

THE EFFECTS OF OCEAN ACIDIFICATION ON FERTILIZATION AND EMBRYOGENESIS IN RELATION TO THE EVOLUTIONARY DEVELOPMENTAL BIOLOGY OF SEA URCHINS IN MOOREA, FRENCH POLYNESIA

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Abstract. Sea urchins are a widespread organism that have an economic importance to humans and play an essential role in their marine community. With anthropogenic carbon emission leading to acidification of the ocean, it is important to gain a better understanding of sea urchins both developmentally and ecologically. This study focused on the effects of ocean acidification on sea urchin fertilization and early development, the evolutionary developmental biology of two urchin species (*Tripneustes gratilla* and *Echinometra mathaei*), and the ecological relationships observed between a variety of urchin species and their marine community. It was found that elevated levels of $p\text{CO}_2$ affected the proportion of eggs that achieved 1st cell division and 2nd cell division, but did not affect the proportion of eggs that achieved fertilization and blastulation. The results of this study predict that *T. gratilla* will be adversely affected by ocean acidification. An abundance survey was conducted to determine relationships between urchin abundance and coverage, with the hope of understanding how a decline in urchin populations may affect their marine community. In addition, it was found that there were significant differences in the time at which the 1st and 2nd divisions were initiated between *E. mathaei* and *T. gratilla*, but not in the time between the 1st and 2nd division in these species. This finding shows that the developmental clocks of these species have diverged over time. Overall, my data on the evolutionary development of *E. mathaei* and *T. gratilla* can be used in conjunction with future research in order to shine light on the evolutionary relationships of sea urchins.

Key words: *echinoids; sea urchins; Tripneustes gratilla; Echinometra mathaei; ocean acidification; evolutionary development; heterochrony; embryogenesis; developmental timing; early development; abundance surveys; Moorea, French Polynesia*

INTRODUCTION

Elevated levels of atmospheric CO_2 have led to an increase in surface sea water's uptake of CO_2 (Caldeira and Wickett 2003). This increase of CO_2 in surface sea water causes higher levels of carbonic acid, which can dissociate to carbonate, increasing the concentration of hydrogen ions. Over time, this process leads to a lowering of the pH of the ocean. It has been predicted that the surface pH of the ocean may decrease by 0.15-0.35 by the year 2100 (Intergovernmental Panel on Climate Change 2007). These seemingly small changes in pH have been shown to have tremendous effects on marine organisms and cascading effects on marine communities (Hoegh-Guldberg et al. 2007, Morita et al. 2009, Gibson et al. 2011).

Closely related organisms can vary in the timing of developmental events, a phenomenon called heterochrony. Variation in developmental timing has evolved in many

organisms. Interestingly, heterochronies have been noted in closely related sea urchins (Raff 1987, Raff 1992). Differences in the timing of developmental events may contribute to differential responses of closely related species to predicted oceanic change. In addition, by studying the heterochronies of closely related organisms, evolutionary relationships can be better understood.

Echinoids, or sea urchins, are among many organisms negatively affected by ocean acidification (Havenhand et al. 2008, Brennan et al. 2010, Reuter et al. 2011). For example, acidification increased energetic costs in urchin larvae thereby inducing a developmental delay (Stumpp et al. 2011). In addition, acidification has been shown to decrease sperm speed and the percent of motile sperm in the sea urchin *Heliocidaris erythrogramma* (Havenhand et al. 2008). Sea urchins are an important model for development and are a critical part of the food web, limiting the biomass of algae and serving as a source of food for many predators (Pearse

2006). Although multiple studies have documented the effects of ocean acidification on urchin development, many species have been understudied or overlooked completely (Havenhand et al. 2008, Todgham and Hofmann 2009, Stumpp et al. 2011, Dupont et al. 2013). This study focused on understanding the effects of ocean acidification on *T. gratilla* because of its economic importance and its noteworthy role in its marine community.

The marine environment surrounding the Society Islands is inhabited by numerous species of echinoids. This study focused on six species of urchins found in the reefs surrounding the island of Moorea. These urchins in order of most to least abundant include: *Echinometra mathaei*, *Echinostrephus aciculatus*, *Echinothrix calamaris*, *Echinothrix diadema*, *Diadema savignyi*, and *Tripneustes gratilla* (Gehlbach 1994). These species are ideal for an evolutionary development study due to their varying degrees of relatedness.

This study addressed four questions: (1) Are the abundances of different species of sea urchins correlated with differing types of coverage? (2) How do the abundances of different species of urchin affect each other? (3) Is the fertilization and early development of *Tripneustes gratilla* affected by ocean acidification? (3) Are there significant differences in the timing of developmental events between closely related urchin species? I hypothesized that acidification will lead to a decrease in the percent of fertilized eggs due to sperm's sensitivity to pH. In addition, due to the disruptive effects changes in pH can have on cellular processes, I hypothesized that the percent of eggs that reached developmental milestones such as first cell division, second cell division, blastulation, and gastrulation will decrease under acidified conditions. Finally, I hypothesized that there would be significant differences in the timing of developmental events between these urchin species.

METHODS

Location

This study took place in the Society Islands on the island of Moorea, French Polynesia (17°30'S, 149°50'W). All of the experimental work took place at the Richard B. Gump research station, which is located on the northwest side of Cook's Bay.

Collection and natural history

The sexes of urchin species are typically indistinguishable based on external characteristics (Hinegardner 1975). Therefore, in order to determine the sex of the urchin species, their gametes must be examined. In an attempt to collect at least five males and five females, I collected at least twenty of each of my focal urchin species (*E. calamaris*, *E. mathaei*, *E. diadema*, *E. aciculatus*, *D. savignyi*, and *T. gratilla*). Many predators of urchins are active during the day. Consequently, many species of urchins tuck themselves into the safety of crevices at this time (Nelson and Vance 1979, Frossard 2011, Young and Bellwood 2011, Kintzing and Butler 2014). In order to feed, the urchins come out of their hiding spots at night. This common predator avoidance behavior shared by many urchins made collection at night more feasible. Therefore, I collected all *E. diadema*, *T. gratilla*, *D. savignyi*, *E. aciculatus*, and *E. calamaris* used for this study between 20:00-23:00 from October 2nd to November 11th, 2017. Because of ease of collection of *E. mathaei* during the day I collected all *E. mathaei* used for this study between 9:00-13:00. I transported all collected urchins to the Richard B. Gump research station where I kept all collected urchins in salt water aquaria with fresh flowing water and ample food (*Sargassum*, *Padina*, and *Halimeda*). During collection, I noted the species, the substrate the urchin was found on, and the depth at which the urchin was found.

Collection sites

All collection of urchins occurred at the reefs of Cook's Bay. There were two main collection sites: the fringing reef adjacent to the Richard B. Gump research station and the back reef near the mouth of Cook's Bay (Fig. 1). All *Echinometra mathaei* used in this study were collected in the coral rubble of the fringing reef. All *Echinothrix diadema* were collected at the back reef of Cook's Bay. *Echinothrix calamaris*, *Diadema savignyi*, and *Tripneustes gratilla* specimens were collected at both the back reef and the fringing reef of Cook's Bay. Maps were made using QGIS using layers courtesy of the Geospatial Innovation Facility, University of California, Berkeley.

Plots for abundance survey

Plots that had already been set up by Hannah Lewis, a fellow researcher, were used for the urchin abundance surveys. Ten plots were located at the northwest side of the mouth

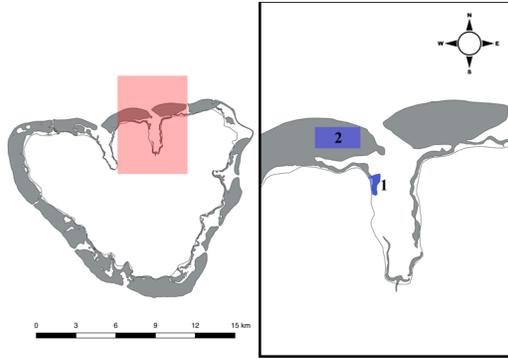


FIG. 1. Map of two urchin collection sites on Moorea, French Polynesia.

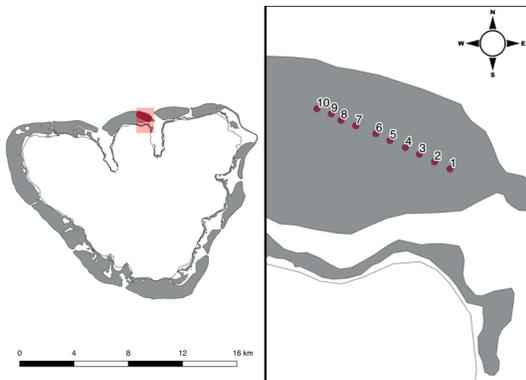


FIG. 2. Map of survey plots on Moorea, French Polynesia.

of Cook's Bay (Fig. 2). These plots were thirty meters long and five meters wide. These plots were marked with buoys made from empty plastic bottles tied to cinder blocks with a rope. These buoys were placed in the corners of the rectangular plots. In addition, the edges of the plots were lined with ropes that were weighted with cinder blocks. Five of the ten available plots were selected based on their variation of coverage. Urchin abundance surveys were conducted for plot numbers 2, 4, 5, 6, and 7 (Fig. 2, Table 3; Appendix A). These plots were ideal for the abundance survey due to their varying amounts of coverage and the presence of a variety of urchin species. Data for each of these five plots was acquired from Lewis (2017). This data included average plot depth and percent coverage of sand, coral rubble, dead coral, *Turbinaria*, *Sargassum*, *Padina*, microalgae biofilm, *Porites*, *Montipora*, and *Pocillopora*.

Abundance surveys

Abundance data was collected by snorkeling the entirety of the five selected plots. Snorkeling occurred on November 16th, 2017 from 19:00 to 22:00. This survey was conducted at night because the nocturnal behavior of many urchin species makes them more visible at this time. Many urchin species exhibit den fidelity and do not travel far from their hiding spots (Nelson and Vance 1979, Carpenter 1984, Hereu 2005), therefore I assumed that this snapshot survey would be fairly representative of the typical abundances of sea urchins in these plots.

Induced spawning

Many urchins use lunar and tidal cycles to synchronize spawning events. *Diadema savignyi* and *Echinothrix diadema* are known to spawn around the full moon, while *Echinothrix calamaris* is known to spawn around the new moon (Coppard and Campbell 2005). I spawned my study species during their appropriate lunar phases.

The following methods for inducing spawning in sea urchins were adapted from Levitan (1993) and Foo et al. (2012). I started by gently shaking the urchins for approximately twenty seconds. This disturbance often triggered a small volume of gametes to be released. This often allowed me to determine the sex of the urchin through the use of a light microscope, before I induced spawning completely. During the visualization of the gametes, I would assess the maturity by looking for swimming sperm and round eggs with small nuclei. If the gametes were deemed to be immature through the absence of swimming for sperm or the presence of an asymmetric shape and/or the presence of a large nucleus (germinal vesicle) for eggs, then these gametes were not used (Fig. 7; Appendix A). If the urchin started to release sperm, I placed its aboral side up on top of a small petri dish. If I did not have the proper ratio of viable gametes I would either quickly collect more urchins and attempt to achieve the proper ratio or proceed with less than five mating pairs in an attempt to limit the time the collected gametes were waiting. If the urchin released eggs, I placed its aboral side down on top of a beaker that was filled with sea water at the current ocean pH of approximately 8.1 (Shi et al. 2010). To induce complete spawning in the urchins, I inserted a needle through the

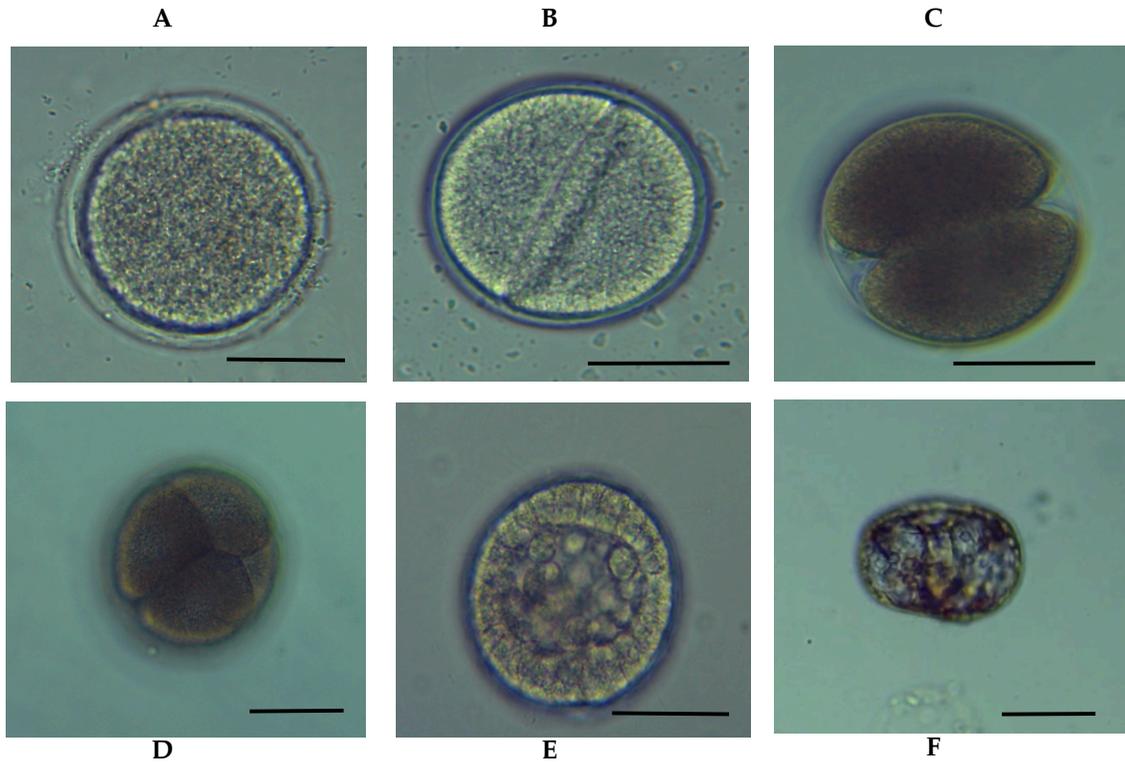


FIG. 3. Early development in *Echinometra mathaei*. (A) Recently fertilized egg with fertilization envelope present; (B) mid-cleavage during 1st division (C) two cell stage after completion of 1st division (D) four cell stage after completion of 2nd division (E) blastula with blastocoel apparent (F) late gastrula with archenteron visible. Scale bar=50µm for A-F.

peristomial membrane and injected 0.5ml of a 0.55M KCl solution in the coelomic cavity. I repeated this injection two more times on each urchin inserting the needle through different spots on the peristomial membrane each time. Once I finished injecting the urchin a total of three times, I gently shook them again to distribute the KCl inside the urchin. I collected the sperm (dry) by pipette and placed it in a labeled micro centrifuge tube.

I placed the micro centrifuge tubes of sperm in a beaker of ice in order to preserve the sperm for the longest period of time. I allowed the female urchin to finish shedding the eggs (approximately 10-30 minutes) and then labeled the beaker accordingly. Once the eggs settled to the bottom of the beaker, I pipetted them into a micro centrifuge tube. I collected as much spawn as possible from each individual.

If the individual spawned a large volume, I distributed it into multiple micro centrifuge tubes. All injected urchins were released back into the ocean at a location far from the

collection sites in order to avoid using the same individuals more than once.

Gametes and crosses

The gametes were stored in sea water taken freshly from Cook's Bay. The storage time of the gametes was minimized and did not exceed four hours. I randomly assigned five males and five females (of the same species) to a breeding pair. Each mixture of gametes had an excess number of sperm (~300 sperm per egg) to ensure the concentration of sperm did not limit the number of eggs fertilized. All gamete mixtures were developed at approximately 19°C.

Fertilization and development in acidified conditions

To determine if elevated levels of $p\text{CO}_2$ decrease the proportion of eggs fertilized, or the portion of eggs to complete 1st cell division, 2nd cell division, blastulation, or gastrulation I

reared mixtures in 1000ppm $p\text{CO}_2$ filtered sea water (FSW). Specifically, I made five random mating pairs formed from five male and five female urchins of the same species. In addition, I made two pseudoreplicates per mating pair per treatment in an attempt to account for unforeseen differences in laboratory conditions. Fertilization of the treatment groups took place in acidified FSW. Fertilization of the control groups took place in current ocean pH FSW. After fertilization, the treatment groups developed in acidified FSW and the control groups developed in current ocean pH FSW for the remainder of the study. First I combined sperm and eggs in a petri dish and diluted the mixture with the appropriate sea water (CO_2 enriched or normal). Then I gently mixed this solution by pipette to ensure it was homogenous. After fifteen minutes (to allow widespread fertilization), I placed a small sample of this mixture on a microscope slide and estimated the proportion of eggs fertilized. Through the use of a microscope, I assessed the fertilization state of the first 100 eggs I detected. If a fertilization envelope was present, the egg was counted as fertilized. If a fertilization envelope was not present, the egg was counted as unfertilized. Because this sample was from a homogenous mixture, this proportion was considered representative of the whole mixture. I conducted visual inspections at 190 minutes, 230 minutes, 1200 minutes, and 1320 minutes after fertilization. At these times, I noted the proportion of eggs that reached the following developmental stages: first cleavage, second cleavage, blastulation, and gastrulation. Completion of the first cleavage was defined as the presence of two cells, completion of the second cleavage was defined as the presence of four cells, blastulation was counted if a blastocoel was present, and gastrulation was counted once an ingression of the primary mesenchyme cells at the vegetal pole formed an archenteron (Fig. 3).

Evolutionary development

To determine the developmental timing of 1st cell division, 2nd cell division, blastulation, and gastrulation in *E. mathaei* and *T. gratilla* I observed their development in controlled conditions. Specifically, I randomly assigned five males and five females, from the same species, to mating pairs. I used a die to assign the individuals to mating pairs. In addition, I had two pseudoreplicates for each cross in an attempt to account for unforeseen differences in laboratory conditions. After crossing these gametes, I observed development in a suitable

and controlled environment. During development, I noted the proportion of eggs that reached certain developmental milestones at various times. These developmental milestones included fertilization, first division, second division, blastulation, and gastrulation. Fertilization was counted if a fertilization membrane was present. The other developmental stages were defined in the previous section. I continued to count the proportion of eggs that reached a certain developmental milestone until over half of the cohort reached the milestone. I then interpolated the time at which fifty percent of the eggs reached that milestone using two data points, one on either side of fifty percent. I also noted any visually apparent differences that I observed between the species. I analyzed the data for significant differences in the timing of developmental events (heterochronies).

Statistical analysis

For the abundance surveys, I analyzed the data for correlations between the abundance values of the six-urchin species and the plot data obtained from Lewis (2017). I also analyzed the data for correlations between the abundance values for each species. I then conducted linear regressions for any relationships between abundance and the plot data with a correlation value (r) greater than the absolute value of 0.70. In addition, I conducted linear regressions for any relationships between abundance values for each species with a correlation value of (r) greater than the absolute value of 0.60. Correlation values calculated through the R package “corrplot” (Taiyun and Viliam 2017).

I used a generalized linear model (glm), in a binomial family, to estimate the probability that each egg reached a certain stage (fertilization, 1st division, 2nd division, blastulation, and gastrulation, 0 = did not, 1 = did). Each generalized linear model (total of 5) included a random effect to account for breeding pair. These models were conducted using the R package “lme4” (Bates et al. 2015). These p-values were calculated using the R package “lmerTest” (Kuznetsova et al. 2016).

For the evolutionary development study, I averaged the time that it took the eggs to reach each developmental milestone, for each pseudoreplicate. I then compared the average values for each developmental milestone between two species using a t-test. In addition, I calculated the time between developmental events for each replicate and species. I then compared the time between developmental

TABLE 1. Correlation values (r) between the abundance of six urchin species and depth/ coverage data. (*) denotes a significant relationship determined in a linear regression.

Abundance of:	<i>D. savignyi</i>	<i>E. aciculatus</i>	<i>E. calamaris</i>	<i>E. diadema</i>	<i>E. mathaei</i>	<i>T. gratilla</i>
Depth	0.77	0.49	-0.34	0.69	-0.11	0.5
Sand	0.35	-0.47	0.27	0.16	-0.3	-0.26
Coral Rubble	-0.24	0.03	0.65	-0.54	-0.76	0.29
Dead Coral	-0.14	0.67	-0.2	-0.17	-0.04	0.68
<i>Turbinaria</i>	-0.19	-0.21	-0.56	0.14	0.86	-0.43
<i>Sargassum</i>	0	-0.32	0.17	-0.48	-0.41	0.41
<i>Padina</i>	0.17	0.39	0.64	0.51	-0.46	-0.46
Microalgae biofilm	-0.69	-0.57	-0.12	-0.51	0.6	-0.55
<i>Porites</i>	0.66	0.05	-0.36	0.17	-0.23	0.77
<i>Montipora</i>	-0.28	*0.96	0.61	-0.04	-0.61	0.25
<i>Pocillopora</i>	-0.56	-0.38	-0.41	-0.35	0.78	-0.38
Live Coral	-0.09	-0.14	-0.75	-0.28	0.58	0.46

events for both species using a t-test. For all statistical analyses, I used an alpha value of 0.05. All statistical tests were conducted in R (R Core Team 2015). All plots were made using the R package “ggplot2” (Wickham 2009).

RESULTS

Abundance across varying coverage

Abundance of *E. molaris* was positively correlated with the proportion of *Montipora* coverage. This strong positive correlation (Table 1) was significant in a linear regression ($F_{1,3}=35.88$, $p<0.01$, Fig. 8; Appendix A). Abundance of *D. savignyi* was positively correlated (Table 1) with depth, but not significant in a linear regression ($F_{1,3}=4.4$, $p>0.05$, Fig. 9; Appendix A). Abundance of *E. calamaris* was positively correlated with the proportion of live coral coverage (Table 1), but not significant in a linear regression ($F_{1,3}=3.82$, $p>0.05$, Fig. 10; Appendix A). Abundance of *E. mathaei* was negatively correlated with the proportion of coral rubble coverage, positively correlated with the proportion of *Turbinaria* coverage, and positively correlated with the proportion of *Pocillopora* coverage (Table 1), but

none of these were significant in a linear regression ($p>0.05$, Fig. 11; Appendix A). Abundance of *T. gratilla* was positively correlated with the proportion of *Porites* coverage (Table 1), but not significant in a linear regression ($F_{1,3}=4.49$, $p>0.05$, Fig. 12; Appendix A).

Relationships between interspecific abundances

Abundance of *E. mathaei* was negatively correlated with the abundance of *E. calamaris* and *E. aciculatus* (Table 2), but neither of these relationships were significant in a linear regression ($p>0.05$, Fig. 13; Appendix A). Abundance of *D. savignyi* was positively correlated with the abundance of *E. diadema* (Table 2), but not significant in a linear regression ($p>0.05$, Fig. 13; Appendix A).

Fertilization and development in acidified conditions

Fertilization did not differ with pCO_2 as syngamy levels were $57 \pm 4.7\%$ at 400ppm and $54 \pm 10.3\%$ at 1000ppm (in a glm, $z=0.4$, $p>0.05$, Fig. 4). First division differed with pCO_2 as $13 \pm 7.8\%$ completed the first division at 400ppm

TABLE 2. Correlation values (r) between the abundances of six urchin species.

	<i>E. mathaei</i>	<i>D. savignyi</i>	<i>E. diadema</i>	<i>T. gratilla</i>	<i>E. aciculatus</i>	<i>E. calamaris</i>
<i>E. mathaei</i>	1	0.08	0.2	-0.41	-0.63	-0.83
<i>D. savignyi</i>	0.08	1	0.84	0.14	-0.08	-0.46
<i>E. diadema</i>	0.2	0.84	1	-0.26	0.04	-0.32
<i>T. gratilla</i>	-0.41	0.14	-0.26	1	0.47	-0.1
<i>E. aciculatus</i>	-0.63	-0.08	0.04	0.47	1	0.47
<i>E. calamaris</i>	-0.82	-0.46	-0.32	-0.1	0.47	1

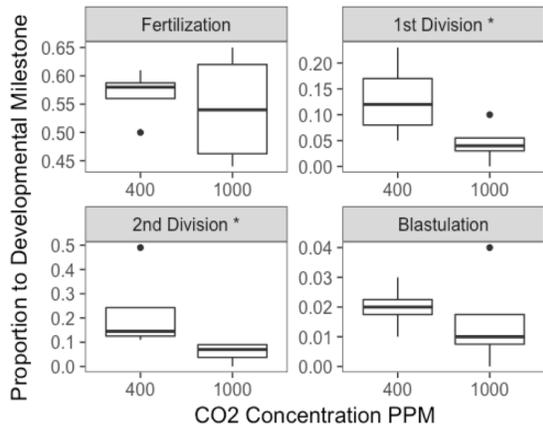


FIG. 4. Boxplot depicting the proportion of cohort that reached multiple developmental milestones in two treatments of varying levels of CO2 concentration.

and $5 \pm 4.1\%$ completed the first division at 1000ppm (in a glm, Est for 400ppm 1.43 ± 0.48 s.e., $z=3.0$, $p<0.05$, Fig. 4). Second division differed with $p\text{CO}_2$ as $22 \pm 18.0\%$ completed the second division at 400ppm and $6 \pm 4.3\%$ completed the second division at 1000ppm (in a glm, Est for 400ppm 1.67 ± 0.48 s.e., $z=3.5$, $p<0.001$, Fig. 4). Blastulation did not differ with $p\text{CO}_2$ as blastula formation levels were $2 \pm 0.8\%$ at 400ppm and $2 \pm 1.7\%$ at 1000ppm (in a glm, $z=0.6$, $p>0.05$, Fig. 4). None of the embryos sampled for any of the replicates proceeded to develop past blastulation. Therefore, the mean values for the proportion of the cohort to complete gastrulation were 0 for both the 400ppm and 1000ppm treatments.

Evolutionary development

Initially, I set out to determine the developmental timing of six urchin species. Unfortunately, due to a variety of different problems, development was only successfully observed and quantified in two urchin species. The species that were successfully used in this study were *T. gratilla* and *E. mathaei*, while the species that I was unable to successfully study included: *E. aciculatus*, *E. diadema*, *D. savignyi*, and *E. calamaris*.

The spines of *E. aciculatus* were very fragile and they were never seen away from their burrows, which made collection extremely difficult. In the end, I was unable to collect enough individuals of this species to study their development. In contrast, I was able to collect a large number of *E. calamaris*. I attempted to spawn a total of fifty-six *E.*

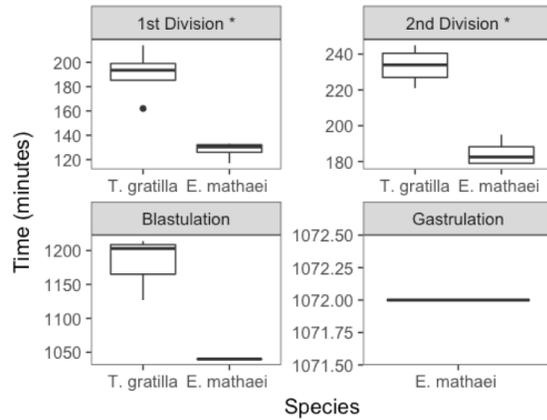


FIG. 5. Boxplots depicting the time to multiple developmental stages in *T. gratilla* and *E. mathaei*.

calamaris, but I was never able to harvest any gametes from these individuals.

I attempted to spawn twenty-five *E. diadema*, and only seven individuals successfully spawned. Unfortunately, five of the seven spawned were females with immature eggs, identified through the presence of a germinal vesicle (Fig. 7). Interestingly, I observed three individuals of this species spawning prior to collection. I then transported these back to the lab, but I was unable to collect any gametes from them.

Over the course of nearly two months, I attempted to spawn a total of seventy-nine *D. savignyi*, and spawning was successfully induced in thirty-four individuals. Unfortunately, the majority of the females spawned immature eggs, identified through the presence of a germinal vesicle (Fig 7). In contrast, the sperm typically seemed to be mature. The only day I successfully spawned

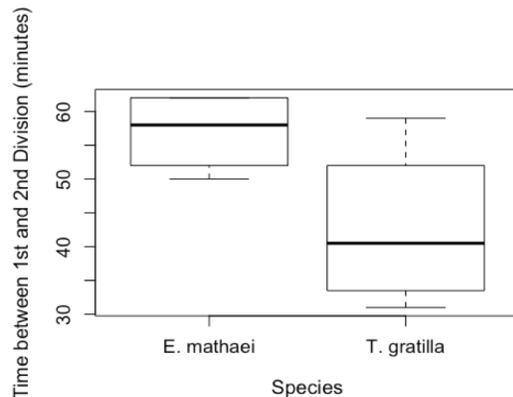


FIG. 6. Boxplot depicting the time between the 1st and 2nd division in *E. mathaei* and *T. gratilla*.

mature eggs was October 7th. Unfortunately, I only had mature eggs from two females and therefore would not have enough replicates for my study.

The time to the 1st division for *E. mathaei* was 128 ± 7 minutes, while the time to the 1st division for *T. gratilla* was 191 ± 21 minutes. These two treatments were statistically different, by t-test ($t_{3,7}=-5.6$, $p<0.05$, Fig. 5). The time to the 2nd division for *E. mathaei* was 185 ± 8 minutes, while the time to the 2nd division for *T. gratilla* was 234 ± 11 minutes. These two treatments were statistically different, by t-test ($t_{5,4}=-7.5$, $p<0.05$, Fig. 5). For *E. mathaei*, the time to blastulation was only successfully measured in one replicate (1040 minutes). While the time to blastulation for *T. gratilla* was 1181 ± 47 minutes (Fig. 5). Therefore, there were not enough data points to run a t-test. For *E. mathaei*, the time to gastrulation was only measured in one replicate (1072 minutes). In addition, all of the replicates for *T. gratilla* ceased to develop past blastulation. Therefore, the timing of gastrulation for *T. gratilla* was not measured and a t-test could not be conducted.

The time between 1st and 2nd division in *E. mathaei* was 57 ± 6 minutes while the time between 1st and 2nd division in *T. gratilla* was 43 ± 12 minutes. The time between 1st and 2nd division was not significantly different between these urchin species ($t_{4,4}=2.1$, $p>0.05$, Fig. 6).

DISCUSSION

Abundance across varying coverage

Although many relationships were strongly correlated, only one of these correlations was significant. This may have been due to the limited number of samples obtained in this survey.

Although not significant, the strong positive correlation between the abundance of *D. savignyi* and depth mostly concurs with previous studies. For example, Gehlbach (1994) found that had *D. savignyi* had a preference for midrange depths between 1.5 and 6.5 meters. The depth surveyed in this study was limited to a maximum of 2 meters due to the survey method. It would be interesting to see how this relationship is affected by an increase in the maximum depth surveyed. It may lead to a bell-shaped relationship which would convey the presence of an optimal range of depths for this urchin species.

The abundance of *E. aciculatus* and the proportion of *Montipora* coverage had a significant positive correlation, which means as

the proportion of *Montipora* coverage increases the abundance of *E. aciculatus* will increase. This is notable because this relationship has not been noted previously. This relationship could be interpreted in two ways, either a behavior of *E. aciculatus*, such as its foraging or burrowing, may be benefiting the colonization and persistence of *Montipora*, or the presence and abundance of *Montipora* is benefiting *E. aciculatus*. Overall, this relationship deserves further research in order to parse out the directionality.

Future research should solidify the presence of these relationships by increasing the sample size. We can use information gleaned from relationships such as these to assist in the conservation of our reefs and sea urchins. Effective conservation measures are especially important in light of predicted oceanic changes.

Relationships between interspecific abundances

Although it was not significant, the strong positive correlation between *D. savignyi* and *E. diadema* is noteworthy. If the sample size was increased and this relationship was determined to be significant, it could mean that either these species are selecting for resources found in similar conditions but that are not the same resource, or these species are both utilizing the same resource and it is not limiting. For example, if these two species were utilizing two different food sources that overlap in distribution, this could be an example of niche partitioning as an evolutionary attempt to minimize interspecific completion. On the other hand, these two species could be selecting for the same habitat, but the factor they are selecting for could be non-limiting such as average depth. Although these urchin species do have a similar diet, Coppard and Campbell (2005) found that *E. diadema* and *D. savignyi* both preferred different species of algal/sea grass.

Although non-significant, the strong negative correlations between the abundance of *E. mathaei* and *E. aciculatus* and the abundance of *E. mathaei* and *E. calamaris* is noteworthy. If the sample size was increased and these relationships were determined to be significant, these species could have similar habitat preferences. *E. mathaei* and *E. aciculatus* have a similar morphology and behavior. These two species of urchins are both rubble dwelling urchins. In contrast, *E. calamaris* and *E. mathaei* exhibit quite different morphologies and behaviors, but could still be competing for

a limited resource. If either of these species are utilizing a similar resource and this resource is limited, this typically leads to competition. Competition between different urchin species has been previously noted. For example, in an experiment by McClanahan (1988), when crevices were limited *E. mathaei* competitively excluded two *Diadema* species.

Future studies should focus on these relationships and determine if a larger sample size leads to significance. Further research into these relationships may lead to a better understanding of niche partitioning and interspecific competition between sea urchins.

Fertilization and development in acidified conditions

The results of this experiment support the conclusion that acidified conditions do affect the early development of *T. gratilla* in that the differences in the proportion of the cohort that reached different developmental milestones between the acidified and normal conditions were statistically significant. Although previous studies have not focused solely on the effects of ocean acidification on the early development of *T. gratilla*, the results of this study show that *T. gratilla* is one of the many organisms that will be adversely affected by ocean acidification. Two different explanations for the difference in proportions between the two treatments exist. The first being that the acidified treatment led to the cessation of development in many fertilized eggs and the second being that the acidified treatment led to a developmental delay. In light of other studies, which have noted developmental delays in echinoids developed in acidified conditions (Kurihara and Shirayama 2004, Stumpp et al. 2011), this seems to be the most likely explanation. Especially because developmental delays and reductions in size have previously been noted in the larval development of *T. gratilla* in acidified conditions (Brennan et al. 2010). A developmental delay may lead to a reduction in the embryos performance, thereby leading to a decrease in the abundance of the adult urchin population.

Ocean acidification is not the only factor threatening the abundance of *T. gratilla*. Overharvesting has led to marked declines in *T. gratilla* populations (Tomascik 1997) and a population crash in the closely related sea urchin species, *Tripneustes ventricosus* (Scheibling and Mladenov 1987). Therefore, ocean acidification and overharvesting may have synergistic effects that could lead to a

population collapse, such as the one produced almost solely by overfishing in *T. ventricosus* (Scheibling and Mladenov 1987). *T. gratilla* grazes day and night and has a substantial impact on the abundance of algae (Tomascik 1997). If the population of *T. gratilla* collapses, this could decrease the threshold of a coral-algal phase shift.

These findings are significant because *T. gratilla* is not only of economic importance, because it is commonly harvested by humans for its gonads, but it also plays an important role in its marine community as both a food source and nutrient cyler. Further research should focus on understanding how exactly ocean acidification will affect the abundance of *T. gratilla*, and how a decline in their abundance may affect their marine community. In addition, future research should focus on how acidified conditions will affect different life stages of *T. gratilla*, such as the pluteus larval stage or the adult urchin.

Evolutionary Development

My developmental timing results are slightly longer than those of Kominami and Takata (1957), who found the 1st division to occur at 70-80 minutes (cf. 128 min), the 2nd division to occur at 115-125 minutes (cf. 185 min), and blastulation to occur at 1080 minutes (cf. 1040 min). Both the results of Kominami and Takata (1957) and my results differ substantially from Ghorani et al. (2012), who found the 1st division to occur at 60 minutes and blastulation to occur at 360 minutes. The timing of sea urchin embryos to developmental milestones is affected by the temperature at which they are reared. Therefore, these discrepancies in our results could have arose from the differences in the temperature at which the embryos were reared (Harvey 1956 and Costello et al. 1957). Kominami and Takata (1957) reared their embryos at 24°C and Ghorani et al. (2012) reared their embryos at 29°C, while I reared my embryos at 19°C.

Due to the lack of any other studies on the timing of early developmental events in *T. gratilla*, I am unable to compare my findings with other results.

Although developmental heterochronies have been noted between different sea urchin species, this study is the first to focus on a comparison of the early developmental timing of *T. gratilla* and *E. mathaei*. The results support the presence of differences in developmental timing between the 1st divisions and the 2nd divisions of these two species. In other words,

heterochronies were found between these two species.

There were significant differences in the time at which 1st and 2nd division occurred in these two species, but the time between the 1st and 2nd division for these two species was not significantly different. This means that the actual developmental time from 1st to 2nd division is not significantly different between these two species, but the time at which they initiate this development is significantly different. This is an interesting finding that shows that the developmental clocks of these species have diverged over time.

This study may act as a stepping stone for other studies. Once the developmental timing for multiple species has been determined these values can be used to understand their evolutionary relationships. Due to the limited number of sea urchins successfully observed in this study, this data will have to be used in conjunction with another study in order to be used to predict evolutionary relationships. Therefore, future studies should focus on determining the timing of developmental events in understudied species of urchins.

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APPENDIX A

TABLE 3. GPS coordinates of the five plots used for the abundance surveys.

Plot	2	4	5	6	7
Latitude	-17°28.92800'	-17°28.88800'	-17°28.86900'	-17°28.85000'	-17°28.82800'
Longitude	-149°49.70400'	-149°49.78500'	-149°49.82800'	-149°49.86700'	-149°49.92300'

TABLE 4. This table denotes time to each developmental milestone for *Echinometra mathaei* and *Tripneustes gratilla* (mean \pm 1 s.d.). Note, the *E. mathaei* values for blastulation and gastrulation are based on only one replicate, therefore standard deviations cannot be reported for these values. In addition, no data is reported for the gastrulation stage in *T. gratilla* because all *T. gratilla* replicates ceased to develop past blastulation.

Stage	<i>Echinometra mathaei</i>	<i>Tripneustes gratilla</i>
1 st Cell Division	128 \pm 7 minutes	191 \pm 21 minutes
2 nd Cell Division	185 \pm 8 minutes	234 \pm 11 minutes
Blastulation	1040 minutes	1181 \pm 47 minutes
Gastrulation	1072 minutes	-



FIG. 7. Comparison of maturity in eggs from *Diadema savignyi*. (A) a mature egg with a small nucleus and round shape (B) an immature egg with a large nucleus (germinal vesicle) with a mostly round shape (C) an immature egg with both a large nucleus (germinal vesicle) and an asymmetric shape. Scale bar=50 μ m for A-C.

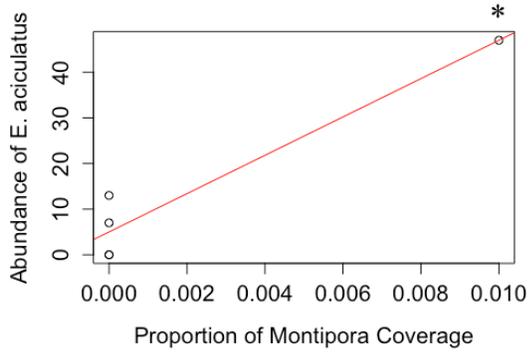


FIG. 8. Linear regression of the abundance of *E. aciculatus* and the proportion of *Montipora* coverage.

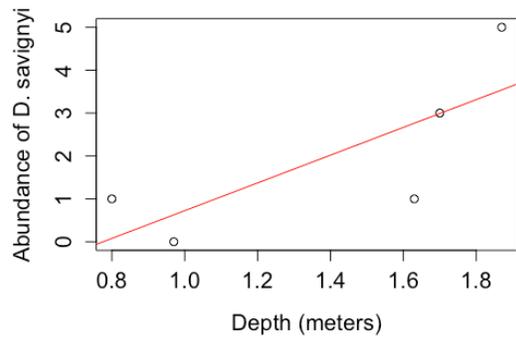


FIG. 9. Linear regression of the abundance of *D. savignyi* and the depth in meters.

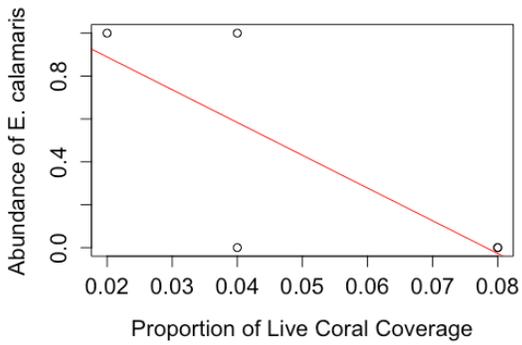


FIG. 10. Linear regression of the abundance of *E. calamaris* and the proportion of live coral coverage.

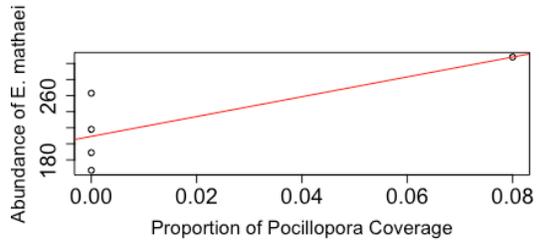
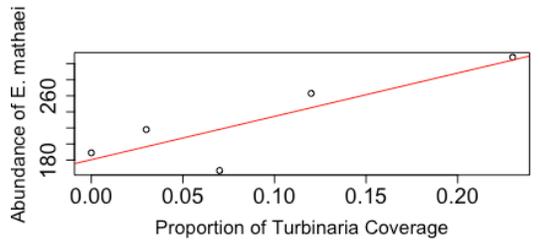
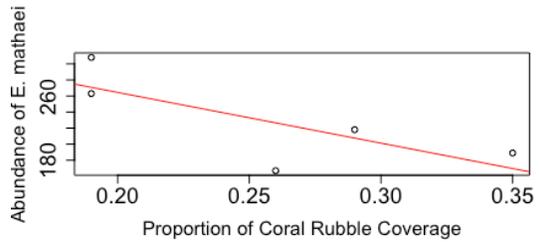


FIG. 11. Comparison of the abundance of *E. mathaei* and the proportion of various coverages using linear regressions.

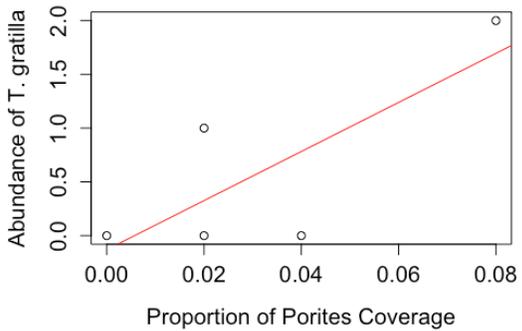


FIG. 12. Linear regression of the abundance of *T. gratilla* and the proportion of *Porites* coverage.

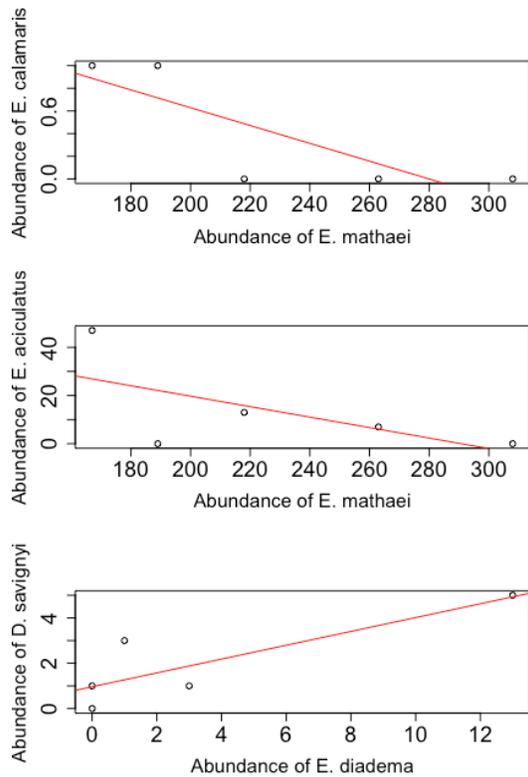


FIG. 13. Comparison of the abundances of multiple urchin species using linear regressions.

APPENDIX B

SPECIES IDENTIFICATION

Echinometra mathaei



This urchin species was typically found underneath and in the holes of dead coral. This species' test can grow to be up to 8cm in diameter (Schoppe 2000). The coloration of this urchin species is variable, but the test is typically a dark color with greater variation in the coloration of the spines. Common coloration patterns of the spines include: black with white tips, purple with white tips, beige with white tips, and green. The characteristic pale ring at the base of each spine can be used to distinguish this species from others. Collection of this species is easy both during the day and night due to their abundance.

Echinothrix calamaris



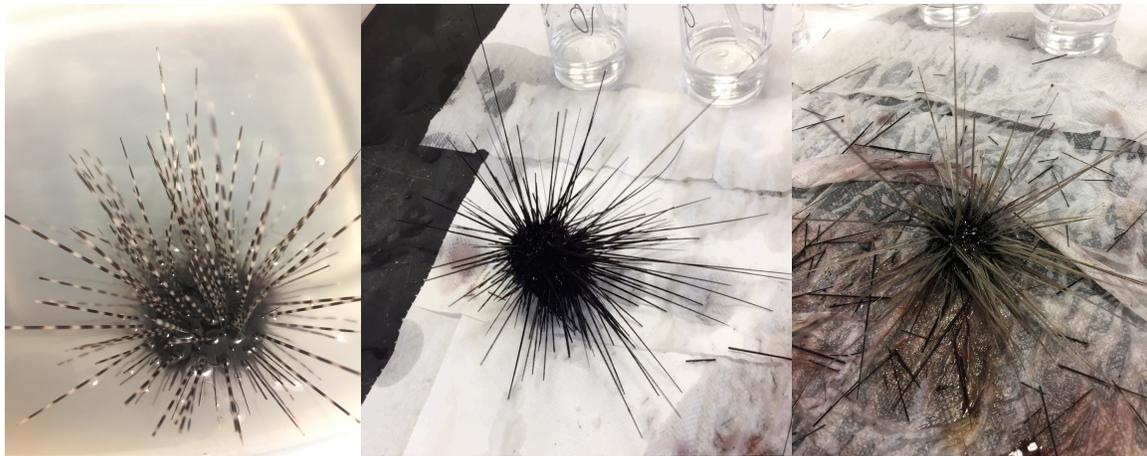
This urchin species was commonly found at the start of the channel, where there are deeper depths and larger crevices in the dead coral. This species has two sets of spines. The longer thicker spines are typically banded, but can also be uniform in coloration. These spines are typically banded with light and dark colors, but can also be completely white, dark brown, or reddish brown. Because these blunt longer spines are hollow, they can be easily broken. The thinner and slightly shorter spines are very sharp and typically darker than the longer blunt spines. These short spines range in color from a yellowish-brown to a dark brown. The anal sac on this urchin is typically light in coloration and is larger than those in *Echinothrix diadema*.

Echinothrix diadema



This urchin species was only found at the back reef on the northwest mouth of Cook's Bay. This species has two sets of spines, but this is less obvious than those found in *Echinothrix calamaris*. These urchins are typically not banded, but can be in juveniles. The spines of this urchin species are typically dark blue or black. A blue sheen can be seen if a light is shined on this urchin. The anal sac on this urchin is usually dark in coloration and is fairly small (typically smaller than those in *Echinothrix calamaris*). The spines of this urchin are not hollow and cannot be easily broken.

Diadema savignyi



This urchin species was commonly found at the start of the channel, where there are deeper depths and larger crevices in the dead coral. The spines of these urchins are hollow, long, sharp, and brittle. The coloration of the spines in this urchin is fairly variable and can range from whitish, grey, black, and banded. This species also has iridescent lines on its aboral surface. These lines are on the interambulacral areas and on the periproct and can be blue or green.

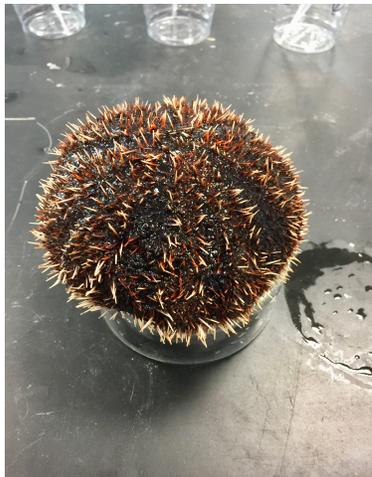
Echinostrephus aciculatus



This species bores into the dead coral and typically remain in their burrows for protection (Guille et al. 1986). Its spines are thin, sharp, and fragile (unlike the thicker and sturdier spines of *Echinometra mathaei*). The coloration of the spines is quite variable, but they are commonly brown, grey, purple, or black. The dark and bare apical disk is a distinctive feature in this sea urchin.

(Photo credit: Kieffer, M. 2008. The sea urchin *Echinostrephus aciculatus* spotted in Zanzibar (Tanzania). [<http://www.flickr.com/photos/mattkieffer/2969289929> – accessed Dec 12 2017])

Tripneustes gratilla



This urchin is typically found on dead coral, coral rubble, or sand. This urchin is commonly hidden by collected debris such as coral rubble, algae, and rocks. This urchin's test is typically bluish-purple while its spines are usually orange, white, orange with white tips, or white with orange tips. Many individuals have long podia that can be seen easily while the urchin is underwater.