Independent Research Projects
Tropical Marine Biology Class
Summer 2012, La Paz, México

Western Washington University
Universidad Autónoma de Baja California Sur

Title **pp**

Summer 2012 Class

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Effects of Human Activity, Light Levels and Weather Conditions on the Feeding Behaviors of *Pelecanus occidentalis* in Pichilingue Bay, Baja California Sur, Mexico

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Keywords: Pelecanus occidentalis, brown pelican, dive, prey capture, light level, human disturbance

Abstract

Evening foraging behavior of *Pelecanus occidentalis* in Pichilingue Bay was studied to determine the effects of weather conditions and human activity. The relationship between peak dive behavior, sunset time, and light levels was also studied. Data collection time took place within roughly a 35-40 minute time period spanning from about ten minutes before sunset to dusk. Dives were counted in one-minute intervals as well as light level recordings. Our studies show that there is in fact a significant influence from light levels past the time of sunset on this foraging behavior. Human activity (number of people and active boats) had no significant effect on the average number of dives per evening. Weather conditions (cloud cover, wind strength, and sea state) had no significant effect on the average number of dives per category.

Introduction

Pelecanus occidentalis (common name: Brown Pelican) is the smallest of the seven pelican species worldwide (Fish and Wildlife 2009). These seven species make up the family Pelecanidae, all of which are known for their totipalmate feet (four toes connected by a web), long bills and gular pouches (Schreiber 1982). *P. occidentalii* are primarily fish eaters, with Pacific coast populations relying largely on anchovies and sardines (Fish and Wildlife 2009). On the west coast these diving birds inhabit southern California through Mexico, Panama, Peru, Chile and the Galapagos Islands (Schreiber 1982). *P. occidentalis* is the only pelican species that plunge-dives for fish (Arnqvist 1992) as opposed to scooping fish from the surface. They are also the only species of pelican that lives and nests in marine environments (Schreiber 1982). These plunge dives act as a clear indicator of hunting attempts by *P. occidentalis* and can be easily counted from a short distance away. There is a small population of *P. occidentalii* that feed daily in an area closely offshore of Pichilingue Beach in Pichilingue Bay. They appear to feed at relatively the same time of day each evening shortly after sunset. By counting dives and observing interactions with surrounding activities, we plan to observe the effects of human activity, varying light levels, and weather conditions on the feeding behavior of *P. occidentalis* in Pichilingue Bay, in Baja, Mexico.

The *P. occidentalis* has faced and is still facing several human based threats to its reproductive success and overall survival. We are interested in these threats as they relate to the feeding behavior of *P. occidentalis.* The most prominent threats *P. occidentalis* now face result from increased human activity in nesting and feeding grounds (Schreiber 1982). Ongoing problems for this species involve their interactions with fishermen. Like other species of the Pelicanidae family, such as the *P. erythrorhynchos, P. occidentalis* can described as being an opportunistic feeder (Findholt & Anderson 1995). Being the only marine species of its family, the *P. occidentalis* have learned that fishermen will often toss excess food overboard from their catch and thus mill around fishing boats as fishing gear is being hauled in (Schreiber 1982). This tameness resultantly attracts *P. occidentalis* to crowded marinas and beach areas in which increased threatening human activity is more prominent.

We took several preliminary observations of our study site at Pichilingue Beach. We observed that on a day-to-day basis there were noticeable fluctuations in the intensity and amount of human activity. The *P. occidentalii* seemed to experience setbacks in their feeding behavior due to activity on the water, especially from motorized vessels. We realized that these fluctuations would allow us to observe differences in feeding behavior of *P. occidentalis* based on varying levels of human interference in the feeding site. We hypothesized that an increase in human activities in Pichilingue Bay, most notably recreational boating, will decrease the overall number of dives in that evening by *P. occidentalii.*

Considerable variation exists in the aerial diving process of *P. occidentalis,* probably accountable through interactions of several factors. Some of these factors that we will be focusing on include: wind conditions, water surface conditions; intensity of the light; depth and clarity of the water (Schreiber et al. 1975). During our preliminary observations of the diving behaviors of *P. occidentalis* in Pichilingue Bay we noticed several events that lead to questions regarding these diving behaviors and potentially influential factors. Our first observation was that a synchronous diving pattern seems to occur close to the same time every evening after sunset. This behavior led us to question the relationship between peak dive rates of the pelicans and the sunset. It is worth noting that the glare on the surface of the water is significantly reduced after sunset due to lack of direct sunlight. In a study on the behavior of birds during a total solar eclipse in Venezuela, frigate birds and pelicans were observed leaving their foraging grounds when the light level dropped enough to mimic sunset (Tramer 2000). We debated if the synchronous diving routine of these birds is triggered by the physical amount of light in the sky or by a certain time after sunset regardless of variations in light. We hypothesize that the feeding behavior is triggered by a certain amount of light in the sky irrespective of sunset time and that we will observe a peak dive rate of *P. occidentalis* during the same light level each evening in Pichilingue Bay.

Our third observation was that weather conditions change the amount of glare on the water and the visibility into the water. We thought it was logical to question whether or not the amount of cloud, wind, and level of sea state affected the peak dive time of the *P. occidentalis.* During preliminary observations we found that partial clouds increased glare on the water and prolonged the time in which bright light still displayed in the sky. We suspect that cloud presence possibly inhibits the birds' ability to spot prey. Therefore, we hypothesize that an increase cloud cover in Pichilingue Bay will shift the peak dive time of *P. occidentilus* farther past sunset than an evening with clear skies. After observing fluctuations in wind strength we hypothesize that an increase in the amount of wind will decrease the average number of dives for the entire data collection time. Additionally, we hypothesize that an increase in sea state intensity will decrease the average number of dives for the entire data collection time.

Methods

In this study we recorded the dive rates during synchronous feedings of *P. occidentalis* and potentially influential factors in Pichilingue Bay from Pichilingue Beach once a day during the evening from 20-28 July 2012. Our data collection time took place within roughly a 35-40 minute time period spanning from about ten minutes before sunset to dusk, based on preliminary observations of synchronous feedings. Each evening we recorded: the number of dives by *P. occidentalis* within a designated time interval, the corresponding light level during each interval, the amount of human activity on the beach and in the water, the sea state, wind condition and weather.

Study Site

Our selected study site, Pichilingue Beach, contained a large expanse of sandy intertidal and subtidal area, making it a popular site for human recreation. The openness of the area allowed for uninhibited viewing of hunting *P. occidentalis*. This also made it a practical site for us to observe the diving habits of these birds from a short distance away. The beach attracted a regular amount of human activity and hunting activity by *P. occidentalis* so it was a logical choice of study site to compare these two events. The popularity of the beach also allowed us to assume that our presence would not affect naturally occurring behavior of *P. occidentalis.*

Dive Counts

To record the number of dives by *P. occidentalis* in a way in which we could observe any trends or patterns over the course of the evening we structured a set of intervals in which we would count dives. We established two separate interval structures during the course of our data collection. We found it necessary to redesign our first recording scheme in order to more accurately and completely record all dives by *P. occidentalis*. In our first set up we followed a one-minute on, two minute off design, using a stopwatch, in which we recorded the number of dives for one minute and then waited for two minutes before recording dives again. Due to the erratic dive pattern of the *P. occidentalis* we found that in our two minute 'off' period we often missed a significant number of data. We therefore redesigned our interval pattern to include all occurring dives by counting them for one-minute intervals every minute for the whole duration of our data collection time.

Light Levels

To be able to compare the dive rates of *P. occidentalis* to the amount of light in the sky we had to have a way to record the light level at each dive interval. The light levels were recorded using a Canon

PowerShot A4000 IS 16.0 megapixel camera by setting a fixed aperture of F3.0 and recording the automatically selected shutter speed calculated by the camera for the amount of light currently in the sky. It was important to record our data from the same location each evening in order to keep our light level readings consistent. Readings were taken from the same patch of beach. The camera was placed facing parallel to the shoreline in a northward direction. The light levels were taken at the beginning of each dive interval.

Human Activity

We wanted to observe any possible correlation between the amount of human activity on the beach and in the water to the overall number of dives by *P. occidentalis* each evening. Upon arrival at our study site each evening we counted the number of disruptive vessels in the bay (jet skis, fishing boats, speedboats, etc.) and estimated the number of people on the beach and swimming in the water. We also noted the noise level on the beach, such as if the nearby restaurant was playing music or, on one occasion, a car alarm going off.

Weather Conditions

In questioning of the effects of weather conditions on the dive rates of *P. occidentalis* we recorded our immediate observations upon arrival at the study site. Sea state was described categorically as ‘flat’, ‘ripples’ and ‘small wavelets’. We assigned ‘calm’ to conditions with flat water to small ripples. ‘Low chop’ was assigned to conditions with small waves with no visible whitecapts. We assigned ‘moderate cop/swell’ to conditions with moderate waves.. The wind conditions were measured on a similar scale including ‘none’, ‘light wind, ‘moderate wind’, and ‘strong wind’. We noted the presence and amount of cloud or haze each evening. We categorically described cloud cover as ‘clear’, ‘hazy’, ‘partly cloudy’, and ‘mostly cloudy’. ‘Clear’ designated days with no clouds or haze present in the sky. ‘Hazy’ described days with indistinctly bordered transparent wisps in the sky. ‘Partly cloudy’ designated days with distinctly bordered opaque cloud formations covering less than half the sky. ‘Mostly cloudy’ designated days with distinctly bordered opaque cloud formations covering at least half of the sky.

Data Analysis

We compared our observed dive rates each evening to each of five potentially influential factors, which include: time of sunset, light level, number of people, number of active boats, sea state, wind conditions, and cloud cover. Peak dive time of sunset and light level were found by calculating the range and mean of the highest 10% of dive counts measured in a one-minute interval. Number of people and number of active boats were each compared to number of dives by a linear regression. An analysis of variance (ANOVA) was calculated for each comparison to determine significance. Sea state, wind conditions, and cloud cover were compared to number of dives by bar graphs with means dives by category. Standard deviation was used to test for significance.

Results

We found that *P. occidentalis* have time and light level windows in which foraging attempts were at their peak. Human activity, measured by number of people at Pichilingue Beach and number of active boats were found to have no significant effect on average number of dives per evening.

We observed *P. occidentalis* in Pichilingue Bay scout by circling above the bay in search of a sufficient food source or wait in the water. When prey was spotted the birds would dive from the air into the water. If successful, the diving individual would return to the surface with a fish in the pouch of its beak, however hunting success was not of interest in this study. There were two styles of dives we observed, neither of which appeared to be significantly advantageous over the other. One style occurred when an individual would hover five to fifteen feet up in the air targeting its prey, then dive straight down into the water, becoming fully submerged. The second style occurred when an individual spotted prey on the surface from a floating position. The individual would then take flight for ten to fifteen feet, barely leaving the water. The individual would then dive into the water at a shallow angle, sometimes spinning 180 degrees. This technique never fully submerged the individual, however it was distinguished from a water landing by a large splash of water.

The number of people present at the beach was found to have no effect on the average number of dives per evening (Table 1). The linear regression gave a slope of -0.08293 and an intercept of 9.53552 (Fig. 1) with a decrease of 82.37% in average number of dives per evening from 30 to 100 people.

The number of active boats in the bay was found to have no effect on the average number of dives per evening. The linear regression gave a slope of 0.1646 and an intercept of 4.0028 (Fig. 2) with a 14.12% increase in average number of dives per evening from 0 to 4 active boats.

The top 10% of dive counts per one-minute interval in relation to time from sunset was found to be between 11 and 24 minutes after sunset (Fig. 3). The average time after sunset for the top 10% of dive counts was 17 ± 4.0 s (mean ± SD). The maximum dives counted in a one-minute interval were 36.

The top 10% of dive counts per one-minute interval in relation to light level was found to be between shutter speeds of 0.01250 and 0.16667 seconds (Fig. 4). The average light level for the top 10% of dive counts was found to be 0.05272 ± 0.04162 s (mean ± SD). Recorded shutter speeds were within a range between 0.001 to 1.0 s and the average light level recorded at sunset was 0.00267 ± 0.00052083 s (mean ± SD).

Cloud cover had no significant effect on the average number of dives per evening (Fig. 5). Clear conditions were found to have an average dive count of 5.9 ± 5.6 (mean ± SD). Hazy conditions had an average dive count of 0 with a standard deviation of 0. Partly cloudy conditions had an average dive count of 5.1 ± 8.0 (mean ± SD). Mostly cloudy conditions were found to have an average dive count of 3.8 ± 5.5 (mean ± SD).

Wind strength had no significant effect on the average number of dives per evening (Fig. 6). Conditions with no wind were found to have an average dive count of 0.11 ± 0.38 (mean ± SD). Light wind condition had an average dive count of 4.9 ± 6.7 (mean ± SD). Moderate wind conditions had an average dive count of 9.06 ± 9.7 (mean ± SD). Strong wind conditions were found to have an average dive count of 0 ± 0 (mean ± SD).

Sea state had no significant effect on the average number of dives per evening (Fig. 7). Flat conditions had an average dive count of 2.8 ± 5.1 (mean ± SD). Rippled conditions had an average dive count of 5.4 ± 7.3 (mean ± SD). Small wavelet conditions had an average dive count of 10 ± 11 (mean ± SD).

Discussion

Our main discovery from this study was that there is a correlation between the light levels after sunset and the peak number of dives by *P. occidentalis.* As our results indicate (Figure 4), there is a window of time in which the pelicans appear to prefer as their optimum feeding time. This peak diving behavior is not immediately cued by the moment of sunset but occurs roughly 15-20 minutes after the time of sunset (Figure 3). There is also a range of light levels in which peak diving behavior was observed. From these results we can confidently fail to reject our hypothesis that the average dive count of *P. occidentalis* is directly affected by the level of light in the sky irrelevant to sunset time. Similar to a study done on foraging areas and feeding habitat selections of *P. erythrorhynchos* by (Findholt, Anderson 1995), our study was conducted in a relatively short amount of time. Therefore, due to the plasticity in resource use of *P. occidentalis*, our results caution against conclusions made from data gathered only during one week of the year.

We cannot confidently give reason for why *P. occidentalis* prefers this certain window of light for optimum feeding, however we have several ideas based on previous studies. (Shreiber 1982) suggests that the aerial diving processes of *P. occidentalis* are affected by several factors, including intensity of light and weather conditions. We noticed that as more time elapsed after the time of sunset the glare on the water surface became less prominent and thus allowed for higher visibility into the water. We suspect that *P. occidentalis* waited until glare from the sun was low enough to maximize prey capture. We also observed that the presence of cloud cover prolonged the time during which prominent glare was present on the water surface.

In a study on bird behavior during a total solar eclipse in Venezuela by (Tramer 2000), the local villagers commented that the frigate birds and pelicans were behaving as they normally did at sunset while the eclipse was taking place. It was also observed that after the solar eclipse ended these same birds returned to the feeding grounds in the bay and resumed foraging (Tramer 2000). This study supports our suspicions that the feeding behavior of *P. occidentalis* is triggered by the amount of light rather than the time of day.

As our results indicate (Figure 5) there was no significant difference found between the amount of cloud cover and the average number of dives. Currently this data leads us to reject our hypothesis that increased cloud cover decreases the average number of dives per evening. However, due to our small sample size we caution against drawing conclusions on the effects of cloud cover on feeding behavior of *P. occidentalis.* Therefore, we cannot currently infer that the prolonged periods of glare created by cloud cover affects the dive counts of *P. occidentalis.*

We found that wind strength had no significant effect on the average number of dives and therefore we reject our hypothesis that decreased amounts of wind increase the average number of dives by *P. occidentalis*. We also found that sea state conditions had no significant effect on the average number of dives and therefore we reject our hypothesis that increased intensity of sea state decreases the average number of dives. Similar to our analysis of cloud cover, wind strength and sea state both had small sample sizes. Therefore, we caution against drawing conclusions on the affect of *P. occidentalis* diving behavior based on these weather conditions.

As our results indicate, we cannot make a confident correlation between increased human activity on and around the study site and the average number of dives in an evening (Figure 1). We therefore we forced to reject our hypothesis that increased human activity would decrease the number of dives in an evening. Similarly, our comparison between the number of boats in Pichilingue Bay and the average number of dives in an evening showed no convincing upward or downward sloping trend (Figure 2). However, it is worth noting that this study was conducted only on a population of *P. occidentalis* that regularly experience contact with humans due to the proximity of their selected feeding grounds to a frequently used beach area. A potentially important factor is that the Baja Ferry terminal is located relatively close to the feeding grounds in Pichilingue Bay and thus a large amount of human traffic is frequently present in the area.

Though we accounted for all active boats and motorized vessels in the bay, we noticed that *P. occidentalis* responded to their presence only when direct interference occurred. We observed several jet skiers on separate occasions making sport of driving directly through flocks of floating *P. occidentalis*. This was the only type of confrontation from which *P. occidentalis* was persuaded to leave their feeding grounds.

In the future we suggest that studies on the diving behavior of *P. occidentalis* should include multiple populations in different locations in order to compare differences in human activity levels and weather conditions. It would be helpful to have a control population that has little to no exposure to human activity. The surrounding landscape in Pichilingue Bay affects the apparent time of sunset and therefore different locations will have different apparent sunset times. These apparent sunset times may influence differences in diving behavior. We also would like to see studies conducted around the feeding behavior of *P. occidentalis* during sunrise light levels as opposed to sunset light levels to see if there are similar behavior patterns.

In summary, our research contributes to the existing knowledge on the foraging behaviors of *P. occidentalis*. Various conditions can affect the aerial diving processes. Our studies show that there is in fact a significant influence from light levels past the time of sunset on this foraging behavior. However, these diving processes were not instantaneously cued by the actual sunset. The peak dive counts occurred in a certain range of light levels well after the exact time of sunset.

Acknowledgements

We would like to thank Benjamin Miner, Deborah Donovan, and Sergio Flores for their guidance and support. Cameron Donegan for the use of her camera. Shauna Roger-Durham for assistance in data collection.

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Tables

Table 1. Linear regression and ANOVA test results for relationships between average number of dives per day and number of people at the study site and number of active boats in the bay. Average number of dives per day was found to have no relationship (p>0.05) to the number of people or number of active boats present.

	Linear Regression			ANOVA
	Slope	Intercept	Percent Change	P-value
Number of People	-0.08293	9.53552	-82.37%	0.06314
Number of Boats	0.1646	4.0028	14.12%	0.8555

Figure Legends

Figure 1- The relationship between the number of people on Pichilingue beach in a single evening and the corresponding average number of dives by *P. occidentalis* for that evening.

Figure 2- The effect of the number of active boats present in Pichilingue Bay on the average number of dives by *P. occidentalis*.

Figure 3- This figure depicts the overall number of dives of *P. occidentalis* in each timed interval as they correspond to the time past sunset in Pichilingue Bay.

Figure 4- The overall number of dives by *P. occidentalis* per timed interval in relation to varying light level. In this figure the smaller shutter speed values indicate a greater amount of light. The average light level at sunset was 0.00267 s.

Figure 5- Average number of dives performed with different levels of cloud cover.

Figure 6- Average number of dives performed in different wind strengths.

Figure 7- Average dives performed in different conditions of sea state.

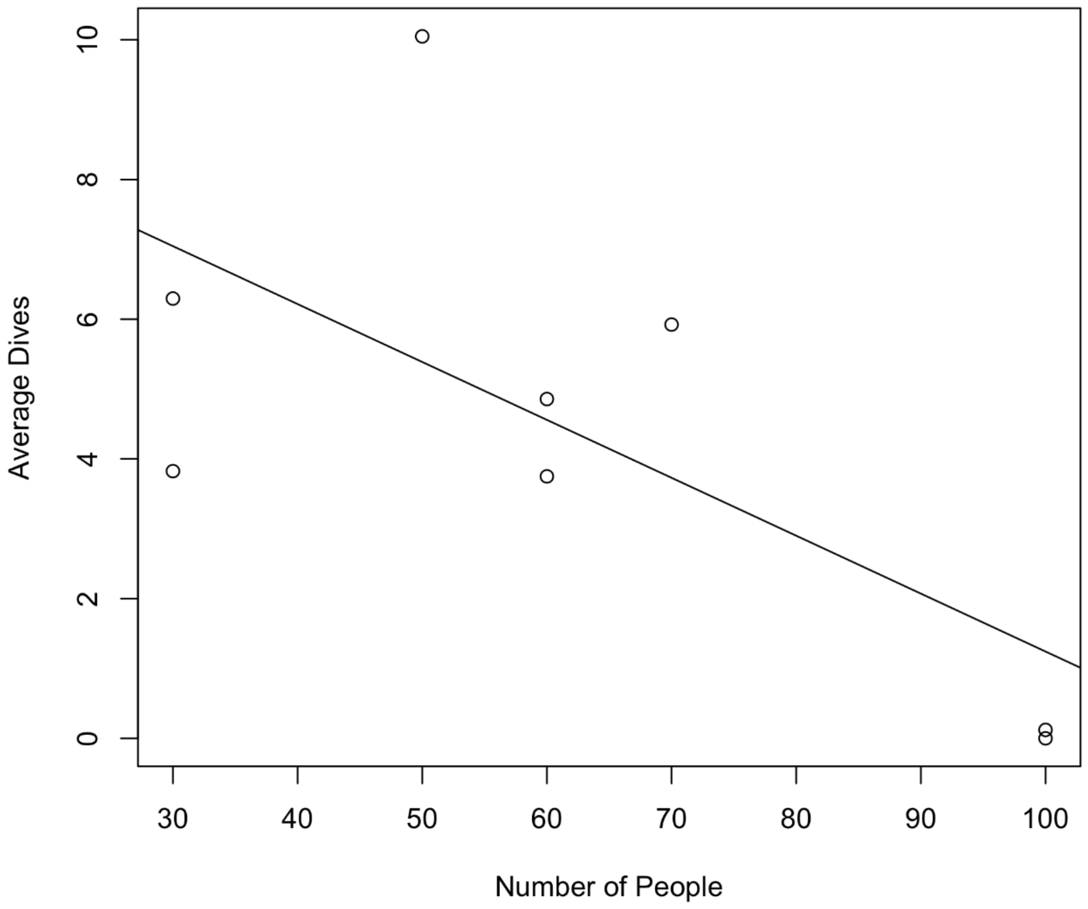

Average Dives
0
2
4
6
8
10
30
40
50
60
70
80
90
100
Number of People

Figure 1

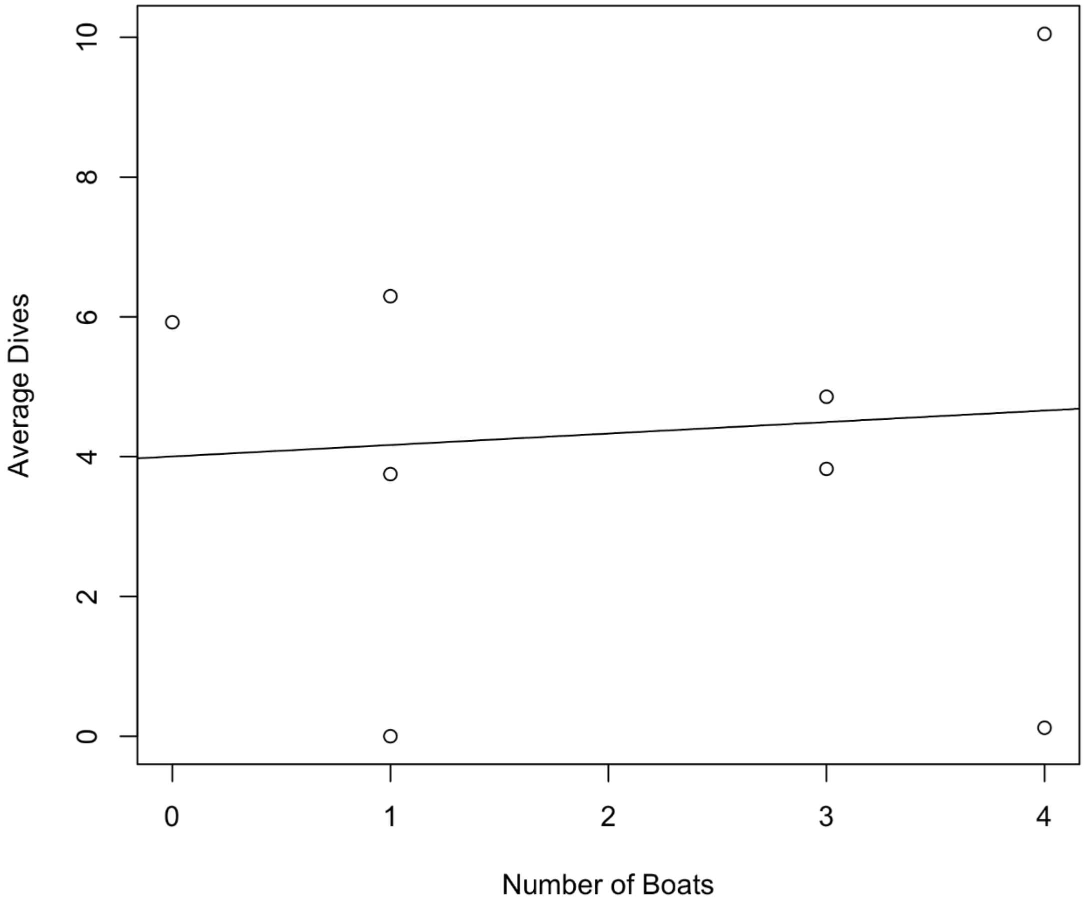


Figure 2

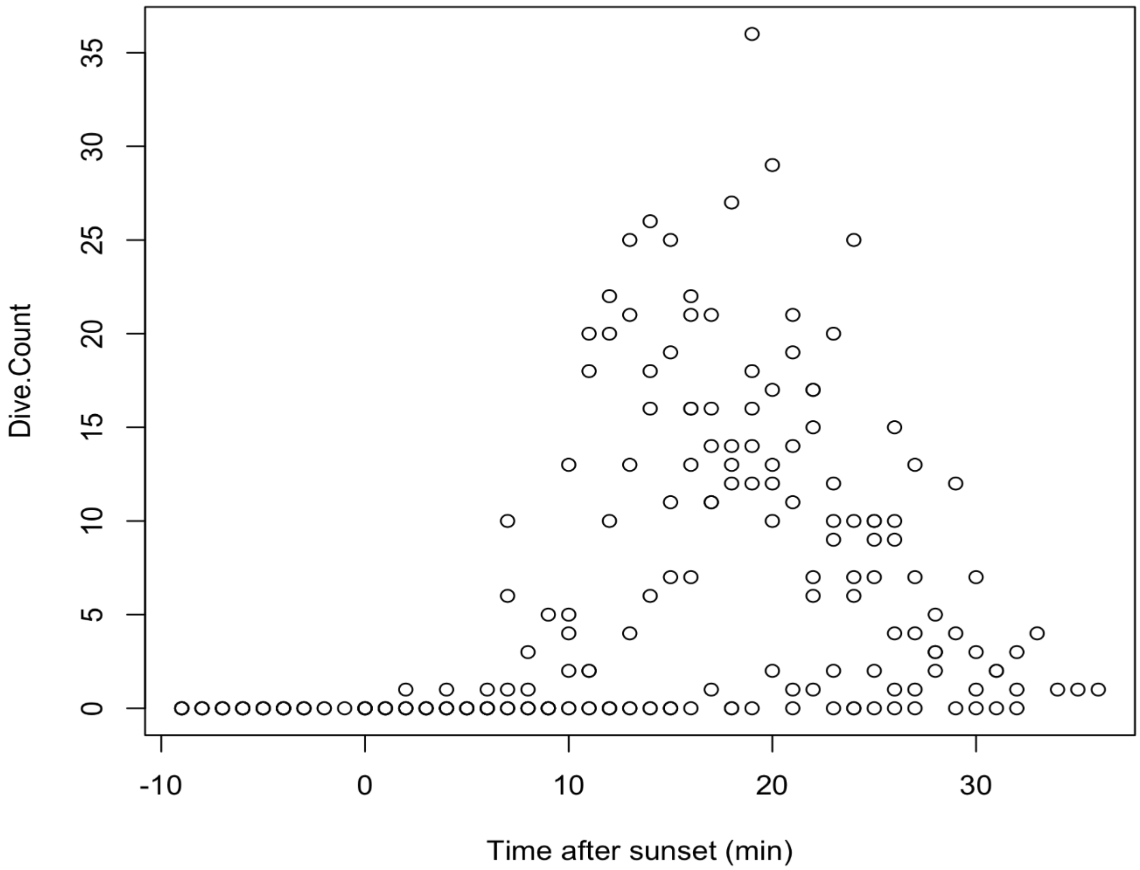
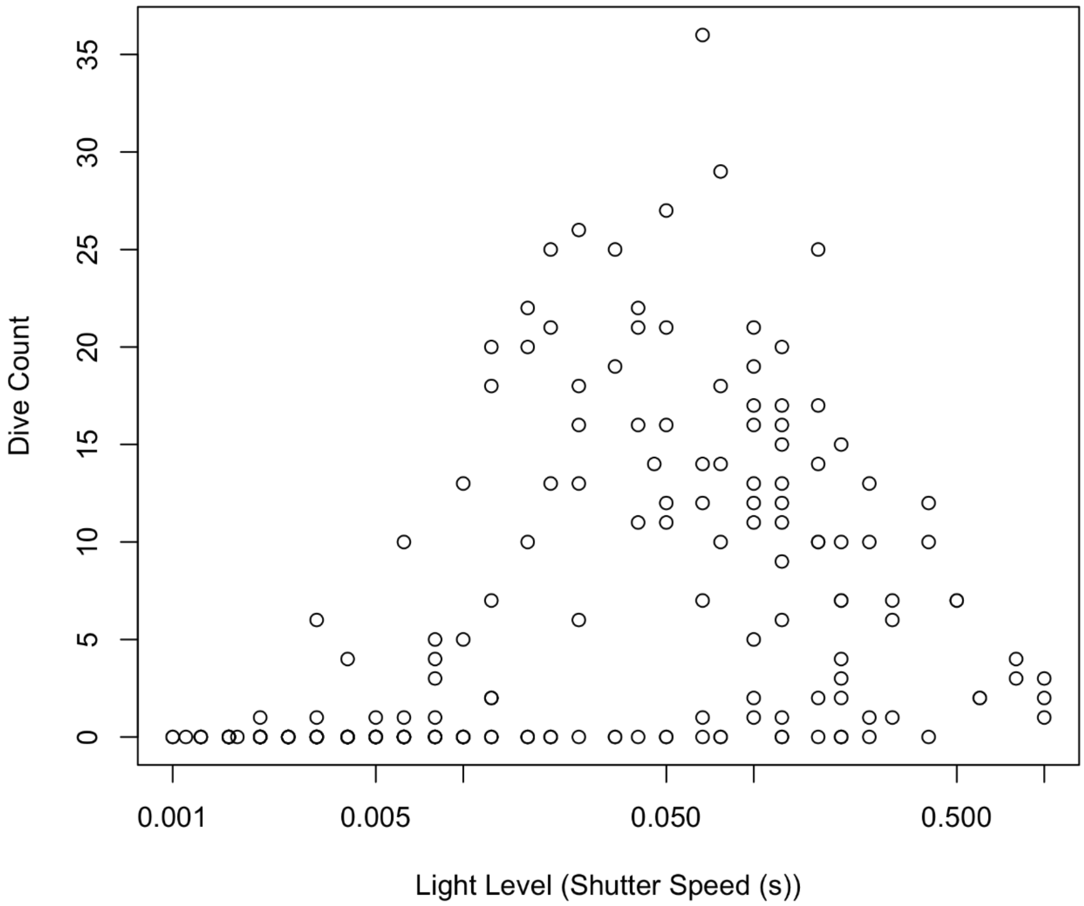

Dive.Count
0
5
10
15
20
25
30
35
-10
0
10
20
30
Time after sunset (min)

Figure 3



Dive Count
0
5
10
15
20
25
30
35
0.001
0.005
0.050
0.500
Light Level (Shutter Speed (s))

Figure 4

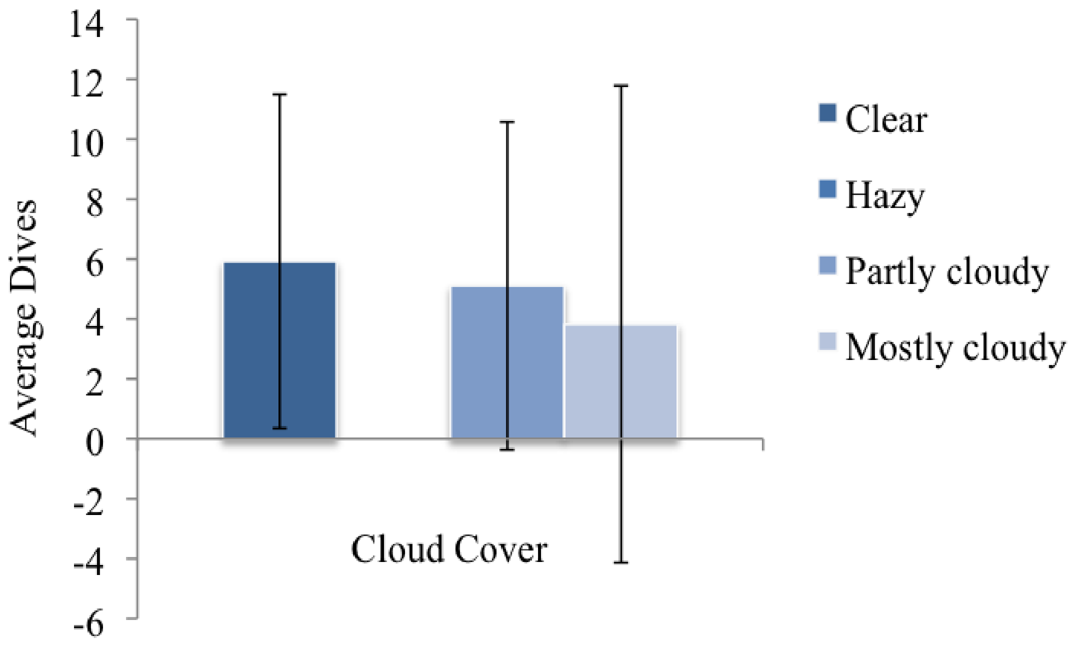


Figure 5. Average number of dives performed with different levels of cloud cover.

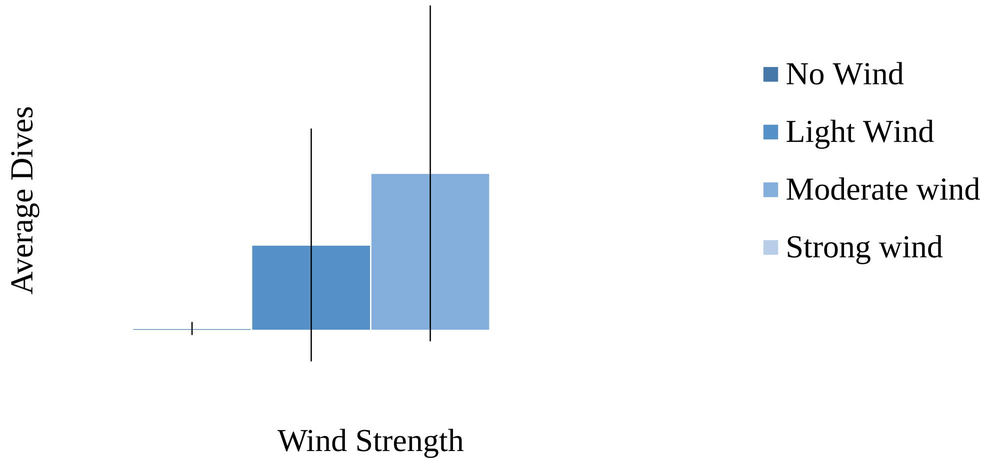

Average Dives
Wind Strength
No Wind
Light Wind
Moderate wind
Strong wind

Figure 6.

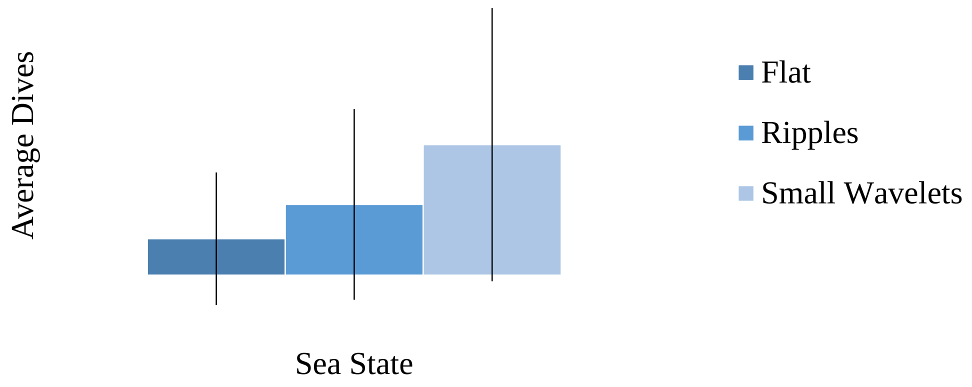


Figure 7

Predation of the brittle star *Ophiocoma alexandri* in the La Paz region, Baja California Sur, Mexico

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Keywords: predation, brittle stars, Ophiuridea, asteriod, chemodetection

abstract

The intent of this study was to evaluate whether three potential predatory sea stars *Heliaster kubiniji*, *Nidorellia armata*, and *Phataria unifascialis* are predators of the brittle star *Ophiocoma alexandri* found in the region surrounding La Paz, Mexico. Previous studies have been conducted on the predatory interactions between sea stars (*Astroidea*) and brittle stars (*Ophiuroidea*). To measure the response of *O. alexandri* to these three potential predators we prepared a sea table with concentric circles marked 10, 20 and 30 centimeters from the center with headings marked in 22.5◦ increments along these circles. A brittle star individual was placed in the middle of the sea table with one potential predator in a corner of the sea table. Once the test individual was placed in the center of the sea table a timer was started and the time, direction and heading of movement recorded. The predator direction was always set at 0°, at each of the circles and the time was stopped when the brittle star reached the 30 centimeter mark on the circle. Additional field observations and measurements were conducted on arm length to assess the degree of predation that *O. alexandri* experience. We found no significant difference in the directional responses of *O. alexandri* when exposed to the three asteroids. This indicates

that *H. kubiniji, N. armata* and *P. unifascialis* are not predators of *O. alexandri*. Of the 53 individuals examined for arm and oral disc length 47% were in the process of regenerating one or more arms indicating that the population studied experiences predation.

introduction

Brittle stars (Ophiuriodea) experience predation from organisms such as fish, crustaceans, and larger sea stars (Lindstrom 1999; McClintok 2008). As a result they have developed anti-predatory: morphologies, camouflage, habitat choices, and behaviors including nocturnal activity and negative phototaxicity (Majer et al. 2009). Brittle stars avoid predation using passive or active behaviors including secreting chemicals, fleeing, or fighting (Pomory 2001).

Brittle stars can shed their arms when stressed or attacked by predators, through a process known as autotomization (Stohr et al. 2012). After autotomization brittle stars are able to regenerate appendages (Wilkie 2001). The energetic cost of regeneration is high, and growth of the appendage requires energy for both the increase in length and the differentiation of cells into structures such as spines and podia (Bowen and Keegan 1983; Sköld et al. 1994; Dupont and Thorndyke 2006). This process often induces the production of chemical damage-released alarm signals in brittle stars as a result of physical injury or tactile sensation of a predator (Majer et al. 2009).

An evolutionary adaptation common in the phylum Echinodermata includes the ability to detect small chemical changes within their environment (Sloan and Campbell 1982; Drolet and Himmelman 2004). Chemodetection allows echinoderms to avoid predators and detect prey. Chemical assessments of the water column are generally conducted by the tube feet of sea stars and brittle stars, and allow predator sea stars to increase their degree of orientation toward prey with increasing accuracy in relation to prey distance (Himmelman and Drolet 2004). Brittle stars are thought to have the highest chemoreception potential within Echinodermata, both in variability and distance from the source, due to the wide varieties of predators that feed on them (Majer et al. 2009). Studies on the predatory sea star *Asteria vulgaris* have shown that their strong sensitivity to biomolecules within the water enables them to locate prey when it is at low densities (Himmelman and Drolet 2004).

Growing interest in the predatory actions of sea stars on brittle stars began after flight behavior and alarm responses were exhibited for the first time in *Pycnopodia helianthoides*, the predatory sun star, and their brittle star prey through the use of time-lapse videography (McClintock et al. 2008). As *P. helianthoides* feeds upon Ophiuriods in the Antarctic, these brittle stars releases a water-born chemical signal. This signal causes individuals to move away from fragments of conspecifics including tissues of tube feet, pyloric caeca, and body walls of the oral disc (McClintock et al. 2008).

Current studies are focused on the predatory sea stars, *Labidaster annulatus*, *Displasterias bradti* and *Perknaster aurorae*, in an attempt to analyze their interactions with brittle star prey (McClintock 2008). While interest in these predatory interactions continues to grow, sea stars from temperate environments have received the most attention, leaving many unanswered questions about these interactions in polar and tropical regions (McClintock 2008). We chose to research the behavioral interactions between multiple asteroid potential predators, the Gulf sun star (*Heliaster kubiniji*), the chocolate chip sea star (*Nidorellia armata*), and the pyramid sea star (*Phataria unifascialis*), and their interactions with the brittle star *Ophiocoma alexandri*.

The brittle star *O. alexandri* is common in the La Paz area of Baja California Sur, Mexico. We have observed large numbers of individuals of this brittle star under rocks in shallow subtidal reefs and rocky substrata. Despite their large abundance and wide distributions little research has been conducted on this organism. Our study aims to elucidate the interactions between possible predator sea stars *H. kuiniji*, *N. armata*, *P. unifascialis*, with the brittle star *O. alexandri*. We hypothesize that *O. alexandri* will show a directional response by moving away from these potential predators. By measuring the direction and velocity that *O. alexendri* travels away from or towards these potential predators we will gain an understanding of both their behavioral responses to potential predators and their ecological importance within these reef ecosystems.

materials and methods

Our study examined the response of brittle stars to potential predators by placing a brittle star *O. alexandri* and potential predators *H. kubiniji*, *N. armata*, *P. unifascialis* in a sea table and recording the behavior of *O. alexandri*. Observations were made on the behavior of individual brittle stars with individual predators, not between individual brittle stars of the same species. This is due in part to the fact that escape adaptations via chemical alarm signals have been observed in *Amphipholis squamata*, *Ophionereis reticultata* and *Ophiactic savyignyi*, suggesting that cross-species recognition of alarm cues is an evolutionarily favorable adaptation and behavioral response to predation (Majer et al. 2009).

Ten *O. alexandri* individuals were collected from the intertidal and subtidal zone near Club Cantamar in La Paz, Baja California Sur, Mexico, after dusk during the last two weeks of July 2012. Different numbers of individuals of the predators *H. kubiniji*, *P. unifascialis*, and *N. armata* were also collected based on the availability of researchers to find them. A sea table was prepared with concentric circles marked at 10, 20 and 30 centimeters from the center. Headings were marked in 22.5◦ increments along the circles to determine the compass heading of the brittle stars movement.

A random number generator was used to determine the corner in which the predator was placed on the sea table. A randomly selected individual of *O. alexandri* was then placed in the center of the tank. The timer was started as soon as the researcher released *O. alexandri* within the center of the centric circles. The position of the predator was selected as the 0° direction, and time and direction of movement was recorded. Time was stopped when the brittle star reached the 30 centimeter mark. A total of ten trials were conducted with each predator. Water was changed between each trial, with new seawater taken directly from the ocean in order to remove traces of the predator and brittle star from the previous trial.

The diameter of the oral disc and the length of arms were also measured for each individual of *O. alexandri*, and any missing arms were noted. We made additional field observations to increase the number of replicates for our missing arms data to assess the degree of predation that the brittle stars experience.

We analyzed the data with a Rayleigh test using circular statistics. We plotted rose diagrams to show average directional movement of individuals.

results

The movement of *O. alexandri* when placed in a sea table with *H. kubiniji* did not show a significant trend in any direction (p-value = 0.6968) (Figure 1). The direction taken by *O. alexandri* when placed in a sea table with the *N. armata* was not significant in any direction (p-value = 0.6068) (Figure 2). When *O. alexandri* was placed in a sea table with *P. unifascialis* no significance was found in the direction of movement of the brittle star (p-value = 0.2558) (Figure

3). A significant difference was observed between the average velocities of the brittle star with *H. kubiniji* and *N. armata* (p-value = 0.03351) (Table 1). Predation of *O. alexandri* was observed in the field through quantifying the missing limbs (one or more missing appendage) of 53 individuals, and we found that 47% of the brittle stars examined in the field had evidence of predation.

discussion

We found that *O. alexandri* on average did not demonstrate directed movement from the three asteroid speciess examined in this study. It is possible that the three asteroids are not predators of *O. alexandri*, or that the brittle star is unable to chemically detect the presence of the asteroids. During our study none of the asteroid species exhibited predatory behaviors towards *O. alexandri*. Upon completion of our trials all species were placed in a holding tank to observe behavior outside of the sea table arena. No brittle stars were consumed during this observation, which leads us to believe that the three asteroids *H. kubiniji*, *N. armata*, and *P. unifascialis* are not predators of *O. alexandri*. The result of this study is interesting when compared to the South American sun star *Heliaster helianthus,* which has been known to feed on motile prey; such as the brittle stars *Ophiocoma pumila* or *Ophiocoma echinata* when stressed (Barahona and Navarrete 2010 ;Stöhr et al. 2012).

In our observation of *O. alexandri* we found that many individuals suffered from predation and were in the process of regenerating their arms or arm tips. The results of this study showed that *O. alexandri* does not move in a direction that is significantly different than the three asteroid species used. We would like to known what species would elicit a flight response for *O. alexandri* and is causing the predation observed within the population studied. Several studies have documented a variety of brittle star predators besides sea stars, and some of these may account for the brittle star predation that we found evidence for. Lindstrom (1999) found that high predation of the brittle star *O. echinata* by the bluehead wrasse (*Thalassoma bifasciatum)*, and the sergeant major damselfish (*Abudefduf saxatilis)*, which occur in Caribbean reef ecosystems. We have observed *Abudefduf troschelli* and *Thalassoma lucasanun* at Cantamar. These two species diverged during closing of the Isthmus of Panama and are closely related (Foster 1987, 1989). Aronson (1998) supports these findings, noting that the primary fish predators of Ophiuroids in Caribbean back-reef habitats are wrasses (*Labridae*) during the day and grunts (*Haemulidae*) and porcupinefish (*Diodontidae*) at night. Hendler et al. (1995) also discovered that brittle stars compose a significant portion of the diets in crabs, shrimps, and 33 species of Caribbean fish. These large amounts of predation on brittle stars and their ability to regenerate limbs led Pomoroy and Lawrence (2001) to calculate that *O. echinata*, a sister species of *O. alexandri*, provides the equivalent of 0.07% of energy supplied to higher trophic levels per day. This information implies that Ophiuroids have the potential to supply significant amounts of biomass to higher trophic levels due to their regenerative abilities (Lindstrom 1999). Therefore future studies should focus on the interaction between cortez rainbow wrasse (*Thalassoma lucasanum)*, and panamic sergeant major (*Abudefduf troschelli*), to determine if these tropical fish are predators of *O. alexandri* and further elucidate this brittle star's role in shallow reef ecosystems in the Gulf of California.

acknowledgements

We would like to thank Ben Miner, Sergio Flores, and Deb Donovan for their guidance and assistance with our study. Gavin Willis was incredibly helpful in data collection, data analysis, and editing. Lastly we would like to acknowledge the staff at Universidad Autonoma de Baja California Sur for their help in the lab.

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tables

Table 1. A comparison of the average velocity of *Ophiocoma alexandri* when exposed to the potential asteriod predators *Heliaster kubiniji*, *Nidorellia armat*, *Phataria unifasciali* (t-value / p-value)

	Heliaster kubiniji	*Nidorellia armata*	*Phataria unifascialis*
Heliaster kubiniji			
Nidorellia armata	2.2356 / 0.03351		
Phataria unifascialis	1.8262 / 0.08654	-0.0196 / 0.9846	

figures legends

Figure 1. The circular histogram of directional movement of *O. alexandri* when placed in a sea table with *H. kubiniji* (p-value = 0.6968).

Figure 2. The circular histogram of the average directional movement of *O. alexandri* when placed in a sea able with *N. armata* (p-value = 0.6068).

Figure 3. The circular histogram of the average directional movement of *O. alexandri* when placed in a sea table with *P. unifascialis* (p-value = 0.2558).

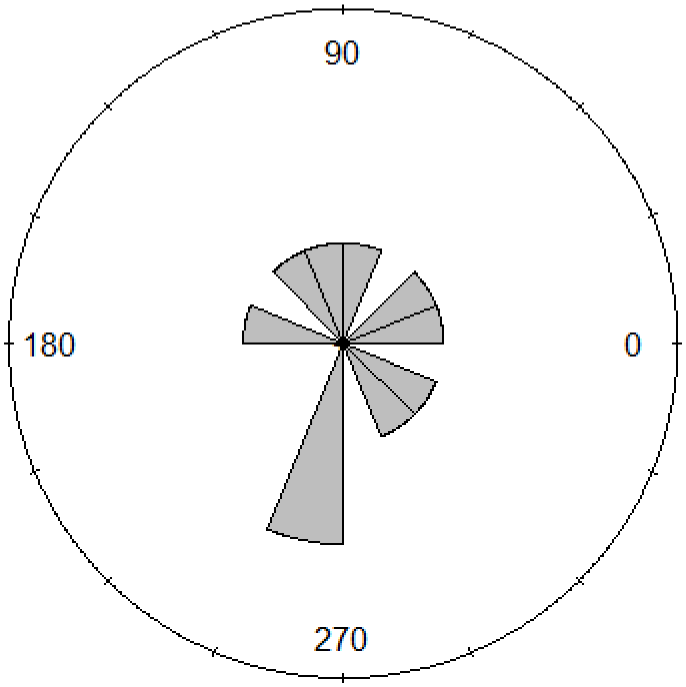

Figure 1

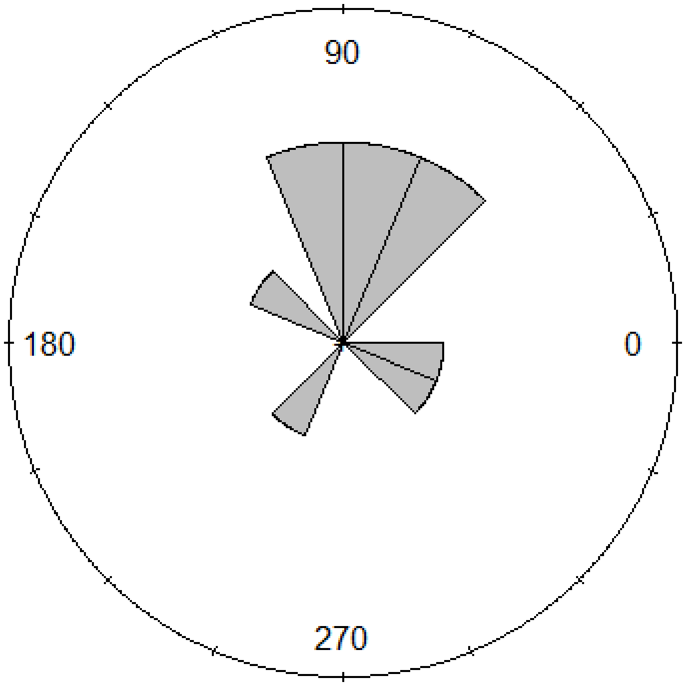

Figure 2

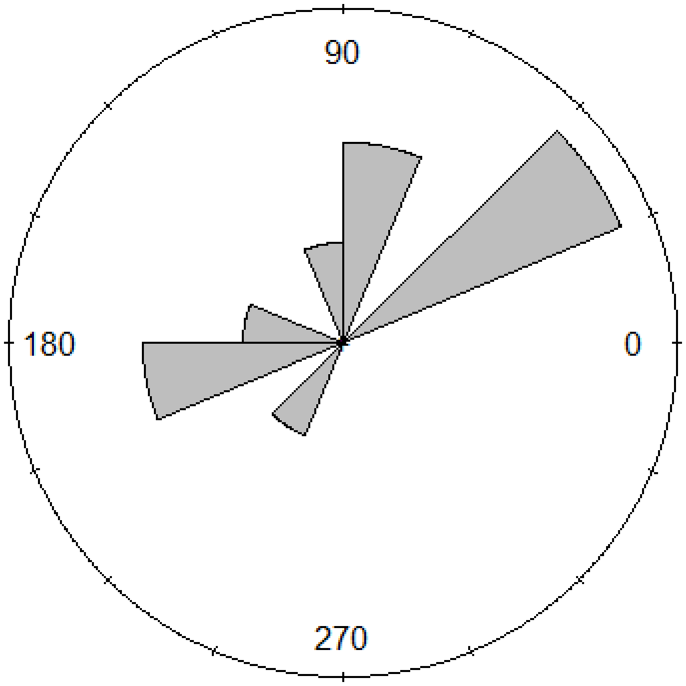

Figure 3

The effect of water temperature and disturbance on the movement of *Nerita scabricosta* in the intertidal zone

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2 August 2012

Keywords: *Nerita scabricosta*, intertidal, behavior, movement, disturbance, temperature.

Abstract

Stimuli that result in the movement of *Nerita scabricosta* were examined over a period of three days. *N. scabricosta* is a common littorine of the intertidal along the coast of Baja California Sur. The intertidal zone, an environment in constant flux, is even more stressful in the tropics due to increased salinity gradients, desiccation, intense solar radiation, and drastic temperature changes. As an extremely abundant species along the coast of Baja California, we were interested in the adaptability of *N. scabricosta* to these factors. We were specifically interested in a unique behavioral trend attributed to *N. scabricosta*, where rather than remaining submerged in the intertidal, snails retreat from the incoming tide. This response in a region where desiccation is extremely likely seems counter intuitive. To determine whether water temperature or level of water disturbance due to incoming tide stimulated snail movement, a fully crossed experiment was designed. The experiment was conducted by applying water via a ketchup bottle to randomly chosen quadrats in either a disturbed manner or gently. Our four treatments were cold/disturbed, cold/calm, warm/disturbed, warm/calm. We also ran trials where snails were randomly assigned either a treatment of splash or no splash and distance travelled after 3 minutes was recorded. We found no significance attributed to either temperature or disturbance as a catalyst for movement, but did find that the presence or absence of water was a factor in the stimulation toward movement. Future studies should focus on the causation of *N. scabricosta* hydrotaxic behavior including possible physiological reasons.

Introduction

The Sea of Cortez is composed of many habitats including pelagic, mangrove, estuary, subtidal, and intertidal ecosystems. Life for organisms in the in intertidal zone is an ever-changing scene of biotic and abiotic factors. Intertidal organisms of the Sea of Cortez not only have to deal with the normal hardships of life in this unique habitat, but also must deal with the added stress of living in the tropics. Moore (1972) examined communities of intertidal organisms at different latitudes, and found that communities located in the tropics were more physically stressed than those located in temperate waters. Intertidal organisms must adapt or acclimatize to such factors as solar radiation, changes in water temperature, desiccation, disturbance, salinity changes, and radical changes in the tidal cycle (Garrity 1984). The physiological and behavioral adaptations that have emerged to cope with the stressors of the intertidal are as different as the species who utilize them.

Snails are commonly studied as a model intertidal organism to determine how they handle the changing environment of the intertidal zone (Garrity 1984; Chapman 1999; Esqueda et al. 2000; Stafford et al. 2007; Vannini et al. 2008; Chapperon and Seuront 2011), and have proved to be extremely adept at coping with the stress of the intertidal. The color and shape of their shells have morphologically evolved to mitigate the effects of heat stress and reduce the need for evaporative cooling (Vermeij 1973; Heath 1975; Britton 1995). Snails have also physiologically adapted to desiccation by controlling their metabolic thermal regulation and increasing their tolerance to thermal and desiccation stressors (Bates and Hicks 2005). Behavioral adaptations to the intertidal include limited movement during stressful periods (Peckol et al. 1989, Lang et al. 1998), selection of favorable microhabitats such as shaded crevices and tidepools (Kensler 1967, Raffaelli and Hughes 1978), and production of mucus holdfasts (Bingham 1972). Snails also close their operculum to prevent evaporation during high temperatures (Garrity 1984).

One example of a snail that utilizes many of the strategies discussed above is *Nerita scabricosta*, an abundant mobile gastropod found throughout Pacific intertidal zones, including the Sea of Cortez (Esqueda 2000). As is true of many intertidal organisms, *N. scabricosta* has its own unique ways of dealing with this stressful environment. To avoid desiccation, these snails can hold water in their shells, which is also used as a cooling strategy. By allowing the water to trickle out of its shell, a snail can lower its body temperature through evaporative cooling (Garrity 1984). Another strategy utilized by *N. scabricosta* is aggregation around other snails and rock crevices with lower temperatures and higher moisture than the surrounding exposed rock during low tides (Esqueda 2000). This behavior also helps protect the snails from wave action and desiccation. *N. scabricosta* typically aggregate around the water line, as opposed to being fully immersed in the ocean or in tidepools (Cates and Donegan, *personal observation*). The snails maintain this position by moving upwards away from the rising tide when splashed by waves and down the rock towards wet surfaces when the tide goes out (Garrity 1984). This hydrotaxis, movement away from water, has been documented in other species of snails, such as *Cerithidea decollate* and high-shore littorinids (Stafford et al. 2007; Vannini et al. 2008). Without the strategies discussed above, *N. scabricosta* would not be able to maintain this behavior of remaining out of the water. Although it is a well documented behavior in many snails, the reasons behind hydrotaxis haven’t been explored extensively to determine the causation of this behavior.

In our study, we explored the behavioral movements of *N. scabriscosta* in a simulated experiment that mimicked incoming tides. Curious as to which factors catalyzed movement, we determined through observation and research that it was likely one of two factors: wave disturbance or temperature change due to the influx of cold water on sun-baked rocks (Garrity 1984; Esqueda 2000; Vannini et al. 2008). By looking at the factors that stimulate movement, we can get one step closer to understanding the causation of hydrotaxis. We hypothesized that the cooler water would stimulate their movement as opposed to disturbance from wave action. To answer our question, we performed field observations and manipulations of the environment by adding cool or warm water in a calm or disturbed manner to *N. scabricosta* covered rocks. We chose to carry out a field experiment because of the low likelihood of being able to accurately replicate the snails' natural habitat in lab. From previous research we thought it likely that uncontrolled variables such as sun exposure, dissimilar substrate, and stress would alter the natural response of snails to our experimental treatments (Chapman 1999).

Methods

To determine which factor or factors stimulate the movement of *N. scabricosta*, we studied their behaviors on the moderately exposed rocky intertidal along the coast of Club Cantamar in Pichilingue, BCS, Mexico. Trials were conducted during low tide over three consecutive days in the afternoon, from 24-26 July 2012. The Beaufort (Beaufort 1805), or wind intensity over the water, was taken each day upon arriving at our site of study to determine water disturbance. Time of day was also recorded prior to each treatment.

To determine whether water temperature or level of water disturbance affected snail movement, a fully crossed experiment was designed. Each of our trial treatments had two components; a component of temperature and a component of disturbance. Disturbance was either present or absent, and temperature was either warm or cold. For each trial, a 9x9 inch quadrat was used and one of four treatments was applied. The four treatments were cold and disturbed, cold and calm, warm and disturbed, and warm and calm. For cold treatments, seawater was taken directly from the ocean to fill spray bottles. Warm treatments were prepared by filling buckets with seawater and allowing them to heat up in the sun until a temperature of at least 88°F was reached, which was approximately 7°C warmer than the ocean water and noticeably warmer to the touch. The temperature of the water for both the warm and cold treatments was measured before trials began. For disturbed treatments, we attempted to mimic the conditions a snail would experience during incoming tide due to increased wave action. We did this by rapidly spraying the snails in the quadrat in short bursts from a squeeze bottle from an angle of 45-90° above the snails, with small breaks between sprays. Calm treatments were accomplished by gently squeezing the spray bottles so a steady, gentle stream of water covered the substrate surrounding the snails.

We ran 9 trials for each treatment over a span of three days, completing 3 trials for each treatment per day. Multiple metrics were recorded for each trial. Prior to applying treatments, we counted all the snails in the quadrat and measured the distance from the snail lowest on the intertidal to the water line. All treatments lasted 3 minutes, during which we observed the reactions of the snails and classified them into four categories. These categories were: no movement, extension of the cephalic tentacles, spinning or rocking, and movement of greater than 1cm away from starting location.

On 30 July, we tested snails' reaction to water was tested to determine if the addition of water alone stimulated movement. We randomly assigned 30 snails to either receive a splash of water, or to remain as they were. Study snails were marked by placing pebbles covered in blue tape next to them so they could be easily seen and their starting location was marked. Each pebble was labeled 1-30 so as to identify each snail and the treatment that they should receive. Once every snail was marked, we briefly squirted water on the snails assigned to the splash treatment from a squeeze bottle and then observed for 3 minutes. After three minutes, we measured how far away from the blue-taped rocks the snails had moved.

Over the three days of experimentation, we also observed the daily behavior of snails in undisturbed, randomly chosen quadrats. We monitored behavior during incoming and outgoing tide, as well as behavioral tendencies of snails in tide pools. In addition to these undisturbed observations, we were also interested in whether a large influx of cold water would affect the behavior of snails in relatively warm tidepools. We accomplished this by pouring a gallon of cold water into a previously chosen tidepool and recorded behavioral observations for 5 minutes.

To analyze our data, we ran an ANOVA statistical test in R to determine if reactions to different water application treatments were significantly different. We also ran a two sample t-test in Excel, and also calculated by hand a Chi-squared test, to determine if snail movement was significantly different between splash/no splash treatments.

Results

Significant differences between our treatments of cold/calm, cold/disturbed, warm/calm, and warm/disturbed were not found. To determine whether only temperature, only disturbance, or a combination of temperature and disturbance had an effect on snail behavior, we separated our treatments into three predictor variables: Temperature, Disturbance, and Disturbance*Temperature. We then categorized response variables: when a snail extended its cephalic tentacles (Tentacles), when a snail rotated (Spin), and when a snail moved greater than 1cm away from its starting location (Crawl). After running an ANOVA analysis on these variables, we found that the only significant trend occurred between Tentacles and Disturbance with a P value of 0.003 (Table 1).

Since temperature and disturbance had no significant effect other than the extension or retraction of tentacles, we tested to determine whether water, regardless of temperature, affected the behavior of *N. scabricosta*. We ran a two sample t-test, as well as a Chi-squared test on our splash/no splash data and both tests found a significant relationship between water presence and the likelihood of snails to move. The two sample t-test returned a significant a p-value of 0.02 (Table 2), and the Chi-squared test returned a significance of P value less than 0.025 (Table 3).

Discussion

Our hypothesis that *N. scabricosta* is stimulated to move in reaction to cold water was unsupported. However, we did find that snails have a higher propensity to movement when they encounter water, regardless of its temperature or the level of disturbance and method of application, than snails that were left dry.

The interaction between wetting the snails and movement leads us to question why the snails moved when they got wet. Snails are ectotherms that rely on their surrounding microhabitat to regulate body temperature (Chapperon and Seuront 2011). When splashed by cool ocean water, it is probable that body temperature is lowered to such a degree that snails might be required to move to a different microhabitat in order to maintain their optimal body temperature. Even when splashed by the warmer water used in our trials, snails may still undergo evaporative cooling to such an extent that their body temperature lowers significantly enough to demand a response. As a tropical snail species, *N. scabricosta* has evolved to tolerate the high ambient temperatures they are exposed to during low tide. Since they are so tolerant to high temperatures, it is likely that these snails are unable to cope with the influx of cool water that arrives with the incoming tide, as it will greatly alter their metabolic regulation. Other snails move away from the rising tide, such as *Cerithidea decollate*, which are mangrove-dwelling snails that aggregate on mangrove trunks above the water line as the tide rises (Vannini et al. 2008). They found that these snails were utilizing an isophasic strategy to relocate to microhabitats with more favorable conditions. *N. scabricosta* is likely performing a similar strategy to maintain its body temperature. Chapperon and Seuront (2011) found thermoregulatory behavior in *N. atramentosa* was flexible and suited many specific habitats. It is possible that the unique water evasion behavior we see in *N. scabricosta* is an example of behavioral flexibility.

We found during our observations that, when vigorously splashed, the snails did not move and rather seemed to cling more tightly to the rocks. During a disturbance event, the possibility of the snails being swept off the rocks was higher than when the water was gently splashing them. Since our disturbance trial mimicked increased wave action, the snails avoided the chance of being swept out to sea by anchoring themselves more securely to the rocks.

Their aggregation behavior around rocks and crevices is another method they may utilize to avoid being swept off rocks. Stafford et al. (2007) found this to be true of high-shore littorinids whose aggregation behavior was affected by wave action, as well as surrounding temperature. They hypothesized that the littorinids were performing hydrotaxis, but they also noted they could be moving along the shore for other reasons, including foraging for food. This latter hypothesis is unlikely to be true of *N. scabricosta* because once the snails reached dry substrate and had stopped moving after a splashing event, we observed them retract their cephalic tentacles and did not see the snails exhibit any foraging behavior. The link between the surrounding environment and cephalic tentacle protrusion is also demonstrated by our finding that during disturbed treatments, a significantly lower number of snails had their tentacles out. This finding also supports the hypothesis that during disturbance events, the snails focus primarily on anchoring themselves to the substrate rather than moving and increasing the risk of being swept away.

Although we now know that *N. scabricosta* are stimulated to move when they get wet, we still have many questions about the physiological processes and causation of this behavior. Future research could determine snail body temperature before and after splashing and see if there is a significant difference that may alter the organism's metabolic function, therefore motivating it to move. The physiological response of anchoring could also be studied by utilizing force meters to see how hard it is to move snails off the rocks pre, post, and during wave disturbance. These future possible experiments would lead to a greater understanding of *N. scabricosta*, which, because of its abundance and dominance in tropical habitats throughout the Baja Peninsula, could result in a better understanding of this ecosystem.

Acknowledgments

We would like to thank Dr. Deborah Donovan for first recognizing the interesting behaviors of *N. scabricosta* and bringing them to our attention and then helping us design our experiment; Dr. Benjamin Miner for his assistance in designing and carrying out our experiment, as well as help with computer program statistical analysis; Gavin Willis for sharing his research on snail trail following with us. Lastly, thanks to Deb Donovan, Kaitlyn Lowder, and Claire Skelly for their constructive feedback in editing this paper.

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Tables

Table 1. ANOVA test analyzing whether disturbance, temperature, or a combination of each, causes a reaction in *Nerita scabricosta*. Classifications of reactions are Tentacles out=Tentacles, Spinning=Spin, and movement of greater than 1cm=Crawl. (df= degrees of freedom, P<0.05 is significant)

	df	F-Value	P Value
Tentacles			
Disturbance	1	10.3899	.002913
Temperature	1	0.1180	.733459
Disturb*Temp	1	0.0432	0.836625
Spin			
Disturbance	1	0.2269	0.6371
Temperature	1	0.0003	0.9873
Disturb*Temp	1	0.3776	0.5432
Crawl			
Disturbance	1	0.9717	0.3317
Temperature	1	1.0805	0.3064
Disturb*Temp	1	2.0151	0.1654

Table 2. Two sample t-Test: Testing locomotive behavior of *Nerita scabricosta* in presence and absence of water. (df= degrees of freedom, P<0.05 is significant)

	Treatment	
	No Splash	Splash
Mean	1	4.133
Variance	7.857	16.267
Observations	15	15
Pooled Variance	12.061	
Hypothesized Mean Difference	0	
df	28	
t Stat	-2.471	
P(T<=t) two-tail	0.02	
t Critical two-tail	2.048	

Table 3. Chi-Squared analysis testing locomotive behavior of *Nerita scabricosta* in presence and absence of water. (df= degrees of freedom, P<0.05 is significant)

df	t-value	P value
1	5.33	<.025

Nocturnal behavior in *Euapta godeffroyi* (accordion sea cucumber): the effects of light exposure and time of day

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Keywords: *Euapta godeffroyi*, nocturnal behavior, circadian rhythms, light

Introduction

Understanding the mechanisms by which biological systems function is essential to conservation of ecosystems, especially delicate ones like coral reefs. One aspect that needs to be understood is the behavior of organisms included in a system. The entrainment of circadian rhythms is influenced by a range of environmental and molecular factors. For example, in mammals, photoreception of light from the surrounding environment dictates time-of-day information that sets daily clocks (Freedman et al. 1999). On the molecular level, a genome-wide analysis linked gene expression to the function of cellular autonomous clocks that ultimately control the timing of most stages of development in *Drosophila* (Ceriani et al. 2002). Circadian rhythms within marine invertebrates have been connected to cyclical factors such as light exposure, lunar activity, and tidal cycles (Last et al. 2009). Patterns of daily rhythms have also been observed in and related to the behavior of many species within the phylum Echinodermata (Singh et al. 1999). These patterns, such as feeding activity, are seasonally affected (Singh et al. 1999, Yang et al. 2006). In some species of the phylum, nocturnal behavior has been observed as the pattern produced by these rhythms.

Among the echinoderms exhibiting daily rhythmic behavior are certain species of sea cucumbers. Understanding the behavior of sea cucumbers is important due to their roles in particle depletion, nutrient cycling, and biodeposition (Singh et al. 1999), consequently serving a cleaning role in an environment (Yang et al. 2006). As slow moving organisms, many sea cucumbers exhibit several defense mechanisms including a thick body wall (Francour 1997) and toxicity (Bakus 1974) to avoid predation. An additional mechanism thought to be employed by sea cucumbers in order to avoid predators is nocturnal behavior (Francour 1997). However, there is indication that a wide array of other factors may cause nocturnal behavior amongst sea cucumbers and other echinoderms.

The feeding activity of sea cucumbers is controlled by a combination of factors such as temperature, photoperiod, and food availability. Singh et al. (1999) studied feeding activity of the species *Cucumaria frondrosa*, which is influenced by day length, chloropigment concentration, and percent organics of the seston. In their study the dominant factor affecting the feeding activity of *C. frondrosa* was the quality of seston, which is characterized by the chloropigment concentration and percent organics. They also proposed these factors are often interrelated, and change simultaneously. This study noted that water temperature, availability of food, and day length are dependent on the season. For example, day length was interrelated to chloropigment concentration.

Given the different factors that influence behavior among sea cucumbers, we were interested in determining if the nocturnal behavior of the apodous holothurian, *Euapta godeffroyi*, was linked to time of day or light exposure. Members of the order *Euapta* move using peristaltic motion, can contract to one-third of their extended lengths, have a thin body wall, and are flexible (Heffernan and Wainwright 1974). Like all apodans, *Euapta* lacks tube feet, papillae, and a radial water canal (Kerr 2001). *E. godeffroyi* is a nocturnal suspension feeder (Kerstitch and Bertsch 2007). Other information pertaining specifically to this synaptid cucumber is extremely limited beyond surveys of distribution, one of which indicated high abundances around Espíritu Santo in the southern Sea of Cortes (Herrero-Pérezrul 2008), an area containing small coral reefs. We observed *E. godeffroyi* nearby in Pichilingue, Baja California Sur in the evening (approx. 20:00 and on) and became interested in studying factors that caused this behavior. We chose to examine the effects of light exposure and time of day on this particular species because we expected these factors to influence *E. godeffroyi* activity. To determine if the nocturnal behavior we observed could be linked to either of those factors, we collected individuals to impose opposing light cycles upon them and recorded their activity.

Methods

We collected *E. godeffroyi* at two different times during our experiment; thus the experiment was blocked in time. Under lab conditions, we imposed two opposite light cycles upon our specimens. To determine if light exposure or time of day were factors influencing their nocturnal behavior, we noted activity for two days in the morning and evening. The procedure for each block was identical with the exception that the first block had observations beginning in the morning and the second block had observations beginning at night. By blocking our experiment in this manner, we were able eliminate the start time as a confounding variable.

For each block, we collected 16 individuals of *E. godeffroyi* at Cantamar in Pichilingue, Baja California Sur. The rocky subtidal collection site was interspersed among corals and was chosen for the large quantity of *E. godeffroyi* found in that area. We transported the specimens to the Universidad Autónoma de Baja California Sur Marine Station in Pichilingue for study. There, identical 100L tanks were set up with unfiltered seawater from the Bay of La Paz and medium sized rocks to mimic *E. godeffroyi*'s environment. Each tank was aerated to ensure that our specimens would receive an adequate oxygen supply through the duration of our experiment. We placed four individuals into each tank and allowed them to acclimate to lab conditions for a minimum of 12 hours.

We imposed two different light cycles upon *E. godeffroyi* –which we called the Normal treatment and the Reverse treatment. In the Normal treatment, the individuals were put in conditions that mirrored the ambient light cycle. Individuals in this treatment were under a light during the day (8:30 to 20:30) to simulate daylight and covered during the night (20:30 to 8:30). In the reverse treatment, we switched the hours that individuals were exposed to light in relationship to the ambient light cycle. During the day they were covered and at night they were under conditions that simulated daylight. Nested in these two levels were the factors of day and night. We randomly assigned treatments to the tanks such that half received the Normal and half the Reverse treatment. We then noted the activity of individuals in each tank twice a day for two days, once in the morning and once at night.

As we observed, we recorded the number of individuals active in each tank. Determining activity was based on the filtering end of the organism. If the tentacles were not visible for an individual, we counted it as inactive. Likewise, if the tentacles were visible but not moving a definitive amount, we counted the individual as inactive. Active individuals were identified by noticeable movement in the feeding tentacles which were usually coupled with peristaltic motion throughout their bodies.

When data collection was complete, we used a χ^2 test to determine if light had different effects on *E. godeffroyi*'s nocturnal behavior. Individuals active under light conditions consisted of those active during the morning in the Normal treatment and those active at night in the Reverse treatment. Individuals active under dark conditions consisted of the individuals active during the night in the Normal treatment and during the day in the Reverse. Because we were also interested in seeing if the nested factors of day and night had an effect, we ran another χ^2 test on the total observed during the day and during the night. Due to the low frequency of active individuals, we used Yates Correction in all of our calculations to correct for small a sample size.

Results

Our results indicate that there was no difference between light and dark conditions, nor was there a difference between activity during the day and night. All of the observed active individuals were seen during the night for the Normal treatment whereas only half were seen at night for the Reverse Treatment (Figure 1), but these differences were not significant (χ^2=0.571; d.f.=1; p=0.450). There was also evidence that activity between when light and dark conditions (active individuals determined using Table 1) was insignificant different regarding the the activity of *E. godeffroyi* (χ^2=0.571; d.f.=1; p=0.450).

Discussion

Neither time of day nor light exposure appear to be factors influencing the nocturnal activity of *E. godeffroyi*. Had a circadian rhythm been the factor responsible for nocturnal behavior, we would have expected to see activity at night regardless of the light cycle. Our data shows that there is no significant difference between the number of individuals active during the day and individuals active at night. By failing to reject the hypothesis that activity is the same during the day and during the night, we can conclude that time of day does not have an impact on the activity of *E. godeffroyi*. We also failed to reject the null hypothesis that light and dark conditions produced similar results. Due to this result, we can conclude that light exposure is not an entraining factor in *E.godeffroyi*. If this experiment were to be conducted again, we would suggest that two additional observations be made half an hour after covering or uncovering the tanks—one in the morning and one at night. Because we made our observations prior to changing the lighting on each tank, no data was collected regarding the immediate effect of light on *E.*

godeffroyi. This data would be helpful in determining if light had an immediate effect on individuals.

While light exposure and time of day can be eliminated as factors affecting the nocturnal behavior of *E. godeffroyi*, research regarding the daily behavior of other marine organisms indicates that there is still a wide array of factors to be investigated. The variety of possible factors influencing entrainment is so abundant that even light exposure can be further analyzed by examining wavelengths associated with individual colors. In the marine alga *Gonyaulax polyedra*, the light receptors entraining the cellular clock are set differently by blue and far red light (Roenneberg and Merrow 2002). Even though *G. polyedra* is a unicellular organism, it is possible that a similar mechanism is at work for *E. godeffroyi* because biological rhythms can be molecular in source. We cannot be certain from our experiment whether or not different wavelengths of light would have an effect on the feeding activity of *E. godeffroyie* because we used exclusively white light.

Temperature is another factor that may be responsible for the entrainment of behavior in *E. godeffroyi*. As we transferred our specimens in and out of tanks, we noticed that they showed heightened activity as they were moved into the warmer water of our transportation containers. While this could be due to the disturbance of physically being moved it is plausible that the variable temperature of the water caused the change in activity. Temperature is linked to activity in other organisms, more specifically, to other species of sea cucumbers. For example, the metabolic rate of *Apostichopus japonicas* increases with rising temperatures but goes into aestivation once low or high temperature thresholds are reached (Yang et al. 2006). These threshold temperatures often coincide with seasonal changes.

The entrainment of behavior due to seasonality is an example of the complexity and interrelated nature of biological rhythms. Inherent in seasonal shifts are changes in temperature, day length, which in turn affects light exposure, and water productivity. The level of productivity in water is important to the species *C. frondosa* because quality of seston is the main factor influencing its seasonally fluctuating activity (Singh et al. 1999). Singh et al. (1999) found that the components of seston quality, percent organics and chloropigment concentration, were positively correlated to day length. These findings exemplify the fact that biological rhythms are complex and cannot be attributed to a single factor. Rather, several factors influence each other to create rhythms in any biological system, contributing to the complexity and the need for understanding such mechanisms.

Acknowledgments

We would like to thank B. Miner and D. Donovan for transportation, set-up, statistical, and moral support. Additionally, we'd like to thank S. Flores-Ramirez for set-up and logistical support, as well as the staff at the U.A.B.C.S. Marine Station for the use of space and equipment.

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Tables:

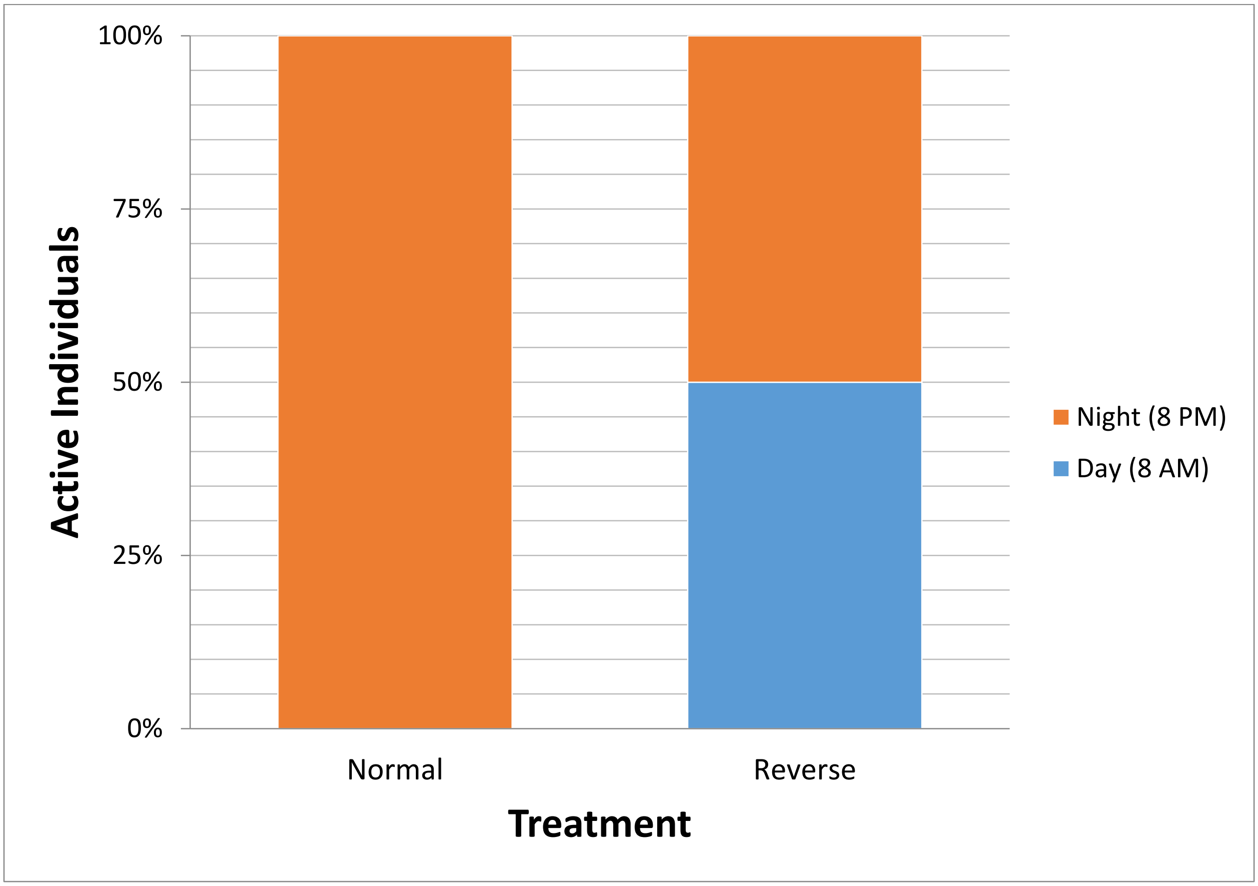
Table 1: Displayed is the number of *E. godeffroyi* individuals that were active during normal and reverse cycles during day (8 AM) and night (8 PM) observations. Light conditions were determined by adding individuals observed during the day in the Normal treatment and at night in the Reverse treatment. Dark conditions were determined by adding in individuals observed during the night in the Normal treatment and during the day in the Reverse treatment

Treatment	Day	Night
Normal	0	3
Reverse	2	2

Figure Legends:

Figure 1. Percentage of observed active *E. godeffroyi* seen at either day or night in the Normal and Reverse treatments.

Figure 1.



Queuing behavior in the intertidal snail *Nerita scabricosta* in the Gulf of California

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abstract

Nerita scabricosta is an isophasic intertidal snail which is common on rocky tropical shores of the Eastern Pacific. It has been observed forming queues (two or more individuals traveling in single file, on the same mucus trail) while migrating between tide levels. This study looked for any hierarchy in the queues. I found that these queues form opportunistically, and are not always led by the same individual. This laboratory study also examined whether these queues are maintained by chemical or visual cues. Although many species of gastropods commonly follow mucus trails, *N. scabricosta* did not demonstrate mucus trail-following behavior while seeking shelter. Instead, they oriented themselves solely by visual clues, following the shell of a conspecific even though it was not producing a mucus trail. *N. scabricosta* is the first gastropod to demonstrate trail following behavior by visually following a conspecific, as opposed to using chemical cues in mucus trails.

introduction

The propensity of snails to follow mucus trails of other individuals has been examined at length. Various species of snails can recognize and follow the mucus trails of both conspecifics (Tankersley 1989; Chapman 1998; Chiussi and Diaz 2002; Edwards and Davies 2002; Stafford et al. 2007; Ng et al. 2011) and heterospecifics (Clifford et al. 2003; Shaheen et al. 2005; Davis-Berg 2011). Motivation for trail-following varies, but includes finding aggregations (Chapman 1998; Stafford et al. 2007), hunting (Clifford et al. 2003; Shaheen et al. 2005; Davis-Berg 2011), finding a mate (Clifford et al. 2003; Ng et al. 2011), and grazing on microalgae trapped in the trails (Edwards and Davies 2002; Davies and Blackwell 2007).

The question of the evolutionary pressures that would lead to locomotion by a mucus-producing foot is an interesting one. Because mucus can act as glue, it allows gastropods to attach to and travel on surfaces or angles that would otherwise be impassible (Davies and Hawkins 1998). This allows them some protection from predators. However, the use of pedal mucus for locomotion costs 12 times as much energy as locomotion with legs for an organism of similar size (Denny 1980), and mucus production accounts for 20-30% of metabolic energy consumption in gastropods (Davies and Hawkins 1998). Since mucus production is so energetically costly, alternative uses of mucus trails would confer a strong selective advantage (Ng et al. 2011).

In an intertidal habitat a single mucus trail can remain in place for two tidal cycles or more, leaving a large temporal window for other individuals to utilize it (Edwards and Davies 2002). Some snail species will devour old mucus trails in order to regain some of the metabolic energy spent creating them (Calow 1974). However, it is more common for snails to use the path of the mucus trail to save energy. Traveling along an old mucus trail reduces the amount of force required for locomotion (Tankersley 1989). Trail following also reduces the amount of mucus that a gastropod must lay down by up to 70 percent (Davies and Blackwell 2007), which is more significant since the metabolic costs of mucus production are 35 times greater than metabolic costs directly associated with locomotion (Davies et al. 1992). Although these following snails are not able to entirely switch off mucus production, the metabolic savings provide a large selective advantage (Davies and Blackwell 2007).

It is generally accepted that gastropods use chemical cues to follow mucus trails. Mucus contains water-soluble biomolecules (Davies and Hawkins 1998) which leave a chemical signature that allows gastropods to differentiate between the trails of conspecifics and heterospecifics (Clifford et al. 2003; Davis-Berg 2011). Left-right asymmetry in the location of sex and defecatory organs in many gastropods allows them to determine directionality of mucus trails (Shaheen et al. 2005), and some species of snails can even determine whether or not an individual whose trail they are using has been feeding (Edwards and Davies 2002). However, Davis-Berg (2011) has observed that the snail *Euglandia rosea* holds its optic tentacles at a smaller angle when following a trail, suggesting that visual cues are also a part of trail-following behavior. It has been shown that snails can associate visual cues with processes such as feeding (Andrew and Savage 2000). Other intertidal invertebrates with developed eyes have demonstrated the capacity to use only visual cues for orientation, especially during times of emersion (Chiussi et al. 2001), and some snails have been shown to use visual information for orientation in their home range, and to only use chemical cues for predator avoidance (Chiussi and Diaz 2002).

Nerita scabricosta inhabits the upper intertidal zone in rocky habitats on the eastern Pacific shore, and is one of the most common intertidal molluscs distributed between Baja California and Ecuador (Brusca 1980; Houston 1980; Garrity 1984; Esqueda et al. 2000). They are an isophasic species, moving up and down the shoreline with changing tidal levels so that they always inhabit the same microhabitat (Garrity 1984). As with many isophasic species, groups of individuals often migrate together (Vannini et al. 2008). *N. scabricosta* often does this in queues: two or more individuals traveling single file along the same mucus trail (author observations). They often join other snails to form large aggregations in shaded and moist microhabitats as protection against desiccation and temperature stress (Garrity 1984; Chapman and Underwood 1996; Esqueda et al. 2000). Other members of this genus have demonstrated a preference for visual cues over chemical cues when seeking shelter (Chiussi and Diaz 2002). In this study I examined the structure of the queues to determine if any hierarchy exists, with certain individuals acting as leaders. I also attempted to determine whether *N. scabricosta* forms these queues by following mucus trails, or by visually tracking other individuals.

methods

Prior to laboratory tests, I performed observations of *N. scabricosta* at Cantamar and Pichilingue, two rocky shores near La Paz, Mexico, with the intention of determining what, if any, factors can be considered the primary cause for their queuing behavior.

In order to investigate queuing behavior, I collected approximately 30 medium-sized *N. scabricosta* snails from a rocky intertidal area near Pichilingue Laboratory at Universidad Autonomous de Baja California Sur. Large individuals were not chosen because they did not exhibit any following or aggregating behavior in the field (author observations). The snails were housed in an aquarium on rocks collected from their habitat. Temperatures of both the air and the water matched the conditions of their habitat at the time of the tests.

My first objective was to determine if the same individuals led the queue each time. Five snails were marked and placed simultaneously near the top of a rock. The snails were all placed the same distance from the water, facing the water, with less than one cm between them. The snails were then observed as they returned to the edge of the water, and each individual was classified as a leader (led a queue), follower (was in a queue but did not lead), independent (showed directional locomotion but was not part of a queue), and inactive (did not move from starting position). This test was replicated ten times with the same individuals, with random placement along the start lines for each replicate. The rock was scrubbed with a brush lightly after each replicate to remove mucus trails (Chapman 1998).

My second objective was to determine which cues *N. scabricosta* uses to form these queues. These tests were run in sunlight on a wet, flat rock. For the first portion of the test, a snail was released on the rock, and allowed to create a mucus trail. The snail was then removed, the rock was rotated by a randomly generated number of degrees, and a second snail was placed at the same location as the first, facing in the same direction as the mucus trail. Directionality of the path that the second snail took was recorded in increments of 15 degrees, with the path of the first snail set as zero. The rock was scrubbed after each replicate. A total of 13 replicates were recorded. For the second portion of the test, a snail was released on the rock facing an empty shell, which was then pulled away by a piece of string in a random direction at a rate similar to that of the snails. The direction of the path that the snail traveled was recorded in increments of 15 degrees, with the path of the shell set as zero. The rock was scrubbed after each replicate, and 13 replicates were recorded.

I analyzed the results in R with a modified Rayleigh test. The direction of the cue (mucus trail or shell) was chosen as the a priori hypothesis for data orientation, and p values from the resulting test were used to determine if the hypothesis was supported (Durand and Greenwood 1958).

results

My field observations found that *N. scabricosta* formed these queues when traveling between tidal levels. Very rarely did the snails travel along dry rock, but rather waited until they were splashed with water before moving. Snails usually formed aggregations, although larger individuals were often found on their own.

In the first set of laboratory tests, none of the snails led a queue significantly more often than any others (Tab. 1). One of the individuals (B) was inactive for half of the tests and didn't lead any queues, but all other individuals led at least two queues (Tab. 1). In a few of the replicates, individuals were observed rotating in one place before orientating themselves towards another individual or individuals, and then forming a queue.

In the second set of tests, among the snails placed directly on a mucus trail, 8% followed the trail directly, and 15% followed within 15 degrees. Among the snails placed next to an empty shell, 46% followed the shell directly, while 54% followed within 15 degrees.

Results of the Rayleigh tests showed that the snails placed on the mucus trail showed no significant orientation towards the trail (Fig. 1). Snails placed next to an empty shell did show a significant orientation towards the shell ($p = 0.0046$) (Fig. 2).

discussion

The findings of the field observations are consistent with previous studies. Snails are more likely to move when desiccation stress is lowered, whether by being splashed with seawater or by elevated moisture levels due to rainfall or humidity (Britton and McMahon 1992; Chapman and Underwood 1996; Bates and Hicks 2005). The purpose of the aggregations that the snails formed is to reduce desiccation stress when moisture levels are lowered (Garrity 1984; Stafford et al. 2007; Vannini et al. 2008). The isophasic supralittoral life pattern demonstrated by *N. scabricosta* is likely a response to the pressures of predation at lower levels and desiccation at upper levels. Since *N. scabricosta* seem to form aggregations based on visual cues, the lack of aggregation-forming behavior in adult individuals is possibly due to a combination of a reduction of desiccation stresses on larger individuals (Chapman 1998) and an inability to detect the aggregations due to eye damage, which is common in intertidal gastropods and often fails to heal properly in adults (Tartakovskaya et al. 2003).

The results of the first laboratory experiment support previous studies, since snails have not demonstrated any social hierarchy or structure (Coffin et al. 2008). Like other aggregations, queues seem to form opportunistically, and it is unlikely that the same individuals form queues together outside of laboratory conditions, since individuals express different aggregation behaviors between tidal cycles (Chapman and Underwood 1996).

The results of the second experiment show that *N. scabricosta* relies on visual cues rather than chemical cues in mucus trails when forming queues. Studies on other species of snails have found no evidence that individuals are visually attracted to other individuals or aggregations, and have suggested that mucus trails are the most important factor in aggregation forming (Chapman 1998; Stafford et al. 2007). The results of this experiment demonstrated that visual cues are sufficient for queue formation, although mucus trails may still play a small role. However, these results are not totally unexpected, as other *Nerita* spp. have demonstrated a preferences for visual cues over chemical cues when navigating their home range (Chiussi and Diaz 2002). This preference is likely related to the fact that these snails spend most of their lives in an emersed habitat where mucus trails lose water rapidly due to evaporation. Although *N. scabricosta* does not seem to recognize the mucus trails of conspecifics, or at least doesn’t follow them while seeking shelter, the reduction of mucus production necessary for locomotion in individuals which join queues provide a definitive selective advantage, since the metabolic energy which they save by this behavior can be used for other physiological processes (Davies and Blackwell 2007).

Some studies have suggested that laboratory studies on snail locomotion are less valid than field observations, since dislodgement causes oriented movement in some species of snail (Petraitus 1982). However, other species demonstrate little to no response to dislodgement (Chapman 1986), and distance and direction of movement by snails has not shown any significant correlation with transplantation (Chapman 1999). Although there exists a possibility that dislodgement or transplantation have some effect on the direction and dispersal of intertidal snails, it is unlikely that it affects the preference of the snails to migrate in groups.

It would be interesting to observe queue-forming behavior during nocturnal tidal cycles, since previous studies have found great reductions in vision and resolution in snails in low light conditions (Zhukov et al. 2002). Further studies on the behavior of *N. scabricosta*, especially with regards to responses to an interruption of visual stimuli during queuing, would provide a greater understanding of the role of mucus trails in the formation of queues.

acknowledgements

Whitney Walters and Deborah Donovan provided helpful comments on drafts of this manuscript. Ben Miner assisted with statistics. Universidad Autonomous de Baja California Sur generously provided use of their Pichilingue laboratory facilities.

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figures and tables

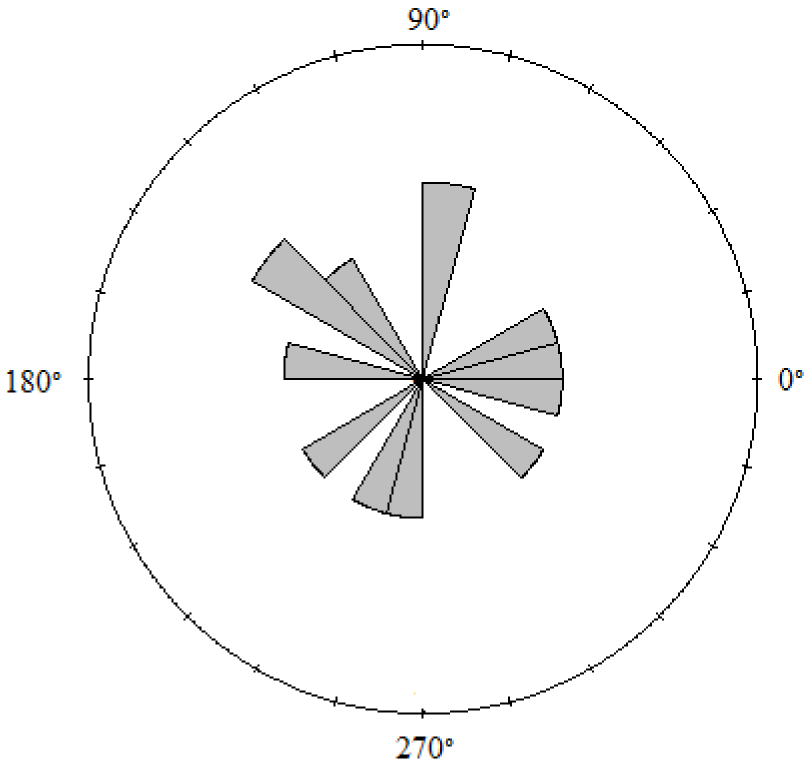

Figure 1.

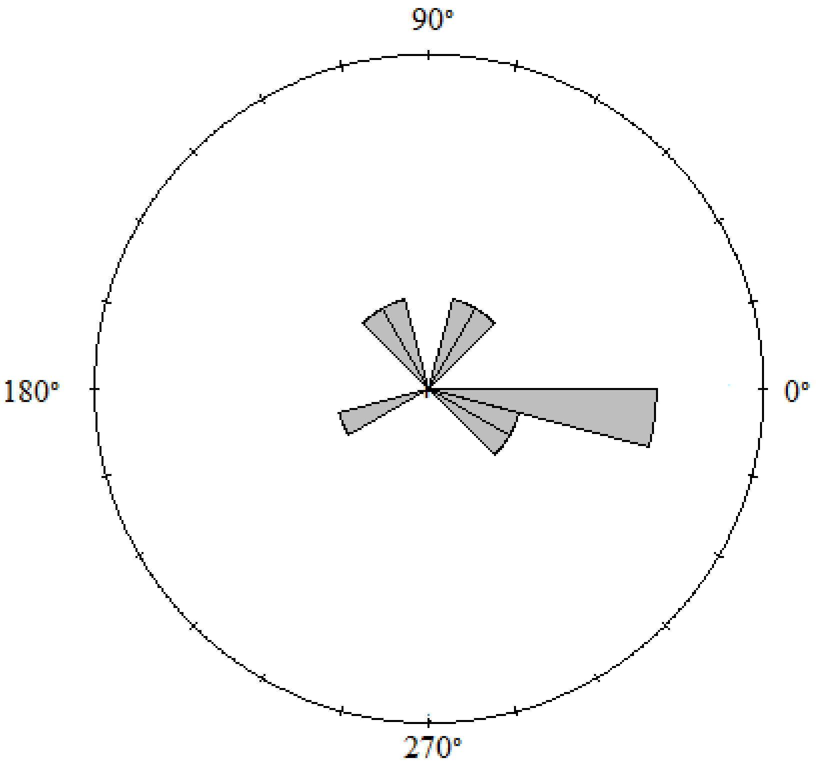

Figure 2.

Table 1.

Individual	Leading	Following	Independent	Inactive
A	3	3	3	1
B	0	3	2	5
C	2	6	2	0
D	4	4	2	0
E	2	5	3	0

figure and table legends

Figure 1. Histogram of the directionality of movement of *Nerita scabricosta* individuals placed on the mucus trail of a conspecific in simulated daytime ebb tide conditions. The direction of the mucus trail is indicated as zero degrees

Figure 2. Histogram of the directionality of movement of *Nerita scabricosta* individuals placed behind the shell of a conspecific without a mucus trail in simulated daytime ebb tide conditions. The direction of the path of the shell is indicated as zero degrees

Table 1. Actions of five *Nerita scabricosta* individuals in queue forming. Categories are leader (led a queue), follower (was in a queue but did not lead), independent (showed directional locomotion but was not part of a queue), and inactive (did not move from starting position). All snails were placed equidistant from the water level, facing the water, approximately one cm from other individuals

The effects of sunscreen on coral bleaching of the genus *Pocillopora* located in Baja California Sur.

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Key words: Coral reefs, Bleaching, Sunscreen, Tourism, Zooxanthellae, *Pocillopora*

ABSTRACT. Coral reefs are one of the most important oceanic ecosystems in the world. They support huge amounts of biodiversity and provide economic income through fishing and tourism. However, aspects of tourism have been thought to be harmful to the health of the corals themselves. Specifically, the chemicals found in sunscreen have been shown to cause coral bleaching. Our study was designed to determine if sunscreen causes bleaching in *Pocillopora* corals off the coast of Pichilingue, Baja California Sur and if increased concentrations induce greater amounts of bleaching. We prepared three concentrations of sunscreen solutions and applied them to branches of coral in the field. Using a quantitative color scale, we determined the percent color change over a six-day period. We did not find a significant difference in color change between corals exposed to the different concentrations. However, a significant decrease in the color of the corals was found, but the causation of this decrease could not be determined. Possible causes for the color change include changes in natural ambient conditions, handling of corals during experimental procedures, or the sunscreen treatments. The latter two causes are examples of how tourist activities could negatively affect coral reefs, necessitating the creation of more Marine Protected Areas (MPAs), stricter regulations in those MPAs, more education for tourists in order to minimize the effects of human influences on corals, and further research into sunscreen formulas that do not contain harmful chemicals.

INTRODUCTION

Coral reefs represent the most productive regions of our world’s oceans. Comprised of many species of coral often cemented together, reefs are locations of high primary productivity that support a wide range of marine life (Levinton 2009). This high level of species richness is not only important for the health of the oceans, but it also provides valuable economic resources for people around the globe. Coastal countries rely on corals as a primary fishing ground for income and millions of people rely on the resources provided by the environment in order to subsist (Roberts et. al. 1998). These coral reef ecosystems are estimated to produce up to 6 million metric tons of fish annually (Munro 1996). Coral reefs also provide costal protection from storms and allow for near-shore habitats such as eel grass beds to exist without the effects of high disturbance (Hoegh-Guldberg 1999).

In addition, coral reefs are a main destination for tourists and can provide economic stimulation to coral reef communities. Unfortunately, tourism is one of the chief causes of damage to coral reefs (Lamb 2011). The continual pressure of tourism on many countries has increased development around coral reefs, such as building marinas, groynes, and causeways that can cause irreversible damage to coral reefs (Wilkinson 2004). Additionally, it has been demonstrated that personal care products, including sunscreens, can cause bleaching in corals, just as temperature anomalies, high irradiance, pollution, and bacterial diseases can (Danovaro et al. 2008). It is estimated that 4,000 to 6,000 tons of sunscreen by-products annually make their way to reef areas and that it only takes 20 minutes in the water before a quarter of the sunscreen applied is washed off a human body (Danovaro et al. 2008).

Corals live a symbiotic life with zooxanthellae, which are single-celled organisms that live in the tissues of corals and other cnidarians. These organisms are part of a group of dinoflagellates that are most often found as plankton. Zooxanthellae is a species best known for the mutual relationship they have with reef building corals. This symbiosis is possible because corals provide a protected environment and compounds necessary for zooxanthellae to photosynthesize (Birkeland 1997). In turn, zooxanthellae provide food as products of photosynthesis to the coral (Birkeland 1997). This gives corals an influx of nutrients so they can secrete the calcium carbonate skeleton that serves as the foundation for coral reefs. Any stress that coral is subjected to will affect the zooxanthellae as well (Birkeland 1997).

Danovaro *et al.* (2008) affirms that sunscreens can cause the rapid and complete bleaching of hard coral, even at extremely low concentrations. The ultraviolet filters in lotions caused the coral to discharge large amounts of mucus, a sign of environmental stress, and to eventually bleach. The research also indicated elevated virus levels in the seawater, suggesting that sunscreens may induce latent viral infections in coral.

Our study was designed to determine if sunscreen has an effect on the *Pocillopora* corals found off the coast of Pichilingue, Baja California Sur, and if so what concentration of sunscreen has the greatest effect. We hypothesized that sunscreen would induce bleaching of the corals and that the higher the concentration of sunscreen, the more extreme the bleaching would be.

METHODS

In this study, randomly selected coral branches in the field were exposed to different sunscreen concentrations. During the following days after the sunscreen application, the corals were observed daily in order to test if they were changing color. After a six day period all data were gathered and analyzed to determine if any recognizable trends occurred between coral color changes and sunscreen concentration.

The three sunscreen treatments were prepared in lab using Banana Boat SPF 50 sunscreen. The sunscreen was measured by mass and 4.96, 10.02, and 15.22 grams were each added to a liter of water, giving final percent masses of 0.49, 0.99, and 1.50 respectively. These concentrations were chosen to replicate actual amounts of sunscreen used by an average person.

In the field, the corals were tagged and treated with the different sunscreen solutions. We tagged 30 branches of coral, all on different heads, near the coast of Pichilingue at the Hotel Cantamar beach. The corals were located on a rocky reef; all at comparable depths between 2 and 5 meters. Due to limited availability the samples were chosen haphazardly. The sunscreen treatments were applied three times over the course of one day, each application three hours apart. We expelled 5 mL of the sunscreen treatment within an inch of the branch being studied. Random numbers were assigned to the corals to determine which ones would receive which of the 3 different treatments.

In order to determine the level of bleaching, we took observations on the color of the coral over a period of six days. Starting 24 hours after the first sunscreen treatment, we made observations of each coral's color. We ranked the coral's color based on a quantitative color scale using three different paint samples. Each paint sample had 5 shades which were numbered from 1-5, 1 being the darkest and 5 being white. Therefore, every consecutive number represented a 20 percent decrease in overall color. The experiment was run blind; that is, the people quantifying the color of the coral were not aware of the concentration of sunscreen that coral had been exposed to. Color observations were taken daily and final color observations were taken 6 days after initial treatments.

To analyze the data, we found the percent color change per day for each coral. We plotted and analyzed with regression the percent color change versus treatment concentration. In order to determine if there was an overall effect of any amount of sunscreen on corals, we also ran a 1-sample 1-tailed t-test to determine significance of overall coral color change.

RESULTS

We found a significant change in the color of the corals over the course of the experiment based on a 1-sample 1-tailed t-test (p=7.9E-7). Of the corals studied, 45 percent experienced a 20 percent decrease in color, 17 percent experienced a 40 percent or more decrease in color, and 38 percent of the corals experienced no change in color (Figure 1). We found no significant difference in the color change between the three sunscreen treatments applied ($F_{1,27}$=0.057, P=0.8) (Figure 2).

DISCUSSION

Coral bleaching occurs when the photosynthetic symbionts of corals (zooxanthellae) become increasing vulnerable to damage by light or other factors. The resulting damage leads to the expulsion of these important organisms from the coral host (Hoegh-Guldberg 1998).

The significant decrease in the color of the corals over the course of the experiment partially supports our initial hypothesis, that sunscreen does have a negative effect on corals. However, due to experimental design, it is uncertain the causation of this decrease as there was not a control treatment. One possible reason for the decline in color could be natural changes in the ambient environment, i.e. changes in temperature, pH, nutrients, salinity, or dissolved oxygen. Hernandez et al. (2010) mentioned that in the tropical east Pacific, coral bleaching is commonly associated with increases in sea surface temperature, but that it can also be due to low temperatures. They also explain that in the beginning of 2008, an anomalously low period of temperature was recorded and consequently a large amount of corals were bleached in different regions in the southern Gulf of California.

Another plausible cause of the decrease in color is the application procedure itself. Tagging and application of the treatments may have caused physical stress in the corals and the zooxanthellae. Local fish in the area were found to be attracted to the color of the tag; this increased attention to the specific corals could also have caused more stress.

Finally, as hypothesized, the sunscreen treatments themselves could have been the cause of the decrease in color. Because of the lack of a controlled treatment, it cannot be determined if sunscreen was the sole reason for the decrease in color of the corals. However, previous studies have determined that specific chemicals in sunscreen can lead to total bleaching of corals (Danovaro et al. 2008). Danovaro et al. (2008) tested sunscreen on corals containing concentrations of UV filters higher than those reported in most natural environments. At the same time, the coral response to sunscreen exposure was not concentration dependent, as the same effects were observed at low and high sunscreen concentrations. Therefore, they hypothesized that UV filters can have potentially negative impacts even at concentrations lower than those used in their study.

Our results indicated that increased concentration of sunscreen did not lead to increased coral bleaching, which does not support our hypothesis. This indicates that it is merely the exposure to sunscreen that initiates the bleaching and that the concentration does not matter. It is therefore plausible that only a small amount of the chemicals in sunscreen are necessary to render the corals vulnerable to the viruses associated with their bleaching.

However, these results may have been due to the random selection, in which some coral heads were at a lower depth than others and vice versa. Corals at lower depths are exposed to lower ambient temperatures. Previous studies have demonstrated that corals subjected to higher temperatures bleach more than corals at lower temperatures when exposed to sunscreen (Danovaro et al. 2008). Previous research has also shown that bleaching occurs more often in shallow waters because the corals are more exposed to frequent changes in temperature, salinity and pH of the water due to the tidal changes (Sulu 2007).

The data presented in this study have important implications for coral reef conservation. As the public realizes the effects of pollution and other harmful actions on corals, more Marine Protected Areas (MPAs) are being developed (Wilkinson 2004). MPAs are an important tool used for coral reef conservation, but they aren't always enough. Many developing countries, where a majority of coral reefs are located, don't have the funds or support needed to properly manage MPAs (Wilkinson 2004). Even in well-managed MPAs, not all damaging actions can be stopped. Tourists can damage reefs just by walking, touching, or collecting coral, as well as boats and anchors causing severe physical damage. Tourism also allows the induction of many pollutants into the coral reef habitats, such as sunscreens (Lamb 2011). If sunscreen exposure or other human contact (i.e. handling the corals while tagging) is responsible for the bleaching of the corals, this would necessitate stricter regulations and more education for tourists visiting coral reefs. In addition, alternative means of sun protection should be emphasized. This could include the use of full body sun-suits (Lamb 2011) or research into sunscreen that does not contain the chemicals that are harmful to corals.

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ACKNOWLEDGEMENTS

We would like to express our gratitude to Benjamin Miner for his close guidance during our project, to Deborah Donovan and Sergio Flores for their feedback throughout the entire process, to Kelly Cates and Cameron Donegan for their revisions of our paper, and to the UABCS laboratory at Pichilingue for the gracious use of their facilities.

FIGURE CAPTIONS

Figure 1. Frequency of *Pocillopora* corals exhibiting 0, 20, 40, and 50 percent decrease in color 6 days after the application of 15 ml of 0.49, 0.99, and 1.50 percent sunscreen by mass treatments off the coast of Pichilingue, Baja California Sur. n=29

Figure 2. The percent color change per day of 29 *Pocillopora* coral branches that were subjected to 15 ml of 0.49, 0.99, and 1.50 percent sunscreen by mass treatments off the coast of Pichilingue, Baja California Sur.

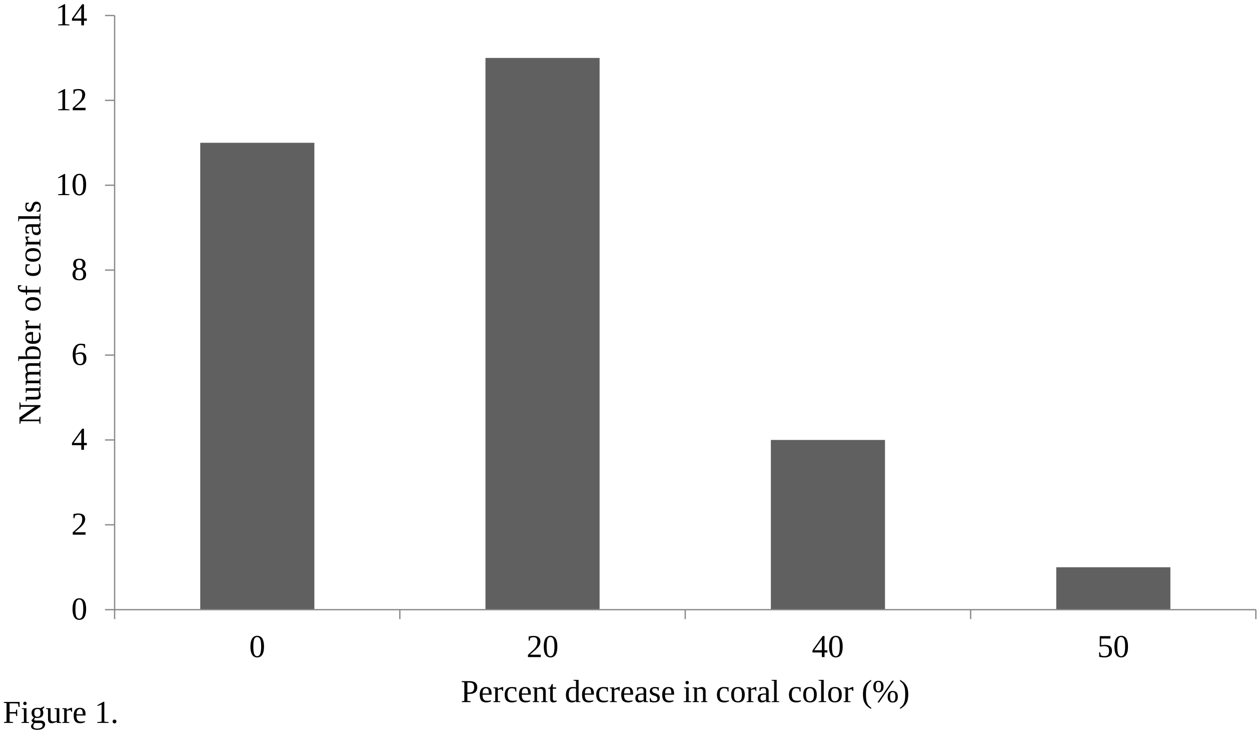
14
12
10
8
6
4
2
0
Number of corals
0
20
40
50
Percent decrease in coral color (%)

Figure 1.

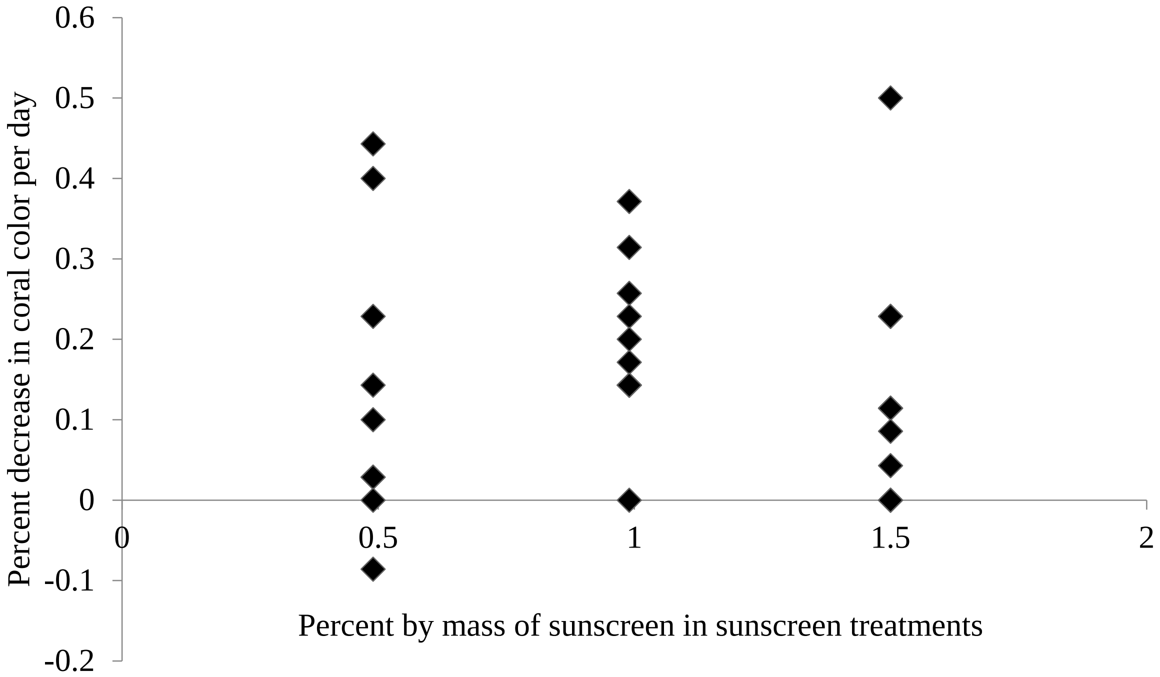
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0
0.5
1
1.5
2
Percent decrease in coral color per day
Percent by mass of sunscreen in sunscreen treatments

Figure 2.

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Effect of tide level on *Uca Crenulata* burrow distribution and burrow shape

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Keywords**:** *Uca crenulata*, California fiddler crab, tide level, burrow, female mate selection

Abstract

Intertidal organisms face greater changes in conditions like salinity and moisture compared to subtidal organisms, and so they must contend with these or risk death. A common behavior that mitigates surface changes is burrowing, which is practiced by *Uca crenulata*, the California fiddler crab. This study in Pichilingue, Mexico examines the effect that tidal zones have on the distribution and shape of *U. crenulata* burrows, including circumference, depth and length. I hypothesized that most burrows would be found at medium tide levels, while there would be no correlation between circumference and tidal height. Also, I hypothesized that as tidal height increased, so would both the depth and length of sample burrows. I laid 11 transects 0.5 m apart parallel to the low tide mark and 18 quadrats were placed along it at each transect to facilitate counting of the number of burrows. Secondly, casts were made of 21 burrows and measured to determine their circumference, depth, and length as well as their height above the low tide line. My data showed significance in all four areas; however, it supports only two of my hypotheses. Both length and depth of burrows increased in higher tidal levels compared to lower ones, which most likely resulted because longer and deeper burrows provide additional protection against desiccation, temperature fluctuations, and salinity changes. More burrows were counted at the low tide level, which does not support my original hypothesis. This proximity may be due to the need for a tide to take out newly-hatched larvae. Finally, circumference was found to increase as distance from the low tide line increased, which suggests that size of crabs also increases. This is supported by studies that showed that fiddler crabs constructed burrows that fit their body size.

Introduction

Intertidal organisms are subjected to a variety of conditions that many subtidal organisms do not have to contend with, such as dessication, changes in salinity, and direct sunlight. As a result, many have developed adaptations such as shell closure, hard exoskeletons that limit cell swelling, movement to match tide levels, dark pigmentation to prevent UV exposure, and burrowing to different depths. The last behavior is utilized by a variety of phyla, such as annelids, bivalves, and arthropods (Willmer et al. 2004).

Where an organism buries itself is determined by a variety of factors: tolerance to salinity and temperature, competitors, availability of food, and tides necessary for reproduction. The effects of these factors vary depending on distance above the low tide mark, and what may be ideal for one species may be detrimental to another (Levinton 2009). An example of this is seen in rocky intertidal areas, where zonation of barnacles and other organisms occurs as a result of different levels of tolerance to desiccation and sun. Hardier species live in higher tidal areas, but these may be out-competed in lower tidal areas (Levinton 2009). Similar cases exist in sandy intertidal zones where adaptations, like such as air-exchanging membranes on some crab legs and blood pigments that store oxygen in lugworms, exist and to allow for high-intertidal living (Levinton 2009).

Once an organism has selected an ideal location in relation to tide height, they must determine how deep they wish to bury themselves to mitigate unfavorable factors. In higher intertidal areas, it is beneficial for organisms to burrow deeper so that temperature is more constant and there is less exposure to light and UV rays, which leads to a moister environment without significant changes in salinity due to evaporation. However, in lower intertidal areas, a deeper burrow can limit feeding opportunities and its construction may be an unnecessary energy expenditure. Therefore, burrowing organisms are faced with tradeoffs when determining their burrow depth , but utilizing the burrowing strategy allows them to live in a more stable environment without sacrificing their beneficial location to tide.

This study focuses on *Uca crenulata*, a burrowing fiddler crab that lives in the intertidal mud flats of estuaries and lagoons along the western coastline of the United States as well as in Mexico (deRivera 2003). Much has been studied in fiddler crabs of the *Uca* genus, including claw waving behavior (How et al. 2007), food availability effects on courtship (Kim and Choe 2003), and ion regulation is small concentrations of seawater (Baldwin and Kirschner 1976). However, relatively little is known about burrowing techniques, burrow shape, burrow distribution and the factors that influence all of these. What has been discovered, however, is that this species burrows in intertidal zones, and these burrows differ between the genders. Males' burrows are typically 15.9 cm deep while females' average 10.7 cm, and the crabs are able to plug these up as the tide rises (deRivera 2003). Since they do not have the constraints of sessile intertidal organisms, individuals are able to select a burrow location that meets their needs of water coverage and food availability, and this is where they are expected to be most abundant. I hypothesized that the highest number of burrows would be found in the medium intertidal range, as this proximity to the water can afford them less exposure to the air which may dry out their gills, yet it also allows them ample time with their burrows uncovered so that they may have time to forage for food and find mates, as both of these are activities are done during the low tide (Murai and Backwell 2006). When the tide begins to rise, however, these activities cease (Hockett and Kritzler 1972). Additionally, since Reanny and Backwell (2007) determined larvae are released on a nocturnal outgoing tide, burrows must be in a location where females may quickly exit the protection of the male burrows and release larvae into the water. I expected a statistically significant positive difference in the average numbers of burrows per square meter found in medium tide level compared to the low, high, and above high tide levels.

Additionally, these crabs are also able to burrow to a depth that promotes less variance in abiotic factors. I hypothesized that burrows would be shallower in the low tide zone because there is less of a need for protection from the changes in salinity and temperature that are more likely in the high tide zone. The length of burrows are hypothesized to also increase as height above low tide increases for similar reasons; ones that are longer are less exposed to the variable intertidal habitat.

I also hypothesized the burrow circumference will have no correlation with height above the low tide mark. The circumference of a burrow is determined by how large the crab residing in it is, and *U. crenulata* dig burrows that fit their size body. As I don’t expect to see significant zonation of sizes of crabs at different tide levels, there should also be no circumference difference at different tide levels.

Methods

Study area

This study was completed in a mangrove channel adjacent to the Universidad Autónoma de Baja California Sur laboratory in Pichilingue, BCS, Mexico. *U. crenulata* were abundant throughout the sand bar that shields the channel from the rest of the bay near the laboratory, but were especially concentrated on a mud flat on the leeward side of the bar. This channel was cut off from the bay at certain times due to tidal flats that were only covered after the tide had substantially come in. This meant the water level remained at low tide for longer periods of time compared to the bay side of the sand bar, and it rose and fell quicker compared to the bay side also.

To carry out my study, I chose a 9 m by 5.5 m mud flat densely populated by *U. crenulata* and performed a series of observations and sampling techniques to determine the distribution of burrows at different tide levels and compare lengths of burrows between these levels. After observation at the site, low and high tide marks were established. Tide levels were defined by dividing the distance between low and high tide marks into three equivalent parts; 1.0 m of the area above high tide was also included, for a total of four defined levels. The low tide level refers to the areas of the mud flat from 0.0 m to 1.5 m above the low tide mark; the medium tide level is from 1.5 to 3.0 m, the high tide level is from 3.0 m to 4.5 m, and the above high tide level is from 4.5 m to 5.5 m above the low tide mark.

Experimental procedure

To determine the distribution of burrows, I laid a transect tape along the low tide line in so that it touched the edge of the water as much as possible along the study area. This was used as a reference point throughout data collection. Quadrats of 0.25 m^2 size were haphazardly laid along the 9 m long transect line and the number of visible burrows were counted until the entire transect line had been covered, which amounted to 18 quadrats per transect. Transects were completed every 0.5 meters up the mud flat until the high tide mark was surpassed at 5.5 m., so a total of 198 quadrats were laid.

To obtain samples of the shape and depth of burrows, they were haphazardly chosen in the sample area and a can of Touch n' Foam Max Fill expanding sealant was used to fill the burrow. Burrow choice was limited to those with a wide enough opening for the application tubing, which was fed down the burrow until either resistance was felt or its length of 14 cm was exhausted. I filled the burrows with foam by slowly drawing the tubing out after I felt confident that enough had penetrated past the end of the tubing to reach the terminal chamber of the burrow, applying foam the entire time to ensure the cast accurately represented the burrow length. Distance to the low tide perpendicular to the transect line was measured, and the dry casts were dug up. The 21 sample burrows were measured lengthwise four times each at approximately 90 degree angles to decide the average length. Depth of the burrow was determined by measuring from the top of the terminal chamber of the burrow to the surface of the mud flat. Circumference was measured just below what would be the entrance of the burrow on the foam cast.

Data were analyzed by fitting a line to each of the four collections. Significance was determined by using an ANOVA regression.

Results

As distance from the low tide mark increased, the number of burrows significantly decreased (Figure 1; Table 1). Five hundred and fifty one burrows were counted in the transect half a meter above the low tide mark, while 149 were counted in the quadrats along the transect laid at 5.0 meters (Figure 1). This is a 73% percent decrease in the number of burrows from 0.5 meters to 5.0 meters above the low tide mark.

As distance from the low tide mark increased, the length of burrows increased (Figure 2). Additionally, the depth of burrows also increased as distance from the low tide mark increased (Figure 3). Both of these trends are statistically significant (Table 2; Table 3). The shortest burrow length was 3.1 cm, sampled from 82 cm above the low tide mark, and the longest burrow was blank percent longer at 33.9 cm at 615 cm above the low water mark (Figure 3). This is almost a 1000% percent increase.

The circumference of burrows significantly increased as distance from the low tide mark increased (Figure 4; Table 4). The smallest sample burrow circumference was 1.2 cm, and this was found at 250 cm above the low tide mark. The sample collected closest to the low tide mark at 48 cm had a circumference of 3.5 while the sample collected at 643 cm had a circumference of 4.5 cm (Figure 4). Circumferences of sample burrows at similar tide heights (231 and 280cm above the low tide mark) were 3.6 and 3.0 cm, respectively (Figure 4).

It is important to note that due to the nature of the cast-making material, insulating foam, the circumference of burrows may have been overestimated, as it expands as it dries. Tests of holes made with a pen showed that these casts had an average circumference that was

1.63 times bigger than the actual circumference of the pen. Additionally, length of burrows may be an overestimate for the same reason. However, the lengths and circumferences of sample burrows measured at different distances above tide height would still have the same proportions.

Discussion

Distance above tide height affected the number of burrows found at low, medium, high, and above high tide levels in significant ways, but not how I expected. More burrows were observed at low tide levels than at each of the three higher levels, which does not support my hypothesis. My data may show this trend for a variety of reasons. Perhaps since this mangrove channel has longer periods at low tide before water rises over the tidal flats, life at low tide for *U. crenulata* here is similar to living in higher tidal levels along exposed beaches. Comparisons between these areas and my study site must be completed to be support this idea, however.

The number of burrows found in the low tide level could also be the result of the need for close proximity to water, which is especially necessary when females wish to release their larvae on tide(Murai and Backwell 2006). If the needed nocturnal tide doesn't reach the burrows out of whence females emerge, they either face a dash to the water while carrying valuable eggs, or they risk reproductive failure. A larger density of fiddler crabs also eases mate searching by cutting down the distance females need to travel to sample a large number of mates (deRivera et al. 2003).

Another explanation is that *U. crenulata* were randomly distributed across the beach, but there were slightly more near the water. With more fiddler crabs burrowing in the low tide level, others followed because many benefits are recognized as a result of more density. Risk of predation to the individual, such as by birds, is lowered as more fiddler crabs congregate together (Murai and Backwell 2006; deRivera et al. 2003).

There was a positive correlation between height above the low tide mark and circumference of burrows entrances. While this is statistically significant, it does not support my hypothesis, as I expected circumferences of burrows to be similar among all tide levels. Circumference of the burrow entrance is related to the diameter, or entrance aperture, of the burrow by the equation circumference=pi*diameter, which works well because burrow tunnels are very circular. Both genders construct burrows that are a good fit for them; this means that they do not have to dig to enter nor is there a large gap around their bodies (deRivera 2005). Since males are on average larger (14.3 mm wide compared to females at 13.9 mm), their entrance diameter is also larger (deRivera 2005). This difference in circumferences at the assorted levels heights above the low tide mark could suggest that the genders segregate themselves, which is unlikely as this leads to a longer, more dangerous mate search. A more likely explanation is that different-sized *U. crenulata* live varying distances from the low tide mark. During my observations at the field site, I noticed that noticeably/considerably more smaller burrows (less than 0.5 cm were found at lower tide levels than at upper ones. However, this was not tested during this study, so it is a possible start for another study. If larger males are living at higher tidal heights, then it is likely they have fought and won their spots, so perhaps higher tidal levels are more prime territory.

Differences in the entrance circumferences of burrows and thus the size of their crabs may be explained by a few different ideas. Firstly, smaller crabs might prefer to be nearer to the water. This would reduce the risk of desiccation of their gills because the soil is moister; additionally, distance they must travel to the water is lessened, which is important because they do not have the speed of larger crabs (deRivera 2005). Secondly, living near other individuals similar to their size allows for easier access to appropriate mates. Females in the *Uca* genus go on long searches for mates that involve watching for waving males and partially placing legs inside burrows before fully entering to mate. Fiddler crabs of the species *Uca annulipes* examine an average of 24 males before deciding on a mate (Backwell & Passmore 1996). deRivera (2005) found that *U. crenulata* females may look at up to 106 individual males before choosing, so a potential mate must have many good qualities before he is selected. A large claw relative to body size signals health and the assurance that this male has fought for prime burrow space (Levinton 2009). Additionally, the female looks at the male's burrow, as this is where she will stay for up to two weeks until her eggs are ready to hatch.

When sampling burrows, females look for ones that are sturdy, are an appropriate depth, and have a diameter that matches their body size, as deRivera (2005) stated that females enter these more than wider or narrower burrows. She also found that burrow entrance diameter affects the incubation time of *U. crenulata* eggs by artificially placing females in burrows of an entrance diameter that they had previously rejected. Females either released their eggs too early in wider burrows or too late in narrower burrows, so they missed the nocturnal tide which meant their attempt at reproduction failed (Reaney and Backwell 2007). Females that were either allowed to stay in their chosen burrow or were placed in a burrow of the same diameter that they had originally picked were able to release their larvae during this tide.

Similarly, burrow depth also influences the incubation rate of *U. crenulata* eggs (deRivera 2005). I hypothesized that depth of sample burrows would increase as height above the low tide line increased, which my data supports. Not only do deeper burrows maintain temperature better than shallower ones, but they also protect from desiccation and changes in salinity. *U. crenulata* need stable environments for successful reproduction, and less variation in abiotic factors are found in deeper burrows, especially in higher intertidal areas (Willmer et al. 2004). Higher intertidal species have other adaptations that perhaps reduce their need for deep burrows; these include blood that binds oxygen for long time periods in lugworms and some crabs that have membranes to allow for air-exchange out of water (Levinton 2009).

Many burrow samples did not curve much and had steep entrance angles, so depth was similar to the length in most cases. I hypothesized that length of sample burrows would increase as distance from the low tide line; my significant data supported this. This trend in length can also be explained by the idea of more stability of abiotic factors as burrow length increases, which becomes more necessary at higher intertidal levels. Both the temperature and the amount of oxygen and other gasses are influenced by length of the burrow, as airflow is decreased the longer they get (deRivera 2005). This air quality can also have an effect on the incubation rate of eggs, so longer burrows may be preferred by females who are plugged in burrows for such a long period of time while their fertilized eggs mature (deRivera 2005).

Many unique aspects of fiddler crabs have been studied, such as sexual dimorphism, male asymmetry, female mate searching, and waving characteristics. Also, larvae release has been a topic of interest (deRivera 2003). However, characteristics of burrows have not yet been fully explored. Studies on burrows such as this one fill in the detail between courting behavior and release of larvae. As deRivera (2005) has pointed out, burrow features affect courtship to the point that a male might be rejected solely because of his burrow and a female's effort at reproduction could fail as a result of an inadequate burrow, this researcher believes more studies on this topic are necessary.

This study focused on burrow length, circumference, and depth as it changed according to distance from the low tide line. However, other factors besides tidal coverage, such as gender, may influence length, circumference, and depth. Male and female burrows differ in size; male burrows are generally longer and they may have more than one terminal chamber, whereas female burrows suffice with one at most (deRivera 2003). This may be explained by knowing that males can mate with more than one female at a time, so each needs a roomier burrow with pluggable chambers for each female (deRivera 2005). In future studies, the length, circumference and depth between burrows of both genders may be studied to determine if there are significant differences in length and depth when burrows are the same distance from the low tide mark.

Acknowledgements

I would like to thank Dr. Deb Donovan for her advice on data collection, information on invertebrates, critiques on my paper, and enthusiasm to help at all hours, including 6:30 in the morning; Dr. Ben Miner for proposal advice, procurement of supplies, and reassuring me that burrow sampling with foam was worth the personal stress of possible crab murder; Dr. Sergio Ramirez for his help throughout the course. I also greatly appreciate Jorge Robles for making extra trips to the lab to collect me; Cameron Donegan for her editing and support throughout the duration of the experiment.

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Tables

Table 1. Results of ANOVA regression analysis with residuals performed on burrow density data in relation to tide level. P value represents a significant result when less than 0.05. (df= degrees of freedom, MS= mean square)

	df	*MS*	*F*	*P value*
Regression	1	78694.59	5.522553	0.046680431
Residual	8	14249.68		

Table 2. Results of ANOVA test regression analysis with residuals performed on burrow length data in relation to tide level. P value represents a significant result when less than 0.05. (df= degrees of freedom, MS= mean square)

	df	*MS*	*F*	*P value*
Regression	1	638.1992	11.12355	0.003683508
Residual	18	57.37371		

Table 3. Results of ANOVA regression analysis with residuals performed on burrow depth data in relation to tide level? P value represents a significant result when less than 0.05. (df= degrees of freedom, MS= mean square)

	df	*MS*	*F*	*P value*
Regression	1	467.4347	10.57755	0.004688413
Residual	17	44.19121		

Table 4. Results of ANOVA regression analysis with residuals performed on burrow circumference data in relation to tide level. P value represents a significant result when less than 0.05. (df= degrees of freedom, MS= mean square)

	df	*MS*	*F*	*P value*
Regression	1	164826.3	4.616904	0.046357887
Residual	17	35700.61		

Figure Captions

Figure 1. Number of burrows graphed in relation to height above tide with linear regression. $R^2 = 0.4084$. ANOVA $P= 0.0467$. P-values of <0.05 are significant.

Figure 2. Length of burrows graphed in relation to distance from low tide mark with linear regression. $R^2 = 0.3819$. ANOVA $P=0.0037$. P-values of <0.05 are significant.

Figure 3. Depth of burrows graphed in relation to distance from low tide mark with linear regression. $R^2 = 0.3836$. ANOVA $P=0.0047$. P-values of <0.05 are significant.

Figure 4. Circumference of burrows graphed in relation to distance from low tide mark with linear regression. $R^2 = 0.2136$. ANOVA $P=0.0463$. P-values of <0.05 are significant.

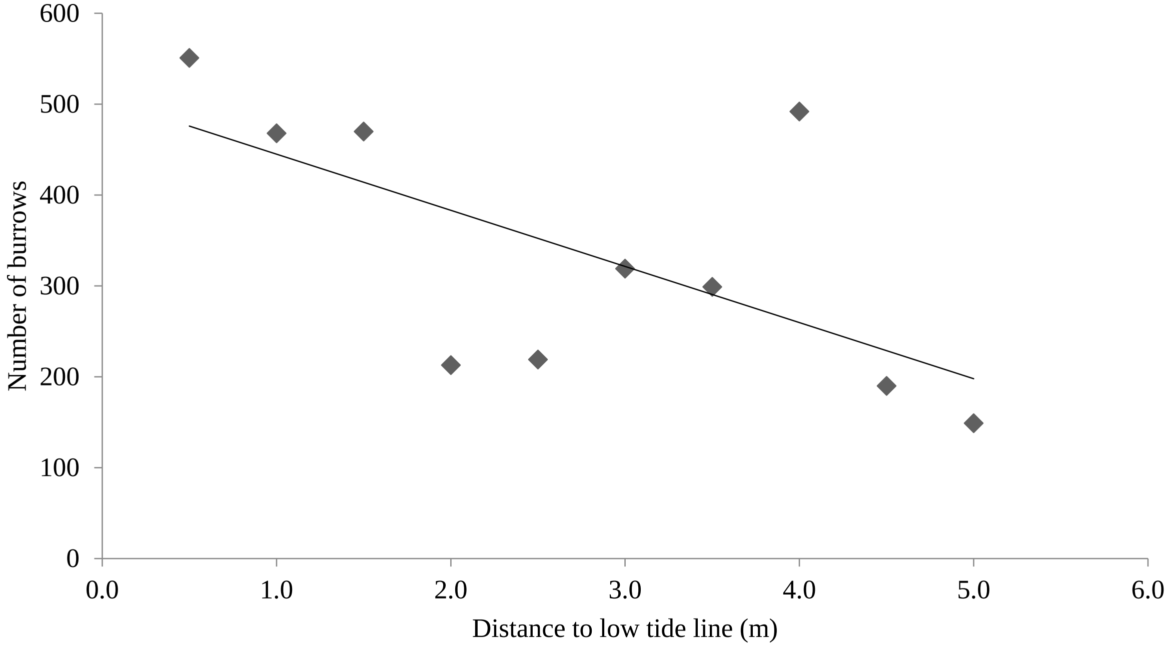
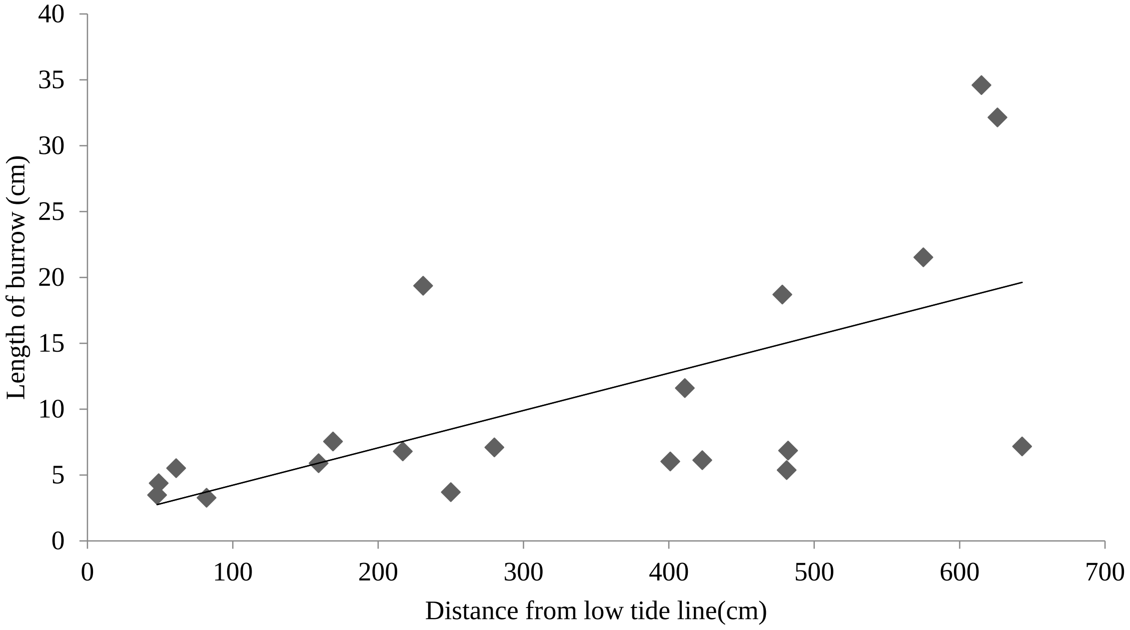
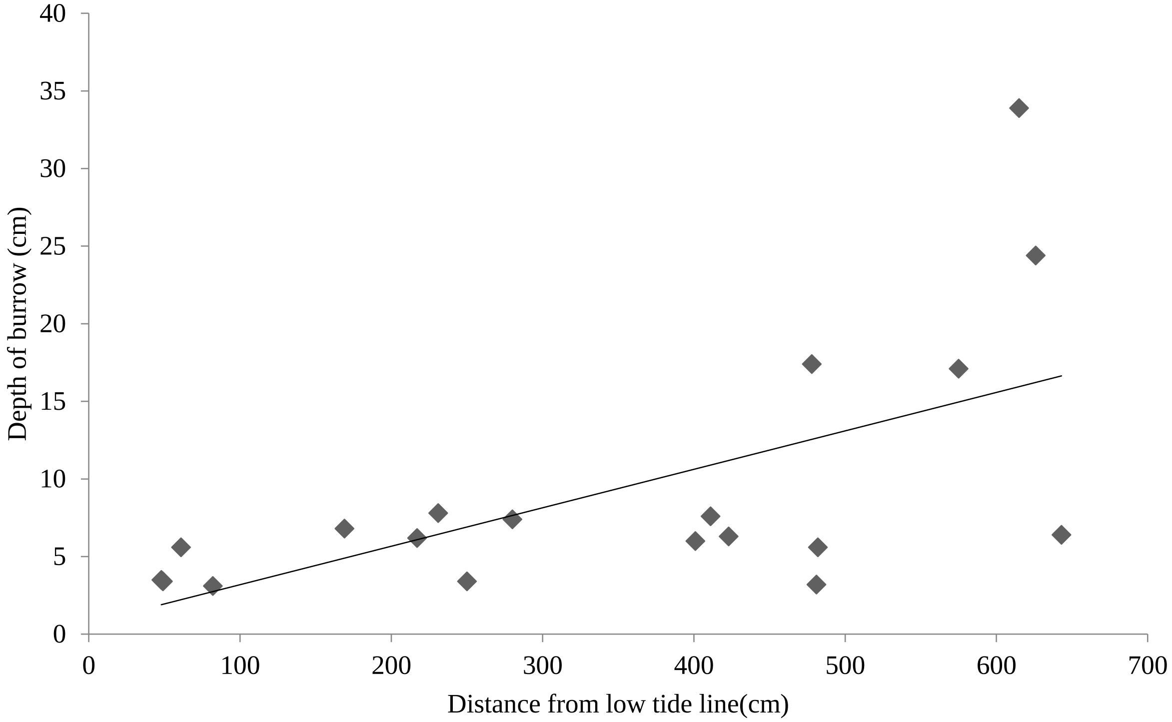

600
500
400
300
200
100
0
Number of burrows
0.0
1.0
2.0
3.0
4.0
5.0
6.0
Distance to low tide line (m)

Figure 1.



40
35
30
25
20
15
10
5
0
Length of burrow (cm)
0
100
200
300
400
500
600
700
Distance from low tide line(cm)

Figure 2.



Depth of burrow (cm)
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500
600
700
Distance from low tide line(cm)

Figure 3.

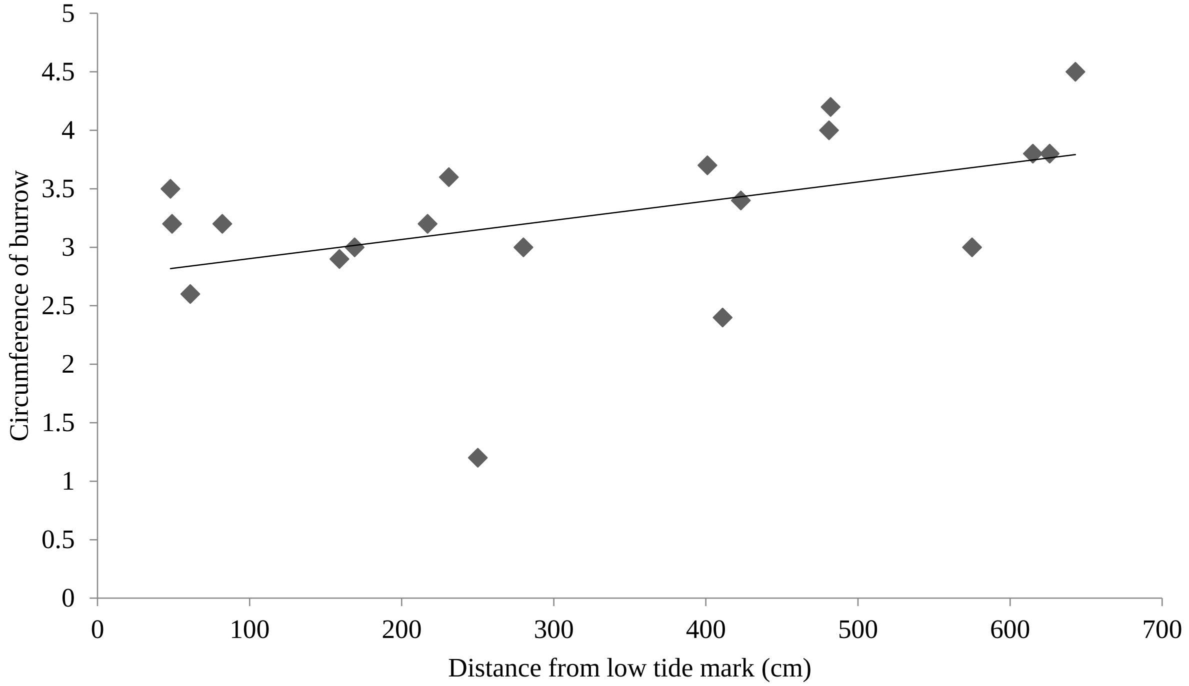

5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Circumference of burrow
0
100
200
300
400
500
600
700
Distance from low tide mark (cm)

Figure 4.

Habitat diversity and substratum composition in relation to marine invertebrate diversity of the subtidal in Bahía de La Paz, B.C.S, México

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Keywords: invertebrate, habitat, substratum, diversity, marine

Abstract

Our study determined whether marine invertebrate diversity is influenced by substrate composition and habitat diversity in the subtidal zone of Bahía de La Paz. In order to sample different marine subtidal habitats we selected three sites: Balandra, Calerita and Pichilingue. We expected that habitat diversity, defined by diversity of substratum composition, would be positively correlated with invertebrate species diversity. We placed three fifteen meter transects at each site and calculated the percent coverage of five different types of substratum. Substratum was characterized as either sand, small rocks, boulders, coral or algae. Samples of invertebrates were taken from three quadrats at each transect. We quantified habitat diversity and species diversity using the Shannon-Weiner Index. According to our results, Calerita was the most diverse habitat. Substratum coverage at Calerita consisted of even amounts of alga, boulders and coral. Balandra was the second most diverse. The site's substratum mainly consisted of large boulders. Pichilingue was the least diverse habitat, dominated by coral. Marine invertebrate species diversity was the highest at Pichilingue, second highest at Calerita and the lowest at Balandra. We found no significant regression between habitat and species diversity indices. However, we found significant regression between percent coral coverage and species diversity, as well as boulder coverage and species diversity. We concluded that coral habitats have a larger capacity for species diversity than we expected. The three-dimensional shape and high production of coral habitats attracts many organisms and can sustain high species diversity. We also underestimated the effect of boulder dominated substratum on the species diversity of an ecosystem. Boulders are normally dominated by highly competitive organisms which have high abundance and alter species evenness. Subtidal boulders experience low frequencies of disturbance and space is rarely available for new sessile species to colonize.

Introduction

Diversity among genes, species and ecosystems can all be used to define biodiversity. Biodiversity on a large scale is shaped by speciation, extinction, genetic drift, gene flow, and changes in geography and climate. Local diversity is shaped over a shorter time frame and is affected by more small-scale alterations in a population. There are many factors that can shift the abundance and distribution of a species allowing for greater diversity to spring up when competition is decreased. We will focus on two influential factors when studying the distribution of biodiversity in the Gulf of California, habitat diversity, defined by the percent coverage of different types of substratum, and the types of microhabitats that compose each observed site.

Characteristics of the substratum are some of the most influential factors when defining an area's biodiversity (Medina 2006). The types of microhabitats present at a site will shape the availability of food, protection, and living space for inhabitants of that site and will therefore affect the area’s biodiversity. Habitat variation also affects the amount of biodiversity seen within a site. Essentially, an increase of species numbers from one place to the next may be explained simply by an increase in the number of microhabitats available (Levinton 2009). This is due to the greater variety of microhabitats provided within a diverse environment. An ecosystem with a large variety of microhabitats has a larger capacity to fulfill the needs of multiple species versus a homogenous habitat, which can only meet the needs of a narrow range of species. With more ecological niches there is less competition for space among species (Yoshida and de Alba 1977).

The purpose of this study was to determine whether marine invertebrate diversity is influenced by substrate composition and habitat diversity in the subtidal zone of Bahia de La Paz. We expected that habitat diversity, defined by high variation in substrate composition, would be positively correlated with invertebrate diversity. Specifically, habitats with more coral coverage will have a positive correlation with species diversity because coral reefs provide a wide range of microhabitats that can support an equally wide range of invertebrates (Levington 2009). Environments that are made up of small rocks and algae will also support high invertebrate diversity. All three of these substrates offer three-dimensional living spaces, in comparison to a sandy or boulder strewn habitat, which only provide a two-dimensional living space. Because of this, boulder strewn and sandy environments will be negatively correlated with invertebrate diversity. Both boulder strewn and sandy environments consist of homogenous habitats. They can only meet the limited needs of a narrower range of species. Because all of the species present in these habitats require the same special resources this may lead to dominance by a more competitive species, in which case abundance of one species would increase and diversity would decrease due to lack of space and resources.

We chose to sample marine invertebrates because their ecological importance makes them a good bioindicator to monitor marine ecosystem health (Nielsen-Muñez & Cortéz 2008). They build up a distinctive and rich ecosystem. With high productivity rates, they can be the food resource for many predators and can constitute complex food chains. Also, their high abundance and relatively low mobility allow us to accurately measure their diversity in a subtidal environment.

Methods

Site descriptions

In order to sample different marine subtidal habitats we selected three sampling sites in Bahía de La Paz, Baja California Sur, Mexico: Balandra, Calerita and Pichilingue. Calerita was chosen to represent the most diverse subtidal habitat. Balandra and Calerita were chosen to represent more homogenous habitats consisting of mainly one type of substratum.

Calculating habitat diversity and substratum composition

To adequately represent the habitat diversity of each site, we laid three fifteen meter transects and recorded the percent coverage of five different varieties of substratum. Substratum was characterized as either sand, small rocks, boulders, coral or algae. By using a one by one meter quadrat to standardize our measurements, we collected this data by taking pictures at every meter mark along the entire length of each transect. Each transect was chosen haphazardly to provide a general representation of the microhabitats present at each site. Transects at each location were laid intending to maintain an approximately constant depth. We measured depth, salinity and temperature in order to ensure that outstanding variables did not vary greatly from site to site.

Calculating species diversity

Samples of invertebrates were taken from three, 1 meter by 1 meter, quadrats from each transect. We measured benthic marine invertebrate diversity by counting the number of species and the abundance of each species of invertebrates in each quadrat. Each quadrat was selected by using a random number generator. In our survey, we did not include invertebrates smaller than one centimeter.

Data analysis

We quantified habitat diversity and species diversity using Shannon-Weiner Index and substratum composition was calculated into percentages. We used Microsoft Excel to analyze the regression of habitat diversity index and substratum composition against marine invertebrate diversity index. Single variable ANOVA's were used in Excel in order to test significance of habitat and species diversity indices between sites.

Results

Habitat diversity and percent composition of substratum

Out of the three sampling sites Calerita had the highest Shannon-Weiner Habitat Diversity Index (1.318). The benthic substratum consisted of mainly corals (37.2%), boulders (29.1%) and algae (21.5%). Balandra had the second highest index (1.036). Boulders covered more than half of the site at Balandra (56.4%). Sand (19.9%) and alga (20.35%) covered areas accounted for the remains of the substratum. The site with the lowest habitat diversity index was Pichilingue (0.837). The site's substratum was composed of corals (70.7%), boulders (20.3%) and small patches of sand (5%) (Figures 1 & 2). The Shannon-Weiner Habitat Diversity Indices were deemed significantly different between the three sampling sites (Table 1).

Species diversity

Marine invertebrate species diversity, quantified by Shannon-Weiner Diversity Index, was the greatest at Pichilingue (3.074). Calerita had the second highest invertebrate diversity index (2.684) followed by Balandra with the lowest index (1.998) (Figure 3). After running a single variable ANOVA test, we deemed the three species diversity indices significantly different (Table 2).

Regression between habitat diversity and species diversity

While examining the data, habitat diversity seemed to be negatively correlated to invertebrate species diversity (Figure 4). However, after regression analysis, we confirmed that there was no statistically significant regression between habitat diversity and invertebrate species diversity (Table 3).

Regression between substratum composition and species diversity

Several correlations were observed between percent coverage of certain types of substratum and invertebrate species diversity. The percentage of coral coverage in each transect was positively correlated to its invertebrate species diversity (Figure 5). This regression was statistically significant (Table 4). Pichilingue, the site with the highest percent coral coverage (70.7%), had the most invertebrate species diversity (3.074). Balandra had zero percent coral coverage and had the least invertebrate diversity (1.998) of the three sites (Figures 2 & 3).

Percent boulder coverage of each transect was negatively correlated to its invertebrate diversity (Figure 6). After running a regression analysis, we confirmed that this negative regression was statistically significant (Table 5). The site with the highest percent boulder coverage, Balandra (56.4%), had the lowest marine invertebrate diversity (1.998). Calerita and Pichilingue had less percent boulder coverage (29.1% & 20.3%) and increasingly larger values for invertebrate diversity index (2.684 & 3.074) (Figures 2 & 3).

The three remaining substratum types that we measured (sand, alga and rock) did not show significant regression to the marine invertebrate species diversity at each site.

Discussion

Our results displayed no regression between habitat diversity and invertebrate diversity (Figure 4). This finding differs from many previous studies of habitat and species diversity, which claim there is generally a positive regression between the two factors (Levinton 2009). However, our results can neither confirm nor deny that a positive regression between habitat diversity and species diversity is a commonly observed trend because we did not find a negative regression between the two factors. We can conclude that the capacity of an area to maintain species diversity is not solely based on large differentiation in substratum, but is rather more dependent on the type of substratum within that area. For example, a site could have many different types of algae, sandy patches and large boulders strewn throughout its area and this site would be considered a diverse habitat. However, these three types of substratum are less able to sustain high species diversity than a coral reef.

Based on our regressions between type of substratum and invertebrate diversity (H') the habitat best suited for supporting high diversity is coral reefs (Figure 5). This is also supported by the fact the Pichilingue, the site with the highest percent coral coverage, has the highest invertebrate diversity (Figure 1 & 3). This may be the case for several reasons the first being, coral reefs provide the most surface area that can be used as habitat. Their irregular shape maximizes the area on the reef that is inhabitable. An increase in habitat abundance leads to a decrease in competition for space among species, allowing for a large number of species to coexist within one habitat. Secondly, a coral reef provides more shelter than other two-dimensional habitats. The reef provides barriers that decrease the strength of wave action and intense sunlight. The reef also provides protection from large predators. Finally, it may be that high diversity centers, like corals, combine stability with higher levels of primary productivity. (Levinton, 2009; Iglesias-Prieto et al., 2004).

The symbiotic relationship coral reefs have with zooxanthellae increases their primary productivity. These zooxanthellae, along with alga colonies, are the main nitrogen and carbon fixers of the coral reef ecosystem. (Iglesias-Prieto *et al*. 2004); In addition, the mucus that is produced by corals is highly energetic and is consumed by fish and invertebrates associated with these colonies. High primary productivity leads to high productivity at subsequent trophic levels.

In short, coral reefs can support high species diversity because of their increased amount of inhabitable area, their high levels of primary productivity and the protection they provide.

Another of our hypotheses stated that habitats including large boulders would have low invertebrate diversity. The results revealed that there was a negative relationship between the boulder-strewn habitats and invertebrate diversity (Figure 6). This may be due to the lack of available space to hide, burrow or inhabit because of the substratum's shape (Sousa, 1979). In this habitat, the two dimensional form obligates the living organisms that occupy the space to be highly competitive specialists. This means that once an organism is attached to a certain place, it would not allow others to inhabit that space. We found that the large boulder strewn habitats have a lower biodiversity and the species that are prevalent dominate the entire habitat.

Marine biodiversity may also be reduced by fragmentation in which previous and continuous habitats are broken up into smaller tracts. An example of fragmentation occurs when large boulders generate large natural barriers for small invertebrates. Boulders also obstruct food that could normally be brought to organisms in the current. Also the number of available mates is decreased, because other individuals are more widely dispersed. We should note that a fragmented habitat may lead to small population sizes that are difficult to sustain over a long periods of time (Levinton, 2009).

Previous studies show that species from the phylum Mollusca and the class Cirripedia are resilient species in habitats consisting largely of boulders; over a large temporal range their abundance remains high and their diversity did not vary. However, other sessile species, such as polychaetes and chitons, showed fluctuation in abundance and diversity over extended periods of time (Chapman 2002). Our results display a similar pattern, for example, at Balandra we observed over 200 barnacles in the three transects. This number is considerably larger than the number of barnacles found at either Pichilingue or Calerita. We can conclude that in habitats with abundant boulders more competitive species, like barnacles and mollusks, are more resilient and therefore flush out less competitive species. This high abundance of competitive species leads to low levels of diversity in the microhabitat. In addition, boulders do not experience high frequencies of disturbance. Wave action rarely affects them and people do not generally turn them over. Because of this there are no other factors that force competitive species out of their niche. The intermediate disturbance hypothesis supports this idea (Sousa 1979). Little species diversity is observed because there are no disturbances to overturn the boulders, open up space, or interrupt the regular distribution of species diversity.

Our results may be used in the future to help conservation efforts select areas that maximize the amount of biodiversity being preserved within a protected area. Utilizing space that is protected is important because there is high demand for the resources provided by the oceans (Ward *et. al* 1999). While conservation efforts are often directed toward a single species and its habitat, if primary focus was shifted to preserving multiple habitats we might be able to conserve more species.

Acknowledgements

We would like to thank Ben Miner, Deb Donovan and Sergio Flores for advising us through the duration of our experiments. Oscar and everyone at UABCS marine lab were also extremely helpful in setting up our experiments and getting all of the equipment we needed. Our editor, Sierra Beckman, deserves many thanks as well.

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Tables

Table 1. This table of values is from a single variable ANOVA where Shannon-Weiner Habitat Diversity Index is the response variable.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.35035353	2	0.175177	10.02185	0.012228	5.1432528
Within Groups	0.10487691	6	0.017479			
Total	0.45523044	8				

Table 2. This table of values is from a single variable ANOVA where Shannon-Weiner Species Diversity Index is the response variable.

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1.779693	2	0.8898463	38.36831	0.000381	5.143253
Within Groups	0.139153	6	0.0231922			
Total	1.918846	8				

Table 3. This table contains values from regression analysis of Shannon-Weiner Habitat Index in relation to Shannon-Weiner Species Diversity Index.

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	0.13463175	0.13463175	0.528200157	0.490948266
Residual	7	1.78421425	0.25488775		
Total	8	1.918846			

Table 4. This table contains values from regression analysis of Percent Coral Coverage in relation to Shannon-Weiner Species Diversity Index.

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.510012239	1.510012239	25.85423874	0.001423545
Residual	7	0.408833761	0.058404823		
Total	8	1.918846			

Table 5. This table contains values from regression analysis of Percent Boulder Coverage in relation to Shannon-Weiner Species Diversity Index.

ANOVA					
	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	1.25281213	1.25281213	13.16702543	0.008413033
Residual	7	0.66603387	0.095147696		
Total	8	1.918846			

Figures

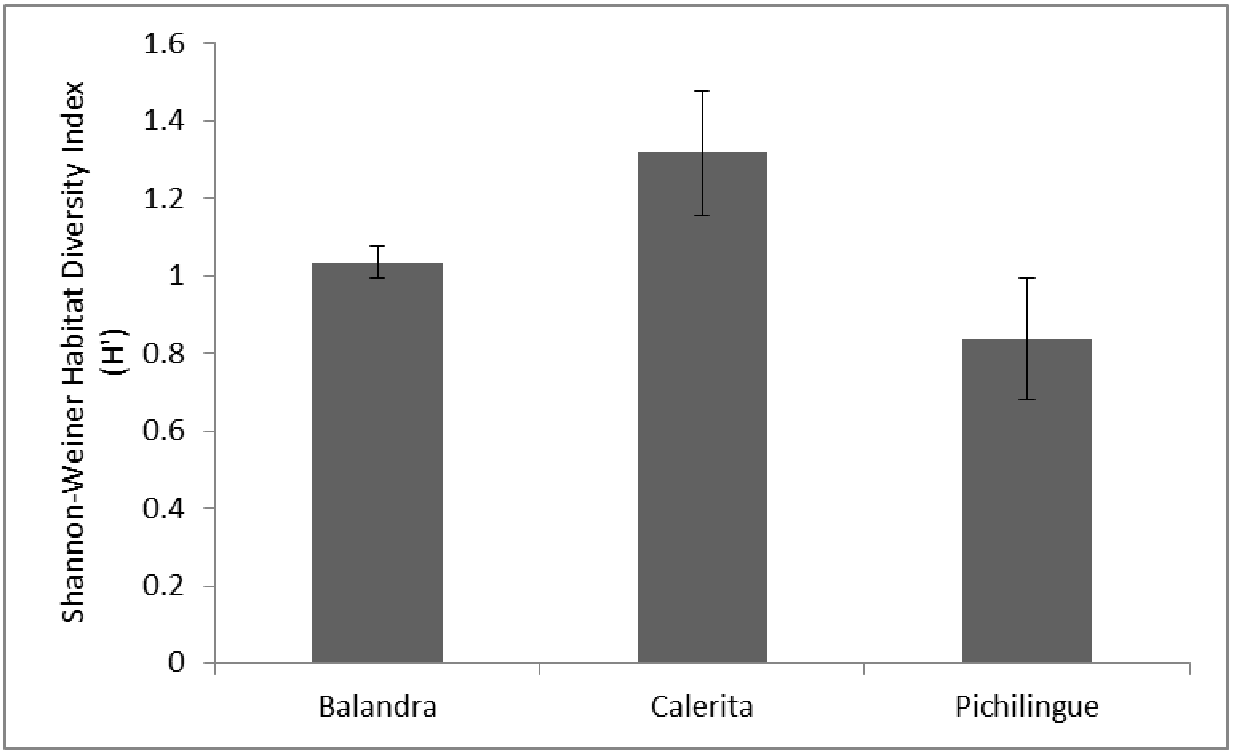
Shannon-Weiner Habitat Diversity Index (H')
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Balandra
Calerita
Pichilingue

Figure 1.

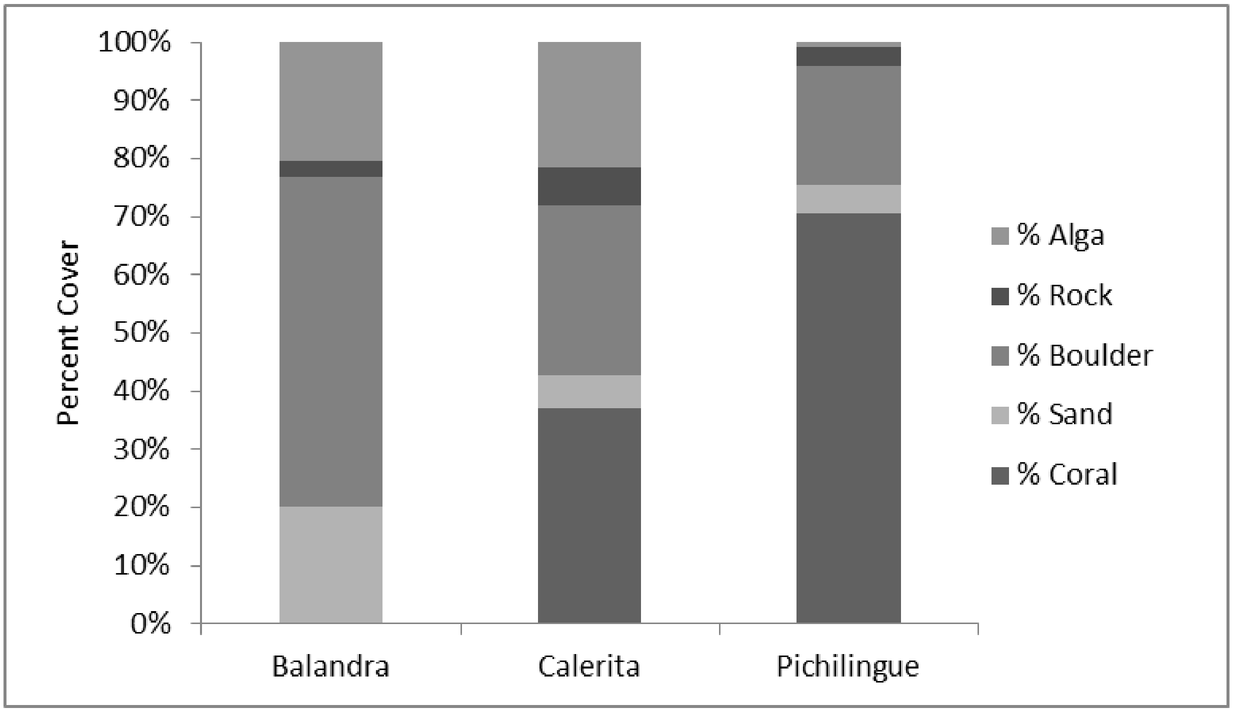

Figure 2.

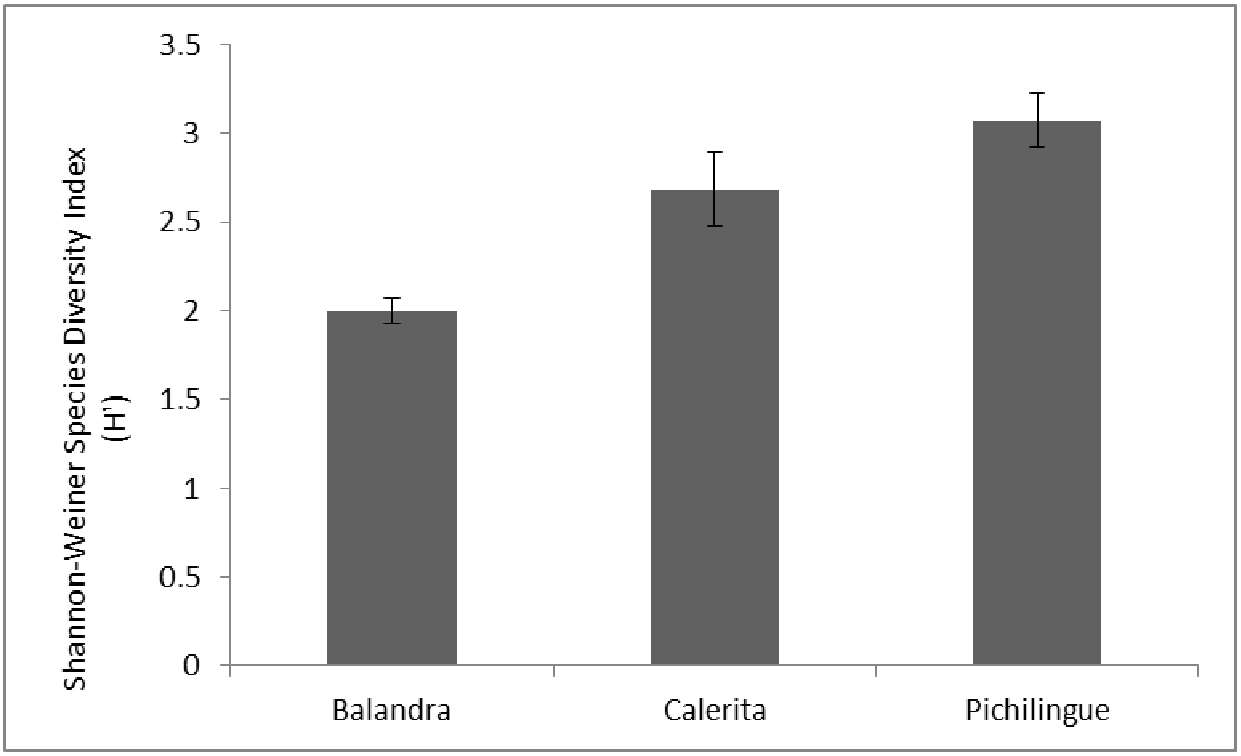

Figure 3.

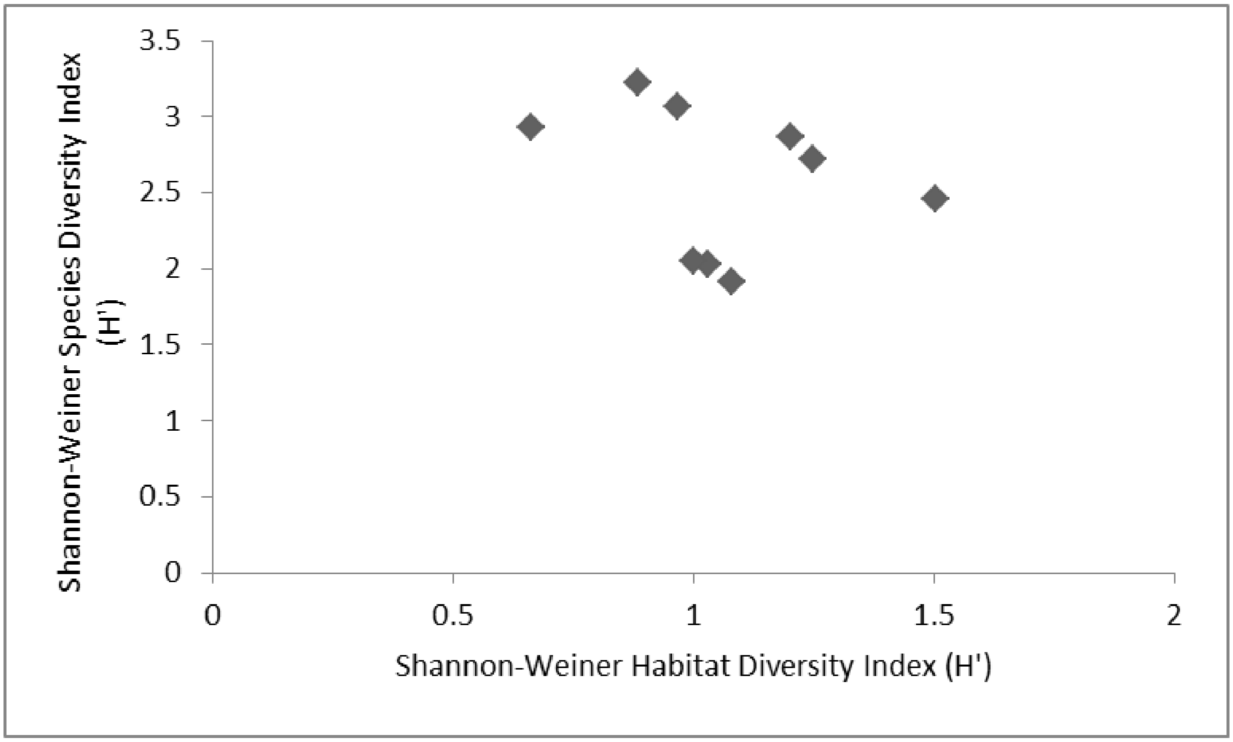

Figure 4.

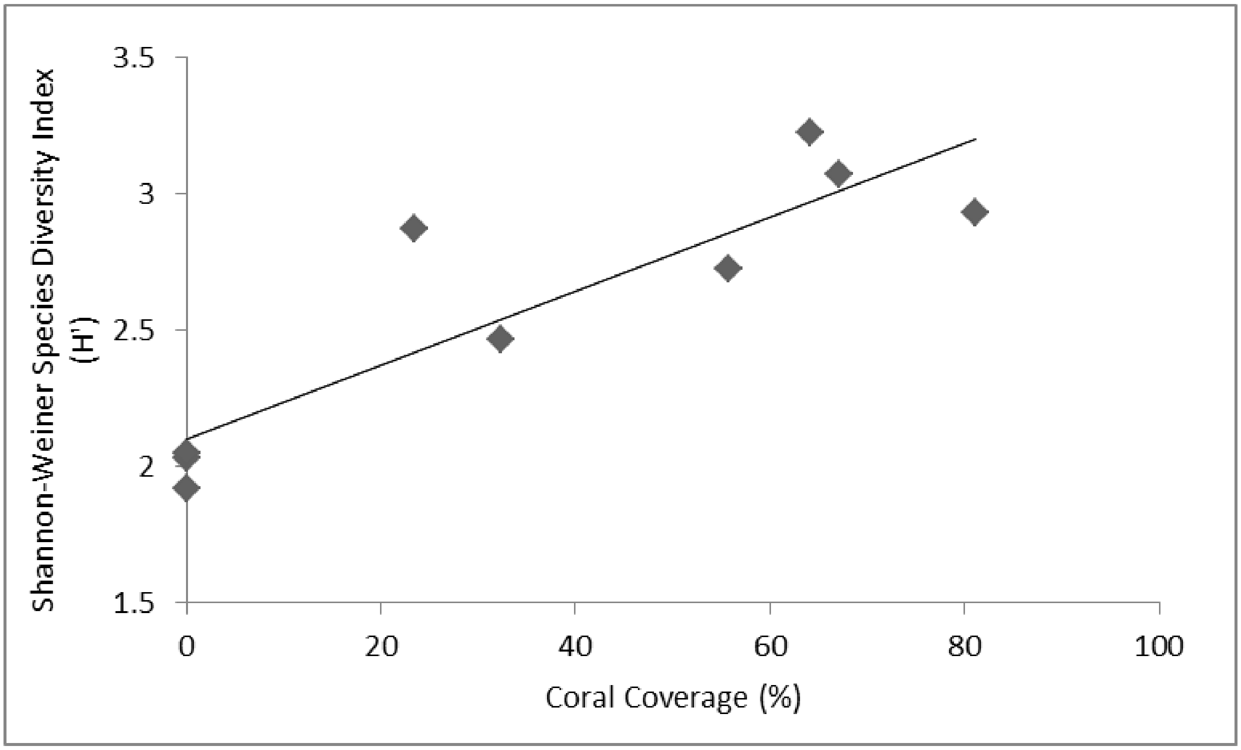

Figure 5.

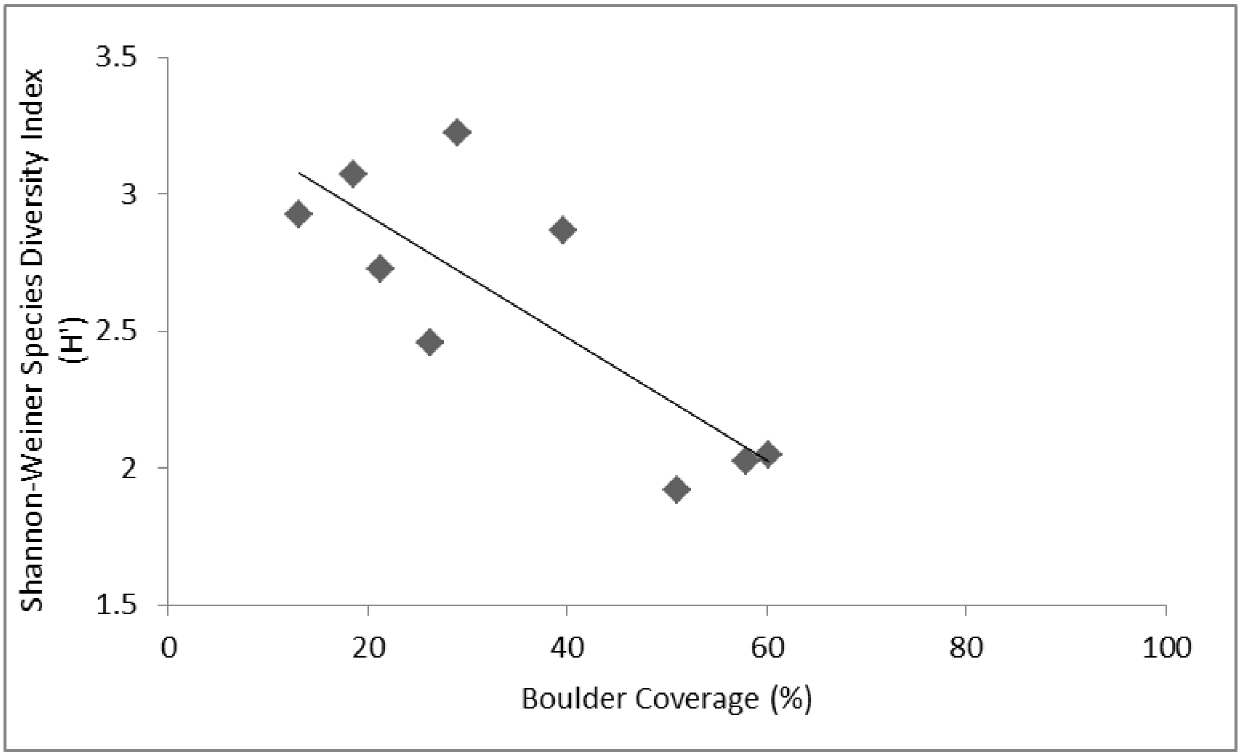

Figure 6.

Figure Legends

Figure 1. This graph shows the difference in habitat diversity indices (H’) between our three sampling sites. Error bars represent the standard deviation of the data.

Figure 2. This graph represents the composition of the substrate at each sampling site. There are five categories of substrate; alga, rock, boulder, sand and coral.

Figure 3. This graph shows the difference in habitat indices (H’) between our three sampling sites. Error bars represent the standard deviation of the data.

Figure 4. This graph represents the relationship between marine invertebrate species diversity (H’) and habitat diversity (H’). There is no statistically significant relationship between marine invertebrate species diversity (H’) and habitat diversity (H’).

Figure 5. This graph represents the relationship between marine invertebrate species diversity (H’) and the percent coverage of coral (%) at all nine transects. The trend line represents a positive linear regression of the data.

Figure 6. This graph represents the relationship between marine invertebrate species diversity (H’) and the percent coverage of boulder (%) at all nine transect. The trend line represents a negative linear correlation of the data.

OPHIUROIDS (Echinodermata: Ophiuroidea) ASSOCIATED WITH SPONGE *Mycale* sp. (Poecilosclerida: Mycalidae) IN THE BAY OF LA PAZ, B.C.S., MÉXICO

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Abstract

Brittle stars are both taxonomic and ecologically diverse. They stars perform important ecological functions in marine communities, and many species develop on sponges forming an ecological community. However, in the Gulf of California there has been little effort to establish an inventory of these echinoderms and their association with sponges. the purpose of our study was to determine the composition of the community of brittle stars on the sponge *Mycale* sp. in La Paz Bay. Ten sponges (*Mycale* sp) from different sizes were collected from each one of the five sites selected in La Paz Bay. Samples were measured and all organisms on them were fixed with alcohol 70%. Later they were conunted and identified. Statistical analysis were made with STATISTICA 8.0 and Excel to evaluate relations between size of the sponges and to determine their ecological status with an Olmstead-Tukey's test. We found six species of brittle stars with a distribution and abundance were greatly different over the bay. There was no evidence of a relation between the volume of the sponges and the number of brittle stars on them. brittle stars seen in this study establish a commensalism facultative relationship with the *Mycale* sp sponges. This way they get protection from predators and a natural source of food (organic particles stuck on the sponges).

Introduction

Brittle stars are both taxonomic and ecologically diverse, in some localities of the Caribbean they can be the most abundant group after the octocorals, nevertheless in the Gulf of California there has been little effort to establish an inventory of these echinoderms (Solis-Marin 1998). It is known that the brittle stars perform important ecological functions in marine communities, being associated with elevated biotic components of reefs and, thus the main reason for important studies of trophic networks (Bejarano-Chavarro et al. 2001). However, the importance of these functions rarely has been demonstrated, and, with some exceptions, their interactions with other organisms have been sporadically documented (Neves & Omena 2003; Ribeiro et al. 2003).

There are many species of brittle star that develop on sponges forming an ecological community, but where the interrelationships between species have not been established. It's been reported that these associations are facultative, and the brittle star can be on or under the sponge. The *Opthiotrix* brittle star uses sponges to protect themselves from currents and to avoid predators. Other studies indicate that brittle stars use sponges as a shelter all day long and they go out to feed themselves at night. One interesting interaction is the one described by Hendler (1984), where he establishes that brittle stars clean the surface of the sponge feeding from stuck particles, thus facilitating the filtration capacity of the sponge.

Since there is currently no inventory of brittle star species in the Gulf of California, the purpose of our study was to determine the composition of the community of brittle stars on the sponge *Mycale* sp. in La Paz Bay, where we search for a homogeneous distribution of brittle stars in the bay. We also relate the size of the sponges with the abundance of brittle stars, so we can determine if the number of brittle stars individuals as well as the number of species depends on sponge size.

Materials and Methods

Study Area

La Paz Bay, Baja California Sur, Mexico, constitutes the most extensive water mass in the oriental coast of the Baja California peninsula (Chávez 1985), and is one of the most important in the Gulf of California because of its constant and highly productive oceanographic conditions (Nuñez-Vásquez et al 2011). The east region is characterized by a predominantly sandy shore of shallow gradient most of which is covered in some parts with algae and primarily of coral *Pocillopora elegans* (Abitia et al. 1996). The west side is characterized by a rocky shore covered primarily of Sargassum (Casas-Valdéz et al. 2006). The area was divided in five sites; 1) San Juan de la Costa, 2) La Paz Marine, 3) Palmilla Beach, 4) Coromuel Beach and Cantamar Marine.

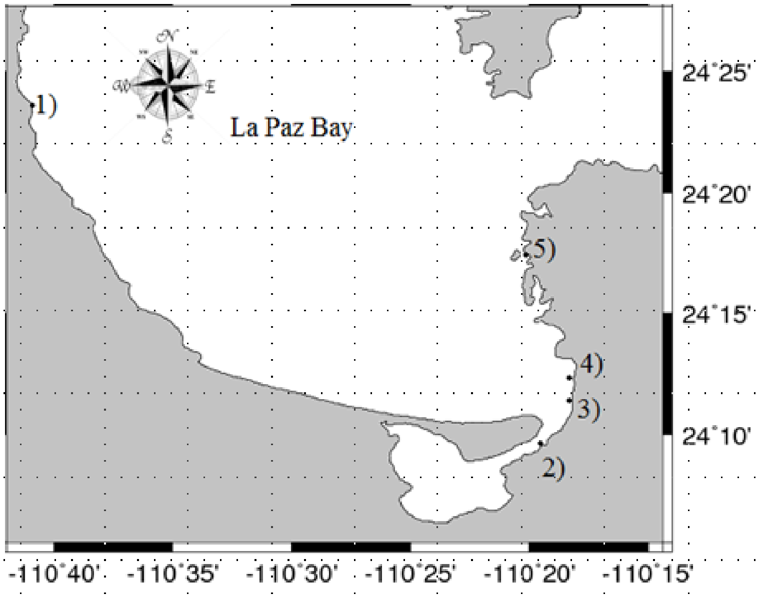

Figure 1. Sample area with its five stations inside the Paz Bay; 1) San Juan de la Costa, 2) La Paz Marine, 3) Palmilla beach, 4) Coromuel Beach and 5) Cantamar Marine

Sample collection

Ten sponges (*Mycale* sp) were collected from each one of the five sites selected in La Paz Bay (San Juan de la Costa, La Paz Marine, Palmilla Beach, Coromuel Beach and Cantamar Marine). The sponges collected were different sizes, three were greater than 30 cm long, three were between 15 and 30 cm, three were less than 10 cm last one was of the most abundant size from that site. With help of a Ziploc bag the sponges were surrounded and then pulled off manually, being careful to not lose the organisms that where on the sponge. The samples were taken to the Faunal Reef Laboratory at the Autonomous University of Baja California Sur where they were fixed with alcohol (70%). Later the measures of the sponges were taken with a rule and the organisms on them were removed away separating them in taxonomical groups. At the same time these were preserved with alcohol (70%). Finally the brittle stars found were counted and identified according to Laguarda-Figueras *et al.* (2009).

Statistical analysis

Normality tests were realized with the software STATISTICA 8.0. The results were non parametric, so we used multiple comparisons between samples through the Kruskal-Wallis test. The size of the sponges was related with the abundance of brittle stars with regressions using

EXCEL 2010 comparing density of each species and the total density of brittle stars with sponge's volume.

We also realized Olmstead-Tukey's test following the methodology of Gonzalez-Acosta et al. (2005) to determine the ecological status of each species. Being the category A) dominant the species that had both density and relative frequency superior to its respective arithmetic mean. B) Common, species with relative frequency superior to their arithmetic mean but density lower. C) Occasional, species with density superior to their arithmetic mean but relative frequency lower. And D) Rare, species with density and relative frequency lower to their arithmetic mean.

Results

In general we found six species of brittle stars divided in four genus and three families; *Ophiothela mirabilis*, *Ophiothrix rudis*, *Ophiothrix spiculata*, *Ophiactis savignyi*, *Ophiactis simplex* and *Hemipholis gracilis*. Their distribution and abundance were greatly different and the species *H. gracilis* was found only in one sponge in Cantamar Marine.

There was no evidence of a relation between the volume of the sponges and the number of brittle stars on them. There was a low adjustment of the model, which presented an r^2=0.25 (Fig. 2).

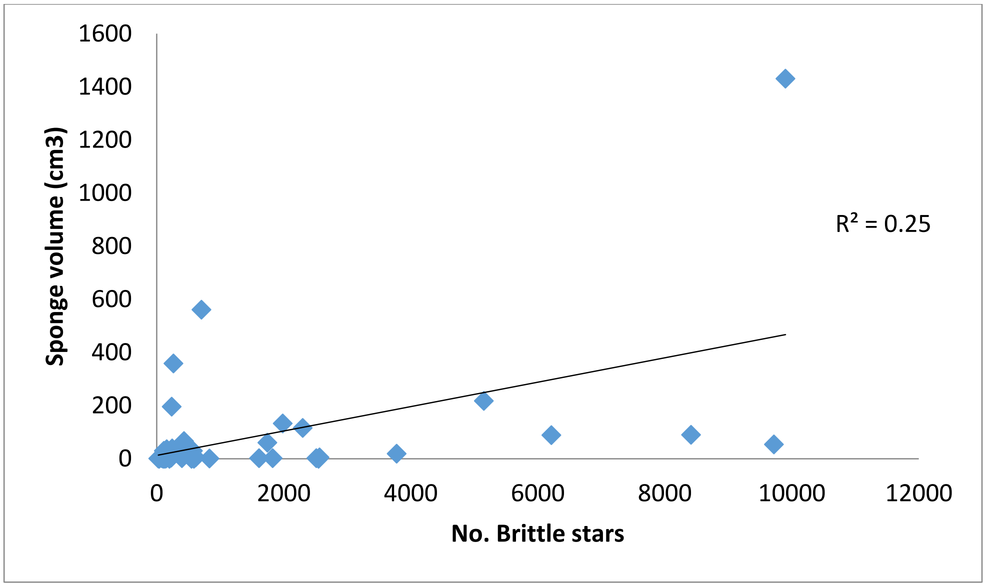

Figure 2. Relation between the number of brittle stars and the sponge volume for the five sites of La Paz Bay.

San Juan de la Costa shows a better adjustment with an r^2 of 0.34 (Fig. 3.1), nevertheless it's not a good model considering the low number of sponges that presented brittle stars.

La Paz Marine improves the adjustment with an r^2 of 0.42 (Fig. 3.2) however it's not a good model too, probably because of the low number of data. The set of data where the volume of the sponges differs greater is the Coromuel Beach Site (Fig. 3.3) which has the lowest value of r^2 with 0.06. Here we could find a sponge with a volume of 1985.28 cm^3 and a tiny one of 254 cm^3. Palmilla Beach on the other side showed a better adjustment with an adjustment of r^2 .87 (Fig. 3.4) however this could not be representative of the site because we were able only to examine four sponges of this site. The last site is the only one that can be considered to explain a direct relation between the size of the sponge and the number of brittle stars with an adjustment r^2 of .99 (Fig 3.5).

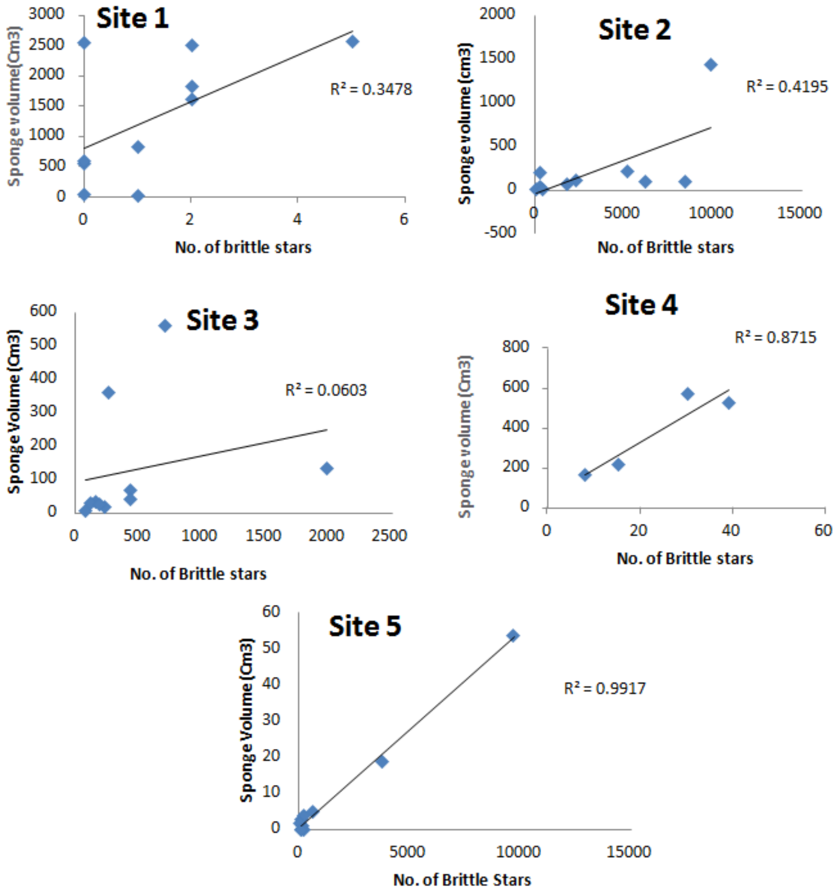

Figure 3. Relation between sponge size (cm3) and the number of brittle stars in the five sites; 1) San Juan de la Costa, 2) La Paz Marine, 3) Coromuel Beach, 4) Palmilla Beach and 5) Cantamar Marine.

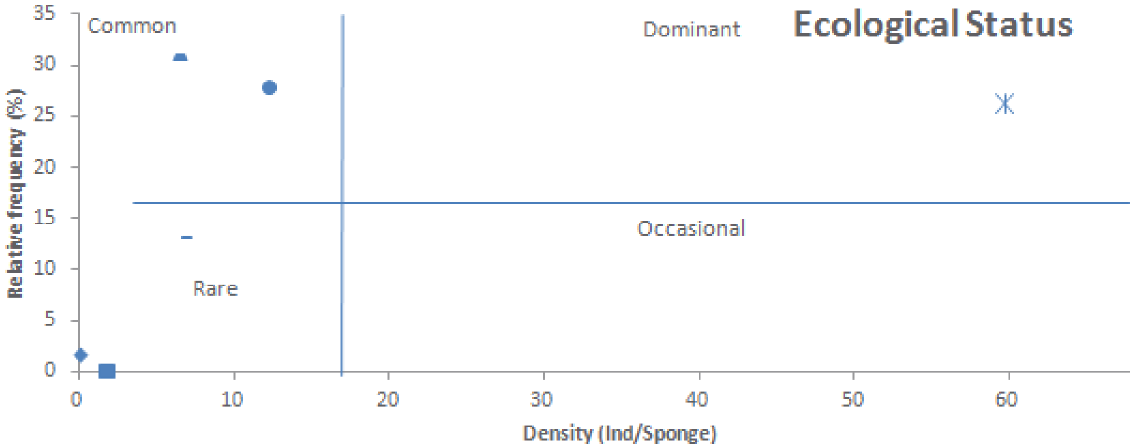

Figure 4.Olmstead-Tukey's diagram of brittle stars that use *Mycale* sp. sponge as a substrate in La Paz Bay; *Ophiotela mirabilis* (Cross), *Ophiothrix rudis* (Circle), *Ophiactis simplex* (triangle), *Ophiothrix spiculata* (square), *Ophiactis savignyi* (line) and *Hemipholis gracilis* (rhombus).

The ecological status of the bay shows that only species that was dominant was (*O. mirabilis*), two were common (*O. rudis* and *O. simplex*), three were rare (*O. spiculata*, *O. savignyi* and *H. gracilis*) without occasional species (Fig. 4).

Discussion

This study revealed a low diversity and richness of brittle stars (only six species) belonging to three genus only; *Ophiothrix¸Ophiactis* and *Hemipholis* being the last one represented by only one individual. This is a big contrast with the 37 species reported for the Gulf of California and the 107 species reported for the Panamic zone (Solís-Marín 1998 and Solís-Marín et al 2005).

Solís- Marín et al. (2005) reported that *Ophiolepis variegata, Ophiocoma aethiops, Ophiocoma alexandri, Ophioderma panamense, Ophiactis simplex* and *Amphipholis platydisca* are the most frequent species in the Gulf of California, being *O. simplex* our only matching species, however, we attribute this difference to the fact that we made our study within the shallow region while the other study was developed in deeper zones.

With the exception of *Hemipholis gracilis* every brittle star identified was already seen on sponges, what suggests there is a non obligated association between the other five species and the *Mycale* sp. sponge, as it has been set out for the relation of other species of brittle stars with sponges (Bejarano-Chavarro et al. 2004; Granja-Fernández & López-Pérez 2011). The encounter of one individual of *H. gracilis* in one occasion using the *Mycale* sp. sponge as a substrate doesn’t represent any kind of relationship or association and we consider it an occasional observation.

Linear regressions didn’t showed any relation at all between size of the sponge and number of brittle stars (Figs 2 and 3) with the exception of the graphs for the site 4 and 5 where the value had a good adjustment (Fig. 3.4 and 3.5). However this could be explained for the fact that Site 4 just had four samples and the data was homogeneous while the Site 5 had three sponges without brittle stars and an homogeneous size within the sponges.

It’s suggested that the species of brittle stars seen in this study establish a commensalism facultative relationship with the *Mycale* sp sponges. This way they get protection from predators and a natural source of food (organic particles stuck on the sponges). However it has not been seen a direct benefit for the sponge of this relationship, and the fact that there is no relation between the size of the sponges and the number of brittle stars suggests that the development of the sponges can go well without the benefits of this symbiotic relationship.

Acknowledgments

We would like to thank our direct advisor PhD. Carlos Sánchez for his support and the facilities of his lab granted to us for this study. Ben Miner for his help in the collection of the samples and his valuable time to correct and be aware of this project.

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Human Disturbance on the Abundance of Six Common Species of Macroalgae in the Bay of La Paz, Baja California Sur, Mexico

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Abstract.

Tourism is a major industry of the southern tip of the peninsula of Baja California. The beaches within the area are heavily-frequented by people who part-take in many tourist activities available. Human disturbance is believed to play a role in the coastal assemblages of algae. Six common species of algae indigenous to La Paz bay were studied including *Ulva lactuca, Codium simulans, Dictyota dichotoma, hydroclathrus clathratus, Hypnea johnstonii,* and *Amphiroa annulata*. The morphology of each of these species varies greatly from one another which may make certain morphologies and life history strategies more tolerant to human disturbance than others. Five locations were visited around the bay of La Paz including the beaches of Calerita, La Concha, Calerita, Pichilingue, and Cantamar. Three transects were placed perpendicular to the shore at each site. Along each transect were placed quadrats noting the presence or absence of each species of interest. Then the percent coverage of each algae was calculated. Human disturbance was estimated by counting the number of people in the first hour of sampling the transects. The number of people was plotted against the calculated percent coverage of each species to see if a correlation between these two variables existed. Then the program R was used to perform an F-test for each correlation. No significance was found for any of the species. However a negative correlation was found for the species that were present at sites chosen. A similar study is called for spaning over a longer time frame to prove if the increased human activity of La Paz bay really has not impacted the benthic algal communities.

Keywords: Baja California Sur Macroalgae Tourism Human Disturbance

Introduction.

In recent years, Baja California Sur has witnessed a substantial increase in tourism. The predicted relatively high anthropogenic pressure on the biodiversity of La Paz bay has become evident (Enrı´quez-Andrade et al. 2005). Souvenir shops, restaurants, and hotels can be found all along the coast line. Tourists also part-take in activities such as snokeling, swimming, fishing, or hiking along the shore. Schiel and Taylor (1999) found that at least 90% of brown algae were removed by groups of hikers along the intertidal in New Zealand. Other studies have shown other forms of human activity to effect coastal benthic algal communities. There is qualitative evidence that many rocky shores in the United Kingdom that have been extensively walked on for several decades have lower levels of biodiversity than in the nineteenth century. It seems even cleaning efforts can impact the beach ecosystems.

Sandy beaches attract high concentrations of tourists. These heavily-used beaches are cleaned frequently by municipal authorities or hotels by removal of rotting seaweed, grasses, dead fish or birds in order to increase the appeal to tourists. However unattractive, natural strandline has many environmental benefits such as enhancement of organic and water content in the upper-shore sand. Enrıquez-Andrade et al. (2005) recognized seaweeds as being a major component to assessing the overall environmental health to coasts along the Gulf of California.

Seaweeds are a group of marine plants which are attributed important ecological roles such as production of atmospheric oxygen and carbon dioxide fixation by photosynthesis in the sea as well as maintaining the health of the areas coast. These have been used as food by humans for over 2000 years and have become very important due to its commercial value as raw material for the production of fertilizers, fodder, medicine and others (Dawes 1987). There are about 140,000 species of algae worldwide (Pedroche et al. 2003). Of which, 6,500 to 7,000 species correspond to marine algae, however, only 40,000 species have been described (Pedroche, et al. 2003). In Mexico, it is estimated that there are about 2,702 species of Rhodophytes, Phaeophytes and Clorophytes (De La Lanza et al. 2000).

The Gulf of California is a subtropical area with special oceanographic conditions. It is a marginal sea of the Pacific Ocean. It has a high diversity of marine flora fauna and high endemism. Because of these characteristics and its relative isolation, it is one of the most studied marine ecosystems of the world. For seaweed richness, it has been estimated that about 580 species of macroalgae have been successfully identified and documented (Readdie et al. 2006). The reasons behind this lack of Rhodophyte and Phaeophyte diversity include abiotic factors including unfavorable substrates, high levels of irradiance, heavy grazing, and freshwater influxes (Pedroche, et al. 2003). Additionally, Valdez and Ramírez (2008) found that regions of lower temperature, high index of upwelling, and high quantities of rocky substrate presented large amounts of seaweed biomass while the opposite was true for areas characterized by high temperatures, sandy substrate, and no evidence of upwelling. In addition, human disturbance should also be considered an important factor affecting algal communities. Pedroche et al. (2003) found in areas hit by high levels of pollution, the abundance of natural macroalgae decreased while the abundance of *Dictyota dichotoma* increased.

In this study, the abundances of six species of commonly found macroalgae in Bay of La Paz were compared to human disturbance. Since knowledge of the biological dynamics is crucial, this allows us to understand how ecosystems respond to natural or anthropogenic disturbance and define what actions should be taken based on this knowledge (Ayala 2001). These species of macroalgae included *Ulva Lactuca*, *Codium simulans*, *Dichtyota dichotoma, Hydroclathrus clathratus, Hypnea johstonii,* and *Amphiroa annulata*. Five sites located around La Paz bay were visited including the beaches of Cantamar, Calerita, Balandra, Pichilingue, and La Concha. These sites, like most of La Paz Bay are composed of sandy and rocky substrates.

Ulva lactuca is most widely known marine Chlorophyte and can be found throughout the world's oceans. The blades of some reach a length of 65 cm, but rarely will its thickness exceed 40 microns (Starr & Taggart 2008). *C. simulans,* another Chlorophyte, is characterized by a spongy, dark green thallus that forms dense circular patches on intertidal rocks. (Readdie et al. 2006). This species is a unicellular, multinucleate organism (Readdie et. al. 2006). We believe that *U. lactuca* has a faster growth rate than *C. simulans* because the thallus of *U. lactuca* is so thin. It was hypothesized that *U. lactuca* to have a greater abundance than *C. simulans* in greater levels of human disturbance because of *U. lactuca's* high growth rate.

The Phaeophtyes chosen were *Dictyota dichotoma* and *Hydroclathrus clathratus.* Based on the study mentioned previously, it was hypothesized that *D. dichotoma* would have a higher abundance than *H. clathratus* in higher levels of human disturbance.

H. johnstonii exhibits a variety of strategies to acquired can be epiphytic on other algae or free-living on rocks or shells. *A. annulata* is a coralline red algae with non-calcified segments between calcified portions on the thallus (Readdie et al. 2006). It was hypothesized that *H. johnstonii* would have greater abundances in higher levels of human disturbance than *A. annulata.*

Methods.

The sites that were sampled included La Concha, Calerita, Balandra, Cantamar, and Pichilingue. The shores of all of these sites consisted of sandy and rocky substrate. Humans frequent most of these natural beaches including Cantamar, La Concha, and Pichilingue. Cantamar and La Concha are beaches located on plots of land owned by hotels. Pichilingue runs parallel to a highway a little north of the hotel Club Cantamar. Tourists are known to frequent these sites. Calerita and Balandra at these locations on the other hand are away from any major roads or hotels, we found species adapted to live in disturbed conditions, mainly Phaeophyta and calcareous Rhodophyta (Ayala 2001). We chose these sites to gain a better understanding of how human disturbance might affect the abundances of the common macroalgae species we selected.

Three transects were randomly placed at each site. Transects ran perpendicular to the shoreline for thirty meters. Half-meter by half-meter quadrats were then placed at each meter of the transects for a total of ninety quadrats at each site. The abundance of each species was measured by noting the absence or presence of *U. lactuca, C. simulans, H. clathratus, A. annulata, D. dichtoma,* and *H. johnstonii*. The level of human disturbance was estimated by counting the number of people at each site for one hour.

From the data collected on the presence or absence of each species, the percent coverage of each species was calculated. Then a Simple Linear Regression was conducted between the percent coverage of each species with the number of people that were counted in the first hour of sampling at each site using the program R. Then the program G*Power 3.1 was used to calculate the power of each regression analysis (Faul et al. 2009).

Results.

After plotting a simple linear regression between the percent coverage and the number of people at each site, an F-test was performed on the data for each species. There appeared to be no correlation between any of the species that were of interest. The species *C. simulans* and *A. anulata* was not observed in the any of the transects. The other four species showed no significant difference (Table 2-5). The slope of each species that was present at each site showed a negative correlation (Figure 2-5). The program G*Power was used to calculate the power of the tests. The power for every regression was 100% at an alpha value of 0.05.

Discussion.

The results from this study do not indicate any evidence to support that people have an effect on the abundances of the algae species selected. *U. lactuca* showed a greater abundance than *C. simluans*, however, *C. simulans* was not observed in any of the transects. The locations of the transects ran along areas comprised mostly of sandy substrata. *C. simulans* is normally found on rocks in the intertidal (Readdie et al. 2006). When the transects of this study ran along rocky substrates, there were greater numbers of seaweed species. However these other species were not of the six species of interest. *H. clathratus* was expected to be in lower abundances than *D. dichtoma* which turned out not to be the case. The third hypothesis regarding *H. johnstonii* being found in greater abundances than *A. annulata* at higher levels of human disturbance also had to be rejected. Other factors may have a greater effect on the composition of the benthic algal community of La Paz Bay.

La Paz bay has a variety of environments, climatic and oceanographic conditions that change year round, and different types of substrata. Norton et al. (1982) noted that the morphology of algae species was a reflection of the environmental conditions in which they matured. Additionally, Littler and Littler (1981) indicated that the algal establishment in the form of mats and tufts seemed to be related to an environment subject to disturbance such as abrasion by sand or because of the instability of the substrate. The areas that were visited around La Paz bay consisted mostly of sandy substrata which may have contributed to the lack of abundance of the species in this study.

This study was conducted in a short time period of only one week. Tourism has been a major impact on the biodiversity of La Paz for many years (Casas et al. 1997). In order for a proper assessment of the impact humans have had on the benthic algal community in La Paz, prolonged experiments are recommended.

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Table 1. Regression results for *Codium Simulans*.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	P-value
Number of People	0	1	00		
Residuals	3	3	0		

Table 2. Regression results for *Ulva lactuca.*

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	P-value
Number of People	5.202	1	5.202	0.0983	0.7744
Residuals	158.727	3	52.909		

Table 3. Regression results for *Dictyota dichtoma*.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	P-Value
Number of People	2.294	1	2.2941	0.1849	0.6962
Residuals	37.215	3	12.4048		

Table 4. Regression results for *Hydroclathrus clathratus*.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	P-value
Number of People	43.028	1	43.028	0.9476	0.4021
Residuals	136.219	3	45.406		

Table 5. Regression results for *Hypnea johnstonii*.

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	P-value
Number of People	12.237	1	12.237	1.0295	0.385
Residuals	35.657	3	11.886		

Table 6. Regression results for *Amphiroa annulata.*

	Sum of Squares	Degrees of Freedom	Mean Square	F-ratio	P-value
Number of People	0	1	0		
Residuals	0	3	0		

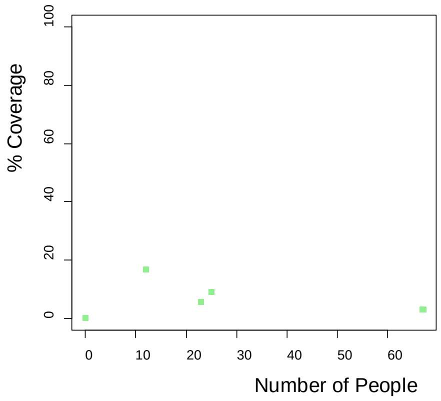

Figure 1.*Ulva lactuca* percent coverage ploted against the Nnumber of people found at each site.

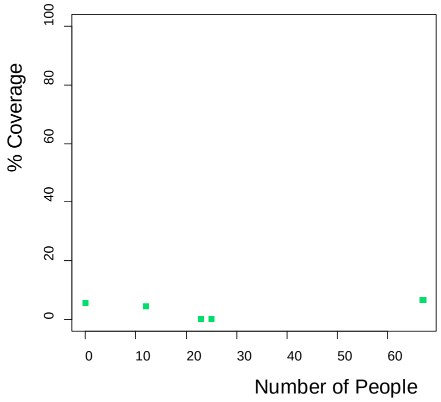

Figure 2.*Dictyota dichtoma* coverage ploted against the Nnumber of people found at each site.

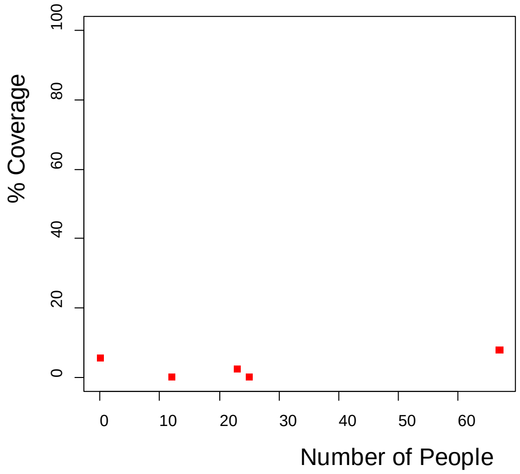

Figure 3. *Hypnea johnstonii* coverage ploted against the Nnumber of people found at each site.

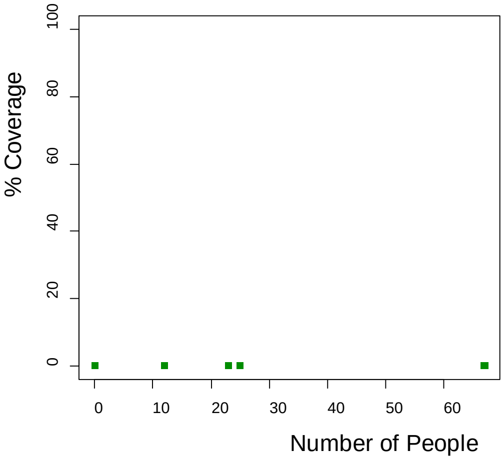

Figure 4. Codium simulans coverage ploted against the Nnumber of people found at each site.

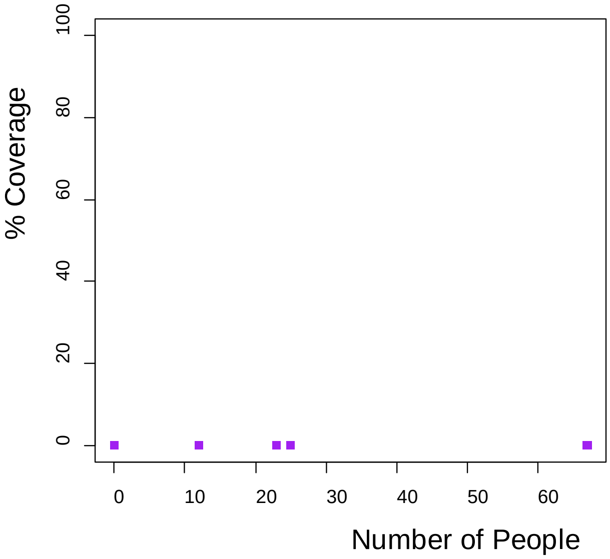

Figure 5.Aphiroa annulata coverage ploted against the Nnumber of people found at each site.