Investigation of *Diadema savignyi*'s role in shaping the abundance of the brown microalga, *Sargassum Pacificum*, in Mo'orea, French Polynesia

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The role of *Diadema savignyi* in controlling the brown microalga, *Sargassum pacificum*, was studied on the barrier reef of Mo'orea, French Polynesia. A survey of the area was conducted to determine if there was a trend between the number of *D. savignyi* and the amount of *S. pacificum* on a coral bommie; no correlation was found. A field experiment involved relocating *D. savignyi* by placing groups of seven on coral bommies in the backreef outside of Cooks Bay. At the end of the experiment, *Sargassum pacificum* cover was compared in urchin manipulated coral bommies and non-manipulated bommies. Manipulated bommies experienced a statistically greater decrease in *S. pacificum* cover. However, the control bommies experienced almost no change, indicating that the increased density of urchins on a bommie caused an increased feeding rate.

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Introduction

Variation in grazing pressure is known to have profound effects on plant communities, and in turn on higher trophic levels (Harper, 1969). Increased grazing pressure can physically remove a species, altering an ecosystem's productivity, or change the limiting nutrients of that ecosystem (Proulx & Mazumder, 1998). Increased herbivore diversity can reduce plant biomass, as the most efficient grazers will dominate, leading to overgrazing. This in turn can depress plant diversity and facilitate the invasion of grazing resistant species (Duffy *et al.,* 2003).

As in the terrestrial realm, marine grazers play an important role in ecosystem stability. Possibly the largest exchange of energy between trophic levels on coral reefs occurs in the interactions between grazers and algae (Hatcher, 1983). This transfer of energy is important for the function of the ecosystem. A change in grazing pressure may result in a so-called coral-algal phase shift; a shift from a coral-dominated reef to a macroalgal-dominated reef (Done, 1992). Coral-algal shifts indicate a decline in coral reef health, and they have been increasingly studied over the past decades as coral health has declined (McManus & Polsenberg, 2004). Coral-algal phase shifts can be caused by outbreaks of coral predators, eroders, over-fishing, habitat destruction, weather-induced bleaching events, and mass coral mortality induced by climate change (Done, 1992). An example of a phase shift occurred in Jamaica during the 1980s (Hughes, 1994). Coral cover in Jamaican reefs decreased from 40-70% to less than 10%, while macroalgae, typically less than 10% in this system, became dominant (Hughes, 1994). Concomitantly, the reefs suffered an almost complete extirpation of the keystone grazer, *Diadema antillarum*. Without this urchin, there was an increase in the amount of macroalgae (Sammarco, 1982). Most of the new alga growth was found on previously bare substrate where the settlement of corals had likely significantly decreased (Hughes *et al*., 1985). Presences of *Diadema* species have been shown to not only reduce algal cover, but also to influence coral recruitment as they create areas for new corals to settle (Sammacro, 1980; Carpenter & Edmunds, 2006). *Diadema* has been observed to have a particularly strong influence on algal distribution in shallower reefs (Morrison, 1988).

The disappearance or growth of urchin populations can have drastic ecosystem effects. In kelp ecosystems, urchin barrens are urchin-saturated areas where urchins have consumed large portions of kelp, thus creating an urchin desert (Watanabe & Harrold, 1991). There are no equivalents of this phenomenon in coral reef ecosystems (North & Pearse, 1970). However, it is possible that after a reef has shifted from coral-dominated to algal-dominated, urchins could reestablish to consume the algae, and thus creating a bare reef (Norström *et al.,* 2009). In contrast to kelp forests, large populations of sea urchins on coral reefs have not been reported to have negative ecosystem effects.

This study has two main components. The first component is a survey to determine the possible relationship between the number of *D. savignyi* on dead coral bommies and the amount of *S. pacificum.* The second is a series of experimental manipulations to understand *Diadema savignyi*'s role in controlling the density of a brown macroalga *Sargassum pacificum* in Mo'orea, French Polynesia. This will determine if an increased amount of *D. savignyi* on a bommie will impact the amount of *S. pacificum* present. The results aim to highlight the effect that large numbers of urchins may have on a coral reef.

Methods and Materials

Site Description

Experiments and surveys were conducted on reefs along the north side of the island of Mo'orea, French Polynesia (17°28'45" S, 149°49'51" W) near the outlet of Cook's Bay (Fig. 1). This marine protected area was chosen for study because of the presence of isolated dead/alive coral bommies (Fig. 2) (roughly 1m apart from each other) and the abundance of *Sargassum pacificum* and *Diadema savignyi*. Due to the proximity to the reef crest, this area is exposed to high water velocities. Water depth in the study area ranges from 0.5-1.5 m. The substrate comprises a gradient of sand and coral rubble. Most of the reef is covered in brown macroalgae, *Turbinaria ornata*, *Sargassum pacificum*, and *Dictyota spp*. The only corals present are *Porites* species. Corallivorous fish are present in the study area as well as juvenile fish, moray eels, and other species of urchins.

Survey

Transect surveys of coral bommies were conducted in the study area from October 21 to 25, 2016. The primary goals of surveys were to characterize the dimensions of bommies, the presence of *D. savignyi* urchins, and the percent cover of *S. pacificum.* Surveys started along a line looking north with a rock (17°28'42.6" S, 149°49'48.3"W) sticking out of the water close to the reef crest and continued west measuring each bommie encountered. Ten different coral bommies were surveyed for three days; each day the survey started closer to the reef crest (north). Sediment was displaced while measuring the bommie, making it difficult to see, so the bommie is surveyed for *D. savignyi* before taking any other measurements. A transect tape was used to measure the circumference of the bommie as well as the height. A piece of small aquarium tubing was used to measure the circumference of each *S. pacificum* about one inch from its base. A ruler was used to measure the height of each plant. Percent cover of *S. pacificum* was estimated by visually scanning each bommie from above (only the top surface of the bommie was considered). R (R Core Team, 2013) was used to run a linear regression to see if *D. savignyi* have an impact on the amount of *S. pacificum*.

Urchin Collection

All *Diadema* urchins used in experiments were collected at night from the reef adjacent to the Gump research station (17°29'26" S, 149°49'33" W) and held for one night before being transported to the backreef. This site offered easier and safer access to urchin habitat at night than the area where surveys were conducted. Urchins were picked off rocks with gloved hands (Kevlar gloves for protection against spines) or long tweezers and then lofted in the water and placed in a colander, and finally, placed in a bucket on dry land. Sizes of urchins collected for study varied from 2.5cm to 9cm test size, larger urchins were preferred for collection. Twenty-five urchins were collected on October 21, 2016 and 42 urchins were collected on November 3, 2016; four color variations were observed white, black, dark purple, and one light green. Urchins were kept in a tank with cylinder blocks that provided places for the urchins to hide during the day. Feeding habits were observed overnight for 2.5 hours with a time-lapse video that took a picture every minute. The camera was aimed at a piece of *S. pacificum* in the tank. *Sargassum pacificum* from the tank was closely examined the next day to characterize *D. savignyi* consumption patterns.

Field Experiment

Field experiments involved placing previously captured urchins at paired experimental and control sites on coral bommies. Cages were not used in this study in order to allow a more natural setting; the bommies are naturally separated from each other and *Diadema* are known not to move more than 1m from their hole (Tuya *et al.,* 2004). Each experimental and control site was marked with numbered flagging. Seven *D. savignyi* were placed on the experimental sites and no *D. savignyi* were placed on the control sites. Each bommie was first surveyed as described above to establish a baseline of *S. pacificum* cover, then urchins were placed and observed for 5 min. to insure they settle into holes and were not washed away by the current. Detailed notes on the appearance of *S. pacificum* were also recorded each visit. Following the experimental addition of urchins, coral bommies were surveyed every other day for the following week. The experiment was conducted in October and November 2016. R (R Core Team, 2013) was used run a linear regression to see if *D. savignyi* have an impact on the amount of *S. pacificum*.

Results

Survey

Thirty-six coral bommies were surveyed; the percent *S. pacificum* cover (42%±30%), number of *D. savignyi* (1.3±1.6), and the volume (6.6 ± 4.3m^3). The size of the coral bommie did not determine the number of D. savignyi as shown by the almost zero correlation (Fig. 3) ($r^2 = 0.019$ p-value = 0.47) and therefore, urchin numbers are not normalized to the size of the bommie in any of the following graphs. No correlation was found ($r^2 = 0.014$, p-value = 0.49) between the amount of *S. pacificum* and the number of *D. savignyi* on a given coral bommie (Fig. 4).

Field Experiment

The control site with no urchins added experienced a 0-10% (1.4% ±14%) change in the cover of *S. pacificum* and the experimental sites with seven urchins added experienced changes in 0-40% (3.8% ±12%) cover. There was a significant difference (Wilcoxon rank sum, W=34, p-value= 0.011) between changes in *S. pacificum* percent cover in control and experimental bommies (Fig. 5). The bommies with *D. savignyi* added had larger decreases (-14%±13%) in the amount of *S. pacificum* cover than the bommies that were not manipulated (-1.4%±13%). None of the test bommies maintained the seven *D. savignyi* added plus the original amount. The number of urchins on the control and tests bommies were not significantly different (Wilcoxon rank sum, W = 22, p-value = 0.74, data not shown). The control and test bommies did not have a constant amount of *D. savignyi* throughout the experiment. The greater the change in *D. savignyi* on a coral bommie, the greater the change in percent cover of *S. pacificum* on that bommie ($r^2 = 0.41$ p-value = 0.013) (Fig. 6).

Laboratory Observation

Captive *Diadema savignyi* were observed simultaneously consuming *S. pacificum* from the top and the bottom of the alga. *Diadema savignyi* consumes only the leaves and the bladders of the alga, leaving the stipe and the holdfasts in place. *Sargassum pacificum* in the field appears to be consumed in this same fashion.

Discussion

Results show that *Diadema savignyi* had a minor impact on the amount of *Sargassum pacificum* on the backreef outside of Cook's Bay in Mo'orea. This was exhibited in the survey results, which showed no correlation between the amount of *S. pacificum* cover and the number of *D. savignyi*, meaning that neither factor had a detectable influence on the other. The small percent change in *S. pacificum* cover for the control bommies shows that *D. savignyi* has a low feeding rate, and thus a minor impact on the amount of *S. pacificum*. In future studies, this problem could be addressed by, weighing *S. pacificum* and reattaching the alga to the bommie then weighing it after.

Diadema spp. are known to prevent coral-algal phase shifts by consuming macroalgae on reefs (McClanahan *et al.,* 1996; McManus & Polsenberg, 2004; Tuya *et al.,* 2004); it is unusual that a strong relationship between the amount of *D. savignyi* and *S. pacificum* was not found. A study conducted in the reefs of the Canarian Archipelago found an inverse relationship between the amount of *Diadema antillarum* and the amount of macroalgae (Tuya *et al.,* 2004). The study explored in this paper had the same sample size as the Canarian Archipelago study, but found a very strong relationship between the macroalgae percent cover and the urchin density ($r^2 = 0.77$ ($p < 0.001$). The Canarian Archipelago study also measured the percent cover of algae using a visual estimate. This study differed because it covered a larger area, 36 sites with two samples at each site averaged together to get a total of 36 samples. The difference in results between this study and mine further proves that *D. savignyi* in the back reef of Cook's Bay, Mo'orea are not the most influential herbivores in the area. Urchin-algal studies often suggest that overfishing of macroinvertebrate-eating fish are the reason for the strong inverse relationship between urchins and macroalgae (Duggins, 1980; Tegner & Dayton, 1981; McClanahan *et al.,* 1994; Tuya *et al.,* 2004). There were multiple pieces of evidence supporting the hypothesis that fish are eating *D. savignyi* in the area; this would explain why a strong relationship between the number of *D. savignyi* and the brown macroalga, *S. pacificum*, was not found. The two groups of seven *D. savignyi* were placed on coral bommies at the south end of the study site where the depth is ~1.5m; two days later, no *D. savignyi* were found on the bommies or adjacent ones, suggesting that they were eaten. For the rest of the experiment, urchins were placed on the north end of the site where the water was shallower ~1m. Most of the seven added urchins were found at the bommies; however not all of them were found, suggesting that there could be predatory pressure on the urchins. One of the experimental bommies had an orange-lined triggerfish, *Balistapus undulatus,* stuck in a crevice (Fig. 7), for at least two days after seven *D. savignyi* were added. This bommie went from having ten *D. savignyi* to two in three days. These fish play a dominant role in controlling urchin populations (McClanahan, 2000; Young & Bellwood, 2012).

Sargassum pacificum is the most abundant macroalga found coral bommies in the area; however *Dictiota* and *Turbinara* are also found. It is possible that *D. savignyi* is consuming other algae on the coral bommie, however *Diadema savignyi* are thought to have a large influence on the distribution of *Sargassum pacificum* (Vaziri, 1995). It is possible that *D. savignyi* are feeding very little, as urchins can go months without eating, but starvation has only been studied in cultured urchins where all food is absent (Spirlet, Grosjean & Jangoux, 2000; Guillou, Lumingas & Michel, 2000). Another possibility is that *D. savignyi* are moving from their daytime hiding coral bommie to a different bommie to feed on at night, but as stated earlier, *Diadema* are known not to move more than 1m from their hole (Tuya *et al.,* 2004).

The control sites had no change in percent *S. pacificum,* possibly because *D. savignyi* are either eating at different bommies, not eating very much, or consuming a different alga. *Diadema savignyi* could be feeding on smaller algae as well. The bommies that had *D. savignyi* added displayed a clear decrease in the amount of *S. pacificum*, and it is possible that it was the change in urchin numbers that caused more feeding. The urchins that I transferred were kept in captivity for one night with a small piece of *Sargassum pacificum*. It is possible that disturbing the urchins caused them to eat more when placed in a natural environment despite previously having food available in the tank. The bommies that had the largest change in the number of *Diadema savignyi* experienced the greatest decrease in percent cover of *Sargassum pacificum*.

Urchins have been studied for use as a biocontrol for invasive algae in Hawaii (Conklin & Smith, 2005; Westbrook *et al.,* 2015). Conkin & Smith investigated the use of lab cultured, Hawaiian native *Tripneustes gratilla* to control invasive algae in Hawaii. This study increased the confidence that the addition of *T. gratilla* will likely be an effective mean of biocontrol against several types of invasive algae. Non-native *Sargassum horneri* is becoming a growing problem in Southern California. This study examined the relationship between urchins and *S. pacficum* here in Mo'orea, in the hope of shedding light on the possible use of urchins as a biocontrol in California. However, *Diadema savignyi* do not eat *Sargassum pacificum* at a quick rate, so if they were to be used as a biocontrol, a large number would have to be used. Because urchins can be detrimental to native kelp forests a native urchin that prefers *Sargassum horneri* to kelp species, would have to used (Rowley, 1989).

Contrary to previous studies, this study shows that urchins do not always have a large impact on the amount of macroalgae. It is important to not overfish the species that play major roles in controlling urchin populations. Further research should explore *Diadema savignyi* populations in comparison to urchin-consumers and how that relationship impacts the amount of macroalgae.

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Figure 1. Map of the study area in Mo'orea, French Polynesia, with the survey area indicated by a red rectangle. Also shown with yellow markers are the UC Gump Research Station and a not fishing buoy and a rock that were used in the field as reference points. Map data: Google, DigitalGlobe, CNES/Astrium.

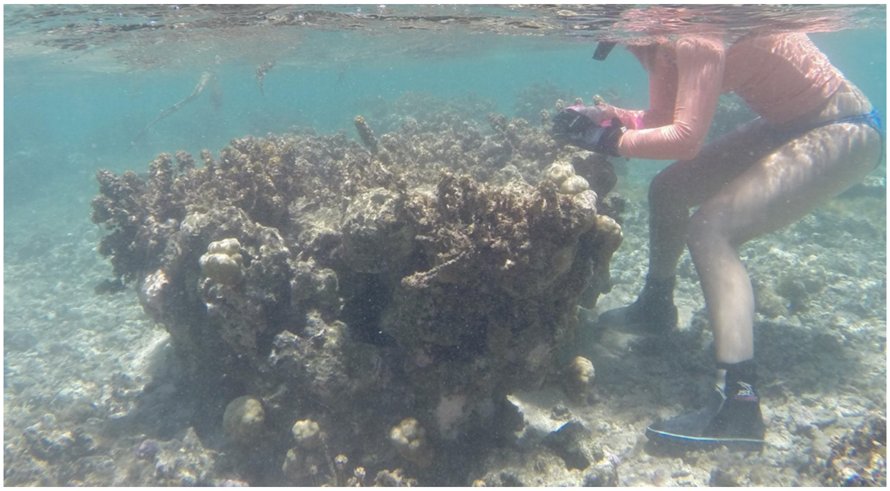

Figure 2. Typical coral bommie found in the backreef with coral rubble substrate and a small amount of *Porties* coral on the sides of the bommie. Photo credit: Mary Mcdonnell.

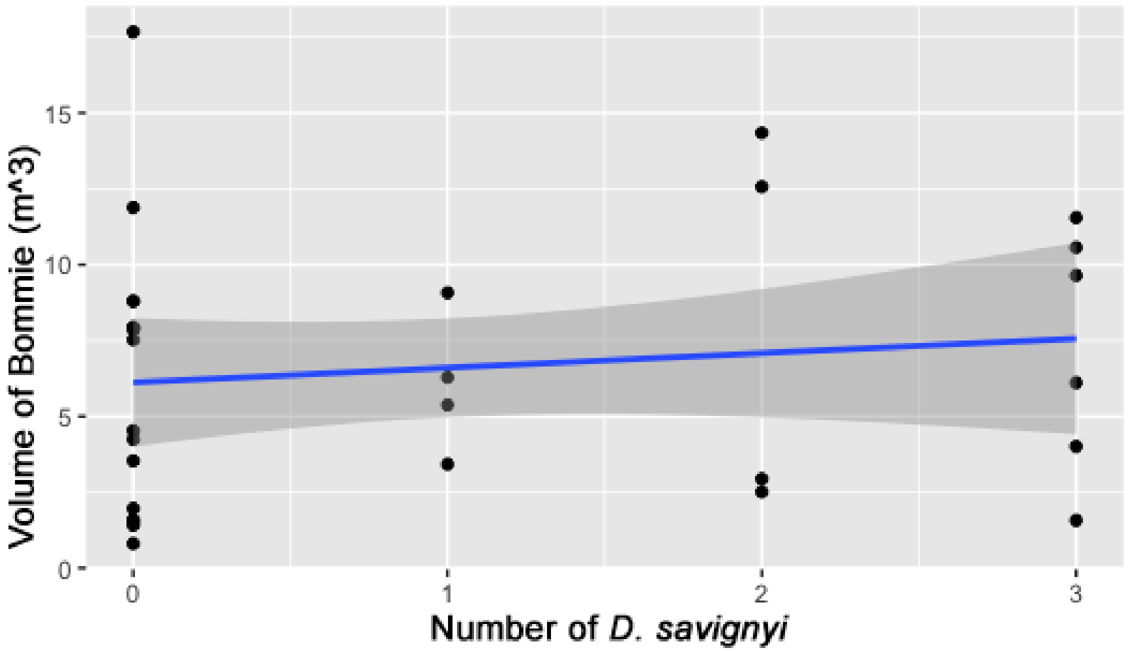

Figure 3. The size of the coral bommie is not related to the number of *D. savignyi* present ($r^2 = 0.019$ p-value = .47). The gray area represents the 95% confidence interval of which the regression line could fall.

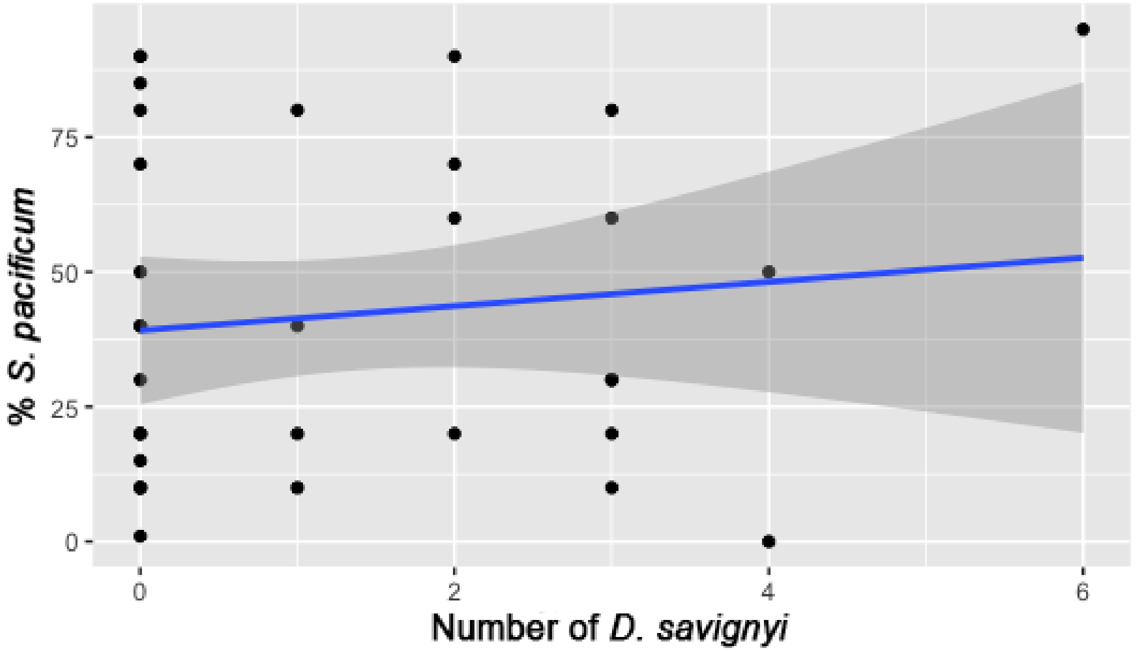

Figure 4. Correlation between percent *Sargassum pacificum* cover and the number of *Diadema savignyi* present on coral bommies during the survey (r2 = 0.014, p-value = 0.49). The gray area represents the 95% confidence interval of which the regression line could fall.

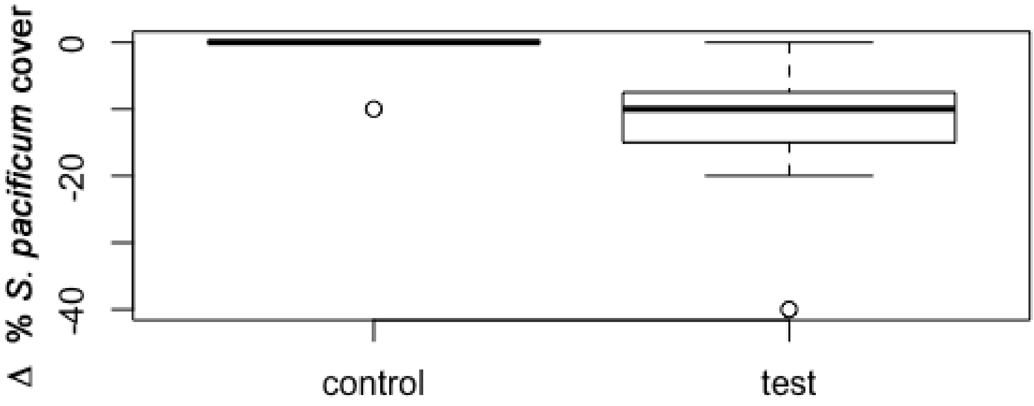

Figure 5. Box plot comparing the change in percent cover of *S. pacificum* of the control and test groups. Control and test group are significantly different (Wilcoxon rank sum, W=34, p-value= 0.011).

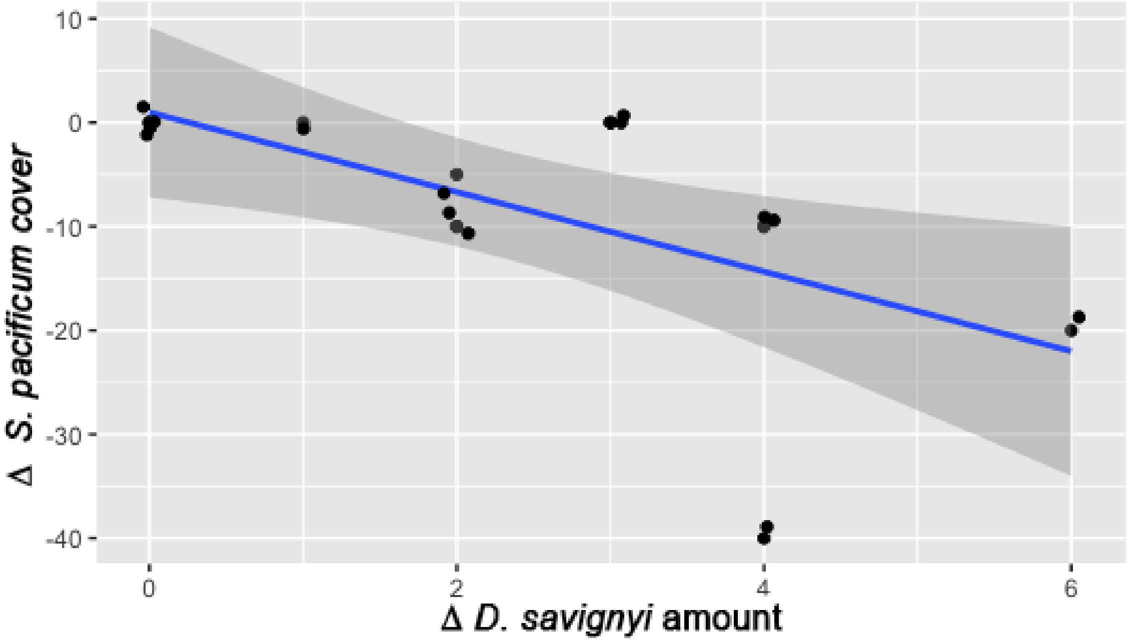

Figure 6. The correlation between the change in *S. pacificum* percent cover and the change in *D. savignyi* abundance on the coral bommies at the end of the experiment ($r^2 = 0.41$ p-value = 0.013). The gray area represents the 95% confidence interval of which the regression line could fall.

Figure 7. The red circle shows *Balistapus undulatus*, Orange-lined triggerfish, stuck in one of the test coral bommies. *Balistapus undulatus* has a large influence on urchin populations.