 100x magnification. These two measures were then used to calculate the area of an ellipse as a proxy for sand dollar disk area.

Data Processing Description

Data Processing:
Data were entered in excel and analyzed in SPSS version 23.

BCO-DMO Processing:
- Adjusted field/parameter names to comply with BCO-DMO naming conventions;
- Missing data identifier "NA" replaced with "nd" (BCO-DMO's default missing data identifier);
- Added a conventional header with dataset name, PI names, version date;
- Added a column for species name and combined two separate data files into one dataset by concatenating

rows;
- Rounded columns: "Avg_Spine_Length", "Avg_sp_length", "Average_spine_length_um" to 3 decimal places (or to the thousandth place).
- Removed columns "SL1", "SL2", "SL3" "DL1", "DL2", "Avg_Spine_Length" and "Avg_sp_length_um" based on submitters feedback.

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Data Files

File
barnes_combined.csv (Comma Separated Values (.csv), 206.44 KB) MD5:61aa33b6b82b91996d717cf11eba00ea Primary data file for dataset ID 877780

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Related Publications

Allen, J. D., & Pechenik, J. A. (2010). Understanding the Effects of Low Salinity on Fertilization Success and Early Development in the Sand Dollar Echinarachnius parma. The Biological Bulletin, 218(2), 189–199. https://doi.org/10.1086/bblv218n2p189 https://doi.org/10.1086/BBLv218n2p189
Methods

Barnes, D. K., & Allen, J. D. (2023). Predators Induce Phenotypic Plasticity in Echinoderms across Life History Stages. The Biological Bulletin, 000–000. https://doi.org/10.1086/725633
Results

Barnes, Danielle K., "Predator-Induced Phenotypic Plasticity Across Life History Stages in Echinoderms" (2020). Undergraduate Honors Theses. William & Mary. Paper 1525. https://scholarworks.wm.edu/honorstheses/1525
Results

Fagerli, C., Norderhaug, K., Christie, H., Pedersen, M., & Fredriksen, S. (2014). Predators of the destructive sea urchin Strongylocentrotus droebachiensis on the Norwegian coast. Marine Ecology Progress Series, 502, 207–218. https://doi.org/10.3354/meps10701
General

Highsmith, R. C. (1982). Induced Settlement and Metamorphosis of Sand Dollar (Dendraster Ecentricus) Larvae in Predator-Free sites: Adult Sand Dollar Beds. Ecology, 63(2), 329–337. Portico. https://doi.org/10.2307/1938950
Methods

Menge, B. A. (1983). Components of predation intensity in the low zone of the New England rocky intertidal region. Oecologia, 58(2), 141-155.
Methods

Merrill, R. J., & Hobson, E. S. (1970). Field Observations of Dendraster excentricus, a Sand Dollar of Western North America. American Midland Naturalist, 83(2), 595. https://doi.org/10.2307/2423965
Methods

Pearce, C. M., & Scheibling, R. E. (1990). Induction of Metamorphosis of Larvae of the Green Sea Urchin, Strongylocentrotus droebachiensis, by Coralline Red Algae. The Biological Bulletin, 179(3), 304–311. https://doi.org/10.2307/1542322
Methods

Pennington, J.T., Rumrill, S.S. and Chia, F. (1986) Stage-specific predation upon embryos and larvae of the Pacific sand dollar, Dendraster excentricus, by 11 species of common zooplanktonic predators. Bulletin of Marine Science, Volume 39, Number 2, pp. pp. 234-240(7).
Methods

Rumrill, S. S., Pennington, J. T., & Chia, F.-S. (1985). Differential susceptibility of marine invertebrate larvae: Laboratory predation of sand dollar, Dendrasterexcentricus (Eschscholtz), embryos and larvae by zoeae of the red crab, Cancer productus Randall. Journal of Experimental Marine Biology and Ecology, 90(3), 193–208.

https://doi.org/10.1016/0022-0981(85)90166-2
Methods

Strathmann, R. (1987). Echinoderm larval ecology. Echinoderm studies 2 (1987), 2, 55-136.
Methods

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Parameters

Parameter	Description	Units
Species	species identification	unitless
Juvenile	Individually identified juveniles	unitless
Treatment	Control or Predator	unitless
Replicate_Number	Replicate	unitless
Date_Settled	Date when juvenile settles in format: YYYY-MM-DD	unitless
Age_at_Settlement	Age when juvenile settled	unitless
Number_Spines	Number of spines present	unitless
SL1_um	Length of longest spine calibrated with ocular micrometer	micrometer (um)
SL2_um	Length of second longest spine calibrated with ocular micrometer	micrometer (um)
SL3_um	Length of third longest spine calibrated with ocular micrometer	micrometer (um)
Avg_sp_length_um	Mean spine length of three longest spines	micrometer (um)
DD1_um	Disk diameter calibrated with ocular micrometer	micrometer (um)
DD2_um	Second disk diameter perpendicular to the first calibrated with ocular micrometer	micrometer (um)
Area_um	Disk area calculated from DD1 and DD2 assuming elliptical shape.	micrometer (um)

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Instruments

Dataset-specific Instrument Name	Olympus
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".

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Project Information

The Ecology of Cloning (Ecology of Cloning)

Coverage: Salish Sea and the Gulf of Maine

NSF Award Abstract:

The recruitment of new individuals into marine communities is critical to determine community structure and yet, many basic parts of the life histories of marine invertebrates remain poorly described. Much of the understanding of the recruitment of marine invertebrates is based on theories centered on per-offspring investment, the dispersal of offspring in the ocean, and the survival of offspring during development. A major, largely neglected, area of research of the recruitment of marine invertebrates is the potential for asexual propagation of offspring during the embryonic and/or larval stages, also called cloning. Cloning is widely known to occur in diverse marine taxa, but the ecological consequences of this phenomenon are poorly understood. This project examines the ecology of cloning in Echinoderms, the taxon in which it is most widely reported. The work identifies the environmental triggers of cloning and tests the consequences of cloning at different stages in the life cycle. The work also trains undergraduate students, K-5 students and adult learners through research experiences, school presentations, and short courses in Maine and Virginia.

This project systematically evaluates inducers and consequences of cloning in multiple species from two temperate coastal habitats. Specifically, the work addresses two primary questions: 1) What are the environmental inducers of cloning and how likely are cloning events to occur in nature? 2) What are the consequences of cloning events for the recruitment of echinoderms into benthic communities? Current understanding of the initiation of cloning suggests that multiple stressors can induce cloning. Yet potential inducers have only rarely been investigated in a systematic fashion and never across multiple species within habitats or across habitats. The current project does both, assessing the potential for variation in biotic (algal food cues and predator cues) and abiotic (temperature, pH and salinity) parameters to induce cloning events in seven species of temperate echinoderms from two coastal areas (the Gulf of Maine and the Salish Sea). In addition to identifying inducers of cloning, very little is known about the fates of clones or the primary larvae that produce them, and therefore the ecological importance of this developmental phenomenon is a black box. As part of answering this second question, the investigators track the fates of clones that are generated at three different life stages (zygotic clones, early larval clones and late larval clones) to metamorphosis and beyond to determine the potential contribution of cloning to juvenile recruitment. By tracking the fates of clones to metamorphosis and in the weeks immediately following metamorphosis, when the vast majority of mortality occurs, they can estimate the degree to which cloning may be a viable developmental pathway for marine invertebrates.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1850837

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