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Abstract

Abudefduf saxatilis, or sergeant major damselfish, are a common reef fish in the Caribbean and western Atlantic that form large feeding aggregations. *Abudefduf saxatilis* are primarily planktivorous, with zooplankton making up over 50% of their diet. Zooplankton are known to have diel movements to avoid predation, which have been shown to be triggered by the presence of ultra violet radiation. Beneath boats along the coast of Bonaire, aggregations of *A. saxatilis* have been observed, but why they prefer these areas over the open water column had not previously been examined. The abundance of zooplankton was estimated beneath boats as well as in the open water, up-current from the boats. Both the abundance and bite rates of *A. saxatilis* were also estimated beneath the boats that corresponded to the estimates of zooplankton abundance. In addition, the bite rates of *A. saxatilis* were estimated in the open water. It was found that the zooplankton abundance ($p < 0.001$) and the bite rate of *A. saxatilis* ($p < 0.001$) were both significantly greater beneath boats than in the open water. Also, a significant correlation was found between increasing abundances of zooplankton and *A. saxatilis* ($p < 0.01$). These results demonstrate that one of the main drivers for the aggregation of *A. saxatilis* beneath boats is likely to feed on the zooplankton, which are in high abundance. In turn, this could alter community structure on the reef due to a decrease in the amount of algae grazing by *A. saxatilis*.



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Introduction

Understanding fish behavior is a key aspect in understanding how a coral reef ecosystem works. A behavior seen in reef fish is aggregating, or gathering of fish into groups. There are three main reasons that fish species form aggregations: protection, spawning, and feeding. Aggregations formed for protection are commonly referred to as schools and most likely occur because they help reduce predation on the individuals in the schooling group (Brock and Riffenburgh 1960). Spawning aggregations occur when individuals of a species of fish gather in large groups in order to release their eggs and sperm into the water column to mate (Tucker et al. 1993). Some common species that form spawning aggregations include Nassau groupers (Tucker et al. 1993) and blue tangs (DeLoach and Humann 1999), as well as cubera, mutton, and dog snappers (Heyman and Kjerfve 2008). Feeding aggregations are less common among fish species, but some examples of these can be seen in some whale sharks (Heyman et al. 2001) and various species of surgeon fish, such as the blue tang (DeLoach and Humann 1999).

Off the western coast of Bonaire, under boats moored above the reef crest, large aggregations of *Abudefduf saxatilis*, also known as sergeant major damselfish, can be seen. *Abudefduf saxatilis* are damselfish that tends to spend the majority of its time in the water column from 0-20 m deep (Feitoza et al. 2003). *Abudefduf saxatilis* form large feeding aggregations of up to 150-200 individuals (Fishelson 1970), but there was no research as to why they would form large aggregations primarily in the shade found under boats rather than out in the open water column. One explanation for why *A. saxatilis* may prefer locations beneath boats could stem from its diet, which is mainly composed of zooplankton, plus a small portion of algae and fish and invertebrate larvae (Randall 1967). In fact, unlike other damselfish, *A. saxatilis* rarely take food from hard surfaces, but rather spend most of their time feeding in the water column (Fishelson 1970).

Behaviors exhibited by zooplankton could possibly be linked to why *A. saxatilis* are found primarily in the shade under boats. There has been a great deal of research done on the migration of zooplankton throughout the water column (Zaret and Suffern 1976;

Ohman et al. 1983; Forward 1988; Speekmann et al. 2000; Leech et al. 2005; Zengling et al. 2013). Zooplankton tend to change their depth in the water column depending on the time of day and exhibit what is referred to as diel vertical migration. It is believed that this behavior evolved so that zooplankton could avoid predators by moving deeper in the water column during the day (Zaret and Suffern 1976; Ohman et al. 1983). Multiple studies found that one of the main factors that determines the depth at which zooplankton can be found in the water column is the intensity of ultra violet, or UV, radiation (Forward 1988; Speekmann et al. 2000; Leech et al. 2005). Presence of UV radiation alerts the zooplankton that it is time to take part in their diel vertical migration in order to avoid the predators that forage for them during the day. Since the research has shown zooplankton tend to migrate vertically in the water column in the presence of UV radiation, it can be reasoned that it is also possible for them to migrate to shaded areas such as under boats (Zengling et al. 2013).

The purpose of this study was to determine whether there was a correlation between the abundance of zooplankton under the boats off the coast of Bonaire and the presence of *A. saxatilis*. The hypotheses were as follows:

H₁: Aggregations of *A. saxatilis* were more likely to be found in areas where zooplankton was abundant

H₂: During the day, zooplankton would be found in a higher concentration in shaded areas, such as beneath boats

This is an important topic to study for multiple reasons. First, there is very little to no research on this behavior that is exhibited by *A. saxatilis*. Most of what is known about *A. saxatilis* concerns their breeding habits, so this could provide an excellent opportunity to gain new insight into their behavior. Secondly, *A. saxatilis*, like other planktivorous fish, are a link between the energy produced by the plankton in the water column and the rest of the reef trophic structure, which makes them an important component of the reef ecosystem (Marcus and Boero 2003).

Materials and Methods

Study site

Data collection took place on the island of Bonaire in the Dutch Caribbean. The study site was the diving location known as Yellow Submarine (12°16'02.44"N, 68°28'19.74"W), which is located just north of the capital city of Kralendijk. The study site was a series of ten, approximately 10 m long boats that were moored approximately 40 m from shore above the reef crest. There was a space of approximately 10 m between each boat. The site has a large population of *A. saxatilis*, which can primarily be found beneath the boats, with a few individuals also found on mooring blocks guarding eggs and in the open water column above the reef crest. An average of 25 individuals was found beneath each boat. The site generally had very little current or wave action, but when a current is present it was typically moving from south to north, parallel to the shoreline.

Data collection

Three different data sets that were collected: zooplankton abundance, *A. saxatilis* abundance, and bite rate of *A. saxatilis*. The abundance of zooplankton was estimated under randomly selected boats throughout the study site and in the open water 5 m up current from each boat chosen. Of the ten boats located at the study site, five boats were tested twice, but never on the same day of data collection. The abundance and bite rate of *A. saxatilis* were estimated underneath boats that corresponded with zooplankton estimates. In addition, the bite rate of *A. saxatilis* was determined in locations in the open water, also corresponding with the sampling locations of zooplankton. Data collection always occurred around midday on days with minimal cloud cover.

Zooplankton was captured using a 20-micron plankton net with an opening that was 30 cm in diameter and a cod end that could hold 400 ml. A rope with a knot tied at 7 m was attached to the net to facilitate the collection of the sample.

Zooplankton collection: beneath boats

A research team of two individuals submerged to a depth of 2 m adjacent to one of the boats. At one end of the boat, one researcher held the net while the other swam, with the rope, along the bottom of the boat until they had reached a distance of 7 m from the net. The first researcher proceeded to release the net while the second quickly pulled it towards themselves with the rope. The opening of the net was quickly pinched off to prevent the escape of any zooplankton and the process was repeated so that there were two passes underneath each boat, equivalent to a 14 m pass. The opening of the net was once again quickly pinched off and the research team swam to the surface so that the sample could be placed into a labeled 100 ml sample bottle.

Zooplankton collection: open water

The team resubmerged 5 m up current from the location of the boat, above the reef crest. If there was no detectable current present, the research team would submerge on the south side of the boat. Once the team reached a depth of 2 m, they performed two more 7 m tows, parallel with the boat just sampled, with the same process as was performed beneath the boats. In total, 15 samples were taken from under the boats and 15 were taken from the open water, up current from the boats, over the course of the experiment.

Zooplankton in the laboratory

The collected samples were taken to the laboratory at CIEE Research Station Bonaire. Each sample bottle was shaken in order to ensure that any zooplankton that had settled to the bottom would be re-suspended in the water. A 30 ml portion of a sample was measured out and poured into a beaker. The zooplankton were euthanized using 5 ml of 95% ethanol. A 1 ml portion of the solution from the beaker was pipetted onto a petri dish with gridlines, which was then placed under a dissecting microscope. The number of zooplankton, which included copepods and the larvae of other arthropods, were observed and recorded. This was repeated nine additional times in order to create ten replicates for

each sample. The ten replicates were then averaged to give the estimated abundance of zooplankton per ml of the sample. This average was then entered into the following equation in order to determine the number of zooplankton per liter of sea water

$$\frac{\text{number of zooplankton}}{1 \text{ ml of solution}} \times \frac{35 \text{ ml of solution}}{30 \text{ ml of sample}} \times \frac{400 \text{ ml of sample}}{1 \text{ tow}} \times \frac{1 \text{ tow}}{\pi(0.15 \text{ m})^2 \times 14 \text{ m}} \times \frac{1 \text{ m}^3}{1000 \text{ L}}$$

Abundance of Abudefduf saxatilis

The abundance of *A. saxatilis* was determined through the use of photography. Before the collection of zooplankton occurred, a photo was taken with a Panasonic Lumix DMC-FT5 waterproof camera under the corresponding boat at a distance as to include all visible *A. saxatilis*. Each photo was later analyzed by visually counting to determine the total number of individuals present under each boat. The total sample size for the abundance of *A. saxatilis* beneath boats was 15.

Bite rate of Abudefduf saxatilis

The bite rate of *A. saxatilis* was quantified two separate ways. One researcher observed a single, random individual for one minute and recorded every time they would bite at the water. A bite was denoted by anytime that the mouth would open in such a way that the lips would quickly extend forward and return back to the starting position. The second researcher had the camera and would video a randomly selected individual for a minute. Each of the videos was then visually analyzed in order to determine the bite rate of that individual. The bite count obtained from each researcher was then averaged to give one number for under each boat and in each open water location. The sample size for underneath boats and in the open water was eight.

Data analysis

The mean zooplankton abundances from underneath the boats and in the open water were compared through the use of a Student's t-test. The data for the abundance of *A. saxatilis* was transformed using the equation $\text{Log}_{10}(x+1)$, where x denotes the number of *A. saxatilis*, in order to create a linear relationship rather than exponential. This relationship was then tested with a linear regression, with zooplankton abundance as the explanatory variable. The mean bite rates of *A. saxatilis* from underneath boats and in the open water were compared using a Student's t-test. The relationship between the bite rate of *A. saxatilis* and the abundance of zooplankton for both data sets were tested with a linear regression, with zooplankton abundance as the explanatory variable.

Results

Zooplankton abundance and location

For zooplankton abundance, there was a total of 30 samples taken, 15 from beneath the boats and 15 from the open water column 5 m up current from each boat. Zooplankton were more abundant under boats than in open water ($t=38.19$, $df=1$, $p<0.001$; Fig. 1).

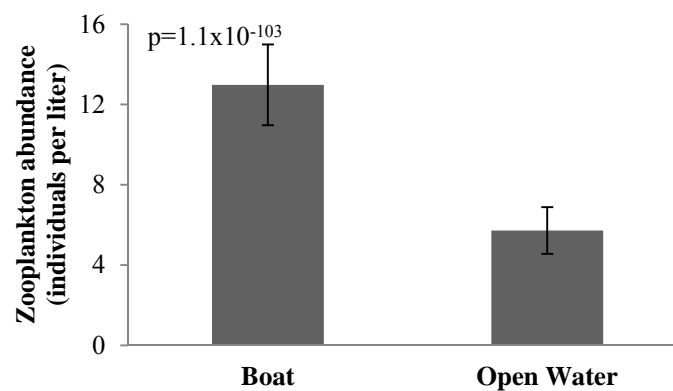


Fig. 1 Comparison of the mean zooplankton abundance, shown in individuals per liter, between two locations, beneath boats and in the open water column above the reef crest ($n=15$ per treatment). Error bars denote the standard deviation from the mean zooplankton abundance for each location.

Abudefduf saxatilis and zooplankton abundance

The number of *A. saxatilis* beneath each boat (n=15) was compared to the abundance of zooplankton and a significant correlation was found between a higher number of individual *A. saxatilis* and a higher zooplankton abundance ($R^2=0.5609$, $df=14$, $p<0.01$; Fig. 2).

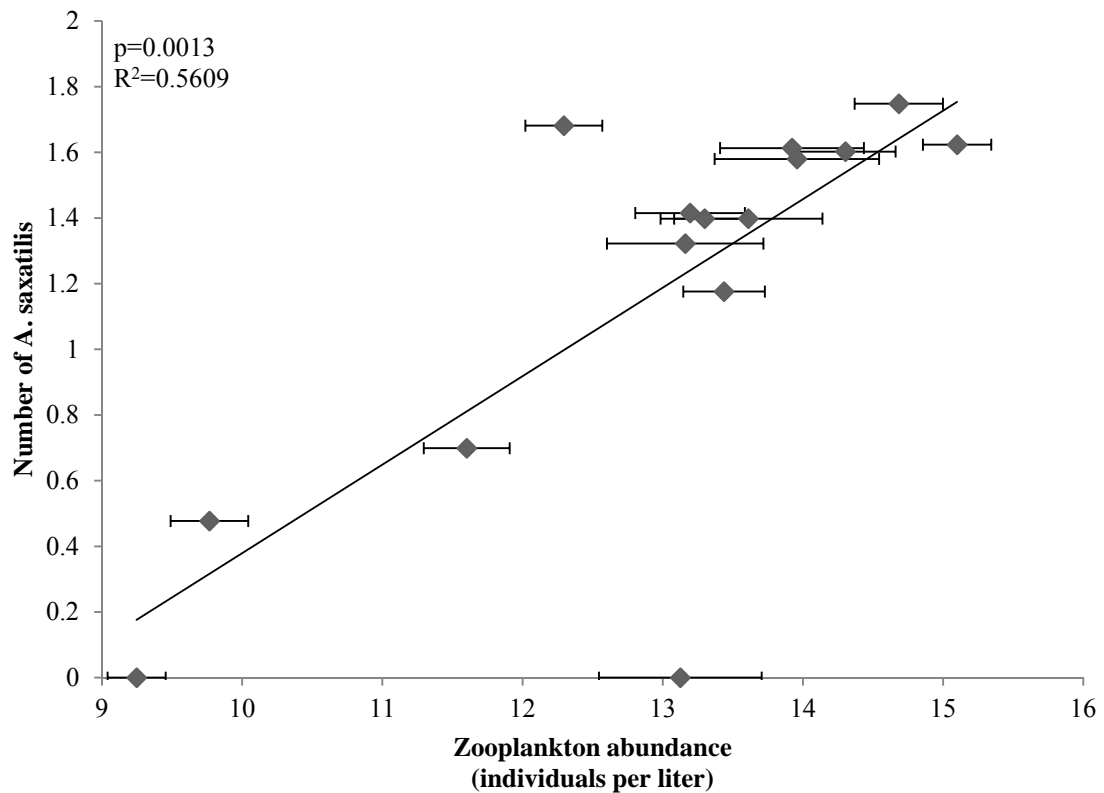


Fig. 2 Relationship between the number of individual *A. saxatilis* and mean zooplankton abundance, shown in individuals per liter, beneath each boat (n=15). The number of *A. saxatilis* has been entered into the equation $\text{Log}_{10}(x+1)$, where x is the number of individual *A. saxatilis*, in order to show a linear relationship rather than an exponential. The error bars denote the standard error from the mean zooplankton abundance beneath each boat.

Bite rate and location

For the bite rate of *A. saxatilis*, measured in bites per minute, there were a total of 16 samples taken; eight beneath the boats and eight from the open water column 5 m up current from each boat. Each sample was an average of two observations; one observation in the field and one video observation. The overall average bite rate of *A. saxatilis* in the open water was significantly less than beneath boats ($t=5.45$, $df=1$, $p<0.001$; Fig. 3).

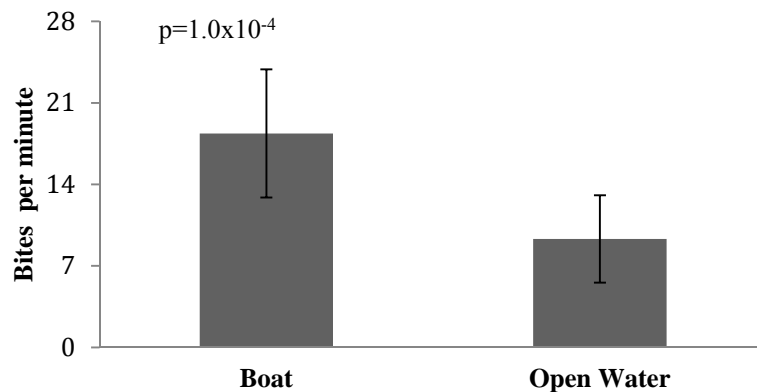


Fig. 3 Comparison of the mean bites per minute of *A. saxatilis* between two locations, beneath boats and in the open water column above the reef crest ($n=8$ per treatment). The error bars denote the standard deviation from the mean bite rate.

Bite rate and zooplankton abundance

The bite rate of *A. saxatilis* in open water ($n=8$) was compared with the abundance of zooplankton and no significant correlation was found ($R^2=9 \times 10^{-5}$, $df=7$, $p>0.05$; Fig. 4a). The bite rate of *A. saxatilis* beneath boats ($n=8$) was also compared to the zooplankton abundance and there was a significant correlation found between a higher number of bites and a higher abundance of zooplankton ($R^2=0.7565$, $df=7$, $p<0.05$; Fig. 4b).

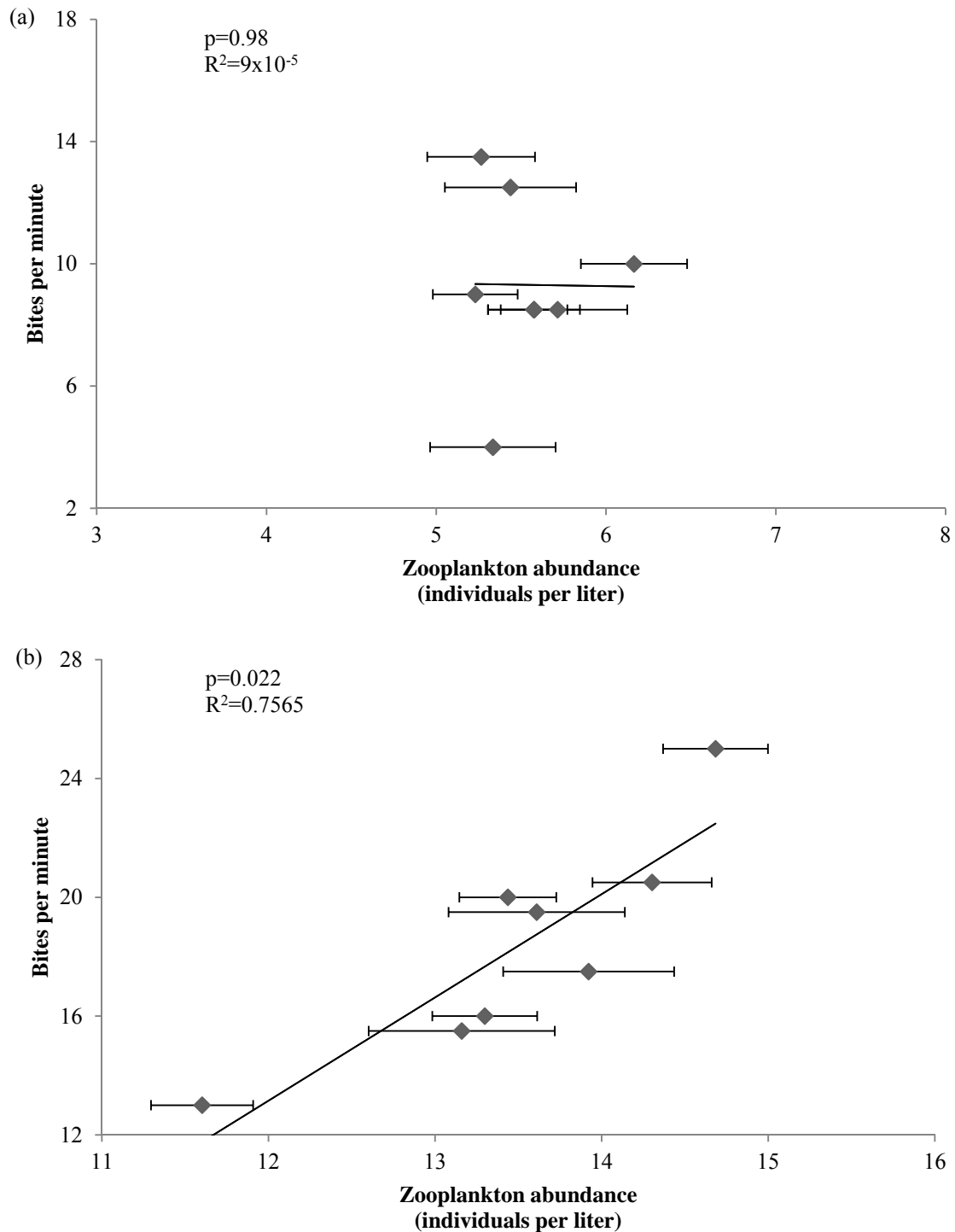


Fig. 4 Relationship between bites per minute of *A. saxatilis* and mean zooplankton abundance, shown in individuals per liter (a) shows this relationship in the open water column above the reef crest, (b) shows this relationship beneath boats. The scale for both for both bite rate and zooplankton abundance is different for (a) and (b) due to great differences in value in the data sets. The error bars denote the standard error from the mean zooplankton abundance beneath each boat.

Discussion

The results of this study showed all of the following relationships were significant: (1) the abundance of zooplankton was higher in the shaded areas beneath boats than in the open water 5 m up current (2) as the abundance of zooplankton increased under boats, the number of individual *A. saxatilis* increased exponentially (3) the bite rate of *A. saxatilis* was higher in the shaded areas beneath boats than in the open water (4) as the zooplankton abundance beneath boats increased, so did the bite rate of *A. saxatilis*. The only relationship that did not show any significant trend was that of zooplankton abundance compared to the bite rate of *A. saxatilis* in the open water, which was expected.

All of the results gained through this study supported the hypotheses that *A. saxatilis* aggregate in areas of high zooplankton abundance and during the day, a higher abundance of zooplankton is found in shaded areas, such as beneath boats. All of the previously stated relationships provide support for these hypotheses, including the relationship between the zooplankton abundance and bite rate of *A. saxatilis* in the open water. This is the case since this shows that the individuals in the open water have various levels of feeding not consistent with the abundance of zooplankton, but when the individuals are found beneath boats, they are there primarily for feeding purposes, and, therefore, have a bite rate proportional to the amount of zooplankton present.

The higher abundance of zooplankton in the low light conditions beneath boats is consistent with the findings of Zengling et al. (2013), that zooplankton exhibit a horizontal migration to shaded areas in conditions of high light intensity.

The results of this study show how large numbers of boats moored above a reef can change the ecosystem structure. The high abundance of zooplankton present beneath the boats provides a large food source for planktivorous fishes during the day. This large abundance of food provides the means for a population increase for species of planktivorous fish. This also means that species, such as *A. saxatilis*, that have more than one food source, would be eating less of their other food source since their primary source of food, zooplankton, is in high abundance. It could be expected that, since *A. saxatilis* is not feeding on algae as much as in a system without high zooplankton

abundance, the algae abundance on the reef would also likely see an increase when compared to an area with fewer boats. This means that the herbivores that would normally be in competition with *A. saxatilis* for algae would have less competition and there would also likely be a population increase within these species. Since there would likely be an increase in the population size among the lower trophic levels, of the reef ecosystem it can also be inferred that an increase could also be seen in the populations of piscivores. This would lead to an increase in the overall biomass of fish in the ecosystem.

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