Independent Research Projects

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Universidad Autónoma de Baja California Sur

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Students:

William Barany
Kelsey Donahue
Holli Fontaine
Nathan Goldschmidt
Philip Kohnken
Luna López Martínez
Geoffrey Mayhew
José Pérez Castillo
Rachel Stendhal
Bernardo Veyrand Quirós
Kira Watts
Bobbie Buzzell
Kiersten Ferguson
Jane Foreyt
Claire Howell
Sarah Lerch
Thomas Marley
Rebeca Meléndez Rosas
Erin Stehr
Jack Stimpson
Valeria Villegas

Faculty:

Alejandro Acevedo-Gutiérrez
Sergio Francisco Flores Ramírez
Deborah Donovan
Benjamin Miner

Does Safe Sea's Sunblock with Jellyfish sting protective lotion make nematocysts' stings hurt less than regular sunblock?

Philip Kohnken
Western Washington University Bellingham, WA 98225
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Abstract

Sunscreen was first brought to the market in the 1960's and then to waterproof sunscreens in the '90s, and soon maybe sunscreen to protect from nematocyst stings. Cnidarians sting from physical and chemical stimulants normally on smaller fish and plankton for protection and food, but sometimes humans get in the way. The clown fish, using mucus can resist these stings from the sea anemone, and Nidaria ltd. scientists mimicked this mucus in the form of a lotion humans can use. Using the stinging hydroid, *Lytocarpus nuttingi*, it was tested whether the sunscreen made a substantial difference in pain versus regular sunscreen. It was found that the protective lotion did significantly reduce the pain of a nematocyst sting from the hydroid. The regular sunblock and bare skin had similar pain levels. The clown fish-sea anemone interaction is an action with an anthozoa, and now proving that the pain was lowered from the hydrozoa suggests that this lotion can be used for protection from many cnidarians. The mucus is also only good enough to protect the clown fish from not dying. Since humans don't die from stings, this mucus will only work to an extent to protect a clown fish. Therefore, the lotion won't completely get rid of the pain from a sting.

Key Words

Lytocarpus nuttingi, nematocysts, Safe Sea sunblock, Clownfish mucus

Introduction

The sun is the provider for our galaxy and humans have learned to utilize its energy for a long time. However, along with this utilization come ultraviolet rays that give skin cancer to those without proper protection. Sunscreen was first brought to the market in the early 1960's, which was only designed to block ultraviolet light. In 1962, the sun protection factor, SPF, was brought to realization along with protection from

ultraviolet-B radiation. In the late 80's, it was brought to attention that ultraviolet-A radiation also plays a role in skin cancer, and sunscreens started to protect that too. As sunscreens became more popular, waterproof sunscreens were in need because they kept washing off whenever people entered the water. So, in the 90's waterproof sunscreens were made.

Having waterproof sunscreens allowed people to go into the oceans and not have to worry about burning when getting out, but there are other dangers within the ocean to worry about. Common dangers to humans in the oceans are the cnidarians: jellyfish, sea anemones, sea nettles, and hydroids. They contain stinging mechanisms, which include nematocysts. Nematocysts are made in nematoblasts, which trigger from either a physical or chemical stimulant. When triggered, the capsule operculum is opened and osmotic pressure is believed to increase, to shoot the nematocysts into the prey, which tend to be smaller fish or plankton. Although, most cnidarians are not lethal to humans, some have caused organ failure and other skin diseases (Newsletters 2001).

One organism capable of not getting hurt by nematocysts is the clown fish. They like to use sea anemones as homes, and since sea anemones use nematocysts, the clown fish coat themselves with mucus that will insulate and protect them from the stinging. In return for the home, clown fish protect the anemone (Drury 2008). Scientists from Nidaria Ltd. in Israel learned a way to replicate this mucus and turn it into lotion humans can use to protect themselves from nematocysts, and they call it Safe Sea.

The point of this experiment was to test whether using Safe Sea makes a substantial difference versus your skin or other sunscreens in protecting against stinging nematocysts using the stinging hydroid, *Lytocarpus nuttingi*, as our stinging organism. The lotion was made to protect from anthozoa organisms because of the sea anemone, and we are using a hydrozoa, so we'll also test if this lotion can work for multiple cnidarians. The stinging hydroid is immobile and uses mucus from its base to attach to the ocean floor, especially bigger rocks. They reside in areas of stronger currents, and it uses its nematocysts to sting prey floating past and filter them to the mouth by their tentacles (Armstrong 2007).

Our hypothesis for this experiment is that the Safe Sea sunblock with jellyfish protective lotion will have a lower pain level than the regular sunblock and control, which is bare skin. Also, we assumed that the regular sunblock and control would be about equal in pain level.

Methods and Materials

Lytocarpus nuttingi was gathered from Calerita, Baja California Sur, Mexico at a depth of 10 to 15 feet. The whole organism was picked up and taken to lab to be placed in a tank of running seawater. With tongs and scissors, *L. nuttingi* was cut into smaller branches, each with enough polyps to sting, while in the seawater.

With a permanent marker, 6 sections were drawn on 3 parts of the arm of both arms: forearm, underside forearm, and bicep. 2 different sunscreens were used: Safe Sea sunblock with jellyfish sting protective lotion (30 spf) and Ultra Defense Sheer Protect (30 spf), both of which are waterproof. In each section of the arm, there were 2 controls blocks, 2 regular sunscreens blocks, and 2 protective sunscreens blocks.

Using q-tips, one person applied the sunscreens to each section of the arm without the one to be stung knowing which sunscreen is where, and recorded on a separate sheet which sunscreen was placed where. After 15 minutes to insure the sunscreen had soaked in, one arm was placed into the tank, which has *L. nuttingi* in it, and the branches of polyps were applied to the blocks of sunscreen and control for 3 seconds each. After one arm was fully stung, the other arm was placed in the tank and stung.

A qualitative scale was used to measure how painful each sting was. 3 was very painful, 2 was painful but bearable, 1 was a little painful, and 0 was not painful at all. This process was completed 3 times to have a total of 36 stings per sunscreen and control.

Results

The protective sunblock of nematocysts affected pain level. Statistical analysis was done on data using the R-program. Sunblock type was the only factor that was significant (Table 1). Other combinations of factors were poor predictors of pain level (Table 1). The control and regular sunblock were close in average pain level, with the

regular sunblock being a little higher (Fig. 1). The protective sunblock significantly lowered the average pain level (Fig. 1).

Discussion

Safe Sea sunblock with jellyfish sting protection lowered the pain of the nematocysts sting from Lytocarpus nuttingi, which is what we expected. Although it lowered the pain, it did not completely get rid of it. The control and regular sunscreen were very close in pain level, which is what we expected.

Nidaria ltd. made a sunscreen with lotion that mimicked the mucus from clown fish when they spread it over themselves to be protected from sea anemones. Sea anemones are apart of the anthozoa phylum, which means that the lotion was made from an organism protecting itself against anthozoa only. But, because the pain was lowered when stung by a hydrozoa, this suggests a wider range in cnidarians for nematocyst protection. On the bottle of Safe Sea sunscreen, it says that it was tested to be effective against Sea nettles, Atlantic Box Jellyfish, and Rhopilema. Now we can add to the list stinging hydroids.

A clown fish emits mucus to be able to survive in the sea anemone. This mucus emitted is meant for a very small organism, so to replicate something that was meant for big things offers challenges. Cnidarians use nematocysts to kill or injure small fish to eat. The clown fish has mucus that protects itself fully, but only enough for it to survive these stings. Humans, being a lot bigger, don't die normally after a nematocyst sting. So, to not fully feel a sting would take a lot stronger proteins than the mucus has to offer. So, we still feel pain after a sting using this lotion, but the pain is less than it would be for skin or regular sunblock.

Regular sunblock had a higher value of average pain, but with error included, regular sunblock and the control can be considered the same. However, after further study to see if regular sunblock hurt more than skin, then it would suggest that buying Safe Sea's protective lotion would be a really good idea if you burn and are going to a place known to have cnidarians that could sting you. While protecting against skin cancer and being water proof, Safe Sea offers sunscreen to protect against different types

of cnidarians. With further experimenting, a sunscreen might be made to fully protect us from all nematocyst stings, just as we found a sunscreen to almost fully protect us from skin cancer.

A couple sources of error occurred when the sunscreen was being applied. The person was blindfolded as to not see which sunscreen was where. However, it was sometimes obvious to tell which sunscreen was which just by the way it soaks into the skin. So, some data points were biased. Also, the polyps from *L. nuttingi* were sometimes not directly placed on the sunscreen and hit some skin. *Lytocarpus nuttingi,* after a couple days, did lose some polyps from its branches, and when the trials were done, the resultant pain could've lessened.

Acknowledgements

I'd like to thank Dr. Ben Miner for helping prepare results, driving me to and helping me get the hydroids from Calerita, and for giving me ideas about the discussion; Dr. Alejandro Acevedo and Dr. Deb Donovan for moral support and funding; Jorge Robles for driving, and WWU for giving me the opportunity to do this experiment; Jack, Luna, Geoff, and Rachael for helping apply sunscreen.

Reference

Armstrong, M. 2007. Wildlife and Plants: Horseshoe crab to ladybug. Marshall Cavendish **9**:526-527.

Drury, F. 2008. Marine symbiosis: clownfish & anemones: the mutual symbiosis between clownfish and their host sea anemones. Gale, Sengage Learning.

External Reflection from a Water Surface. ScubaGeek. Available from http://scubageek.com/articles/wwwatw.html.

Jellyfish Sting Newsletters. 2001. University of Maryland School of Medicine **24**.

Nematocysts. The Jellies Zone. Available from **http://jellieszone.com/nematocysts.htm**.

Smith, Shawn M. A Brief History Regarding Sunscreen. EzineArticles. Available from **http://ezinearticles.com/?expert=Shawn_M_Smith**.

The Safe Sea Story: stop the stinging. DiveSports. Available from **http://www.divesports.com/Safe-Sea-Jellyfish-Protectant-Story-a/142.htm**.

What is the history of sunscreen? WiseGeek. Available from http://www.wisegeek.com/what-is-the-history-of-sunscreen.htm.

Table 1. Results from the three-factor ANOVA. The three main factors were the arms with 2 variables, position with 3 variables, and sunblock with 3 variables. The other factors were combinations of the three main factors. Df represents degrees of freedom, SS sum of squares, MS mean squares.

	Df	SS	MS	F-value	P
arm	1	1.12	1.12	2.67	0.11
position	2	0.22	0.11	0.26	0.77
sunblock	2	19.06	9.53	22.67	<0.0001
arm x position	2	0.52	0.26	0.62	0.54
arm x sunblock	2	0.02	0.01	0.02	0.98
position x sunblock	4	1.39	0.35	0.83	0.51
arm x position x sunblock	4	0.76	0.19	0.45	0.77
residuals	90	37.83	0.42		

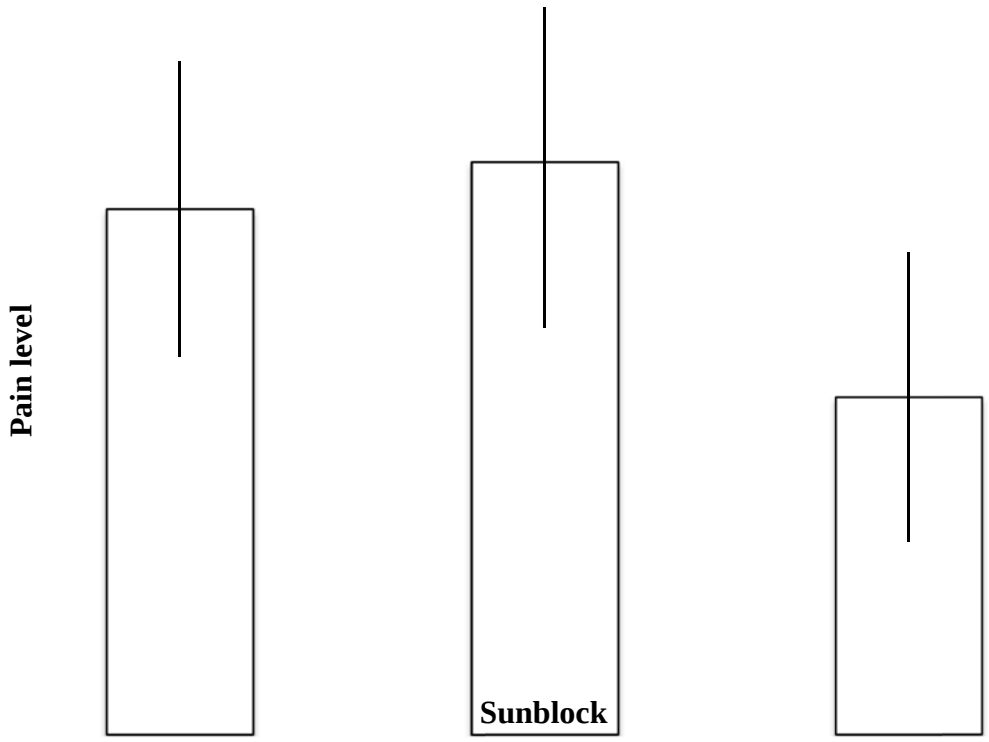

Figure 1. The pain level associated with each sunblock and control after the nematocysts were applied from *Lytocarpus nuttingi*. 36 stings per sunblock were done, and qualitative measurements were made to rank the pain of the sting on a scale of 0-3, 3 being the most pain.

Analysis of the territorial behaviors and responses to nest disturbances of *Abudefduf troschelii* (Panamic sergeant major)

Jane Foreyt, Claire Howell, and Valeria Villegas

Department of Biology, Western Washington University, Bellingham, WA 98225;
Department of Biology, Humboldt State University, Arcata, CA 95521;
Departamento de Biología Marina, Universidad Autónoma de Baja California Sur, La Paz, B.C.S, México C.P. 23080

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Abstract: *Abudefduf troschelii* (Panamic sergeant major) lives along coastlines from the Gulf of California to the Galapagos Islands. To cope with environmental pressures, the fish engage in colonial nesting, fiercely defend their nests, and prioritize threat removal. We tested the prioritization responses of *A. troschelii* to different disruptions in their nests during breeding season. This was done by introducing 6 different organisms/objects (*Pharia pyramidata*, *Heliaster kubiniji*, *Echinometra vanbrunti*, *Tripneustes depressus*, a rock, and sand) into their nests (*N*= 5 for each) and observing both the time the fish first attempted to remove them and the time it took for complete removal. We found that *A. troschelii* prioritizes its removal efforts by attempting to remove living organisms before abiotic objects. Prioritization activity maximizes the probability of egg survival. Total time of removal depended on if the organisms stuck to the rocks. Among those that did not stick there was no difference between organism and object removal times, but the fish's behavior during the removal time demonstrated a system of prioritization. *A. troschelii* first prioritized defense against fish, then the removal of biotic, and finally abiotic intrusions.

Key Words: *Abudefduf troschelii,* prioritization, territoriality, colonial nesting

INTRODUCTION

Context-dependent decision making is an important trait for species living in complex environments (Leese *et al.,* 2009). Fish living in habitats such as high diversity rocky areas near shore must be able to respond to a combination of factors, including predation and resource availability, in a manner which maximizes benefits. Decision making and defensive prioritization are particularly pertinent behaviors surrounding nesting. Adaptive strategies and behaviors employed during these times reflect environmental pressures such as competition for space, competition for mates, and

predation of eggs (Foster, 1989). This is exemplified by the colonial nesting behaviors of *Abudefduf troschelii*, the Panamic sergeant major of the Gulf of California.

Abudefduf troschelii is among the most common reef fish in the Gulf of California and is distributed as far south as the Galapagos Islands (Thomson *et. al.,* 2000). It is distributed throughout rocky, shallow subtidal areas and feeds on algae and small invertebrates. In the early summer season, males group together to form nesting colonies; each male clears an area approximately 1–2 m^2 of rocks, sand, and algae and guards it fiercely from any intruders throughout the entire breeding and nesting season (Haley and Müller, 2002). Once a male chooses a mate and the female lays her eggs in his territory, he fertilizes the roe and continues to defend the nest until the eggs are hatched by the end of the summer (Thomson *et al.*, 2000).

Colonial nesting and territoriality are two of the most apparent nesting behaviors of *A. troschelii* which result from the pressures of their environment (Leese *et al.*, 2009). Tyler (1995) observed nesting behaviors of *Abudefduf abdominalis,* a Hawiian damselfish with nearly identical behavior and ecology to *A. troschelii* (Thomson *et al.,* 2000), and concluded that colonial nesting reduces predator interactions with eggs. Communal nesting grounds also result in greater mating success, because large groups of males send off stronger signals to females and are more likely to attract them than solitary males (Foster, 1989).

Increased territoriality enhances protection of the eggs from predators; however, it must be balanced with the cost of defense to the fish, as well as time losses and distractions from mating (Leese *et al.*, 2009). Many factors in *A. troschelii* habitats, such

as the fish's health and the simultaneous arrival of a predator or intruder and a mate, influence the fish's response. In this intricate environment, it is advantageous for the fish to prioritize its concerns and respond accordingly.

In this study we were interested to see how *A. troschelii* prioritized their reactions to various disruptions in their nesting sites. We observed the reaction times of *A. troschelii* individuals when six foreign objects (two carnivorous species of sea stars- *Pharia pyramidata* and *Heliaster kubiniji;* two herbivorous species of urchin- *Echinometra vanbrunti* and *Tripneustes depressus*; an algae-covered rock; and handfuls of sand) were introduced to their nesting territories. The time it took to remove each was recorded. We hypothesized that *A. trochelii* would respond to the placement of any foreign object (living or inanimate) by attempting to remove it, and that the fish would prioritize the removal of living objects over the removal of inanimate objects. Among the living organisms, we also hypothesized that carnivorous species would be perceived as a greater threat to the nest and would thus be removed before herbivorous ones.

METHODS

This study was conducted in the middle of *Abudefduf troschelii* breeding season in the near-shore rocky habitats at Club Cantamar in Pichilingue, Baja California Sur, Mexico. One individual of each of the following four organisms were collected to test the responses of *A. troschelii* to biotic intrusions of their breeding territories: *Pharia pyramidata* (yellow spotted star), *Heliaster kubiniji* (Gulf sun star), *Echinometra vanbrunti* (a common Pacific sea urchin), *Tripneustes depressus* (brown urchin). Abiotic intrusions used were a small, algae-covered rock and handfuls of sand. Trials were run

prior to data collection in order to determine acceptable organism and rock size for feasible removal. *P. pyramidata* was placed upside down (to prevent it from quickly adhering to the rocks) in the center of one *A. troschelii* territory, the observers swam a sufficient distance away so as not to distract the fish, a stopwatch was started, and the behavior of the fish was observed. Times were recorded when *A. troschelii* made its first attempt to remove *P. pyramidata* and again when the intruder was completely removed. This procedure was repeated five times for each of the six different organisms/objects (N= 5 for each species). The same organism for each species and the same rock were used for each of their five respective replicates, and a new handful of sand was gathered for each sand replicate. A total of 30 different breeding territories were observed. Figures were created to reflect the time of initial interest and total removal time, and significance of results was calculated with a one-way ANOVA test.

RESULTS

There was a considerable difference between the times of *Abudefduf troschelii's* first removal attempt of live organisms compared to the abiotic objects. The mean response times differed significantly among the organisms/objects (one-way ANOVA $F_{5,24}$=36.14, p<0.001). The mean for all four live organisms was 6.85 ± 4.17 seconds (all values are means ± standard deviation), and between the two abiotic objects the mean response time was over 9 times longer (64.6 ± 39.6 seconds) (Figure 1). The shortest response time evoked by an object (sand) was 36.6 ± 19.3 seconds, which was 182% longer time than the longest response time evoked by an organism (*Pharia pyramidata*) at 13 ± 6.32 seconds.

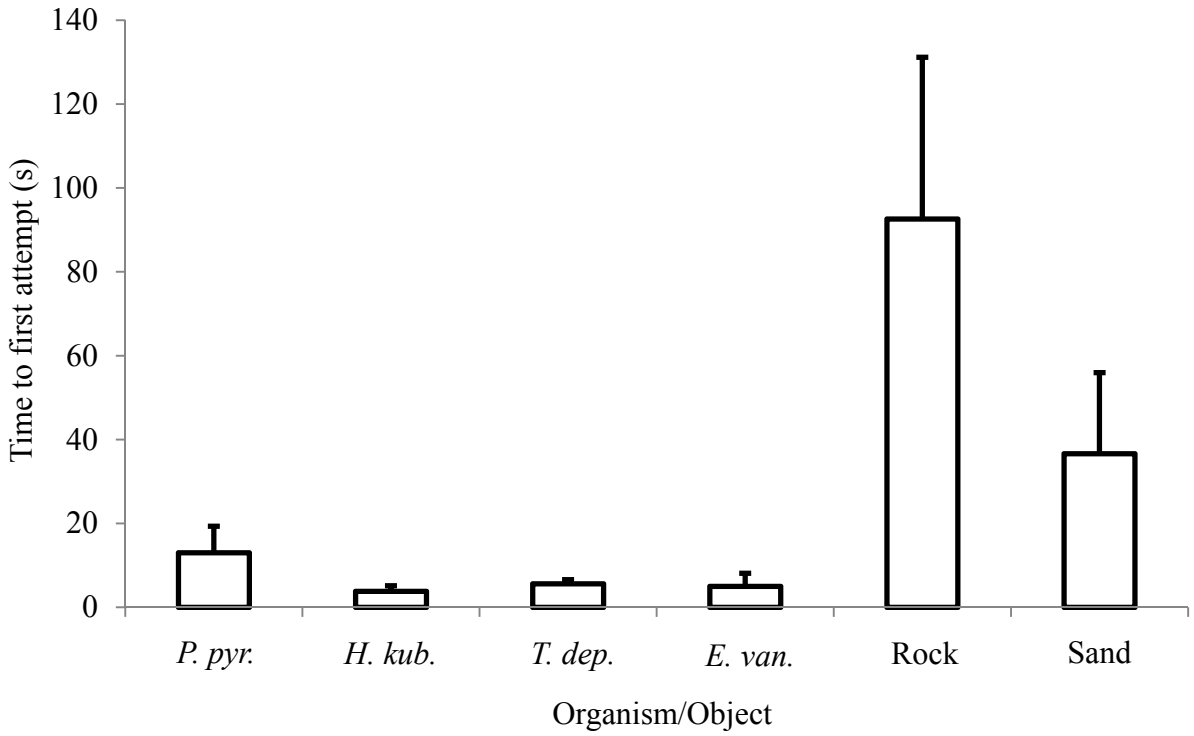


Figure 1. Mean times to the first attempt by *A. troschelii* to remove the following organisms/objects from their breeding territories: *P. pyramidata, H. kubiniji, T. depressus, E. vanbrunti,* a rock, and handfuls of sand. Five territories were tested for each of the six intrusions (*N*= 5 for each species), and the same organisms and rock were used for each of their five respective replicates. Error bars indicate standard deviation.

The total removal times for the organisms and objects were on average similar, regardless of the nature of each intruder. Two distinct sets of data are present among the total removal time data: one where removal times were less than 180 seconds and the other where removal times were greater than 350 seconds (Figure 2). In general, it took fewer than 180 seconds for *A. troschelii* to remove the organisms/objects once the fish displayed interest in their presences; the mean removal time for the intrusions with faster removal times was 47.4 ± 25.2 seconds. There were a few cases (only organisms) where removal times ranged from 350 to 1200 seconds; the mean removal time for the intrusions with slower removal times was 852 ± 291 seconds. Of the organisms which

exhibited these longer total removal times, *Heliaster kubiniji* averaged the longest (1140 ± 84.9 seconds) and *Echinometra vanbrunti* averaged the shortest (600 ± 340 seconds), nearly half the average total removal time of *H. kubiniji*.

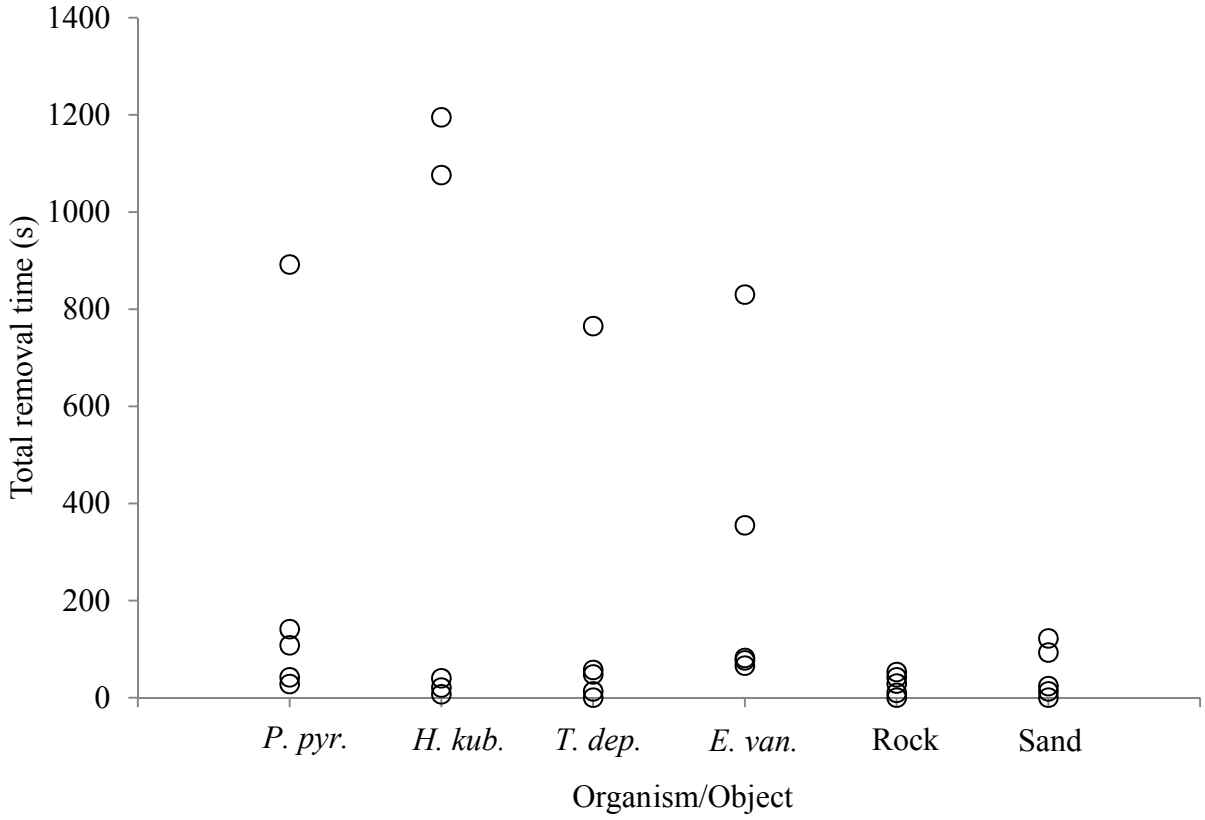


Figure 2. Raw data of total removal times from *A. troschelii* breeding territories for each replicate of each organism/object (*P. pyramidata, H. kubiniji, T. depressus, E. vanbrunti,* a rock, and handfuls of sand). A different territory was used for each replicate (*N*= 5 for each species), and the same organisms and rock were used for each of their respective five replicates.

When the data from the organisms which adhered to the substrate were excluded (Figure 3), there was no significant difference between mean total removal times of biotic and abiotic objects (one way ANOVA test $F_{5,18}$=1.68, p=0.19). The absolute maximum and minimum removal times belonged to the two sea star species. The rock and sand removal times were between those of the local minimum and local maximum mean removal times of the two urchins. The removal times of *E. vanbrunti* were most constant

(standard deviation= 8.19), whereas the removal times of *P. pyramidata* and the sand were highly variable (standard deviations= 53.7 and 53.8, respectively).

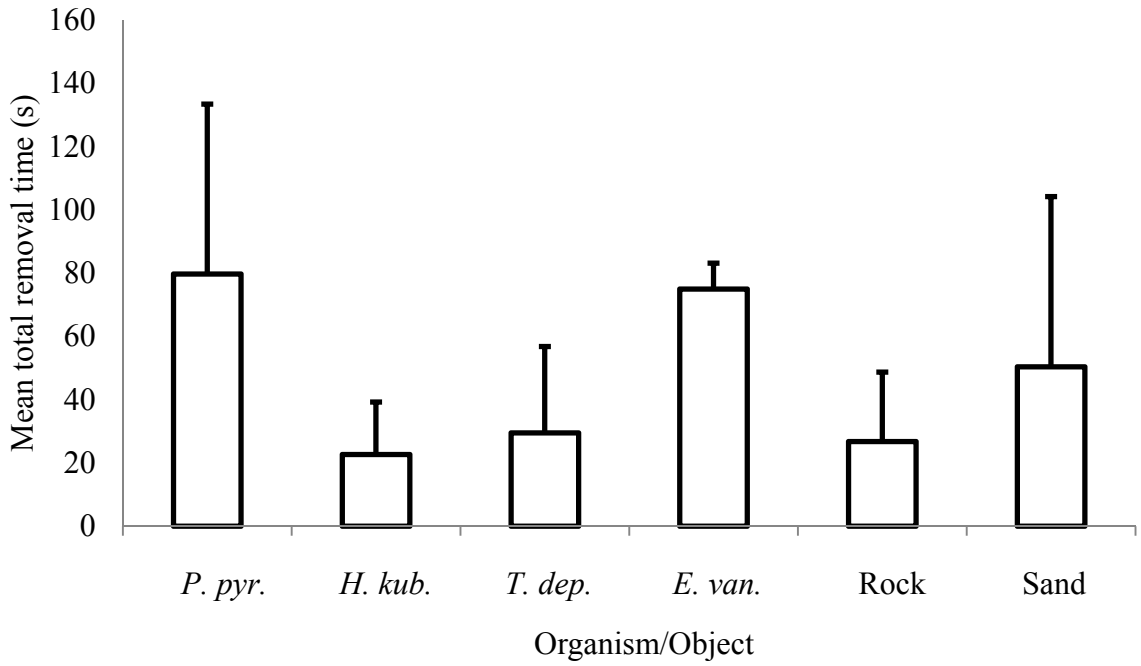


Figure 3. Average total removal times of the organisms (*P. pyramidata, H. kubiniji, T. depressus, E. vanbrunti)*, rock, and sand. The data from organisms which stuck were excluded from this analysis; *N*= 3 (*H. kubiniji* and *E. vanbrunti*), *N*= 4 (*P. pyramidata* and *T. depressus*), and *N*= 5 (rock and sand). Each intrusion was tested in a different territory. Error bars indicate standard deviation.

DISCUSSION

Many coral reef fish species exhibit territorial behaviors, which are defined as active defensive behaviors against intruders (Letourneur, 2000). *Abudefduf troschelii* is an aggressively territorial species during its nesting season (Haley and Müller, 2002). Our results showed that when foreign objects are placed in a nesting site, the fish respond differently, depending on the nature of the objects. Intrusions by living organisms such as sea stars and urchins elicit more rapid removal responses (initial attempt) than intrusions

by inanimate objects such as rocks and sand (Figure 1). This demonstrates the ability of *A. troschelii* to prioritize threat level responses. Our findings are in agreement with those of previous studies. For instance, Haley and Müller (2002) observed behavior of the beaugregory damselfish (*Stegastes leucostictus*) and concluded that the male can prioritize threats from egg predators by assessing the number of predators and their own vulnerability to the predators.

Another potential explanation for the discrepancy between response times to living organisms and inanimate objects (in addition to prioritizing their responses based on threat assessment) could be that placement of the living organisms in nests is more obvious to *A. troschelii* than the inanimate objects. The shapes of the organisms are more foreign to the fish than sand or rocks, the fish may be able to detect slight movements these echinoderms make, and/or the fish may be able to detect scent or chemical differences in the water due to the presence of foreign organisms in their nests. All of these factors could contribute to *A. troschelii* not noticing the rock or sand as quickly as the live organisms, hence the longer mean times to first attempts of removal.

The two distinct groups of total removal times in Figure 2 reflect different scenarios within the processes of removal. In the shorter time period, the fish was able to physically pick up the intrusion and either toss, drag, push, or dust it out of the territory. Sea stars were generally dragged because of their larger sizes, and the urchins were picked up by a spine and carried out. The fish would attempt this almost as soon as the organism was placed in the territory; it was clearly a difficult task because of the sea stars' weights and the urchins' sharp spines. If *A. troschelii* was distracted for a significant amount of time, the sea star or urchin had an opportunity to adhere to the rock.

This accounts for the longer set of removal data. In this situation, *A. troschelii* was unable to lift the intruding organism out of the nest, so other methods of removal had to be employed. If *H. kubiniji* adhered to the rock, for instance, the fish would pester it with nips and tugs until the star voluntarily left the area. The other species were also tormented until they either intentionally vacated the nest, or they lost their hold on the rock and the fish was able to remove them.

While an organism was within the nesting territory, the fish devoted nearly all of its attention to the removal efforts, only pausing to defend its territory from other fish. Although we were not scientifically examining the responses to trespassing fish, we observed that if a fish were to swim nearby or enter a territory, *A. troschelii* would temporarily cease trying to remove the intrusive organism/object and chase the fish instead. The protective behaviors of *A. troschelii* reflect a system of prioritization. *A. troschelii* devoted a significant amount of effort to the removal of the living organisms. However, these organisms did not present the instant priority given to approaching fish. This observation fits with that of Leese *et al.* (2009) in the nest defense tactics of *Stegastes leucostictus* (bicolor damselfish) against *Thalassoma bifascitum* (bluehead wrasse).

The removal process of the abiotic intrusions followed a different pattern from the living organisms, although the total time for removal was similar. Abiotic intrusions represented a minimal cost of energy or harm to *A. troschelii*. To remove the rock, the fish nudged it out of the nesting areas in a process which would have taken only a few seconds to complete had the fish devoted a consistent amount of attention toward its removal. After first noticing the rock, *A. troschelii* began nudging it but was easily

distracted by intruding fish, attempting to attract a mate, and/or patrolling the nest periphery for other threats. It would readdress the rock between distractions until removal was complete. These actions imply that the rock was viewed as an inconvenience as opposed to an actual threat to the nest. A similar attitude was shown toward the sand. Between distractions, *A. troschelii* dusted the sand off the rocks by fluttering their fins.

We concluded that *A. troschelii* has a system of prioritization of removal efforts to threats in their nesting territories. The fish prioritized removal of the following disturbances in descending order of importance: other fish, living organisms such as sea stars and urchins, and finally inanimate objects such as rocks and sand. These findings supported our first hypothesis that *A. troshelii* would attempt to remove disturbances to its nesting site and that removal efforts would be prioritized toward the removal of living species. We found no evidence to support our second hypothesis that *A. troschelii* would remove carnivorous intruders faster than herbivorous ones.

Our conclusion that the fish have a system of prioritization relied heavily on our observations during the total time of removal. In order to provide more support for our conclusions, the proportion of time the fish spent actively attempting removal should be quantified in follow-up experiments. Further studies could also explore the process by which the fish identifies threats.

REFERENCES

Brusca, R.C. & Brusca, G.J. (1990). *Invertebrates*. Sunderland, Massachusetts, USA: Sinauer Associates, Inc.

Foster, S. A. (1989). The implications of divergence in spatial nesting patterns in the geminate Caribbean and Pacific sergeant major damselfishes. *Animal Behavior, 37*, 465-476.

Haley, P. M. & Müller, R. C. **(**2002). Territorial behaviour of beaugregory damselfish (*Stegastes leucostictus*) in response to egg predators. *Journal of Experimental Marine Biology and Ecology, 273*, 151-159.

Leese, M. J., Snekser, J. L., & Itzkowitz, M. (2010). Interactions of natural and sexual selection: damselfish prioritize brood defense with male–male competition or courtship. *Behaviour*, *147*, 37-52.

Letourneur, Y. (2000). Spatial and temporal variability in territoriality of a tropical benthic damselfish on a coral reef (R´eunion Island). *Environmental Biology of Fishes, 57*, 377–391.

Thomson, D.A., Findley, L.T., & Kerstitch, A. N. (2000). *Reef Fishes of the Sea of Cortez: The Rocky Shore Fishes of the Gulf of California.* Austin, Texas, USA: First University of Texas Press.

Tyler, W. A. (1995). The adaptive significance of colonial nesting in a coral-reef fish. *Animal Behavior, 49*, 949–966.

Plant decomposition in relation to bioturbation by *Uca crenulata* in a mangrove forest in Mexico

Holli Fontaine

Department of Biology, MS 9160, Western Washington University, Bellingham, WA 98225

Abstracts:

Bioturbation increases microbial action in wetlands, but the relationships involved are not yet well understood. This paper is designed to help understand the relationship between degree of bioturbation, measured in terms of crab burrow densities, and rate of organic decomposition. I have taken soil samples from transects along the lower intertidal zone of a fringe mangrove forest near La Paz, Baja California Sur, Mexico. Leaves from the mangrove tree *Rhizophora mangle* were buried in the soil samples and left to decompose for a controlled period of time in the lab. The samples were taken in such a way that they did not come into contact with any of the burrows. The soil samples were compared to each other in terms of abundances of burrows made by *U. crenulata,* sediment size and rate of decomposition. No correlations were found between any of these factors. This data supports the prediction that the microbial effect caused by bioturbation is only seen within very close proximity to individual burrows and number of burrows have no effect.

Introduction:

Mangrove forests are the second most productive ecosystems in the world, producing up to 2.27 g/m^2 of new organic matter per day (Lugo and Snedaker 1974). All of this productivity leads to large quantities of plant debris, which eventually will be broken down and decomposed into inorganic nutrients (Marchand et al. 2003). It is

because of this nutrient cycling that the detritus in mangrove forests is an important part of maintaining their habitat stability, as well as those of adjacent coastal ecosystems (Middleton and McKee 2001).

Rates of leaf litter decomposition in mangrove forests depend partly on the bioturbation (reduction and transport of organic matter, specifically soils) by macrofauna. This behavior affects the nutrient cycling process by increasing microbial abundance and activities due to complex biogeochemical interactions (Bertics and Ziebis 2009; Lugo and Snedaker 1974). As an example, bioturbation by crabs in Australian swamps increase turnover of mangrove leaf litter by as much as 750% (Middleton and McKee 2001).

Fiddler crabs are among the most abundant bioturbating macrofauna in coastal areas. The activity of these crustaceans is important to nutrient recycling, organic matter degradation and primary productivity (Bertics and Ziebis 2009). *Uca crenulata* is the only fiddler crab found in the western United States and is common throughout Baja California and the corresponding coastline of mainland Mexico (DeRivera 2003). These crabs consume leaves close to their burrow entrances (Nobbs 2003) and take uneaten fragments into their burrows (Middleton and McKee 2001). The leaf matter in these burrows degrades more quickly than those placed on the soil surface. Part of the reason for this is that mangrove tissues are more rapidly digested by microbes after being broken down into smaller particles by the crabs (Middleton and McKee 2001).

Although it is well accepted that burrowing organisms such as *U. crenulata* affect litter decomposition, studies relating to this topic have only just begun (Bertics and Ziebis 2009). It seems quite plausible that there would be a direct relation between the amount of bioturbation that goes on in an area and the efficiency of litter decomposition.
The purpose of this study is to test for such a relationship using burrow densities of *U. crenulata* in a mangrove forest in the Gulf of California. I hypothesized that I would see a significant positive correlation between the number of burrows in the area and the rate of decomposition in soil samples.

Methods:

Study area:

In this experiment I collected soil samples from the middle of a fringe mangrove forest in Balandra Bay, near La Paz, Baja California Sur, Mexico. The forest appeared to have a roughly equal representation of two mangrove species, *Rhizophora mangle* and *Laguncularia racemosa*. All sampling was done in muddy, exposed areas between mangrove trees.

Distribution sampling:

Before collecting soil samples, I conducted several transects in the area to characterize the general patterns of burrow densities. The transects started a couple of meters below the water line and perpendicular to the shore line. At every meter, I found the number of burrows within a 20 cm^2 quadrant. I also measured the distance from the center of the quadrant to the nearest mangrove tree. Each transect continued until I consistently started getting zero burrows.

Soil sampling:

Soil samples were taken along transects that were set up along the lower intertidal zone, where leaves degrade faster than higher up in the intertidal (Middleton and McKee 2001). Transects were also performed below the water line to keep moisture levels saturated for all samples because moisture increases decomposition rates (Lugo and Snedaker 1974). A 20 cm^2 quadrant was put down every half meter and I recorded how many burrows were within it. I used this size quadrant because it gives a close proximity to where the sample would be taken, but is also large enough to fit over a dozen burrows. I collected the soil samples in the middle of the quadrants, making sure not to go directly through a burrow opening. I also collected a few random soil samples to be used in determining how long the experiment should run.

Collection apparatus:

Aside from a thin layer at the surface, mangrove sediments are anaerobic (Bertics and Ziebis 2009). In order to keep anaerobic conditions in my samples, I constructed 33

cm long PVC pipes that were hammered into the soil until they passed the water line. The top portion of the apparatus was allowed to fill with water before I capped it. Once all samples in the transect were prepared in this manner, I dug them up and quickly capped the bottom end. Each apparatus was labeled at the top with the sample number and the number of burrows before being hammered into the ground.

Leaf sampling:

In the area between where I took my transects, I collected leaf samples from a single *R. mangle* tree. The leaves were selected to be similar in size (0.63-0.81 grams), in good condition and from the same area of the tree. At the lab they were washed and dried before being weighed. They were also labeled to distinguish them from other leaf pieces that may have been in the soil samples.

Decomposition:

After collection, all soil samples were taken to the lab. I submerged one leaf into each sample from the top, making sure that the leaves were completely covered by soil before capping them. As an extra insurance against water leaking out and air leaking in, I also duct taped both ends of the apparatus. I let the samples sit upright in an un-air-conditioned portion of the lab because heat speeds up decomposition rates (Lugo and Snedaker 1974). The placement of the containers was randomized so that microhabitats within the lab did not skew the data.

After a 48 hour period, I began opening up one of the extra soil samples every day to check decomposition rates. I designed to end my experiment after finding a leaf with 25% weight degradation or more. This occurred after 5 days.

Analysis:

Once I declared my experiment over, I collected the leaves from each soil sample, one by one. When collecting the samples I noticed that the size of the sediment particles changed and that the amount of soil that actually ended up in the samples varied. I noted the height of the soil in the apparatus as well as the sediment size for each sample. I also took a photo of each leaf next to the soil from which it came as visual data. Once this was

done, I collected all leaf samples and very carefully washed and dried them. The leaves were then weighed and compared to burrow density in terms of percent degradation. I then found the T-value and P-value of the data to determine what the relationship was between them.

Results:

Decomposition and burrow density:

The relationship that I found between number of crab burrows in 20 cm^2 quadrants of the soil samples and the percent of leaf tissue that was decomposed is shown in figure 1. The percentage of tissue that was decomposed ranges from just under ten to almost 90 percent. The number of burrows ranged from 0 to 8. There are no visible patterns in this data. The T-value of these data is -0.631, indicating that the slope of the line of this relationship is zero and the P-value of these data is 0.54, further indicating that there is no correlation between these data.

Sediments:

The correlation between the size of sediment and the height of soil collected in the soil samples was -0.60152. Areas with larger sediment particles, sand, yielded smaller samples. Areas with pure silt mud soils yielded full samples. This makes sense because the smaller the soil particles, the more water will be retained. These water particles create hydrogen bonds with the soil and PVC to keep it all together. I am not including this data because I found no correlation between sediment size or soil height with the percent of plant tissue that was decomposed.

Burrow distribution:

I collected data on the distribution of the *U. crenulata* burrows in my study areas. I found that the crabs were very limited in where they would build their burrows. I did not find any burrows more than 2 meters upland of the water line or more than 3.5 meters away from the nearest mangrove food source. The highest densities were found close to a mangrove tree and below the water line. I was not able to take data any farther than two

meters below the water line. However, my data predicts that I would find even greater densities deeper into the water (see Figure 2). The correlation between burrow density and distance from the lower intertidal line was -0.7837. The correlation between burrow density and distance from the nearest mangrove tree was -0.6944.

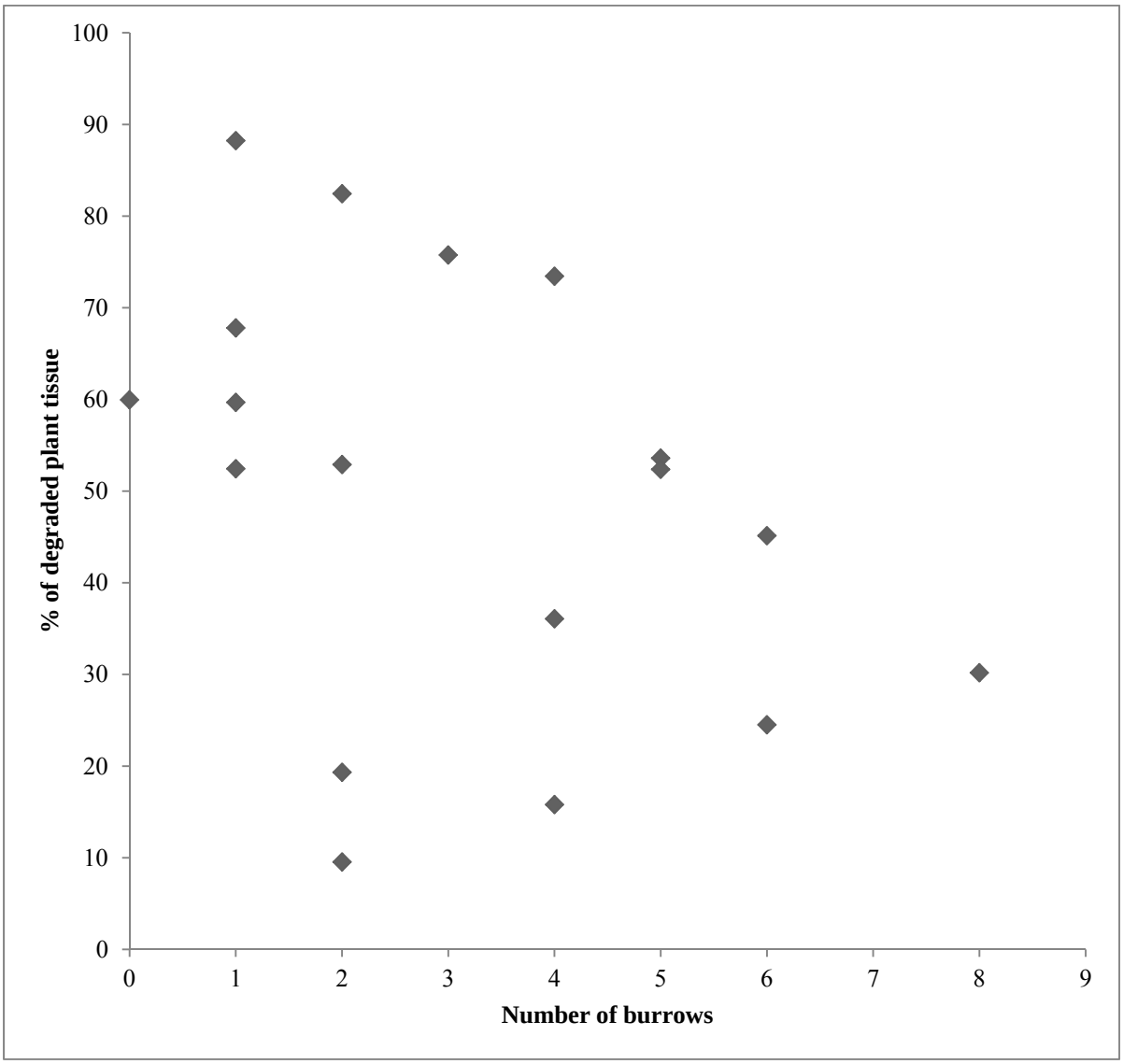


Figure 1 Soil samples were collected with various proximities to crab burrows and were left to degrade *Rhizophora mangle* leaves for five days. The T-value of these data is –0.631 and the P-value is 0.54.

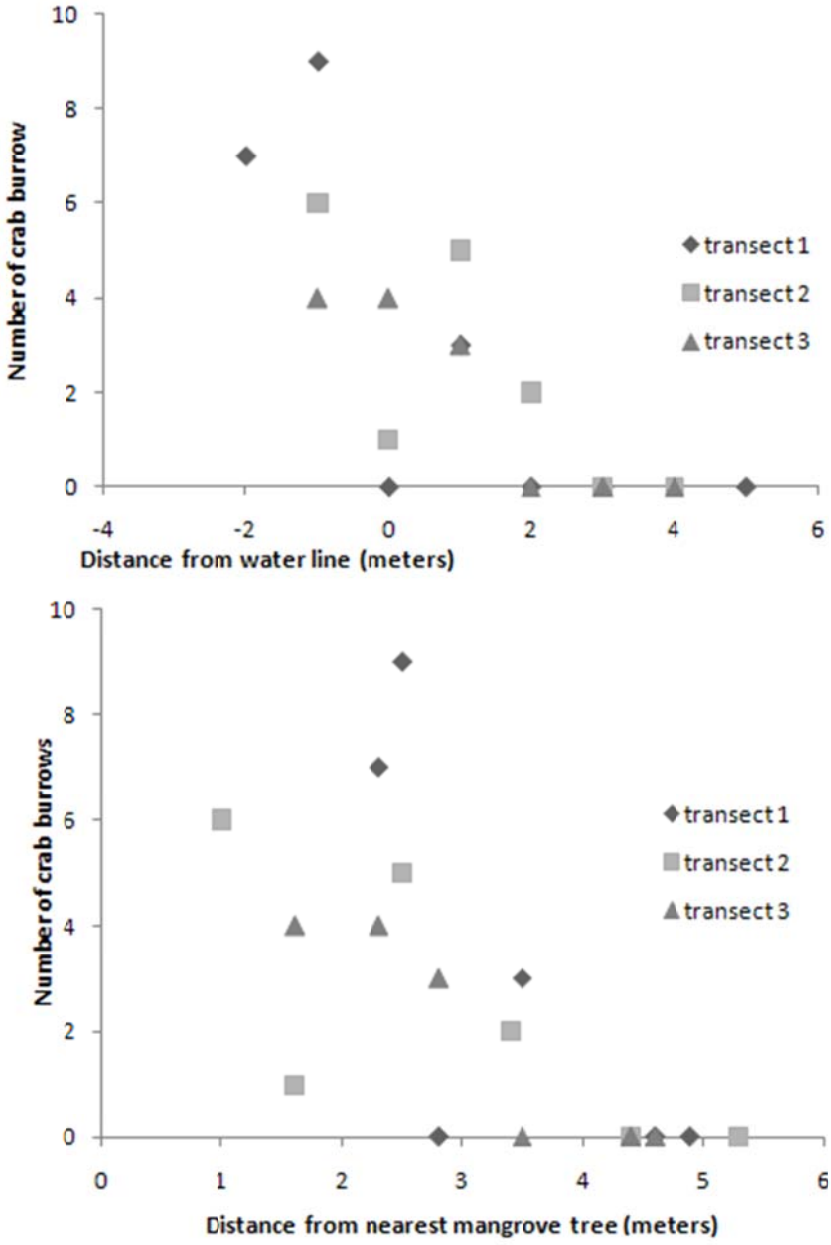


Figure 2 Three transects were performed perpendicular to the water line in the middle of a mangrove forest at Balandra Bay, Baja California Sur, Mexico. The transects were done about an hour after low tide, so the water line in these data approximates the lower boundary of the intertidal zone. At each meter of the transects, the number of *U. crenulata* burrows in a 20 cm^2 quadrant were counted. (a) The correlation between the distance of the water line and the burrow density is -0.7837. (b) The distance from the center of each quadrant to the nearest mangrove tree was recorded. The correlation of these data is -0.6944.

Discussion:

Decomposition and burrow density:

I did not establish a correlation between decomposition rate in mangrove soil and the number of *U. crenulata* burrows. However, Bertics and Ziebis (2009) have shown that microbial abundance is greater within *U. crenulata* burrows than on the surface. This is because burrows provide a better environment for decomposition, offering additional surfaces for microbial colonization and chemical reactions. Also, the physical stability within the burrows relative to the frequently disturbed sediment surface allows for better microbial development (Bertics and Ziebis 2009). On top of this, bioturbation allows oxygenation through gas exchanges between atmosphere and sediment (Marchand et al. 2003). This is important because degradation of plant material in anaerobic environments is generally slower than under aerobic conditions (Middleton and McKee 2001).

My data show that this relationship between microbes and burrows does not exist on a large scale. I set up my soil samples to not directly include burrows in order to keep conditions anaerobic and limit oxygen as a contributing variable to the data. However, *U. crenulata* burrows are built at a 45° angle and are J-shaped (DeRivera 2003), making it difficult to completely exclude them from the samples. The wide variety of decomposition rates in my soil could be explained by various proximities to individual burrows. I know that at least one of my samples went directly through a burrow, because I found a live crab in the middle of it. This same soil sample also had the one of the highest rate of decomposition, with 72.4% of the original plant disuse broken down by microbes.

Alternatively, it is possible that the relationship between *U. crenulata* burrows and microbial action is completely different in Balandra Bay from the location of the Betics and Ziebis study, at Catalina Harbor, Catalina Island, CA, USA. Their study site was a finely sandy beach (Bertics and Ziebis 2009) with little localized flora (personal observation from photograph). It is possible that the difference in physical and biological properties of these two habitats have a significant effect on the relationship between decomposition and bioturbation by *U. crenulata.*

Burrow distribution:

I found a strong negative correlation between the densities of *U. crenulata* burrows and distance from the shore. In fact, I found no burrows beyond two meters of the shoreline. This differs from the distribution patters of *U. crenulata* studied at Catalina Harbor, Santa Catalina Island, CA, USA (Bertics and Zeebis 2009). They had even distribution of burrows through the entire length of 10 meter transects from the shoreline. A hypothesis for the differences among my study and Bertics and Zeebis (2009) is that water retention in the soil accounts for the difference. *Uca* species have been noted for being limited in physiological tolerances to moist habitats (Nobbs 2003).

Works Cited:

Bertics, V. J. and W. Ziebis. (2009) Biodiversity of benthic microbial communities in biotubated coastal sediments is controlled by geochemical microniches. The ISME Journal 3:1269-1285

DeRivera, C. E. (2003) Causes of a male-biased operational sex ration in the fiddler crab Uca crenulata. Japan Ethological Society and Pringer-Verlag Tokyo. 21:137-144

Lugo, A. E. and S. C. Snedaker (1974) The ecology of mangroves. Annu. Rev. Ecol. Syst (Volume: Pages)

Marchand, C., E. Lallier-verges, F. Baltzer (2003) The composition of sedimentary organic matter in relation to the dynamic features of a mangrove-fringed coast in French Guiana. Estuarine, coastal and shelf science 56:119-130

Middleton, B. A. and K. L. McKee (2001) Degradation of mangrove tissues and implications for eat formation in Beliean island forests. Journal of Ecology 89:818-828

Nobbs, M. (2003) Effects of vegetation differ among three species of fiddler crabs (Uca spp.) Journal of Experimental Marine Biology and ecology. 284:41-50

Rivera-Arriaga, E., A. L. Lara-Domínguez, G. Villalobos-Zapata and A Yáñez-Arancibia (2003) Trophodynamic ecology of two critical habitats (seagrasses and mangroves) in Términos Lagoon, southern Gulf of Mexico. [*Ecología trofodinámica*

de dos hábitats críticos (pastos marinos y manglares) en la Laguna de Términos, sur del Golfo de México] Fisheries Centre Research Reports. 11(6)

Effects of Increased Temperature and Acidification on coral *(Pocillopora damicornis)* of Different Depths in the Sea of Cortez

Sarah Lerch, Kelsey Donahue, Kiersten Ferguson

Western Washington University

Abstract

Climate change in our environment is a topic of concern as human induced greenhouse gas (GHG) emissions are rising. This rise in GHG levels has caused warmer ocean temperatures and an increase in acidity levels. These two abiotic factors have been known to have a negative effect on coral reef ecosystems within tropical regions. Studies have shown that coral bleaching is a consequence of these stressful conditions. Coral bleaching occurs when the symbiotic algae within the coral are expelled or lose pigmentation due to stressful environmental conditions. Our experiment examined thermal stress and acidification effects on the coral *Pocillopora damicornis* in the Sea of Cortez. We measured the effects of elevated temperature and acidity levels on the corals from both shallow and deep water habitats. We hypothesized that both shallow and deep water fragments would bleach equally with elevated acidity levels and that bleaching rates for the deep coral fragments would be higher under elevated temperatures. We collected 16 corals from both shallow (1.5m) and deep (3.5m) habitats and placed them in four different seawater baths: normal acidity (pH 8) with high temperature (32°C), normal acidity (pH 8) with normal temperature (28°C), high acidity (pH 7) with normal temperature (28°C), and high acidity (pH 7) with high temperature (32°C). After 12

hours of exposure time in the treatments, the corals were returned to their normal temperature and pH environment. Photos were taken of the fragments over a 4-day period and their color change was evaluated. Temperature and time were the only significant variables that affected color change, while pH and depth were insignificant. Our hypothesis stating that corals from both depths would bleach equally at elevated acidity levels was accepted while our hypothesis stating that corals from different depths would bleach differently to elevated temperatures was rejected.

Key Words: Coral bleaching, *Pocillopora damicornis*, climate change, pH, temperature

Introduction

Anthropogenic influences since the mid-20th century have had drastic affects on the environment. Though natural disturbances such a volcanic events have contributed to climate change, the dominant influence on climate change in the last 50 years has been human impact (Karl and Trenberth 2003). According to the Intergovernmental Panel for Climate Change (IPCC) Fourth Assessment Report under the AR4 Synthesis Report "there is *high agreement* and *much evidence* that with current climate change mitigation policies and related sustainable development practices, global GHG emissions will continue to grow over the next few decades" (IPCC 2007). As greenhouse gases (GHG) continue to accumulate in the atmosphere, global temperatures are rising and tropical sea temperatures are predicted to increase by 1-2°C by 2100 (Bijlsma et al. 1995 *fide* Hoegh-Guldberg 1999). Increased levels of atmospheric CO_2 will also result in higher CO_2 levels in the ocean leading to acidification (Anthony 2008, Hoegh-Guldberg 2007).

Future changes in ocean temperature and acidity due to climate change will have profound impacts on coral reefs. The loss of pigment related to a coral's symbiotic algae, zooxanthellae, is called bleaching, and can be caused by the loss of pigment from the zooxanthellae itself, or by the loss of the zooxanthellae from the coral (Buddemeier & Fautin 1993). Corals are known to bleach when they exceed the annual maximum temperature for their habitat (Brown 1993), and an average increase of 1-2°C could cause this to happen more frequently. It has been found that the thermal tolerances of reef building corals are likely to be exceeded every year for the next few decades (Hoegh-Guldberg 1999). Acidification of the ocean will also lead to less carbonate availability to biological systems. The CO_2 absorbed into the ocean reacts with water to create carbonic acid, this acid then dissociates into bicarbonate ions and protons that react with more bicarbonate ions. This chain reaction leads to a reduced rate of calcification for reef building corals (Hoegh-Guldberg 2007), and could also reduce the growth of coral structures.

Both temperature change and acidification caused by climate change pose threats to the health of reefs worldwide. The consequences of coral bleaching range from both impacts on local and global economies to ecosystem impacts. Many economies are reliant on or are integrated with coral reef systems, and local economies surrounding reef systems rely on them both as fisheries and as an income source via tourism (Hoegh-Guldberg 1999). The estimated cost of losing 58% of coral reefs would result in a 90 billion dollar loss for the tourism industry (Hoegh-Guldberg 1999). Coral reefs also provide high rates of primary productivity within regions where productivity is generally low. Productivity in open oceans around reefs can fall as low as 0.01 g C m^{-2} day^{-1} while

productivity within the reef can be around 280 g C m^{-2} day^{-1}, many thousands of times higher (Hatcher 1988 *fide* Hoegh-Guldberg 1999). Reefs also provide habitats to many organisms and protect adjacent coastlines from wave action that can lead to erosion and damage habitats such as mangroves. Thus, coral communities are integral to the welfare of many systems, both economically and biologically, making their health and conservation all the more important.

Our experiment examined the affects of high temperature and low pH on a stony coral *Pocillopora damicornis*. The corals were obtained from the tropical Sea of Cortez, an area expected to be affected drastically by climate change, with up to a 2°C increase in the next century. Another point of interest in this region stems from a period of El Niño Southern oscillation in 1998 that resulted in a loss of over 60% of the coral (La Jeunesse 2006), a devastating event. To better understand how climate change or particular events such as the 1998 El Niño year will affect these fragile and important ecosystems, corals were obtained from both shallow water and deep water habitats within the subtidal zone. We wanted to answer the question as to how corals living in different conditions would respond to acidification and thermal stress. To answer this question we subjected coral fragments of two depths to four different treatments. Two treatments had higher acidity with normal or increased temperature, and two treatments had normal acidity with normal or increased temperature.

We hypothesized that corals living at different depths would respond differently to the stresses of temperature change because they live in different conditions. We predicted that corals from both depths would respond with equal amounts of bleaching to increased acidity, and corals at deeper depths would respond with higher rates of

bleaching to the elevated temperature than those at shallow depths. We hypothesized this because the temperature change for the deeper dwelling corals is even farther outside of their natural temperature fluctuations than for the corals in shallower, warmer waters.

Methods

We chose the coral *Pocillopora damicornis* for this experiment because of its convenient distribution within the site area, growth structure, and abundance in the tropical Gulf of California. We obtained coral fragments from the bay in front of Hotel Cantamar in La Paz, Baja California Sur, Mexico. Each fragment ranged from roughly two to seven inches in length, and was broken off using plastic forceps to reduce damaging the tissues of the coral as much as possible. Sixteen pieces of coral were gathered within a small area of the bay from depths of 1.5 and 3.5 meters, each collected from a different randomly selected colony to represent an individual. After retrieving each coral fragment from its respective colony, we placed them in labeled Styrofoam cups filled with seawater and stored them in a cooler during the collection period. We used Styrofoam cups in an attempt to minimize tissue damage from the coral pieces touching different surface areas and each other. The cooler kept the corals at their natural temperature during the collecting period.

After transport to the lab, we affixed each coral fragment to a labeled plastic stopper top by wrapping aluminum foil around both the base of the coral and the stopper. We then took photos of each fragment to document their initial pigmentation. We prepared four different seawater treatments to simulate stressful conditions for the corals,

combining the variables of pH and increased water temperature. The first treatment was initially at 28°C and a pH of 8 (normal temperature and normal pH i.e. the control treatment), the second treatment was initially at 28°C and at a pH of 7 (normal temperature and lower pH), the third treatment was initially at a temperature of 32°C and at a pH of 8 (high temperature and normal pH), and the fourth treatment was initially at a temperature of 32°C and a pH of 7 (high temperature and lower pH). We measured pH using pH strips. The pH of 8 was chosen because it was the pH of the seawater in the corals natural environment, slightly basic. The temperature of 28°C (room temperature) was used to simulate the temperature of the water of their environment, though this was above their natural habitat temperature of 25°C. We did this because no efficient modes of cooling the water were available in the lab. Each of the temperatures used was the initial temperature, as all the treatments cooled to 25°C by the end of the corals exposure time of 12 hours. Eight coral fragments, four from shallow water and four from deep water, were placed in each treatment and left to soak overnight.

After the 12-hour soak in the simulated conditions, we placed the coral fragments into a small seawater bath to rinse them of their treatments. We then took photos of each fragment and transferred them to a larger aerated seawater tank. We took photos every 24 hours for four days to track the change in coral pigmentation. At the end of the experiment, we analyzed the photos with Photoshop (CS4). We recorded the mean color value for each RGB color (red, green, and blue) for 5 haphazardly chosen circular areas on the image of each coral fragment. Each circular area had a diameter of 64 pixels. For each color value we averaged these five circular spots in order to get one datum per coral fragment per time. On the 4th day we observed the fragments under a dissecting scope to

determine whether the polyps had bleached or died.

To determine whether treatments differed from one another we analyzed the data with a linear mixed effects model. Color values, red, blue, and green, were the response variables and temperature, pH, depth, and time were our predictor variables. Time was treated as a random effect because we repeatedly sampled each coral. The model was fully crossed and non-significant interactions were removed.

Results

We found varying trends within the data for the rates of color change for *Pocillopora damicornis* when fragments collected from shallow and deep areas were subjected to increased temperature and acidity. General trends showed that each coral became lighter in color over the course of the experiment. Figure1 shows the effects of varying pH and temperature on the RGB color values of the coral fragments. As color values increase the amount of the given color decreases, therefore increases in color value may be interpreted as bleaching or dying. Each data point represents the averaged mean color value of the corals collected in shallow and deep waters for each day data was taken. The error bars represent the standard deviations within the data. The trend seen in both the pH and temperature graphs is that the amount of color change for all colors increases as the length of time increases. For the variable of pH, the amount of color change for the three colors is similar for the fragments in both the pH 7 and the pH 8 treatments, with a slight increase in color change for the corals in the treatment with pH 7 at the four-day mark. Between each color, red showed the most color change, green the

second most, and blue the least. For the variable of temperature, although all the colors experienced color change, the coral fragments exposed to the temperature of 32°C had a greater amount. In keeping with the results found for pH, the color red showed the most color change while green and blue showed less respectively.

Figure 2 shows the change in color values as the variable of depth changes. Each point represents the averaged mean color values of either shallow or deep water corals for the day data was taken. The error bars represent standard deviations. Little difference can be seen in the trends for the deep and shallow corals. Again red showed the most color loss while green and blue showed less respectively.

Table 1 shows the statistical data taken from an ANOVA analysis of the data. The table reveals the contributing factors to the various outcomes within the experiment. When the p- value for a certain factor exceeds 0.05 this shows that the factor being analyzed does not contribute to the observed outcome. Table 1 shows that temperature and time were both significant influencing factors, with p-values of 0.003 and 0.004 respectively, for the loss of the red from the corals. The table also shows that temperature and time, with p-values of 0.006 and 0.003 respectively, were significant factors in affecting the amount of color loss in the green values. Unlike red and green, the amount of color loss within the blue values was only affected by time, which had a p-value of 0.004. When observed under the dissecting scope at the end of the experiment we found that all the coral fragments had died.

Discussion

As our results showed, all the coral fragments collected consistently became lighter in color as the experiment continued. This lightening of color indicates that the corals were either bleaching or dying. It was found that all the coral fragments ultimately died, indicating that color change in this case was either an indicator of death or a combination of bleaching and death. Corals do not bleach instantly, rather it takes time after a disturbance for them to lose pigmentation. This was what we observed in our experiment. After the initial 12-hour exposure time, the corals slowly lost their color and then died during the next four days. Hoegh-Guldberg (2007) found that corals may survive a bleaching event and recover their zooxanthellae symbionts after mild thermal stress, but typically they will show reduced growth and fecundity afterwards. Our experiment has shown that when exposed to more extreme thermal stress in addition to the stresses of the laboratory environment the coral *Pocillopora damicornis* does not merely bleach, but dies.

We hypothesized that corals from both depths would show increased rates of bleaching due to increased temperature, and that hypothesis was accepted. We also expected that corals from deeper habitats would show higher rates of bleaching compared to corals from shallow habitats, and this hypothesis was rejected. ANOVA analysis of our data found that the only significant factors affecting the corals were temperature and time, meaning that the high temperature treatments and time had greater effects on coral bleaching and death than any of the other factors. Temperature has a drastic affect on the relationship between corals and zooxanthellae. The coral's symbiotic dinoflagellate, zooxanthellae, are vital to the corals health because they trap nutrients and solar energy

giving the coral 95% of its metabolic needs (Hoegh-Guldberg 2007). Under heat stress, zooxanthellae will either leave the gastrodermal cells resulting in less pigment, or the gastrodermal cells will separate from the coral host along with the zooxanthellae (Lesser 1997). In either event, increased temperature conditions damage the PSII and electron flow that initiates the Calvin cycle, damaging their photosynthetic mechanism (Jones 1998). This alters the symbiotic relationship between the algae and the coral because the coral can no longer receive the photosynthetic products (sugars and amino acids) that it normally acquires in exchange for essential plant nutrients (Hoegh-Guldberg 1999).

Our hypothesis that corals living in deeper depths would respond with higher rates of bleaching to thermal stress was rejected. Our results showed bleaching and death of the deep and shallow water corals at similar rates. Research has found that corals that live within habitats, such as shallow waters that are more prone to stressors, bleach less than corals found in subtidal environments (Buddemeier and Fautin 1993), but this was not what we observed in our experiment. Other studies have shown vertical distribution patterns to be highly predictable, producing obvious zonation patterns in distribution of zooxanthellae species (Jackson 1991). Our shallow coral fragments expelled their zooxanthellae and/or lost pigmentation at similar rates as the deep-water fragments. This suggests that there were no differences in thermal tolerances between depths. We may have also found no significance in depth because we collected fragments from areas that were not far enough apart in depth, with only a two-meter difference between the shallow and deep collecting sites.

Bleaching within the two pH treatments showed similar trends for all corals, accepting our hypothesis that both shallow and deep water corals would react similarly to

increased acidity levels. The experimental outcomes were somewhat different than expected: pH had no affect on bleaching. We found that acidification does not have an influence on coral bleaching or death. Rather, acidification impacts the growth rates and structural integrity of corals by decreasing their ability to calcify (Hoegh-Guldberg 1999). This reduction in calcification also leads to a decrease in skeletal density of coral communities and outward growth, which then allows for erosion to occur at increased rates (Hoegh-Guldberg 2007). While acidification is detrimental to coral health and productivity, it does not impact the relationship between coral and their zooxanthellae.

Most information suggests that the capacity for acclimation by corals has already been exceeded, and that adaptation will be too slow to avert a decline in the quality of the world's reefs (Hoegh-Guldberg 2007). Corals are unable to keep up with the rate of ocean warming and though they will not become extinct their health and distributions will be severely compromised for many hundreds of years unless warning is mitigated (Hoegh-Guldberg 1999). Studies such as this one must be done in order to better understand the relationship between corals and the abiotic variables climate change has put in flux: ocean temperatures and pH. Coral reefs serve as not only important economic and biological systems but also as some of nature's most astounding creations. To allow these beautiful and beneficial habitats to waste away would be a crime against both nature and future generations. More research must be done in order to preserve and maintain the health of coral reefs worldwide or these wonderful habitats may be horribly damaged for the foreseeable future.

References

Anthony K.R.N., D.I. Kline, G. Diaz-Pulido, S. Dove, O. Hoegh-Guldberg. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. The National Academy of Sciences **105:** 17442-17446.

Bijlsma, L., et al. 1995. Coastal zones and small islands. In 'Climate Change 1995—Impacts, adaptations and mitigations of climate change: scientific-technical analyses: the second assessment report of the Inter-Governmental Panel on Climate Change'. Cambridge University Press: New york. pp. 6-12.

Brown B.E. 1993. Coral bleaching: causes and consequences. Coral Reefs **16:** S129-S138.

Buddemeier R.W., D.G. Fautin. 1993. Coral bleaching as an adaptive mechanism. BioScience **43:** 320-326.

Hatcher, B.G. 1988. Coral reef primary productivity: a beggar's banquet. Trends in Ecology and Evolution **3:** 106-111.

Hoegh-Guldberg O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Reserve **50:** 839-66.

Hoegh-Guldberg O., et al. 2007. Coral reefs under rapid climate change and ocean acidification. Science. **318:** 1737-1742.

Intergovernmental Panel on Climate Change (IPCC). 2007. Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment

Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. Cambridge, UK.

Jackson, J.B.C. 1991. Adaptation and diversity of reef corals. Biological Science **41:** 475-482.

Jones R.J., O. Heogh-Guldberg, A.W.D. Larkum, and U. Schreiber. 1998. Temperature-induced bleaching of corals begins with impairment of CO2 fixation mechanism in zooxanthellae. Plant, Cell and Environment **21:** 1219-1230.

Karl T.R., and K.E. Trenberth. 2003. Modern global climate change. Science **302** 1719-1723.

LaJeunesse T.C., H. Reyes-Bonilla, M.E. Warner. 2006. Spring "bleaching" among *Pocillopora* in the Sea of Cortez, Eastern Pacific. Springer-Verlag DOI:10.1007/s00338-006-0189-3.

Lesser M.P. 1997. Oxidative stress causes coral bleaching during exposure to elevated temperatures. Springer-Verlag **16**: 187-197.

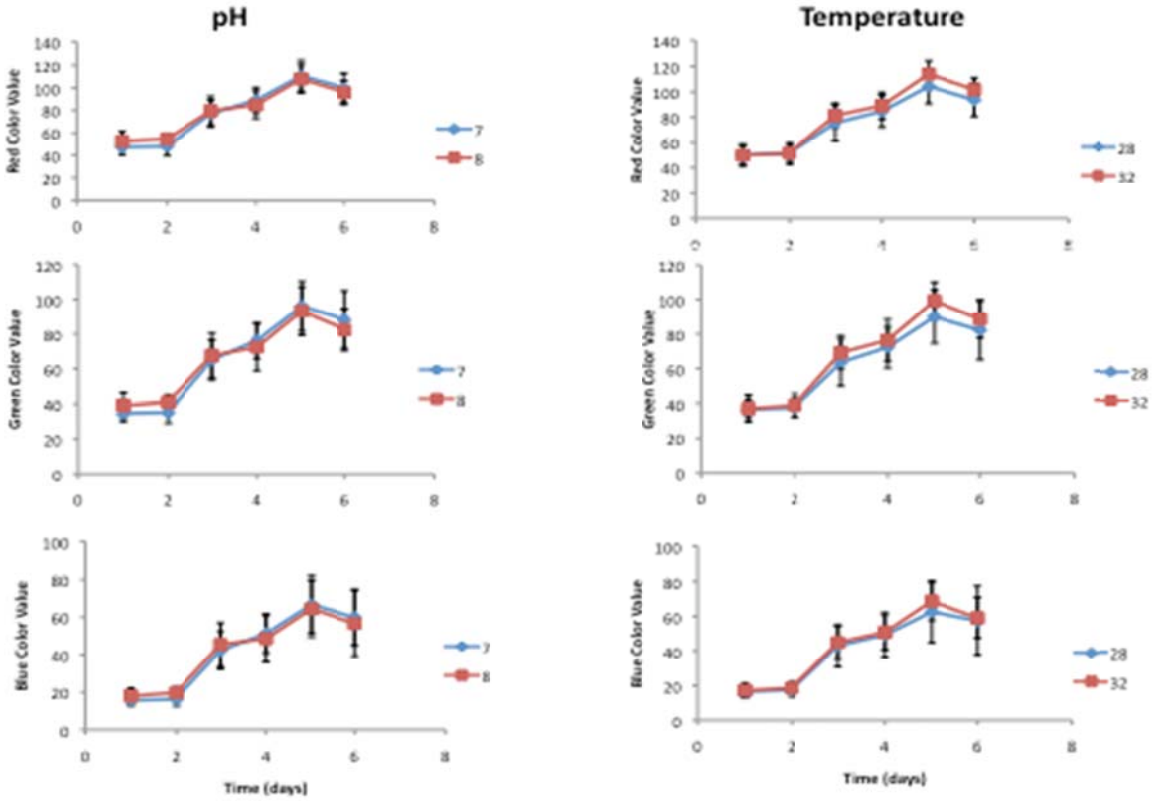


Figure 1. Red, green, and blue color values plotted against time (days) for the variables pH and temperature for all corals *(Pocillopora damicornis)* studied. Points are means with error bars representing standard deviations of the means. Higher color values indicate reduced color or pigment (bleaching).

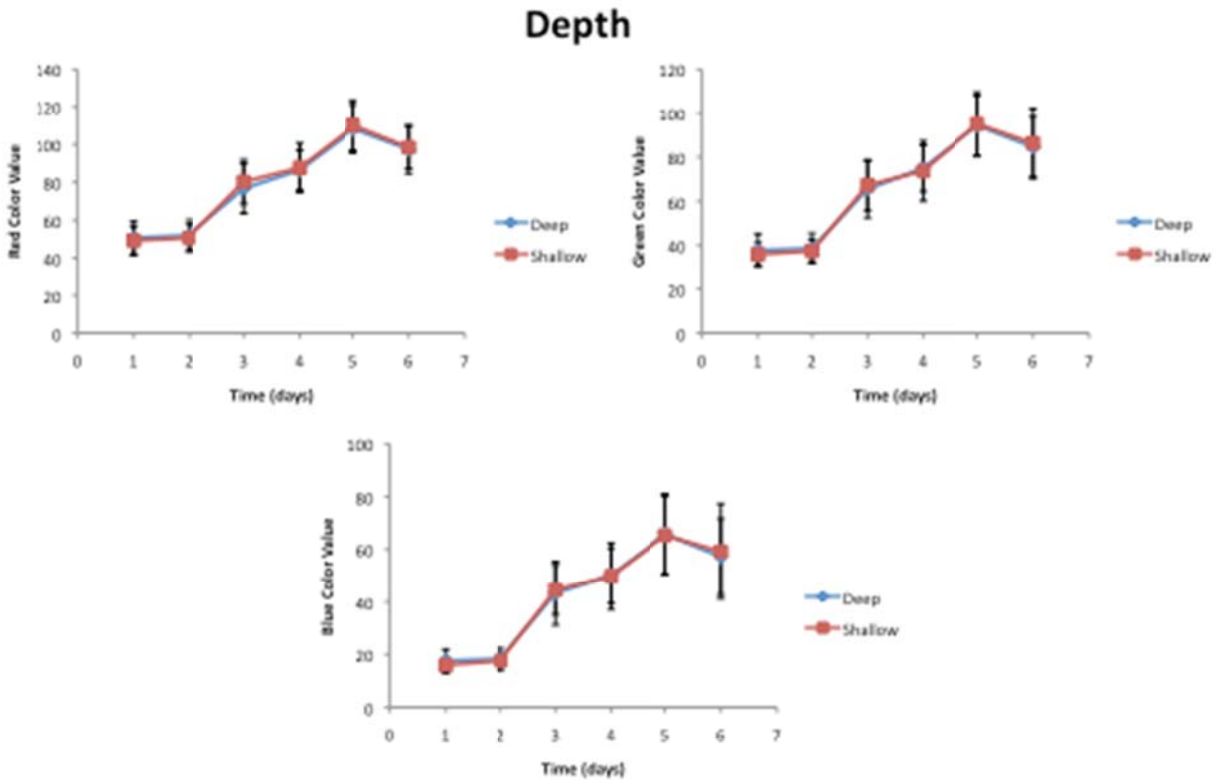


Figure 2. Red, green, and blue color values plotted against time (days) for the variable of depth for all corals *(Pocillopora damicornis)* studied. Points are means with standard deviations represented as the error bars. Higher color values indicate less color or loss of pigment (bleaching).

Table 1. Statistical data obtained from ANOVA analysis using R showing which predictor variables affected the response factors of the color variable red, green, and blue (RGB). Highlighted values are significant.

	Red DF	**Red t-value**	**Red p-value**
Depth (m)	183	0.506	0.614
pH	183	0.361	0.718
Temperature (°C)	183	2.997	0.003
Time (Days)	4	6.083	0.004
	Green DF	**Green t-value**	**Green p-value**
Depth (m)	183	-0.006	0.995
pH	183	0.044	0.965
Temperature (°C)	183	0.006	0.006
Time (Days)	4	6.312	0.003
	Blue DF	**Blue t-value**	**Blue p-value**
Depth (m)	183	0.014	0.989
pH	183	0.074	0.941
Temperature (°C)	183	1.326	0.187
Time (Days)	4	6.067	0.004

Wildlife forensics on cetaceans of the Baja California Peninsula

Luna N. López-Martínez[1], Geoff Mayhew[2] & Jack Stimpson[2]

[1]*Departmento de Biología Marina, Universidad de Baha California Sur, La Paz, B.C.S., México*

[2]*Biology Department, Western Washington University, Bellingham, WA 98225, USA*

Abstract

Cetaceans have important ecological roles, as they consume large biomass from most trophic levels and play a key role in nutrient cycling. Threats to these organisms in turn can have profound consequences on aquatic ecosystems. Anthropogenic impacts directly influence habitat quality and food abundance. Thus, populations that have fallen below a critical limit are important to monitor in order to assess their viability. Conservation efforts may be supplemented by genetic tools that make analyzing genetic diversity much less invasive and accurate. The usage of the Polymerase Chain Reaction allows for poor DNA to be amplified creating a new area of study regarding deceased animals. We employed wildlife forensics on beached cetaceans off the coast of the Baja California peninsula which was declared a biosphere reserve by the Mexican government. Our study demonstrates how the genetic analysis of beached cetaceans can be used to monitor patterns of mortality amoong populations in the area in an effort to prioritize conservation efforts. We found seven different species experiencing mortalities in the area. *Lagenorhynchus obliquidens* and *Delphinus delphins* were found to experience mortalities concentrated along a common genetic lineage. Conversely, *Tursiops trancatus* exhibited mortalities that were spread out evenly among different genetic lineages. The

results of studies such as this are important in monitoring the success of reserves and ensuring the conservation of the species and unique populations within them.

Keywords: Wildlife forensics, cetaceans, conservation, Baja California peninsula, mitochondrial control region

Introduction

Many species of cetaceans have a wide distribution in more than one ocean, and some individuals migrate over an extensive range (Hoelzel 1998, 2004). Where and when they can be found are largely influenced by food abundance, refuge from predators, and preferences for particular birthing habitat (Flores et al. 1996, Hoelzel 1998, Kerosky et al. 2008). Their presence in a given area has obvious ecological impacts such as consumption at most trophic levels (top-down effects). They also play a key role in nutrient cycling when they defecate and provision nutrients to benthic communities in the form of food bits or entire carcasses. In essence, they ultimately have a great influence on the aquatic communities they inhabit (Bowen 1997).

Conversely, threats to cetacean populations can in turn have profound consequences on aquatic ecosystems. Anthropogenic impacts such as hunting, fishing, tourist activities, aquaculture, boat traffic and pollution (Guerrero et al. 2008) directly influence their habitat quality and food abundance. These impacts can potentially alter cetacean

abundances and distributions. Acknowledging these stresses make it necessary to monitor the viability of their populations and ensuring the conservation of cetaceans as well as the ecosystems in which they play a role (Kerosky 2008).

A number of cetacean species are listed as at risk or endangered by the IUCN, meaning their populations have fallen below a critical limit (INE 2007). From the perspective of conservation efforts, it is important to maintain a particular population size because as populations decrease, finding mates becomes more difficult. Additionally, it is evident that a level of genetic variability must exist to ensure viability in the future (Gilpin and Soulé 1986, Booy et al. 2000). The development of genetic tools has made it increasingly easier to gauge the genetic diversity for a population of organisms (Hoelzel 1998) and supplement strategies for conservation.

However, collecting tissue samples for DNA analysis in the field is difficult (DeSalle & Amato 2004), and may require destructive methods such as darting that are not advised on already stressed populations (Hoelzel 1994). One alternative method is the collection of tissue samples from deceased cetaceans. Though the genetic material may not be as high of quality, the usage of the Polymerase Chain Reaction (PCR) can amplify regions of DNA to identify the species and the population from which the sample was taken (Piggott & Taylor 2003, DeSalle & Amato 2004). With additional data it can also provide insights to the amount of genetic variability within populations (Kerosky 2008).

Our study focuses on cetacean populations of the Baja California Peninsula. The area is highly productive and harbors cetaceans from both tropical and temperate waters (Urbán 1993). In addition to attracting cetaceans, the high diversity and abundance of economically significant organisms encourages extensive human exploitation of the area including over fishing, tourism, and aquaculture (Moore & Clarke 2002). It is clear that cetacean populations are affected by anthropogenic sources of stress. A notorious case is that of the vaquita (*Phocoena sinus*), a species of porpoise endemic to the northern Gulf of California, which has been driven near extinction chiefly due to bycatch (D'Agrosa et al. 2000). Such scenarios must be remembered for sustainable management of other cetacean populations.

Despite the fact that the region off the coast of the Baja peninsula was declared a biosphere reserve by the Mexican government's National Institute of Ecology (Instituto Nacional de Ecologia or INE) in 1988, human impacts have not been well quantified. Surveys are required to understand what species of cetaceans are in the area as well as their rates and causes of mortality in order to prioritize conservation efforts. We employed wildlife forensics on beached cetaceans to identify the species in the area and to determine whether or not observed mortalities occurred among populations.

Materials and methods

Samples

During 2006-2009, tissues samples were collected from cetacean carcasses washed up on the Pacific coast of the Baja California peninsula and fixed in 95% ethanol. From these, we obtained skin samples of 81 individuals that were found beached along a 42 km stretch along the coast of Isla Magdalena facing the Pacific Ocean.

DNA extraction

We minced the skin tissues and set them to incubate overnight in an SDS (20%) and proteinase K digestion. Subequently, we purified the DNA by performing two phenol:chloroform:isoamyl alcohol (25:24:1) extractions and precipitated via centrifugation with sodium acetate (3.0 mM) and chilled ethanol (100%). Our DNA samples were then analyzed quantitatively and quantitatively via electrophoresis in a 1% agarose gel stained with ethidium bromide.

Amplification of mtDNA

We performed the Polymerase Chain Reaction (PCR) on the mitochondrial (mt) DNA control region (D-loop), a sequence approximately 550 base pairs (base pairs) long. This was performed in 50 μL volumes containing 3.5 mM $MgCl_2$, 23.75 μL deionized water, 10 mM each dNTP, 5 μL reaction buffer, 10 μM forward primer t-Pro whale (5' TCA

CCC AAA GCT GRA RTT CTA 3'), 10 μM reverse primer Dlp5-H? (5' CCA TCG WGA TGT CTT ATT TAA G 3'), 0.25 units of Taq DNA polymerase, and 2.5 μL DNA sample. Thermal cycling consisted of an initial 7 minute denaturation period at 94ºC, followed by 32 cycles of 30 seconds at 94ºC for denaturation, 60 seconds at 53ºC for primer annealing, and 40 seconds at 72ºC for elongation, and concluded with a 7 minute final extension step at 72ºC. We verified the PCR products on 1% agarose gels stained with ethidium bromide.

Genetic sequencing and analysis

Our PCR products of adequate quality were sent to MacroGen Inc. (Korea) for dideoxy determination sequencing. We compared our acquired sequences to the consensus sequences of GenBank (http://www.ncbi.nlm.nih.gov/genbank/) via BLAST for species identification (http://blast.ncbi.nlm.nih.gov) and then we aligned sequences using the clustalX software (Thompson et al. 1997). Using the FindModel program (www.hiv.lanl.gov/ content/sequence/findmodel/findmodel.html), we evaluated substitution models for input into Mega Software (Kumar et al. 2008) for evaluation of evolutionary relationships among sequences and phylogenetic reconstruction (using a neighbor-joining model).

Results

Of the 81 individual DNA samples, 42 successfully produced PCR products and 38 could be identified. Seven species were found in the sample: *Delphinus delphis* (Common Dolphin), *Grampus griseus* (Risso's Dolphin), *Tursiops truncatus* (Bottlenose Dolphin), *Delphinus, capensis* (Long-Beaked Dolphin), *Pseudarea crassideas* (False Killer Whale), *Stenella coeruleoalba* (Stripped Dolphin) and *Lagenorhynchus obliquidens* (White-sided Dolphin) (Figure 1). *Lagenorhynchus obliquidens* was the most represented, with 11 mortalities. *Lagernrhynchus obliquidens*, *D. delphis*, and *T. truncatus* sequences were acquired in adequate numbers to construct phylogenies in conjunction with sequences acquired from GenBank (Figure 2, Figure 3, and Figure 4). The majority of sequences acquired were from samples take during 2009, so trends in mortality could not be observed through time.

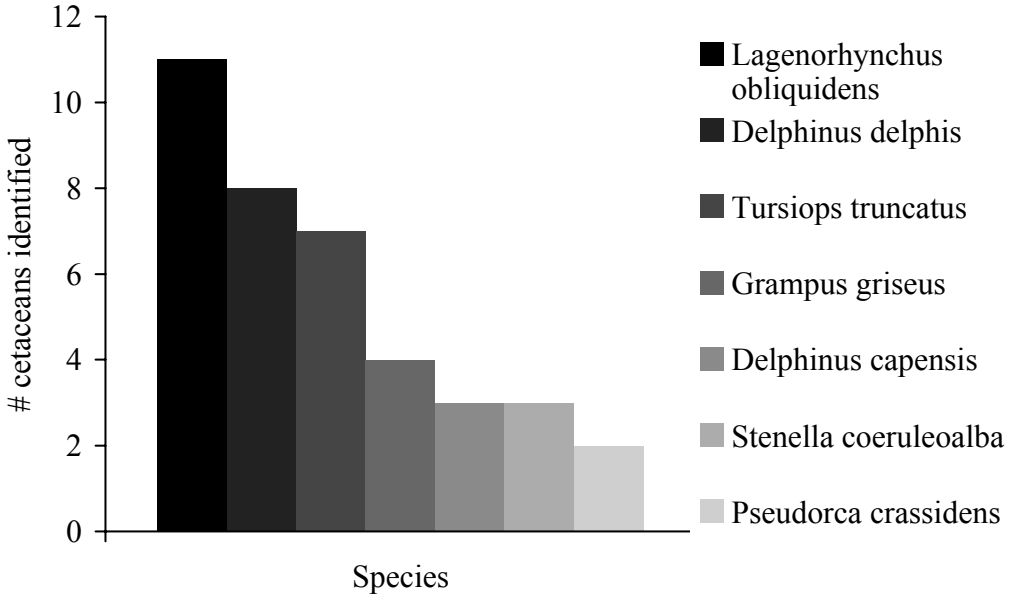


Figure 1. Number of cetaceans identified (via wildlife forensics), organized by species, using skin samples taken from beached carcasses on the Pacific coast of Baja California Sur between 2006 and 2009. Seven species were identified by comparing acquired sequences with consensus sequences from the GenBank database.

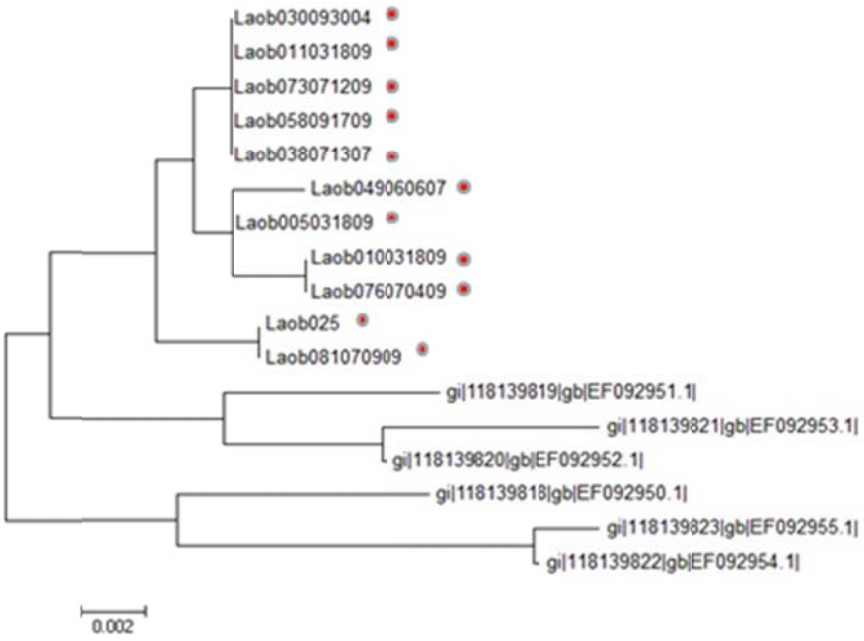


Figure 2. Neighbor-joining phylogenetic tree of *Lagenorhynchus obliquidens,* relating sequences of the mitochondrial control region based on a Tamura-Nei substitution model. Red dots indicate sequences acquired from carcasses sampled on the Pacific coast of Baja California Sur.

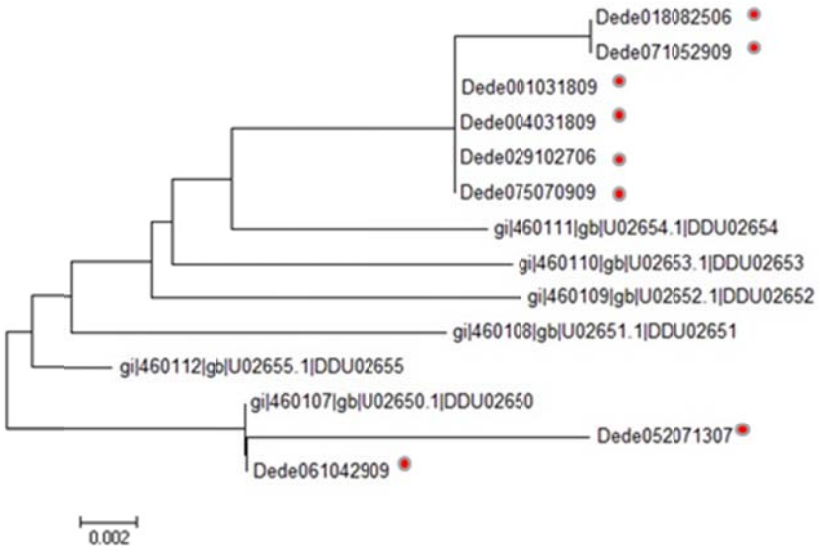


Figure 3. Neighbor-joining phylogenetic tree of *Delpinus delphis,* relating sequences of the mitochondrial control region based on a Tamura-Nei substitution model. Red dots indicate sequences acquired from carcasses sampled on the Pacific coast of Baja California Sur.

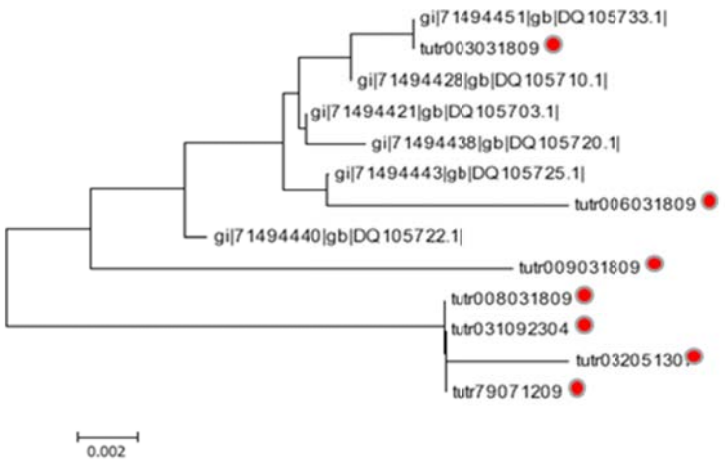


Figure 4. Neighbor-joining phylogenetic tree of *Tursiops truncatus,* relating sequences of the mitochondrial control region based on a Tamura-Nei substitution model. Red dots indicate sequences acquired from carcasses sampled in on the Pacific coast of Baja California Sur.

The reconstructed phylogeny of *L. obliquidens* displays our acquired sequences on a separate lineage to those of other Pacific populations found on GenBank. Moreover, it shows there was little variation in nucleotide substitutions in the D-loop region. The phylogeny of *D. delphis* placed our sequences in two distinct lineages, and one of which showed very limited genetic variation. Conversely, sequences from *T. truncates* were placed in various lineages when compared with those of the database and showed a great amount of variation.

Discussion

Our results suggest that dolphinids maintain high species diversity in the area, evident by the seven species of dolphins identified in our assessment along the western coast of California Baja Sur. Among the different species found in our study, two represented significantly higher proportions of the total mortalities. The two species that displayed the highest rate of mortalities were *L. obliquidens* and *D. delphis*. Both these species showed mortalities for specific local populations, and we can conclude that these populations are not genetically diverse due to the representative neighbor-joining phylogenic tree. The mortalities and genetic diversity of these populations may mean that certain populations of this species are more influenced by anthropogenic or natural impacts than other populations of these species (Hoelzel 1998).

The other type of results we gathered concerned mortalities of a species that was mixed throughout a diverse genetic range. The results for *T. truncatus* may imply that there are factors impacting the entire species evenly and may not be due to a lack in genetic diversity in one population. The phylogenic tree shows larger genetic variation in these populations as lengths of the lines are greater between individuals.

For the conservation of populations displaying heavy impact, it is important to identify which groups of cetaceans are suffering in a lack of genetic variation. Populations with critically low numbers and genetic variation may suffer by not being viable or able to resist disease (Kerosky 2008). By analyzing the genetic makeup of deceased cetaceans, it

becomes possible to monitor populations in a non-destructive manor in an effort to identify which populations are at greater risk. The result of this monitoring would allows the development of more effective management plans that can treat specific populations, rather than the whole species, as specific units of management for conservation (Hoelzel 1998).

This study was not intended to quantify human impacts on cetacean populations, but was designed to detect trends in mortality that might suggest anthropogenic sources. It must be stated that our data does not represent actual mortality rates and only identifies the species that have been found washed ashore. The causes of death, anthropogenic or natural, cannot be extrapolated either. This requires inspection for wounds such as gunshots, boat-strike damage, or net entanglement (Moore 2002, Kerosky 2008). However, a more extensive study that focuses on the causes of death of individuals while simultaneously determining which populations experience such mortalities could better supplement management efforts. Such research would highly benefit the conservation of cetaceans in the waters of the Baja California peninsula. Despite the fact they live in areas declared marine reserves, the success of the reserves cannot be determined without monitoring the species that inhabit them. Laws regarding the management of species within these reserves are only as effective as the extent to which they are enforced (Hoelzel 1998).

References

Bowen, W. D. 1997. Role of marine mammals in aquatic ecosystems. *Mar. Ecol. Prog. Ser.* 158: 267-274.

D'Agrosa, C., Lennert-Cody, C.E., Vidal, O. 2000. Vaquita bycatch in Mexico's artisanal gillnet fisheries: driving a small population to extinction. *Conservation Biology*.14(4): 1110-1119

DeSalle, R., Amato, G. 2004. The expansion of conservation genetics. *Nature Reviews Genetics* 5(9): 702-712.

Guerrero-Ruiz, M., Urbán-Ramírez, J. and L. Rojas-Bracho. 2006. Las Ballenas del Golfo de California. Instituto Nacional de Ecología (INE-SEMARNAT). México. 526 pp.

Hoelzel, A. R. 1994. Genetics and ecology of whales and dolphins. *Annu. Rev. Ecol. Syst.* 25: 377-99.

Hoelzel, A. R. 1998. Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. *The Journal of Heredity* 89(5): 451-458.

Instituto Nacional de Ecologia (INE) 2007

Flores-Ramírez, S., Urbán-Ramírez, J.,Villarreal-Chávez, G. and R. Valles-Jimenez. 1996. Cambios espaciales y temporales de la estructura comunitaria de los cetáceos en Bahía de La Paz, B.C.S, México (1988-1991). *Ciencias Marinas* 22(2). pp. 151-173.

Kerosky, S., Munger, S., Hildebrand, J. 2008. Cetacean research and conservation: a summary of current efforts and future needs. Scripps Instituion of Oceanography. University of California, San Diego.

Kumar, S., Dudley J., Nei, M. & Tamura, K. 2008. MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. *Briefings in bioinformatics* 9: 299-306.

Moore, S.E., Clarke, J.T. 2002. Potential impact of offshore human activities on gray whales (Eschrichtius robustus). *J. Cetacean Res. Manage* 4(1): 19-25

Piggott, M. P. & Talor, A. C. 2003. Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. *Wildlife researce 30: 1-13*

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25(24): 4876-4882.

Creosote Acts as a Teratogen and Delays Development in Embryos of the Sea Urchin *Echinometra vanbrunti*

Thomas Marley, Rachel Stendahl, and Kira Watts

Department of Biology, Western Washington University, Bellingham, WA 98225

Abstract. Creosote is the most widely used wood preservative in North America, and as such, it is often found in and near marine environments, where toxic chemicals can leach into the water. To gain a better understanding of creosote's effects on the development of a tropical intertidal sea urchin, *Echinometra vanbrunti*, we exposed sea urchin embryos to the toxicant and monitored their growth. We collected and spawned 2 female and 3 male urchins from a beach in Baja California Sur, Mexico, and fertilized the eggs in 2 control solutions and 6 creosote concentrations, ranging from 0.15 to 3.0 ppm creosote. The urchins displayed development abnormalities at all concentrations above the control, and the rates of embryo development were significantly lower than the control at concentrations of 0.75 ppm and higher. Abnormalities included asymmetrical embryos and exogastrulation. This data supports the toxicity of creosote to developing embryos and provides relevant information for updating regulation standards.

Keywords: creosote, teratogen, development, sea urchin embryology, *Echinometra vanbrunti*, pollution, polycyclic aromatic hydrocarbons

Introduction

Coal tar creosote, a varying mixture of more than 1000 chemicals—including polycyclic aromatic hydrocarbons (PAHs), cresol, and phenols—has been used extensively as a wood preservative, especially in and near marine environments (Stratus Consulting 2006). Creosote production in the United States began in 1917, and by 1992, total annual production had exceeded 2.96 billion pounds (USITC 1994, cited in ATSDR 2002). Because the leaching rate of creosote is enhanced by factors such as heat, warm water, and flow rate (Stratus Consulting 2006), tropical shores, especially those with large tidal fluctuations, are particularly susceptible to pollution from creosote-treated posts, poles, and pilings.

The most prominent contributors to creosote's toxicity are PAHs (ATSDR 2002), fat soluble molecules which accumulate in invertebrates lacking the cytochrome P450 metabolic pathway, which fish and other vertebrates use to metabolize PAHs to an excretable form (Marty *et al*. 1997). PAHs have, however, been demonstrated to be carcinogenic to vertebrates, including humans (Bos *et al.* 1984; Mastrangelo *et al.* 1996). This raises much concern because many other sources of PAHs exist, including crude oil, fossil fuel exhaust, charred meat, and anywhere else organic material is burned with insufficient oxygen for complete combustion (ATSDR 1995). Though these other sources do not contain concentrations of PAHs as high as those found in creosote, they are much more prevalent in our everyday lives. Also, catastrophic events can release extremely large volumes of pollutants into the ocean (i.e. the 2010 Gulf of Mexico oil spill) which result in higher environmental concentrations of PAHs than creosote leaching. To

understand how PAHs might affect an ecosystem, creosote is an accessible and relevant replacement to use in laboratory testing.

Because of their proximity to sources of creosote leaching, littoral organisms are exposed to higher concentrations of the toxicant, and the teratogenic effects of creosote on sea urchin embryos are very similar to those caused by exposure to isolated PAHs (Pillai *et al*. 2003), further demonstrating that creosote's toxicity comes from PAHs. Additionally, environmental levels (as low as 0.5 ppb) of benzo(a)pyrene (BaP), a representative PAH, can induce teratogenic effects in embryos of *Strongylocentratus purpuratus* (Hose *et al.* 1983), a littoral sea urchin inhabiting the Pacific coast of North America.

Sea urchin embryology is well documented and has helped elucidate processes of morphogenesis common to all deuterostomes (Kominami and Takata 2004), and the 48-hour sea urchin assay is a common toxicological test (Bellas *et al.* 2005; Maciorowski *et al.* 1982). In the present study, we used *Echinometra vanbrunti* to test the toxicity of creosote taken from a utility pole on the shore of the Bay of La Paz, Baja California Sur, Mexico.

Creosote is certainly toxic to sea urchin embryos, causing exogastrulation of the blastulae via a β-catenin dependent pathway (Pillai *et al*. 2003), but less data is available concerning how creosote affects the rate of development and the prevalence of developmental abnormalities during the first 48 hours after fertilization. To approximate environmental conditions, we fertilized sea urchin embryos in filtered sea water (FSW) containing various concentrations of creosote (0.15 – 3.0 ppm) and periodically monitored their development for 48 hours in order to determine how creosote affects the

rate of development and incidence of malformed embryos of *Echinometra vanbrunti* as compared to a control. *E. vanbrunti* is an intertidal purple urchin and one of the most common urchins inhabiting the Sea of Cortez (Brusca 1980), and its preference for shallow and even exposed rocky habitats in tropical regions may increase its exposure to creosote.

Methods and Materials

Treatment preparation

We collected creosote directly from a telephone pole approximately 50 meters from the Bay of La Paz. We first created two 100-ml control treatments, one containing only filtered seawater (FSW) and one comprised of 0.1% ethanol in FSW. Next, we dissolved 3 mg of creosote tar in 1 ml of ethanol and then added FSW to a total volume of 1 L, resulting in a stock creosote solution with 3 ppm creosote and 0.1% ethanol. Using this stock, we created 100 ml of the following dilutions for each of our 6 creosote treatments: 5% (0.15 ppm creosote, 0.005% OH), 10% (0.3 ppm creosote, 0.01% OH), 25% (0.75 ppm creosote, 0.025% OH), 50% (1.5 ppm creosote, 0.05% OH), 75% (2.25 ppm creosote, 0.075% OH) and 100% (3 ppm creosote, 0.1% OH). Each of these 8 treatments we stored in a sealed flask until ready for use.

Animal collection and spawning

We collected 6 purple urchins, *Echinometra vanbrunti*, from a rocky section of the beach at Club Cantamar, Baja California Sur, Mexico, and we prepared a 0.5 M KCl solution by dissolving 3.7280 grams solid KCl in 100 ml distilled water.

To induce spawning in the urchins, we injected each animal with approximately 1 ml 0.5 M KCl using a hypodermic needle. Once injected, the ripe urchins began releasing gametes, and we then sexed each animal. For the two ripe females, we placed each animal with its aboral side down on top of a beaker full of FSW to collect the eggs. After each urchin stopped releasing eggs into the water, it was placed in a bucket for return to the wild. The eggs remained in the bottom of the beaker, and after gathering eggs from both females, we carefully poured about half the water out for transport to the lab. For the three ripe males, we collected the sperm with a mouth-pipette and transferred the sperm to a lab tube, which was kept on ice for transport to the lab. We returned the adult urchin's to their original habitat shortly after we spawned them.

Fertilization

After letting the eggs settle in the beaker, we measured the concentration to be approximately 20% eggs, and then we homogenized this solution by stirring and added 5 ml to each of our eight 100-ml treatments, resulting in an egg concentration just less than 1% in each. We then added 2 drops of sperm to 100 ml of FSW and slowly added 20 drops of this sperm dilution to each treatment while stirring constantly in an effort to minimize the occurrence of polyspermy.

Observations

We used a 50 µl micropipette to transfer samples from the treatments to microscope slides for viewing under a compound microscope. We conducted initial observations 10 minutes after introducing the sperm to verify fertilization, using this as our 0-hour check. Then we made observations of all 8 treatments at hours 4, 18, 24, 28, 40, and 48. We took digital photographs of each slide and quantified the results by

analyzing the first 20 fertilized embryos on each picture, starting on the left side of the frame. For those 20 embryos, we counted how many were deformed and how many appeared normal (compared to controls), and we also rated each embryo's developmental stage according to the following scale:

0 – Still inside fertilization envelope (pre-hatch)

1 – Ciliated blastula (post-hatch)

2 – Gastrula (invaginated or evaginated archenteron)

3 – Prism stage

In addition to the photographs, we also captured video of the slides to determine if the embryos were alive (moving) or not.

Statistical Analysis of Developmental Rating

Differences between treatment groups were analyzed by one-way analysis of variance (ANOVA), and groups that differed significantly ($p<0.01$) were analyzed by Dunnett's test (familywise error rate = 0.05) for multiple comparisons against a control. Minitab 16.1.0 software was used for all statistical analyses.

Results

At the end of the 48-hour test, nearly half of the embryos sampled from the 100% (3.0 ppm creosote) treatment were malformed, while all embryos from both control samples were normally developed (Figure 1). In general, our final samples showed a trend of increasing developmental abnormalities as creosote concentration increased, with the 5% and 10% (0.15 and 0.30 ppm creosote) treatments displaying similar incidences of abnormalities and the 25% and 50% also forming a similar pair.

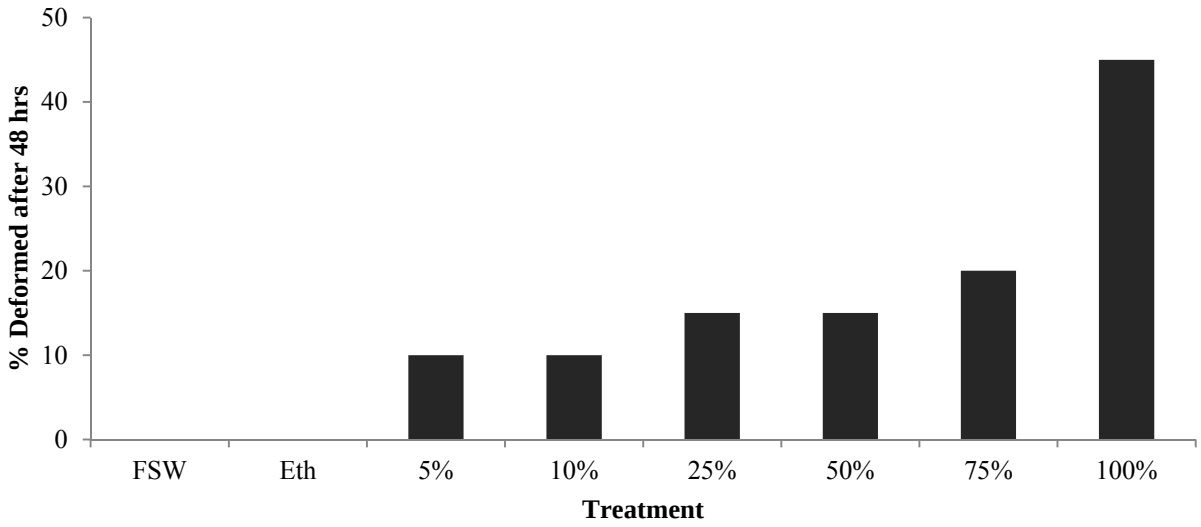


Figure 1. Percentages of abnormally formed sea urchin embryos after being subjected for 48 hours to 8 treatments (n=20 for each): FSW (pure filtered seawater), Eth (0.1% ethanol), 5% (0.15 ppm creosote, 0.005% ethanol), 10% (0.3 ppm creosote, 0.01% ethanol), 25% (0.75 ppm creosote, 0.025% ethanol), 50% (1.5 ppm creosote, 0.05% ethanol), 75% (2.25 ppm creosote, 0.075% ethanol), 100% (3.0 ppm creosote, 0.1% ethanol).

The deformities present in our experiment were mostly uneven cell divisions and the resulting asymmetric blastulae and gastrulae (see S1). We also found evidence of exogastrulation in which the archenteron was evaginated, as previously described by Pillai *et al*. (2003). This abnormality was not (yet) fatal to the embryos, however, and they were able to continue development, albeit quite slowly.

During the first 4 hours after fertilization, all embryos sampled from each treatment were at stage 0 (Figure 2). By hour 18, a clear trend had developed in which increased creosote concentration correlated to decreased stage of development. After 24 hours, the average stages for both control samples and the 5% sample were all near 3, with the 10% treatment only slightly lower (not significant), and all other samples displayed a negative correlation between creosote concentration and developmental stage. Samples taken at later times showed results similar to the 18- and 24-hour checks. By the end of 48 hours, there was no significant difference between the average ratings of the

control groups and the 5% and 10% treatment, and the other treatments lagged far behind in development, though the embryos were still alive.

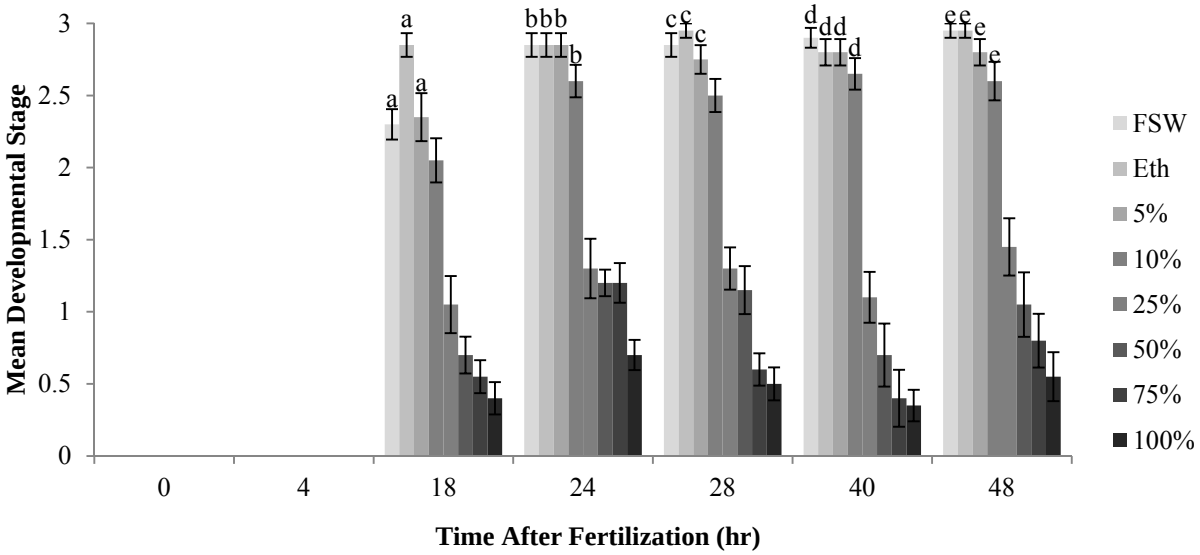


Figure 2. Mean stage of development for samples from 8 treatments (see Figure 1) taken at various times after fertilization. Letters above the bars indicate groups of values which are not significantly different from the ethanol control (Eth) as determined by Dunnett's tests with familywise error rates of 0.05.

Discussion

These results confirm that creosote is detrimental to development of sea urchin embryos. At 3 ppm, nearly half of the sampled embryos were malformed, and at concentrations as low as 0.15 ppm, we found an increase in the number of deformities, though higher concentrations were necessary to significantly affect rate of development. The developmental stage of the 10% (0.3 ppm creosote) treatment was not significantly different from the control at the end of 48 hours, but the stage of the 25% (0.75 ppm creosote) was about half the value of the control. Thus, we determined the no-observed-effect level (NOEL) for creosote with relation to rate of development of *E. vanbrunti* to be 0.3 ppm creosote, though the NOEL for developmental abnormalities appears to be lower. Our best estimate of the lowest-observed-effects level relative to rate of

development is 0.75 ppm creosote, but finer resolution of treatment concentrations may show this value to be even lower.

Pillai *et al*. (2003) determined the EC_{50} to cause exogastrulation in *Strongylocentrotus purpuratus*, the California purple urchin, to be 1.57 ppm creosote, but it appears that our data show this value for *E. vanbrunti* to be over 3 ppm. This discrepancy could be due either to species-related differences or to the source of creosote used—we used creosote which had been weathered for several years, while Pillai *et al*. (2003) used freshly produced creosote. It could be that the weathered creosote has already leached much of its toxic content into the environment.

PAHs, the largest contributors to the toxicity of creosote, are only slightly soluble and do not readily leach from creosote, and 12-year-old marine pilings can still leach up to 75% as much creosote as freshly treated wood (Stratus Consulting 2006). However, if our data lead to an EC_{50} which is double that of another study, then we surmise that our creosote has already lost a significant portion of its PAHs, the urchins we used are more resistant to the toxicant, or some combination of these two possibilities. There is also the possibility that the composition of the creosote used differs vastly between studies. Investigation into the cause of the difference between results may shed light on the amount and content of toxicant leached into the environment or perhaps some toxicologically relevant difference between the two species.

Creosote is highly toxic to a variety of marine species (Ownby *et al.* 2002; Suchanek 1993), especially during development (Barron *et al.* 2003; Bellas *et al.* 2005; Hose *et al.* 1983). Our study provides further evidence of this susceptibility, specific to early embryos of *E. vanbrunti*, a common urchin in the Gulf of California. Such

information is important for policy revision and regulation of toxic compounds used in areas vulnerable to pollution.

References

ATSDR. 1995. Toxicological profile for polycyclic aromatic hydrocarbons. US Department of Health and Human Services: Agency for Toxic Substance and Disease Registry. ATSDR Publication TP69.

ATSDR. 2002. Toxicological profile for wood creosote, coal tar creosote, coal tar, coal tar pitch, and coal tar pitch volatiles. US Department of Health and Human Services: Agency for Toxic Substance and Disease Registry. ATSDR Publication TP85.

Barron, M. G., Carls, M. G., Heintz, R., and Rice, S. D. 2003. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. *Toxicological Sciences*. **78**: 60-67.

Bellas, J., Beiras, R., Mariño-Balsa, J. C., and Fernández, N. 2005. Toxicity of organic compounds to marine invertebrate embryos and larvae: a comparison between the sea urchin embryogenesis bioassay and alternative test species. *Ecotoxicology*. **14**: 337-353.

Brusca, R. C. 1980. *Common Intertidal Invertebrates of the Gulf of California*. Ch 26. University of Arizona Press, Tuscon, AZ.

Bos, R. P., Theuws, J. L., Leijdekkers, C. M., and Henderson, P. T. 1984. The presence of the mutagenic polycyclic aromatic hydrocarbons benzo[a]pyrene and benzo[a]anthracene in creosote P1. *Mutagenic Research*. **130**: 153-158.

Hose, J. E., Puffer, H. W., Oshida, P. S., and Bay, S. M. 1983. Developmental and cytogenic abnormalities induced in the purple sea urchin by environmental levels of benzo(a)pyrene. *Archives of Environmental Contamination and Toxicology*. **12**: 319-325.

Kominami, T. and Takata, H. 2004. Gastrulation in the sea urchin embryo: a model system for analyzing the morphogenesis of a monolayered epithelium. *Developmental Growth Differential*. **46**: 309-326.

Maciorowski, L. W., Little, L. F., Raynor, R. C., and Sims, J. L. 1982. Bioassays: procedures and results. *Water Pollution Control Federation*. **54**(6): 830-848.

Marty, G. C., Short, J. W., Dambach, D. M., Willits, N. H., Heintz, R. A., Rice, S. D., Stegeman, J. J., and Hinton, D. E. 1997. Ascites, premature emergence, increased gonadal cell apoptosis, and cytochrome P4501A induction in pink salmon larvae continuously exposed to oil-contaminated gravel during development. *Canadian Journal of Zoology*. **75**: 989-1007.

Mastrangelo, G., Fadda, E., and Marzia, V. 1996. Polycyclic aromatic hydrocarbons and cancer in man. *Environmental Health Perspectives*. **104**(11): 1166-1170.

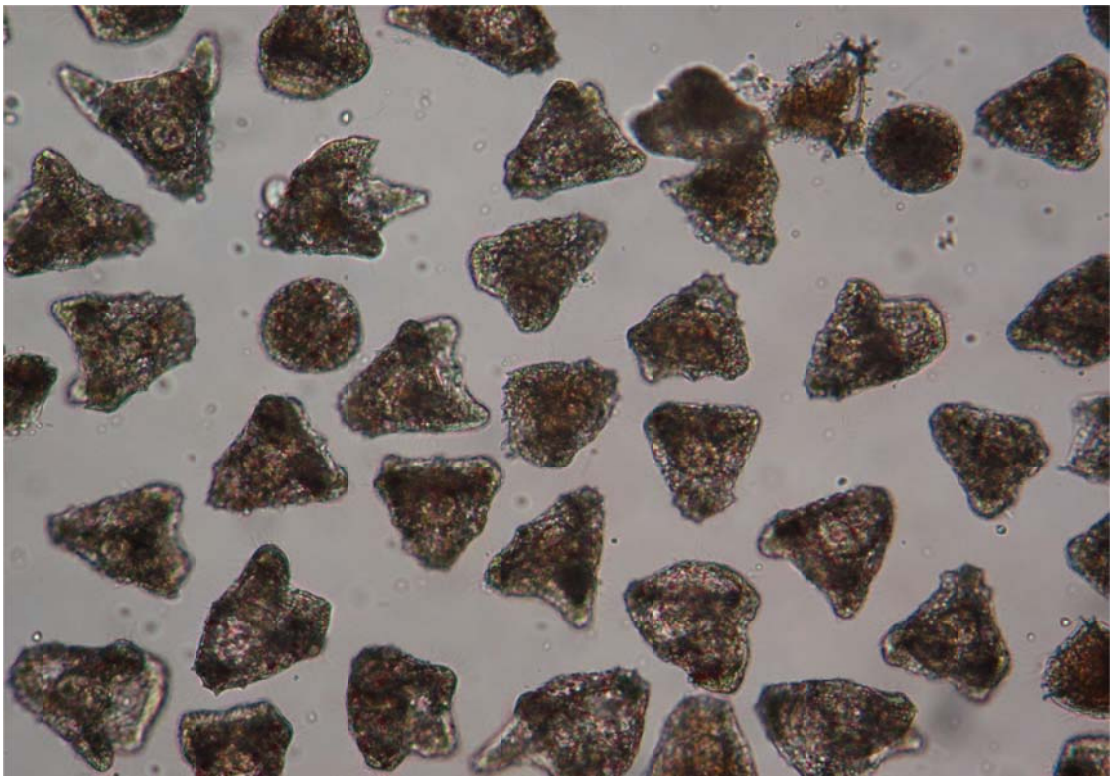
Ownby, D. R., Newman, M. C., Mulvey, M., Vogelbein, W. K., Unger, M. A., and Arzayus, L. F. 2002. Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote-contaminated sediments. *Environmental Toxicology and Chemistry*. **21**(9): 1897-1902.

Pillai, M. C., Vines, C. A., Wikramanayake, A. H., and Cherr, G. N. 2003. Polycyclic aromatic hydrocarbons disrupt axial development in sea urchin embryos through a β-catenin dependent pathway. *Toxicology*. **186**: 93-108.

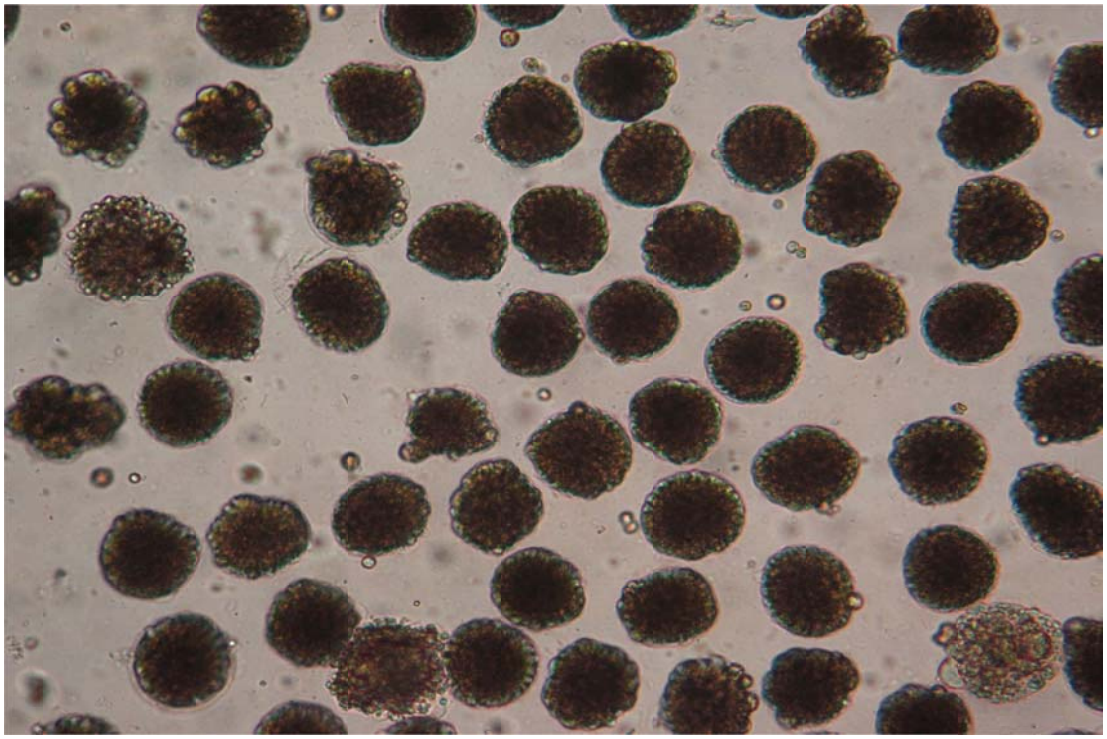
Stratus Consulting. 2006. Creosote-treated wood in aquatic environments: technical review and use recommendations. Stratus Consulting, Inc., Boulder, CO.

Suchanek, T. H. 1993. Oil impacts on marine invertebrate populations and communities. *American Zoologist*. **33**(6): 510-523.

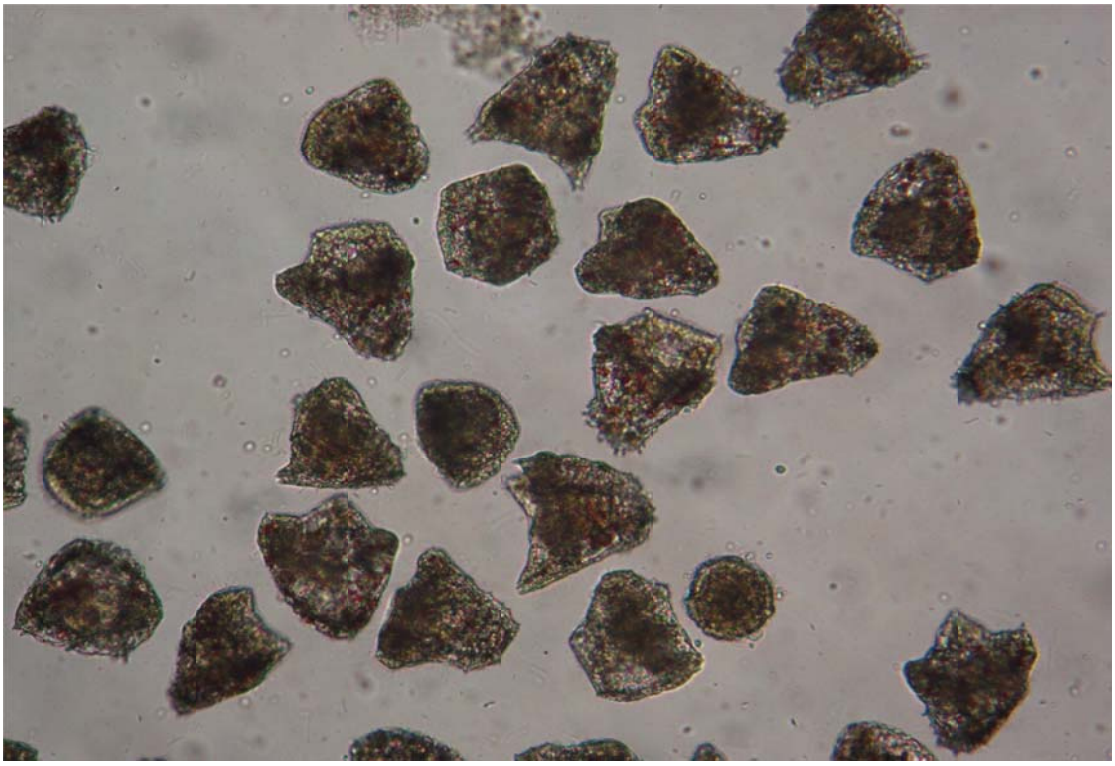
USITC. 1994. Synthetic organic chemicals: United States production and sales, 1992. United States International Trade Commission, Washington, DC. USITC Publication 2720.



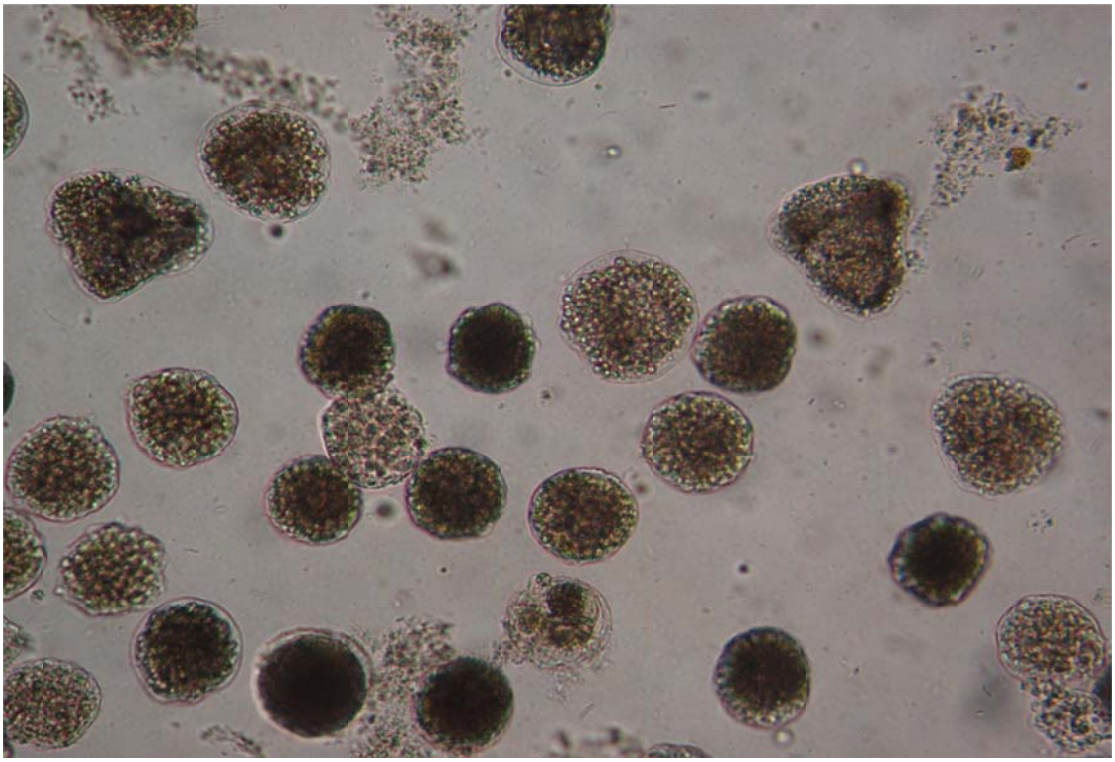
Picture 1. Sea urchin embryos from the FSW control 48 hours after fertilization



Picture 2. Sea urchin embryos from the 100% (3.0 ppm) creosote solutions 48 hours after fertilization



Picture 3. Sea urchin embryos from the 10% (0.3 ppm) creosote treatment 48 hours after fertilization



Picture 4. Sea urchin embryos from the 25% (0.75 ppm) creosote treatment 48 hours after fertilization

Coral reef fish diversity of Calerita Beach, La Gaviota Beach, and the Club Cantamar beach of Baja California Sur, México

Bobbie Buzzell[1], José Pérez-Castillo[2], Bernardo Veyrand-Quirós[2]
[1]Department of Biology. Western Washington University Bellingham, WA 98225;
[2]Universidad Autónoma de Baja California Sur carretera al sur Km 5.5 C.P. 23080 B.C.S., Mexico

Abstract

Many studies have investigated diversity of various types of organisms, because diversity reflects upon the stability and structure of whole ecosystems. The purpose of this study was to define the fish diversity of local coral patches in Baja California Sur. We attempt to answer if human activities are affecting the fish diversity of Calerita Beach, La Gaviota Beach, and the beach of Club Cantamar. Since human activities have been a major influence on many coastal systems, we hypothesized that there would be a positive correlation between human activities and reef fish diversity. Calerita was the sample site with the least amount of human influence, La Gaviota served as the medium, and Cantamar was the location with the greatest amount of human influence. Transects of 30 meters were laid parallel to each other, approximately 5 to 10 meters away from each other in each study site. Underwater sketch boards were used to record each species and number of individuals observed within 2 meters of either side of the transects. The Shannon-Weiner index, Pielou evenness index, Simpson index, and richness were calculated for each study site. PRIMER was used to find the taxonomic diversity of each site. Calerita had the greatest fish diversity of all three sites, and La Gaviota and Cantamar had similar diversity levels. There was a distinct difference between the Shannon index values of Calerita and the other two sites. Each index, and the taxonomic

diversity found by PRIMER , supported our hypothesis. Indeed, there was a correlation between the degree of human influence of the sites and fish diversity. This accentuates the idea that humans are affecting coastal systems, and that this may pose an immediate concern to conserving the local corals.

Keywords: Coral reef, fish diversity, species richness, species evenness, ecological index, Shannon-Weiner index, Simpson index, Pielou index, taxonomic diversity.

Introduction

While global warming may be a long term consequence, marine habitat devastation by human influence is a more immediate outcome. Human activity has become a dominant disturbance affecting the organisms in local marine habitats, especially tropical coral reefs. In Baja California Sur, many coastal paradises have become the prime location for tourist attractions, boat docks, and other commercial and recreational activities. This poses a grave concern for conservation of coral reefs, and other marine systems. Our prime question is how does human activity affect the fish species diversity in the local coral reefs?

Coral reefs are fragile systems that can support some of the most diverse fish species (Sale, 2004). Corals feed on zooplankton, but their primary source of energy is from their symbiotic, photosynthesizing zooxanthellae protozoa (Connell, 1978). The primary productivity of corals is strongly coupled to secondary productivity in the tropics (Gray, 2001), which accounts for the high biomass and diversity observed in coral reefs. How coral reefs become and maintain highly diverse communities is a controversial

topic, but we can conclude that high diversity is a result of niche specialization among organisms (Ogden and Quinn, 2002). Another component that contributes to high diversity is intermediate disturbance, because no disturbance would result in highly competitive interactions, causing dominance among only a few species (Syms and Jones, 2000). However, too much or too little disturbance results in negative effects on the coral reef populations. Recent studies have shown that corals in the Gulf of California have become decreasingly able to recover from disturbances such as hurricanes and El Niño events. Such staggering recovery is leaving these ecosystems more susceptible to human manipulation (Alvarez-Filip, 2006). Reef fishes are an important group to study, because they reflect upon the colonial structure of coral reef ecosystems. Fish fauna found in the Gulf of California occurs mainly in rocky, reef-type geology regions. The structure of reef fish communities are considered the most complex and variable in the world due to the range of opportunities offered by the environment (depth, protection by refugees, nutrients, temperature variations) for the development of fish (Areola-Robles, 1998).

In this experiment, we attempt to distinguish between the species diversity of three locations that are all in close proximity of each other- Calerita Beach, La Gaviota Beach, and the beach at Hotel Club Cantamar. We define species richness as the total number of species present in one study site. Species equitability, or evenness, is how evenly the individuals are distributed among the different species (Clarke, 2001). The terms diversity and species richness are often confused, because both determine the number of species. However, the term diversity is used not only to describe the number of species, but also to describe whether or not species are evenly represented in the community. To clarify in this context, diversity refers to the expression or index of the

relationship between the number of species and the number of individuals (Spellerberg and Fedor, 2003).

The purpose of this study is to define fish species diversity of three local coral reefs, and to bring evidence of how decreased fish species diversity is caused by human activities. We believe there is a positive relationship between the two components, and we expect to detect a decrease in species diversity with increased local human activity. Transects were a simplistic method for this experiment, and allowed us to gather a broad range of information to be analyzed by PRIMER and manipulated in excel.

Study Area

Punta Galerita Beach, also known as Calerita, is located on the peninsula of La Paz, in front of the San Lorenzo Seaway at 24^0 21' 1.82'' N and 110^0 17' 0.07'' W, southwest of the La Paz Bay (Figure 1). Calerita is characterized by sandy, rocky bottom boulder, with a gentle slope and very dynamic waves (Rodríguez-Morales, 1997). This study site served as the most distant location affected by human activities. Cantamar Beach is property of Club Cantamar Hotel, and is near the city of La Paz- 18 km from State Highway No. 11. The beach is situated on the coast of the southeastern region Bay of La Paz between the ferry port and Pichilingue beach. The study site is located between coordinates 24º 16' 43.22" N and 110º 20' 2.70" W (Figure 2). It is distinguished as sandy bottoms, and areas covered by coral. Cantamar has little tidal exchange with shallow depths that do not exceed 5 meters. Since this beach is located near a hotel, the corals here have continuous contact with human influences (litter, pollution, tourists, boats, etc…).

Beach La Gaviota is located in front of the island "La Gaviota", and is accessed over the hill from Pichilingue beach. La Gaviota served as the location with intermediate to high disturbance levels caused by humans. Litter was prominent, but there was also a high frequency of passing boats. It is located between 24º 17' 8.92" N and 110º 19' 58.67" W (Figure 3). This beach is shallow with a rocky bottom, and has certain areas covered by coral. Dynamics of waves and tides are very low, with depths no greater than 4 meters in the section we surveyed.

Methods

All surveys occurred between the hours of 10:00am and 1:00pm to limit time as a variable in the experiment. Three transects of 30 meters were laid parallel to each other, approximately 5 to 10 meters away from each other in the subtidal zone of each study site. As we would lay transects behind us, we recorded each species and number of individuals observed within 2 meters of either side of the transect. Underwater transects were used to record this data. Any unknown species was photographed to be identified later. After completing the first transect, we rotated positions to complete the process again, and then once more with a consecutive rotation. This gave us three replicates for each area observed along the same coral reef.

For each species, the number of individuals observed was summed and divided by three to obtain an average. The program PRIMER was used to analyze the data, and attain the taxonomical diversity of each study site. Delta + represents how branched the taxonomic diversity is. In other words, an observed higher Delta + value, the greater the diversity in species, Genus, Family, Suborder, and Order, and vice versa for smaller Delta

+ values. Each of the taxa was assigned the following weight by PRIMER: species, 20; Genus, 40; Family, 60; Suborder, 80; and Order, 100.

Several indices were used to distinguish diversity of the three study sites. The Shannon-Weiner diversity index highlights the species richness and equitability factors of diversity to varying degrees.

$H' = -\Sigma_i p_i \log(p_i)$

The proportion of the total count coming from the ith species is represented as p_i. We used Pielou's evenness index to express equitability. H_{max} represents the maximum possible value of the Shannon diversity index.

$J' = H'/H_{max} = H'/\log S$

The Simpson index is also employed here. While there are a several forms of the equation, we used the equation appropriate for smaller samples.

$\lambda' = \{\Sigma_i N_i(N_i - 1)\}/\{N(N-1)\}$

The Simpson's index is a "dominance index", because its largest values correspond to communities whose total abundance is dominated by one, or a very few, of the species present (Clarke and Warwick, 2001).

Results

Calerita had an average of approximately 19.33 species, and Cantamar and La Gaviota had the least amount of species, with averages of 12 and 11.33 species, respectively; the p value of the richness graph was 0.002 (Figure 4). In Calerita, we observed a Shannon-Weiner index of 1.99, while Cantamar and La Gaviota presented similar indices of 1.55. The Shannon index graph had a p value of 0.01 (Figure 5). The

Pielou evenness of Calerita was 0.76, Cantamar's was 0.62, and La Gaviota's was 0.64. For this index, p was equal to 0.57 (Figure 6). The Simpson index showed that there was a higher probability of finding more dominant species in Calerita than the other two sites, with a p value of 0.16 (Figure 7). There was a positive relationship between the high dominance value in Calerita and the site's high species richness (Figure 7; Figure 4).

Calerita had the greatest taxonomic diversity with a delta of 88.62. La Gaviota had the next greatest delta of 81.47, and Cantamar followed in close proximity, with a delta of 81.47 (Figure 8). Suborders had the greatest variation of all the taxa. There were six different suborders present, and Labroidei was the most abundant suborder. The four species with greatest dominance was *Stegastes rectifaenum, Abdefduf troschelii, Thalassoma lucasanum y Stegastes acapulcoensis* (Table 1). Overall, Calerita had the greatest fish diversity of all three communities.

Discussion

The aforementioned results give clear evidence that our hypothesis is supported. The fish species richness was greatest in Calerita (Figure 4), which suggests that the site is indeed less impacted by humans. Calerita is a more remote beach in comparison to La Gaviota and Cantamar, and is why fewer people may go there. The Shannon index was reflected more by the richness of the sites, than evenness. Statistically, the probability of our null hypothesis being true for species evenness is greater than 50% (Figure 6), thus there is a large probability that the evenness values of the study sites are similar. This is why evenness does not affect the Shannon index of this study. As observed in the Simpson index, the probability of finding more dominant species in Calerita is greater

than Cantamar and La Gaviota (Figure 7). This is consistent with the species richness in Calerita, because the more species accounted for, the more dominant species there may be among the community. Taxonomic diversity was also greatest in Calerita, and this shows that there is a large amount of genetic variation among this community. Even in small samples, these records show variations in the species composition among sites, and can be explained by minute differences in the structure of the community, (Ormond y Roberts 1997, Almany 2004).

As previously discussed, the intermediate disturbance hypothesis is an important process for achieving a high level of diversity (Syms, 2000), and we can see that the excess disturbance caused by humans in La Gaviota and Cantamar is detrimental to the fish diversity of these areas. Conversely, Calerita's high diversity may be a result of intermediate disturbance. After perturbation of a community, there will be a flux of stabilization to reach equilibrium amongst the ecosystem (Connell, 1978). In a sense, we can say the high diversity of coral reefs is a result of constant equilibrium processes from a source of intermediate disturbance.

Although there is a strong connection between the degree of human activity and the fish diversity for each site, there are other factors not included in this study that may affect the fish diversity. The dynamics of environmental conditions around the reefs are also an important factor in the differences in species richness of fish. Differences in species are affected by the size of the reefs, because larger reef areas will have a greater amount of primary productivity (Williams, 1986). Calerita´s larger area of reef area consists of more food and shelter for fish species, and allows for less interspecific competition.

According to Arreola-Robles (1998) there are a variety of environments with a complex mix of conditions in the Gulf, such as currents, physical structure and the area covering the reef. These factors have been identified previously as the cause of the differences in ecological indices across different studies (Williams, 1986). Reefs in the region of La Paz are generally of two kinds- protected and exposed. Protected reefs (Cantamar and La Gaviota) are characterized by limited influence of currents, winds and waves. Exposed coral reefs are influenced by strong currents and winds (Arreola-Robles, 1998). Exposed beaches like Calerita will have a greater flux of nutrients, because of currents. Howerver, La Gaviota and Cantamar are more enclosed in the Bay than Calerita, and this may limit the flux of nutrients into the systems. Herbivorous fish, such as *Stegastes punctatissima* and *Canthigaster rectifraenum,* are more abundant in regions of no currents, because limited or nonexistent currents allow for the flourishment of algae, and limited plankton populations that would otherwise attract many carnivorous fish (Arreola- Robles, 1998). The structure of the fish community can also be determined by seasonality. For example, the coral reefs of the Gulf of California experience a higher abundance of *Stegastes rectifraenum, Cirrhitus rivulatus* and *Myripristis leiognathos* during the summer months *(*Arreola-Robles, 1998).

García-Charton (1995) states that one of the determining factors shaping the community structure is the physiographic structure of the reef. Corals are a topographically heterogeneous substrate, and this is utilized by many species of reef fish for protection, but some species do show preferences for certain species of corals. Tilman (2001) found that *Thalassoma lucasanum* prefer corals of the Genus *Pavona* (Espinoza 2005). Thus, type of coral could influence the location of which the species inhabit. In

our study, *Pocillopora elegans* was the prominent coral observed, so we may have only found the species that prefer this species of coral. For future studies, it would be important to incorporate coral reefs with a wide array of different coral species.

Studying the fish diversity of coral communities reflects upon entire ecosystems, and large diversities are often correlated with healthy, productive ecosystems. Understanding this relationship creates roots for conservation, because destroying these types of ecosystems could create a chain reaction among other ecosystems. This could affect the fishing industry as a source of resources for the country's economy (Arreola-Robles, 1998). So not only do reef fish depend on the productivity of coral reefs, but human society does as well, and this is why sustainable management needs to be directed at these ecosystems.

Literature Cited

Alvarez-Filip L, Reyes-Bonilla H. 2006. Comparison of community structure and functional diversity of fishes at Cabo Pulmo coral reef, western Mexico between 1987 and 2003. Proceedings of 10th International coral Reef Symposium. 216-225.

Arreola-Robles JL. 1998. Diversidad de peces de arrecife en la región de La Paz, B.C.S. Mexico. Undergraduate Thesis. CICIMAR-IPN. Mexico. 75 p.

Clarke KR, Warwick RM. 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. 2nd Edition. Primer-E Ltd. Plymouth Marine Laboratory, UK.

Connell JH. 1978. Diversity in tropical forests and coral reefs. Science. 199: 1302-1310.

García-Charton, J. A. 1995. Relaciones con el habitat y factores ambientales que determinan la estructura especial del poblamiento ictiológico de los fondos rocosos infralitorales del mediterráneo occidental. Memorias de grado de licenciatura. University of Murcia, Spain. 143 p.

Gray JS. 2001. Marine diversity: the paradigms in patterns of species richness examined. Science. 65(2): 41-56.

Nelson JS.1994. Fishes of the World. 3rd edition. New York: John Wiley and Sons, Inc. 600 p.

Ogden JC, Quinn TP. 2002. Migration in coral reef fishes: Ecological significance and orientation mechanisms. Fairleigh Dickinson University Teague Bay, Christiansted, St. Croix U.S. Virgin Islands 00820 USA.

Rodríguez-Morales EO. 1997. Variación interanual de las macroalgas en punta Galeras, B.C.S. Undergraduate Thesis. Universidad Autónoma de Baja California Sur. 70 pp.

Sale, PF. 2004. Connectivity, recruitment variation, and the structure of reef fish communities. Integr. Comp. Biol. 44:390-399.

Spellerberg IF, Fedor PJ. 2003. A tribute to Calude Shannon (1916-2001) and a plea for more rigorous use of species richness, species diversity and the 'Shannon-Wiener' Index. Global Ecology and Biogeography. 12: 177-179.

Syms C, Jones GP. 2000. Disturbance, habitat structure, and the dynamics of a coral-reef fish community. Ecology. 81(10): 2714-2729.

Williams, D. McB. 1986. Temporal Variation in the Structure of the Reef Slope Fish Comunities (Central Great Barrier Reef): Short-Term Effects of *Acanthaster planci* Infestation. \Marine Ecology Progress Series, 28: 157-164.

Figure and Table Legend

Table I

Species	Genus	Family	Suborder	Order	Calerita Average	Cantamar Average	Gaviota Average
Holocanthus passer	*Holocanthus*	Pomacanthidae	Percoidei	Perciformes	10.67	1.33	*3.33*
Haemulon sexfasciatum	*Haemulon*	Haemulidae	Percoidei	Perciformes	43.33	2.67	*3.67*
Microspathodon dorsalis	*Microspathodon*	Pomacentridae	Labroidei	Perciformes	7.00	2.00	*3.00*
Stegastes rectifaenum	*Stegastes*	Pomacentridae	Labroidei	Perciformes	74.67	72.33	*76.33*
Abudefduf troschelii	*Abdefduf*	Pomacentridae	Labroidei	Perciformes	42.00	84.67	*60.33*
Thalassoma lucasanum	*Thalassoma*	Labridae	Labroidei	Perciformes	245.00	41.00	*16.33*
Myripistis leiognathus	*Myripistis*	Holocentridae	Holocentroidei	Beryciformes	10.33	0.00	*0.00*
Sargocentron suborbitalis	*Sargocentron*	Holocentridae	Holocentroidei	Beryciformes	43.67	0.67	*0.00*
Ophioblennius steindacneri	*Ophioblennius*	Blenniidae	Blennioidei	Perciformes	0.67	2.00	*1.00*
Arothron meleagris	*Arothron*	Tetradonthidae	Tetraodontoidei	Tetraodontiformes	1.00	0.33	*0.33*
Diodon holocanthus	*Diodon*	Diodonthidae	Tetraodontoidei	Tetraodontiformes	2.00	1.00	*0.33*
Cirrhitichthys oxycephalus	*Cirrhitichthys*	Cirrhitidae	Percoidei	Perciformes	0.33	0.00	*0.00*
Kyphosus elgans	*Kyphosus*	Kyphosidae	Percoidei	Perciformes	8.67	0.00	*0.00*
Stegastes acapulcoensis	*Stegastes*	Pomacentridae	Labroidei	Perciformes	34.00	0.00	*24.00*
Canthigaster puntatissima	*Canthigaster*	Tetradonthidae	Tetraodontoidei	Tetraodontiformes	1.00	3.33	*0.00*
Epinephelus labriformis	*Epinephelus*	Serranidae	Percoidei	Perciformes	1.33	0.33	*1.67*
Cirrhitus rivulatus	*Cirrhitus*	Cirrhitudidae	Percoidei	Perciformes	1.67	1.33	*0.00*
Thalassoma gramaticum	*Thalassoma*	Labridae	Labroidei	Perciformes	37.33	7.67	0.00
Sargocentron suborbitalis	*Sargocentron*	Holocentridae	Holocentroidei	Beryciformes	21.67	0.00	0.00
Fistularia commersonii	*Fistularia*	Fistulariidae	Syngnathoidei	Gasterosteiformes	0.67	0.00	0.00
Gymmomuraena zebra	*Gymmomuraena*	Muraenidae	Muraenoidei	Anguilliformes	0.33	0.00	0.00
Sphoeroides annulatus	*Sphoeroides*	Tetradontidae	Tetraodontoidei	Tetraodontiformes	2.00	0.00	0.33
Sphoeroides lobatus	*Sphoeroides*	Tetradontidae	Tetraodontoidei	Tetraodontiformes	1.33	0.00	0.33
Gymnothorax castaneus	*Gymnothorax*	murenidae	Muraenoidei	Anguilliformes	0.67	0.00	0.00
Mycteroperca rosacea	*Mycteroperca*	Grammistinae	Percoidei	Perciformes	0.33	0.00	0.00
Needlefishes		Belonidae	Belonoidei	Beloniformes	6.67	0.00	0.00
Johrandallia nigrirostris	*Johrandallia*	Chaetodontidae	Percoidei	Perciformes	1.00	0.00	0.00
Scarus ghobban	*Scarus*	Scaridae	Labroidei	Perciformes	0.00	0.33	2.33
Chromis atrilobata	*Chromis*	Pomacentridae	Labroidei	Perciformes	0.00	18.33	0.00
Bodianus diplotaenia	*Bodianus*	Labridae	Labroidei	Perciformes	0.00	0.33	0.00
Serranus psittacinus	*Serranus*	Serranidae	Percoidei	Perciformes	0.00	0.00	0.33
Labrisomus xanti	*Labrisomus*	Tripterygiidae	Blennioidei	Perciformes	0.00	0.00	0.33
Hoplopagrus guentherii	*Hoplopagrus*	Lutjanidae	Percoidei	Perciformes	0.00	0.00	0.33

Figure 1

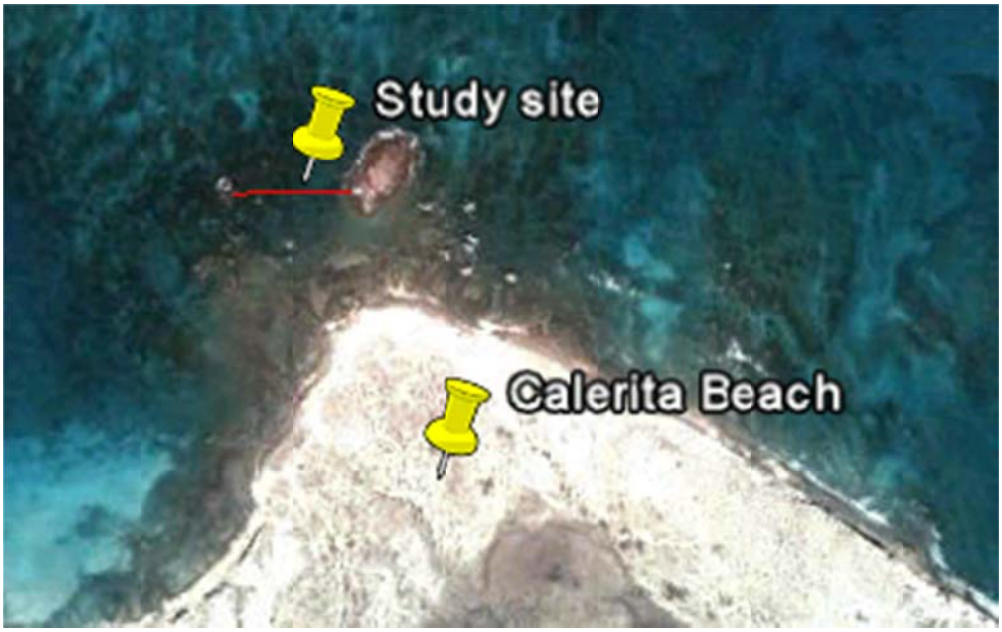


Figure 2

Figure 3

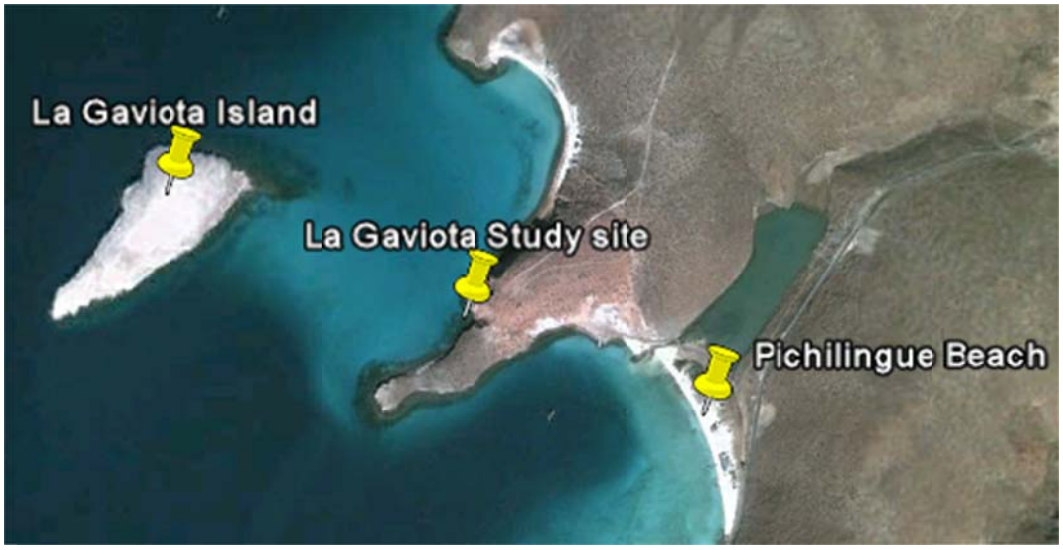


Figure 4

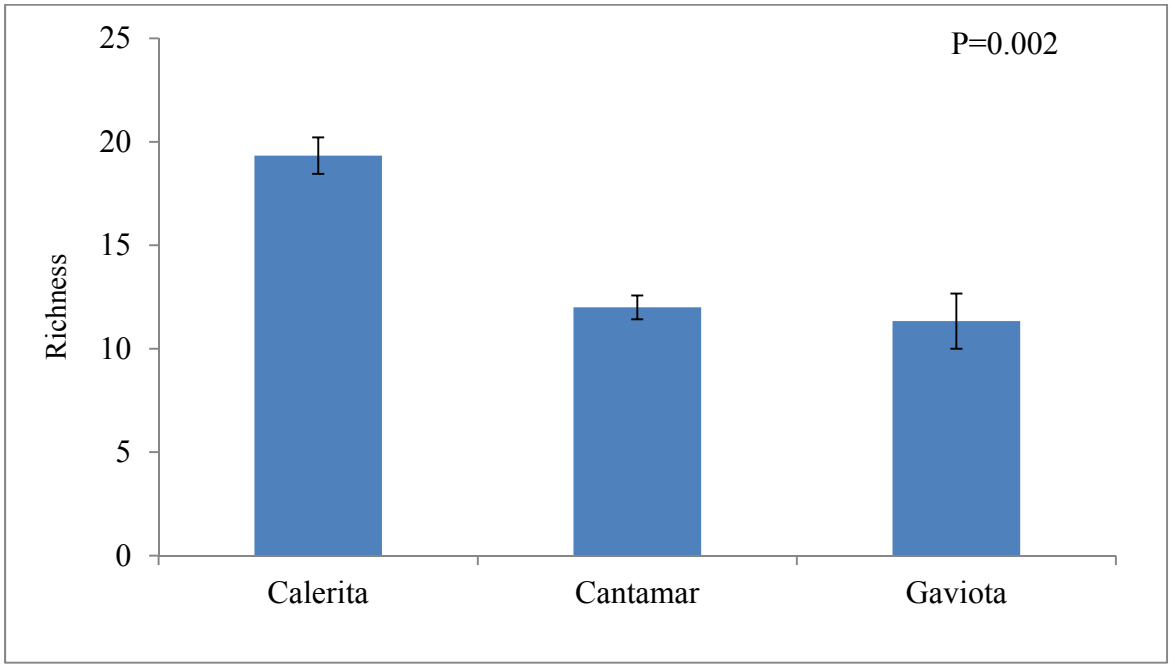


Figure 5

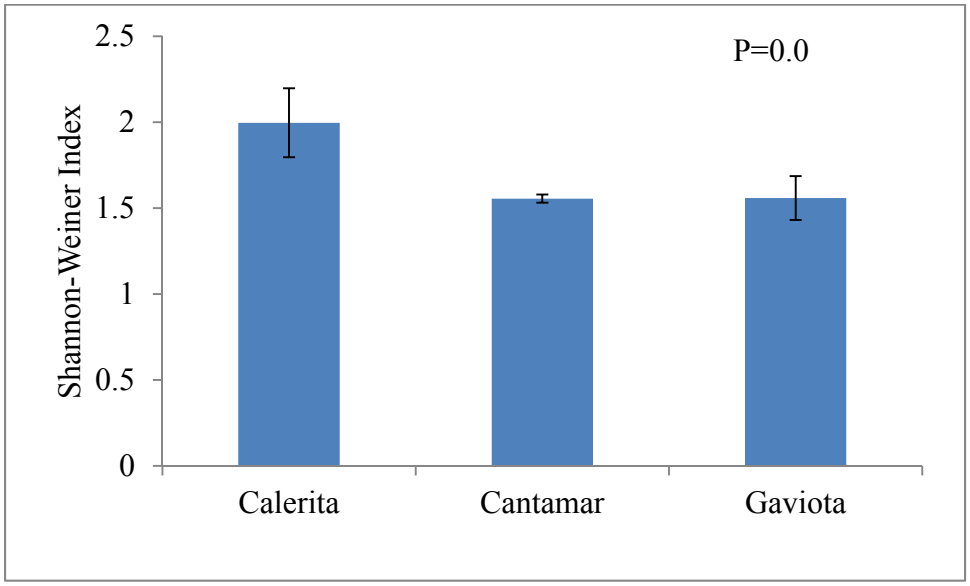

2.5
2
1.5
1
0.5
0
Shannon-Weiner Index
P=0.0
Calerita
Cantamar
Gaviota

Figure 6

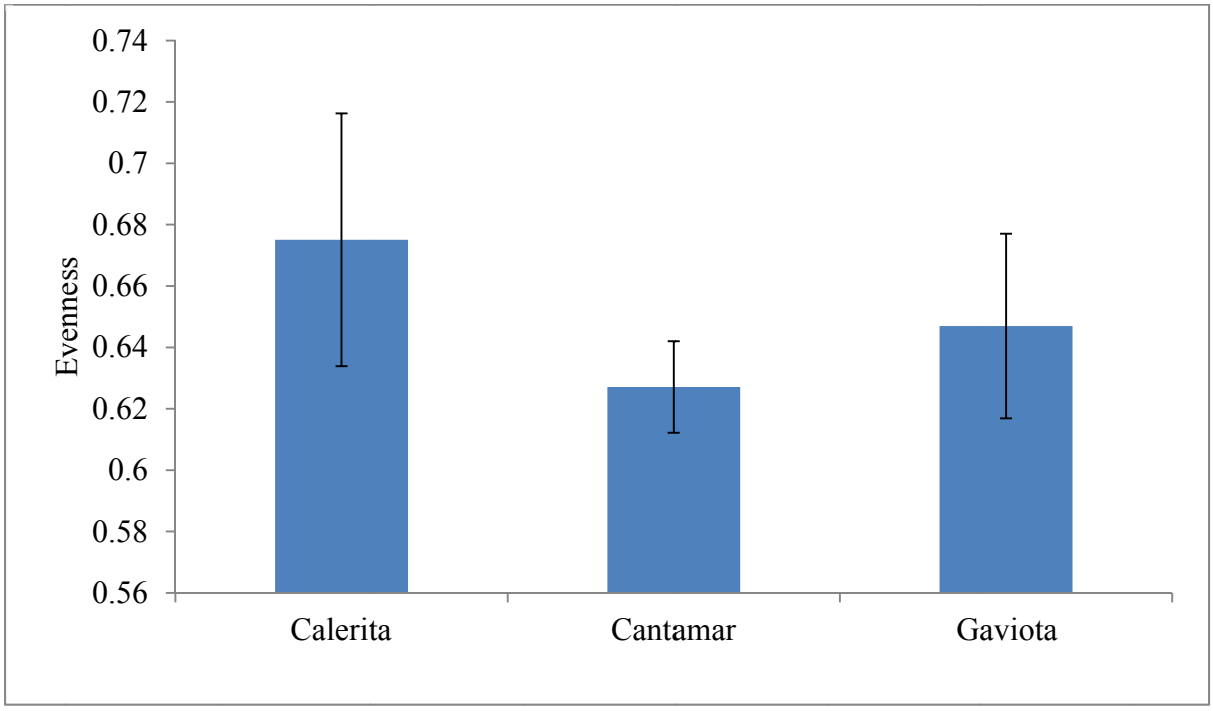

0.74
0.72
0.7
0.68
0.66
0.64
0.62
0.6
0.58
0.56
Evenness
Calerita
Cantamar
Gaviota

Figure 7

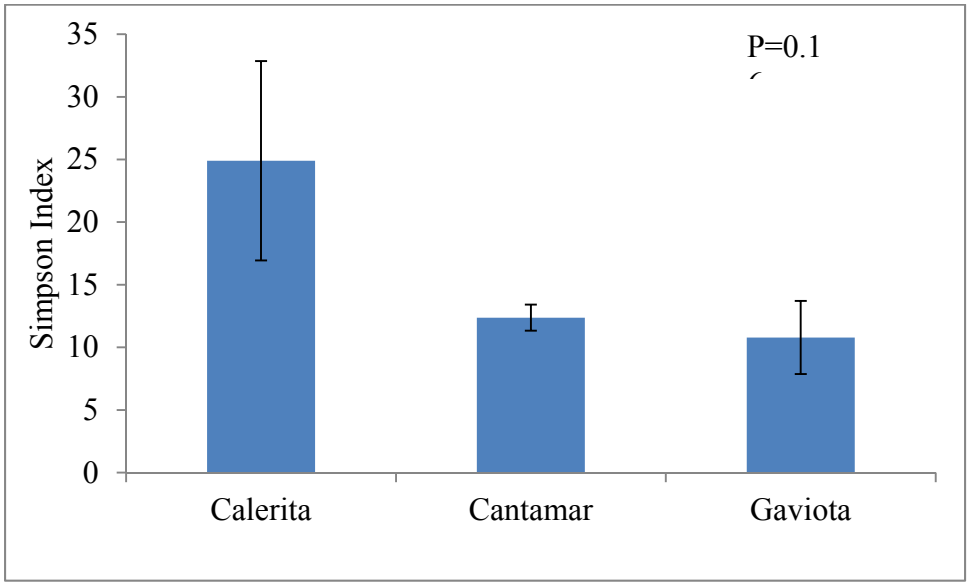
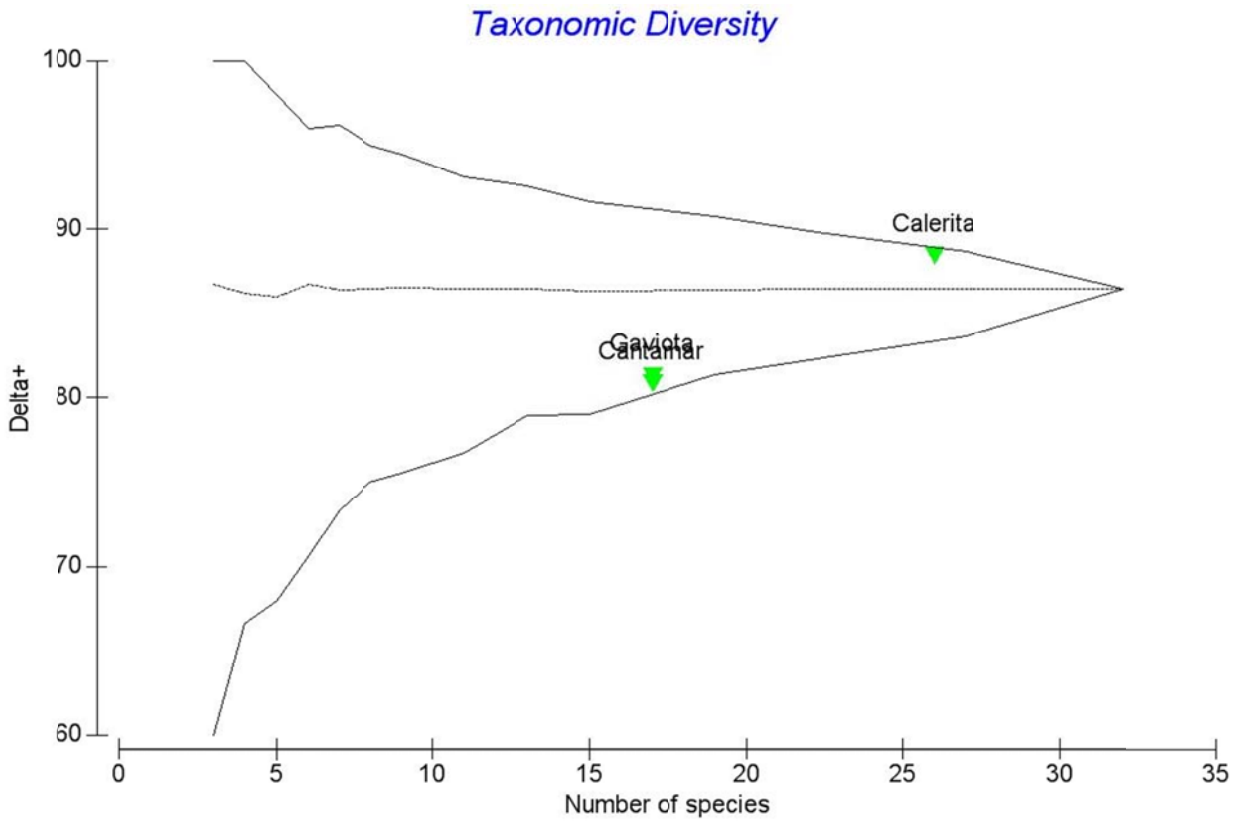

35
30
25
20
15
10
5
0
Simpson Index
P=0.1
Calerita
Cantamar
Gaviota

Figure 8


Taxonomic Diversity
100
90
80
70
60
Delta+
Calerita
Gaviota
Cantamar
0
5
10
15
20
25
30
35
Number of species

Community diversity changes within three different microhabitats throughout the day

William Barany[2], Nathan Goldschmidt[2], Rebeca Melendez-Rosas[1] and Erin Stehr[2]

Universidad Autonoma de Baja California Sur[1]
Canetera al Sur Km, S.S., La Paz, Baja California Sur, Mexico
Western Washington University[2]
Bellingham, WA 98225, USA

Abstract:

The Gulf of California supports a diverse assemblage of marine organisms that form distinct communities within the three dominant subrata in the littoral zone; rocky, sandy, and coralline. Each of these microhabitats can occur in close proximity to one another but support strikingly different communities. These differences are primarily attributed to topographic complexity and the availability of niches. The sandy substratum is unstable and offers few areas for residence or predation avoidance for epifaunal organisms. Conversely, the rocky substratum is stable and topographically complex, offering many cracks and crevices for organisms to colonize. Coral-covered rocks offer an intermediate habitat. The dynamic coral and its commensals actively defend this substratum from epifaunal invertebrate colonization.

The purpose of this study was to determine how community diversity differs between these three microhabitats throughout the day. Snorkel surveys were performed in the morning, afternoon, and night for three consecutive days and species presence and abundance were tallied. With these data the Shannon Index scores were calculated and used as a metric of biodiversity to make comparisons between microhabitats and time of day. Within each microhabitat, biodiversity was highest during the morning.

Additionally, the rocky microhabitat had the highest biodiversity while the sandy microhabitat exhibited the lowest level of biodiversity, supporting our predictions.

Additionally, there was a noticeable increase in invertebrate abundance during the night. Sixteen *Dolabella auricularia,* nocturnally foraging mollusks, were collected and subjected to varying laboratory conditions to observe behavioral responses to light exposure and time of day. The nocturnal behavior of *D. auricularia* was dictated by the time of day rather than light exposure.

Keywords: Microhabitats; Species abundance, richness, and diversity; Shannon Index; *Dolabella auricularia*; epifauna; diurnal and nocturnal behavior

Introduction:

The extensive shoreline of the Gulf of California supports a diverse mix of Californian, Panamic, and Indopacific zoogeographical fauna, including some 271 species of reef fishes (Arreola-Robles & Elorduy-Garay 2002; Thomson et al. 2000). Though the majority of the intertidal zone of the Gulf is characterized by rocky substratum, the Gulf also contains both sandy beaches and coral-covered rock. Each of these habitat types can be found in close proximity to one another, and while physical and chemical parameters may be similar between them, the richness, abundance and biodiversity of the communities they support can be surprisingly different (Arreola-Robles & Elorduy-Garay 2002).

Species living in the littoral environment, over time, become adapted to different microhabitats because of the fundamentally different environmental niches each contain. Each substratum (Sandy, Rocky and Coral) offers different conditions for marine species.

Rocky, sandy, and coral substrata, even when occurring nearby one another, have different rates of sedimentation, light periods, wave energy exposure, and trophic resources (INVEMAR 1998; Thomson et al. *2000*).

In addition to the substratum, light availability is a primary factor in determining marine communities. The Gulf of California is a tropical sea with high light availability year round, resulting in huge amounts of primary production during the day. This creates abundances of biomass that can sustain diverse and multi-trophic communities. During the nighttime however, organisms must have a specific set of physiological and behavioral adaptations different from diurnal species to be able to detect predators as well as find food and mates. Most of the reef fish species found in the Gulf of California are diurnal, that is, only active during the day. This precludes them from actively utilizing the nighttime marine environment (Hobson 1975).

Sandy substratum presents fundamental challenges to resident species. Organisms who permanently reside here must be either highly camouflaged or able to burrow and dig into the sediment to avoid predation. Other factors affecting organisms in the sandy habitat are grain size, depth, available organic material, gas exchange by sediment and upwelling (Arango 1996; Corpes 1992).

Globally, coralline habitats show a great diversity and abundance of both fish and invertebrate species. These communities are dependent on light, which decreases with depth. The great diversity within coral habitats is attributed nutrient abundance and protection from predation. Importantly, the Gulf of California does not contain any true coral reef habitat north of Cabo Pulmo. The coral habitats found north of this are

classified as rocky reefs and have comparatively lower biodiversity rates due do differences in nutrient cycling and light availability (Fernández et al. 2007).

The most diverse microhabitat within the Gulf of California is the rocky shore. The highly stable substratum and complex topography provides expansive surface area to primary producers, as well as hiding places to avoid predation, especially for juvenile fish. Rocky shores are equally important to invertebrate species. The heterogeneity of this substratum is readily colonized by invertebrates that are able to hide in crevices and caves to avoid predation (Fernández et al. 2007; Menge & Branch 2001; Brusca 1980).

This study investigates how marine epifaunal communities vary within these three microhabitats throughout the day. We hypothesize that overall, each microhabitat will support a greater diversity and abundance of species during the day compared with the night. Additionally, we hypothesize that the rocky microhabitat will support a greater diversity of species than the other two microhabitats for the above mentioned reasons.

Methods:

Field survey:

The field sites were all located near Cantamar beach on the Pichilingue peninsula of Baja Califonia Sur, Mexico. We placed one ten meter transect in each of the three microhabitats which were defined by their underlying substratum; sandy, rocky and coral. These transects were anchored for the duration of the study. The locations for surveying were chosen because all three microhabitats were proximal to each other and at a similar depth to limit confounding variables.

We snorkeled along each transect three times a day; 08:30 (n=8), 14:30 (n=11) and 21:30 (n=7) for three consecutive days. We placed a 1 m^2 quadrant at the beginning of each transect to aid in the estimation of the observation area. We observed epifauna within 1 m of the transect and recorded species presence and abundance on underwater slates. We used the mean of these observations for analysis. Unknown, or unrecognized, species were photographed for identification later. Species were identified using photographic guides (Thompson et al, 2000; Brusca, 1980; Gotshall, 1998; Kerstitch and Bertsch, 2007; Humann and Deloach, 2004).

We tabulated all of the observational data from each survey and calculated several different metrics of biodiversity; species richness, species evenness, and the Shannon Index. To make comparisons and determine trends between the biodiversity of each microhabitat, we used the Shannon Index score due to its sensitivity to both species richness and evenness. We performed an analysis of variance (ANOVA) using both microhabitat type and time of day as interacting factors predicting the response variable of the Shannon Index score.

Laboratory exposure:

Several of the species observed were seen exclusively during the 20:30 sampling time; these species were predominantly invertebrates found in the rocky habitat. We chose the nocturnal forager, *Dolabella auricularia,* as a representative of these species in order to observe behavioral responses to light exposure and time of day. We set up eight microcosms at the Universidad Autonoma de Baja California Sur (U.A.B.C.S.) research station on the Pichilingue Peninsula by filling 1000 L tanks with unfiltered seawater and aerating them to ensure an adequate oxygen supply. We then collected small to medium

sized rocks from the intertidal zone of Cantamar beach and placed them in each tank to simulate the crevices *D. auricularia* inhabits during the day. We then collected sixteen individuals from rocky habitats near our study site and transported them to the U.A.B.C.S. research station. We placed two individuals in each tank and allowed them to acclimate to laboratory conditions for approximately 12 hours.

We then randomly selected four tanks to cover up; excluding light in order to mimic nighttime conditions. After three hours we uncovered the tanks and recorded the number of individuals that were visible in the tanks. We then switched the covers to the other four tanks. Beyond intital observations, we repeated this process and recorded observations four times throughout the day; 14:00, 17:00, 20:00 and 23:00.

To determine if *D. auricularia* responded to light exposure or time of day, or both, we performed ANOVA's on both predictor variables independently and with interaction.

Results:

Analysis of field surveys:

Species richness was determined by counting the number of individual species present in each microhabitat. We also calculated the Shannon Index of biodiversity for each survey to gain a more holistic view of epifaunal community composition within each microhabitat. We calculated the species evenness from each survey using the ratio of the observed Shannon Index to the Ideal Shannon score. Each microhabitat had the highest Shannon Index score during the morning (Table 1). Additionally, the rocky

microhabitat had the highest mean species richness, diversity, and Shannon Index score throughout the day compared to the sandy and coral microhabitats (Table 1).

Because each microhabitat offered distinct substrata, and surveys were completed at three distinct times of day, both of these predictor variables were used to test the response of community biodiversity, represented by the Shannon Index score. We ran an ANOVA with the interacting factors of microhabitat substratum and time of day and found a significant difference in Shannon Index score between the three microhabitats (Table 2, $p=0.04$). Both of these factors together explained the difference in biodiversity between the three microhabitats.

Analysis of laboratory exposure:

We ran an ANOVA with interaction between the predictor variables of time of day and light exposure to determine if there was a significant effect on *D. auricularia* behavior. We found no significant effect on behavior from these interacting factors, nor light exposure on its own (Table 3, $p=0.78$). However, time of day had a highly significant effect on *D. auricularia* behavior (Table 3, $p<0.001$).

Discussion:

The primary difference between the three microhabitats was the composition of the substrata. Substratum composition is linked to marine species abundance and diversity. The underlying principle behind this link is that different substrata offer

varying levels of primary productivity, niche abundance, shelter and protection from predation (Brusca, 1980).

Our rocky microhabitat was topographically complex. There were cracks and crevices that organisms could use as shelter from predators and sites for residence and reproduction. The substratum also provided a diverse array of niches that fish and invertebrates were able to establish as their territory.

Rocky microhabitats have high levels of primary productivity, which encourages an influx of primary consumers to feed (Hannan et al. 1982). Secondary consumers then feed on the primary consumers and in this fashion the microhabitat can support a multi-trophic community. Our prediction that the rocky habitat would support the greatest diversity of epifauna during all times of day was supported by our data. The rocky microhabitat contained the greatest species richness (~12) among all surveys when compared to the coral microhabitat (6) and sandy microhabitat (4). These data are in agreement with prior research that show rocky reefs support a great variety of marine life, commonly including hundreds of species of fish, invertebrates and algae. Complex assemblages of mobile and sessile invertebrates enhance this diversity (Hannan et al. 1982).

Our coral transect exhibited very low numbers of epifaunal invertebrates. The entire transect was dominated by the elegant coral (*Pocillopora elegans*) that served as the substratum. *Pocillopora elegans* chemically discourages invertebrates from colonizing on or near coral structures through the use of allelochemicals (Jackson *et al.* 1975). In addition, coral is a dynamic living organism. Invertebrates that require a stable,

solid substratum to attach to cannot bind to the coral. Since invertebrates, other than the coral itself, were virtually eliminated from our transect we saw correspondingly lower numbers of total epifaunal individuals and lower levels of biodiversity. In addition, the coral offered little refuge space for larger fish so most of those observed were passing through in schools (primarily *Abudefduf troschelii*), resting near the coral or feeding on the zooxanthellae (primarily *Stegastes rectifraenum*).

The sandy microhabitat had, as predicted, the lowest levels of species diversity. Invertebrates that require a rigid substratum are unable to reside in sandy habitats since they are dynamic as a result of wind and wave action. In addition, sandy habitats have lower levels of primary productivity when compared to rocky and coral habitats (Brusca 1980). As a result, there is little food available to encourage the presence of secondary consumers and their predators.

When we compared diversity between the daytime and nighttime, we found significantly lower levels of diversity during the night (Figure 1). The primary reason for this reduction is that most of the fish species observed in the Gulf of California are diurnal. This means that they are active during the day and inactive during the night. This behavioral category includes the taxonomic groups of Labridae (wrasses), Balistidae (triggerfishes), Chaetodontidae (butterflyfishes) and Pomancentridae (damselfishes) (Hobson 1975). When we swam the transects at night we did not see these individuals because they were hidden in their nocturnal refuges.

In contrast, during the nighttime we found a higher abundance and diversity of epifaunal invertebrates. This is because many of the invertebrates that occupy our study

region are nocturnal, that is active during the night. This includes the purple sea urchin (*Echinometra vanbrunti*), the slate pencil urchin (*Eucidaris thouarsii*) and the blunt end sea hare (*Dolabella auricularia*) (Brusca 1980). For example, within the coral microhabitat we observed an eleven-fold increase in *E. thouarsii* abundance during the night compared with the day.

To better understand why some invertebrates become active during the night, we performed a behavioral experiment on *D. auricularia*. The results of our experiment indicate that *D. auricularia* becomes active during nighttime hours regardless of the presence or absence of light (Table 3, Figure 2).

This behavior may represent a circadian response by *D. auricularia* as other invertebrates have demonstrated similar responses (Sokolove et al. 1977). More research must be done in order to accurately determine the mechanism that causes these organisms to become active. We do know that *D. auricularia, E. vanbrunti* and *E. thouarsii* are all nocturnal grazers (Brusca 1980). Importantly, the increase in diversity of invertebrates at night was not enough to counteract the dramatic decrease in fish diversity. As a result of this, Shannon Index scores within all microhabitats were lower at night than during the day (Fig., 1).

The results of our experiments can be applied to regions where the natural coastal ecosystems have been destroyed or disturbed as a result of human activity. We found that manmade reefs like the one that we surveyed at Cantamar beach can support high levels of epifaunal diversity. If a natural coastal habitat, such as a mangrove habitat, is destroyed in the wake of development, it is encouraging to find that there are measures

that can be taken that can reestablish at least some levels of diversity to the subtidal zone. This can be as straightforward as spreading boulders along the sea floor.

Acknowledgement:

We thank each of our professors for providing logistical support as well as technical guidance. Specifically, A. Acevedo-Gutierrez for helping us through early trials and tribulations in topic selection and experimental design as well as collection of the hares. B. Miner for providing statistical guidance. S. Flores-Ramirez for providing constructive criticism, and J. Robles for transportation expertise.

References:

Arango, C. P., and Solano, O. D. 1996. A community analysis of soft bottom megafauna (Crustacea, Molusca) from the Southwestern region of Santa Marta, Colombian Caribbean. Bulletin of Marine and Coastal Research. **28:**125

Arreola-Robles, J. L., and Elorduy-Garay, J. F. (2002). Reef fish diversity in the region of La Paz, Baja California Sur, Mexico. *Bulletin of Marine Science,* **70:** 1–18.

Brusca, R. C. 1980. Common intertidal invertebrates of the Gulf of California. 2nd edition. University of Arizona Press, Tucson.

Corpes (Consejo Regional de Planificacion de la costa Atlantica). 1992. El Caribe colombiano. Realidad Ambiental y Desarrollo. Cons. Reg. Planif. Costa Atlánt., Santa Marta.

Derby, C. D. 2007. Escape by inking and secreting: marine molluscs avoid predators through a rich array of chemicals and mechanisms. The Biological Bulletin **213:** 274-289.

Fernández, J., Arévalo, J., Delgado, J., and Otto, O. 2007. Canarias. Ecología, medio ambiente y desarrollo. Ed. Centro de la cultura popular canaria.

Gotshall, D. W. 1998 Sea of Cortez marine animals a guide to common fish and invertebrates Baja California to Panama. Sea Challengers, Monterey.

Hannan, J., and Smith, A. 1998. Rocky reef habitats. Fisheries NSW. Sydney. **3:**19-24.

Hobson, E. 1975. Feeding patterns among tropical reef fishes. American Scientist. **63:** 381-392

Humann, P., and Deloach, N. 2004. Reef fish identification Baja to Panama. New World Publications, Jacksonville.

INVEMAR (Instituto de Investigaciones Marinas y Costeras). 1998. Informe del estado de los ambientes marinos y costeros. Ambiental marine system, programa SINAM. Colombia, Santa Marta.

Jackson, J. B. C., and Buss, L. 1975. Allelopathy and spatial competition among coral reef invertebrates. Proceedings from the National Academy of Science. **72:** 5160-5163

Kerstitch, A., and Bertsch, H. 2007. Sea of Cortez marine invertebrates a guide for the Pacific Coast, Mexico to Peru. Sea Challengers, Monterey.

Menge, A. B. 1995. Indirect effects in marine rocky intertidal interaction webs: patterns and importance. Ecological Monographs **65:** 21-74

Menge**,** B., and G. M. Branch. 2001. Rocky Intertidal Communities. *In* M. D. Bertness, S. D. Gaines, and M. E. Hay (eds.), Marine community ecology*,* pp. 221–251. Sinauer Associates, Sunderland.

Sokolove, P.G., Beiswanger, C.M., Prior, D.J., and A. Gelperin. 1977. A circadian rhythm in the locomotor behaviour of the giant garden slug *Limax maximus*. Journal of Experimental Biology **66:** 47-64.

Thomson, D. A., Findley, L. T., and Kerstitch, A. N. 2000. Reef fishes sof the Sea of Cortez. The University of Texas Press, Austin.

Tables:

Table 1. Mean metrics of biodiversity during the morning, afternoon, and night within three different microhabitats (n=3).

Microhabitat	Metric	Morning	Afternoon	Night	Mean
Sandy	Richness	6.50	5.00	0.50	4.00
	Evenness	0.85	0.73	0.00	0.53
	Shannon index	1.60	1.19	0.00	0.93
Rocky	Richness	11.00	13.00	11.50	11.83
	Evenness	0.79	0.71	0.56	0.69
	Shannon index	1.90	1.80	1.36	1.69
Coral	Richness	7.50	6.67	4.00	6.06
	Evenness	0.65	0.69	0.70	0.68
	Shannon index	1.32	1.24	0.97	1.18

Table 2. ANOVA table for the interaction between the two predictor variables of microhabitat and time-of-day on the response variable of Shannon Index score.

	Df	Sum Sq.	Mean Sq.	F value	P value
Microhabitat	2	2.03	1.02	14.26	<0.001
Time	2	2.3	1.15	16.17	<0.001
Microhabitat:Time	4	0.98	0.25	3.4	0.043
Residuals	12	0.85	0.07		

Table 3. ANOVA table for the interaction between the two predictor variables of time of day and light exposure as well as their individual effects on *D. auricularia* behavior.

	Df	Sum Sq.	Mean Sq.	F value	P value
Time	1	9.03	9.03	22.73	<0.001
Light	1	0.03	0.03	0.08	0.78
Time: Light	1	0.03	0.03	0.08	0.78
Residuals	28	11.13	0.40		

Figure legends:

Figure 1. Shannon Index scores at three different times of the day within three different microhabitats. An ANOVA model with an interaction between the predictor variables of microhabitat and time detected a significant difference in Shannon Index scores ($p=0.04$).

Figure 2. The behavioral response of *Dolabella auricularia* to the time of day (Day<8PM<Night). Number out is defined as the mean number of organisms outside of the rocky hiding place during behavioral observations ($n=16$, $p<0.001$).

Figure 1.

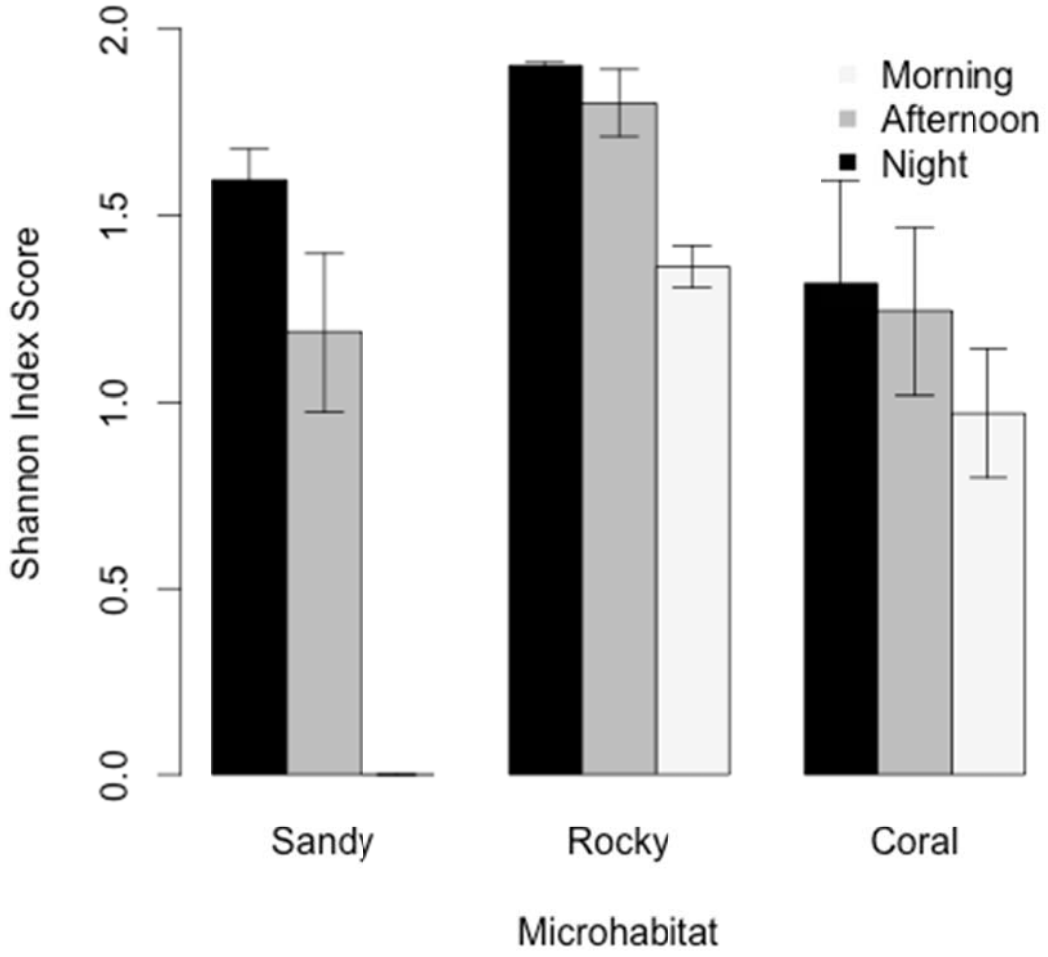
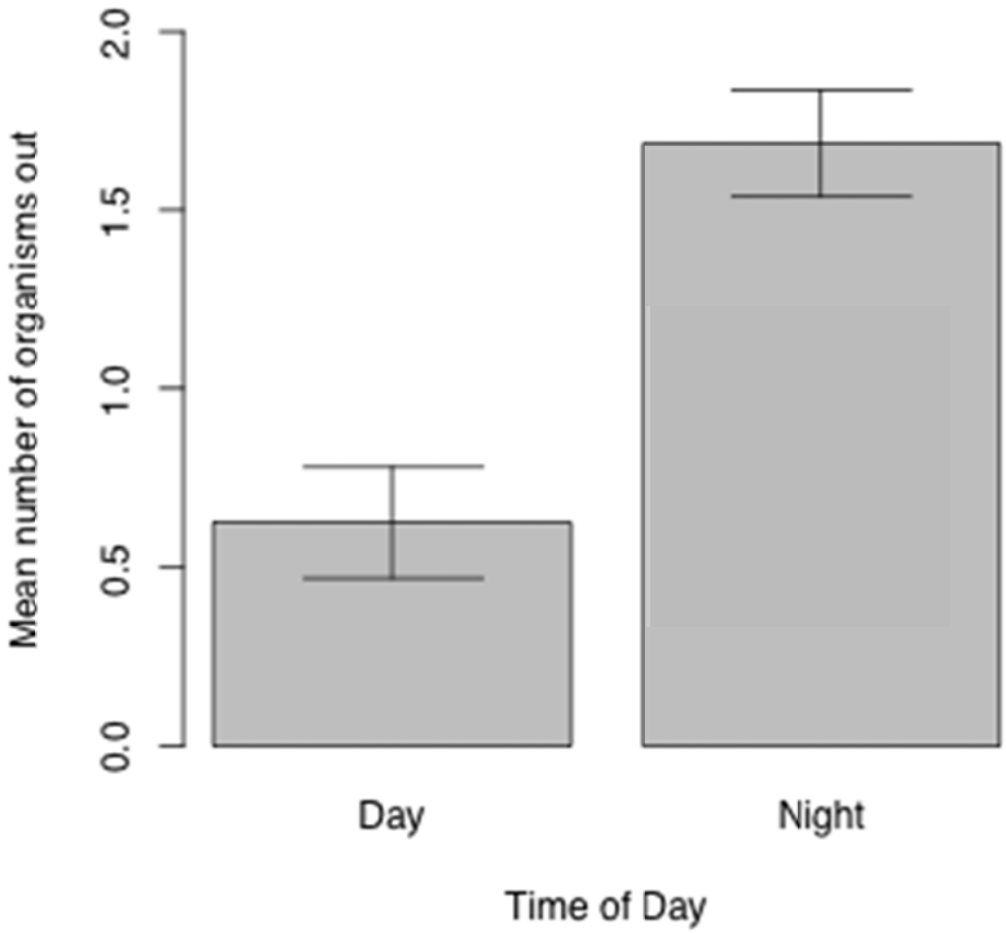

Shannon Index Score
0.0
0.5
1.0
1.5
2.0
Morning
Afternoon
Night
Sandy
Rocky
Coral
Microhabitat

Figure 2.


Mean number of organisms out
0.0
0.5
1.0
1.5
2.0
Day
Night
Time of Day