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Effect of polyvinyl chloride on settled community biodiversity and invasive species: Analyzing diversity differences in La Paz Bay

Key words: Anthropogenic disturbance, pollution, non-indigenous species, biological invasions, settlement

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Effect of polyvinyl chloride on settled community biodiversity and invasive species

Jaclyn Stapleton and Laura Anthony

Abstract

As anthropogenic disturbance increases in the marine environment such as plastic and metal pollution, many habitats are facing new problems. One of the issues with little research is the settlement of species on pollution and outcompeting other species. This can decrease biodiversity of areas with excessive amounts of pollution, as well as provide an opportunity for non-indigenous species to spread to new areas due to their high tolerance of harsher substratum. These high tolerance species that can settle and grow more easily on plastics that leach toxic chemicals as they biodegrade and inhibit settlement. Our study analyzed if the biodiversity and proportion of invasive species on plastic differed from natural substrata. We focused on Cantamar beach in the Gulf of California where there are several polyvinyl chloride (PVC) pipes in the water. The settled macroalgae, macro-invertebrate, and surrounding fish populations were analyzed along these pipes and compared to nearby natural substrata of the same depth. Time for settlement was also accounted for by placing paired PVC pipes and scrubbed rocks around the area below the tidal line for six days. These replicates were removed and species were counted. The difference in biodiversity on the PVC and natural substrata for both the pre-settled and the experimental were not found to be significant. However, *Acanthophora spicifera,* an invasive red algae in the Gulf of California, was found on the PVC pipes. The proportion of this species was found to be significantly greater on PVC than natural substrata. We conclude that the lack of significant difference could be due to a difference in the time of settlement for each substratum. Our results show that plastic pollution is a viable reason for the growth of invasive species.

Resumen

A medida que aumenta la perturbación antropogénica en el entorno marino, como la contaminación de plástico y metales, muchos hábitats se enfrentan a nuevos problemas. Uno de los temas que tiene poca investigación es el asentamiento de especies en la contaminación y la competencia con otras especies. Esto puede disminuir la biodiversidad de áreas con cantidades excesivas de contaminación, y también brinda una oportunidad para que las especies no indígenas se propaguen a nuevas áreas debido a su alta tolerancia al sustrato más duro. Estas especies de alta tolerancia pueden asentarse y crecer más fácilmente en plásticos que lixivian químicos tóxicos a medida que se biodegradan e inhiben la sedimentación. Nuestro estudio analizó si la biodiversidad y la proporción de especies invasoras asentadas en el plástico difieren de los sustratos naturales. Nos enfocamos en la playa de Cantamar, en el Golfo de California, donde hay varias tuberías de cloruro de polivinilo (PVC) en el agua. Se analizaron las macroalgas, macroinvertebrados establecidos y las poblaciones de peces circundantes a lo largo de estas tuberías, y se compararon con los substratos naturales cercanos de la misma profundidad. También se tuvo en cuenta el tiempo de asentamiento colocando tubos de PVC emparejados y rocas lavadas alrededor del área debajo de la línea de marea durante seis días. Estas réplicas se eliminaron y las especies se contaron. La diferencia en la biodiversidad en el PVC y los sustratos naturales tanto para el pre-establecido como para el experimental no fue significativa. Sin embargo, se encontró *Acanthophora spicifera*, una alga roja invasora en el Golfo de California, en las tuberías de PVC, y se encontró que la proporción de esta especie era significativamente mayor en PVC que en los sustratos naturales. Concluimos que la falta de diferencia significativa podría deberse a una diferencia en la cantidad de tiempo de para cada sustrato. Nuestros resultados muestran que la contaminación plástica es una razón viable para el crecimiento de especies invasoras.

Introduction

The marine environment has experienced a significant amount of anthropogenic disturbance, including pollution (Occhipinti-Ambrogi 2006). Pollution has increased in the marine environment due to an increase in the manufacturing of materials such as plastics and metals (Barnes 2002), which can cause a multitude of problems. While issues such as entanglement and ingestion have received much attention (Young et al. 2009, Jambeck et al. 2015), problems such as the settlement of algae and invertebrates on pollution has been less publicized. Many types of pollution cover natural substratum and can prohibit species from settling on their natural habitat (Piola & Johnston 2008). Several also leach various chemicals as they biodegrade that both harm species and prevent their settlement (Li et al. 2015). Because pollution is harsh substrata, settlement of more competitive species, such as invasive species, becomes more prominent on many pollution types that are in the ocean (Piola & Johnston 2008). Thus, the diversity of the region is lowered. Non-indigenous species are transported to new areas via pollution because they are able to tolerate harsher conditions and can then become invasive (Barnes 2002, Occhipinti-Ambrogi 2006). In addition, previously non-indigenous species can become invasive as pollution increases as they outcompete native species (Piola & Johnston 2008).

The transport of species can be from both the fouling of ship hulls and ballast water, as well as the movement of marine debris. Ship hulls are often covered in anti-fouling paint, so any species able to tolerate these chemicals are often able to outcompete native species (Piola & Johnston 2008). Similarly, drift debris can move species that are able to tolerate different types of pollution to new areas where they can again become invasive (Barnes 2002). Invasive species

are an issue because they can take up nutrients and exclude native species, thus changing an entire ecosystem (Ávila et al. 2012).

Plastics both provide a unique substrate and release chemicals as they degrade (Fotopoulou & Karapanagioti 2015, Li et al. 2015). Polyvinyl chloride (PVC) ranges from soft to hard, and was found to have a rough and uneven surface with ER, allowing for easier settlement (Li et al. 2015). In addition, as plastics such as PVC degrades, new functional groups are exposed on the surface and interact with chemical pollutants, which can then be released in pH changes such as in the stomach of organisms (Fotopoulou & Karapanagioti 2015). This breakdown can be prevented by biofouling as it shields plastics from ultraviolet radiation, however toxic leachates in the plastic can prevent settlement of certain species (Li et al. 2015). For example, PVC was found to be the most toxic out of five different studied plastics to settling barnacle nauplii, likely because many of the chemicals are broad-spectrum biocides (Li et al. 2015). Overall, new plastics were found to be hazardous to the settlement of marine organisms (Li et al. 2015).

Due to the harmful components of plastics, it is likely that many species will not be able to settle on such plastics so other more competitive species can settle. This can both decrease biodiversity of the area and lead to the invasion of nonnative species (Barnes 2002). Biological diversity is vital for healthy ecosystems because it increases the likelihood of maintaining functional groups during disturbances such as natural disasters (Elmqvist et al. 2003). Areas more prone to invasion typically have high boat traffic where ballast water dumps non-indigenous (Piola & Johnston 2008). In addition, more stagnant waters such as shallow water and tidal pools are more likely to be harmed by the leaching of chemicals from plastics (Li et al. 2015), which might allow for invasive species to outcompete native species more easily. The

Gulf of California has both boat traffic and is more enclosed than the coast, and has thus experienced several outbreaks of invasive species (Aguilar-Rosas 2013). Many algae species have become invasive such as *Acanthophora spicifera,* a red algae that was introduced into the Gulf of California in 2006 (Ávila et al. 2015). *A. spicifera* has negatively impacted La Paz Bay, Baja California Sur, Mexico. This red alga is a problem to native species due to its high morphological plasticity, reproductive strategies, and its adaptability to a variety of water conditions (Ávila et al. 2012). With its ability to grow and reproduce in a multitude of environments, its main impact on native species is its use of space. *A. spicifera* has a large biomass which outcompetes and smothers native algae and invertebrates (Ávila et al. 2012). Other species such as *Sargussum muticum, Sargassum filicinum, Grateloupia turuturu,* and *Grateloupia lanceolata* are non-indigenous species of algae that have been seen in the Gulf of California (Aguilar-Rosas et al. 2013).

Because pollution has been seen to pose such a harsh environment for species, we wanted to know if biodiversity is lower on pollution such as polyvinyl chloride than on natural substrata. We focused on Cantamar beach in La Paz Bay, Baja California, Sur because we located pre-settled PVC pipes in the water. We hypothesize that biodiversity of macro-invertebrates and macroalgae will be lower on the PVC pipes that are in Cantamar beach than on the nearby natural rocks of the same depth. Due to the possibility of organisms to easily outcompete others on pollution, we also analyzed if there was a difference in the proportion of non-indigenous species growing on PVC is higher than on natural substratum. To account for the possible time difference of pre-settled substratum at Cantamar beach, PVC and scrubbed rocks were also placed around the area to compare settlement over time.

Methods

Our study consisted of an observational field study as well as an experimental field study conducted in the Gulf of California at Cantamar beach. We chose this site because it had PVC pipes with unknown settlement in the environment as well as rocky substratum nearby. Also, Cantamar beach was one of the most accessible locations available. Our goal was to determine the biodiversity and the extent of invasive species that grew on PVC pipe compared to natural substratum by looking at both pre-settled PVC pipe and rocks as well as monitoring the settlement of algae and invertebrates on newly placed PVC pipe and rocks.

We performed a transect along two of the PVC pipes already at Cantamar beach and performed one meter species counts every ten meters along the pipes. These seven areas were paired with the nearby rocks at the same depths of the same 1000cm^2. Pictures were taken along the transect sections as well as the rocks and species were identified and counted. Fishes within half a meter of the pipe were also accounted for in the species counts.

Fifteen replicate PVC pipes and scrubbed rocks were also placed around the area below the low-tide line to control for time of settlement. The pipes were 15cm long with a 10cm diameter, and the rocks were found that were approximately 15cm by 10cm to pair with each pipe. The pipes were filled with sand and capped with duct tape so as to sink, and each pair was labeled on the bottom with a number 1 through 15. The beach was split into a grid and a random number generator was used to pick the location where each pair of PVC pipe and rock was placed. The painted numbers were placed against the sand so as not to attract species. The pairs were checked for settlement every twenty-four hours and removed after five days to identify and count settled species of macroalgae and macro-invertebrates.

The biodiversity and proportion of invasive species were statistically analyzed using R-studio. The Shannon Diversity Index was calculated for each experimental sample and the mean Shannon Index on PVC was compared to the mean Shannon Index on natural substratum using a two-sided t-test. The Shannon Index was also calculated for invertebrates on the pre-settled PVC pipes and the pre-settled natural substratum and compared in the same way. The percent cover of macroalgae species was calculated on the pre-settled PVC and natural substratum and Simpson's Diversity Index was used to compare the biodiversity of algal species, again a two-sided t-test was used to compare the means. One algal species was identified as nonnative, and this species was used to find the proportion of invasive algae. The proportion of invasive species on pre-settled PVC was compared to the proportion of invasive species on natural substratum using a generalized linear model.

All pre-settled organisms were not disturbed during this experiment beyond taking pictures and setting down temporary PVC quadrats. The rocks that were a part of the experimental design were returned to the water after collected. In addition, any organisms settled on the experimental PVC pipes that could be removed were placed back in the intertidal zone before the PVC was disposed of. All species were identified using photo identification books (Brusca 1980, Humann 2004, Readdie 2006, Kerstitch 2007).

Results

Thirty-one invertebrate and algae species were identified across both subtrata. 93.5% of species were identified as native, 3.22% were identified as non-indigenous, and 3.22% as cryptogenic (Table 1). In this scenario, we defined cryptogenic as any species that was unable to

be identified such as tube worms that would not leave their shells or species that have not been recorded as native or nonnative.

No significant difference was found between the biodiversity of macroalgae and macro-invertebrates on the experimentally placed PVC pipe and rocks (Fig. 1a) ($t_{(2)16.588}$ = -0.55682, P-value = 0.5851). The mean Shannon Diversity Index for the PVC pipes was 0.0516 while the mean Index for the rocks was 0.0977. All species were identified as native species to the Gulf of California, thus no proportion of invasive species was calculated for the experimental treatments.

The hypothesis that the biodiversity of macro-invertebrates and surrounding fishes on pre-settled PVC pipe would be different than that of pre-settled natural substrata was rejected (Fig. 1b) ($t_{(2)11.859}$ = 0.206, P-value = 0.841). The mean Shannon Index of the PVC was 0.228 while the mean Shannon Index of the natural substrata was 0.270. All invertebrates and fishes were identified as indigenous species to the Gulf of California or were cryptogenic on the pre-settled pipe and natural substrata, thus no proportion of invasive species was calculated for the macro-invertebrates and fishes. There was also no difference in diversity between the macroalgae growing on the pre-settled PVC and natural substrata (Fig. 1c) ($t_{(2)11.285}$ = -0.391, P-value = 0.703). The mean Simpson's Diversity Index for the PVC was 0.598 while the mean Simpson's Diversity Index for the natural substrata was 0.643.

An invasive species of algae was found on the pre-settled PVC pipe, thus the proportion of invasive algae was calculated from the percent cover of algae on the pre-settled PVC and natural substratum and a significant difference was found (Fig. 1d) (z = 3.501, P-value = 0.00437). No invasive algae were found on the natural substrata but an average of 36.4% of algae cover was *Acanthophora spicifera,* an invasive species of algae in the Gulf of California.

Discussion

Overall, no differences in biodiversity were seen between either the pre-settled or experimental PVC and natural substrata were seen. This result could show that Cantamar beach is enclosed enough to result in harmful impacts on settlement from the leaching of chemicals as seen by Li et al. (2015) with the aversion of barnacle nauplii to settle on PVC. Thus, no species appears to be outcompeting others more on the PVC pipe than on the natural substrata. This is interesting given the analysis of the harmful effects of biodegrading plastics. However, PVC was found to be the least biodegradable than high-density polyethylene and polyethylene, two other common plastics (Fotopoulou and Karapanagioti 2015). Therefore, it is possible that PVC does not expose as many functional groups that collect harmful chemicals over time and does not leach out as many chemicals that can suppress settlement as other plastics (Li et al. 2015).

The non-indigenous species found on the PVC pipe, *A. spicifera,* is native to Florida and the Caribbean and has been a successful competitor in many habitats where it has been introduced. It was first seen in the Port of Pichilingue of the Gulf of California in 2006 (Ávila et al. 2012). It has been observed that *A. spicifera* can have negative effects on native algae when in high concentrations (Ávila et al. 2012). It is possible that the PVC provides a harsher substratum for native species (Li et al. 2015), and thus this non-indigenous species is able to grow in abundance on the harsher substrata. This species was not observed on the natural substrata within the randomized quadrats, but it has been observed in the area growing on corals. As we do not know how long the pre-settled PVC pipes have been at Cantamar beach, it is possible that *A. spicifera* has not yet had time to dominate the PVC pipes, but it is evident that the species is much more able to grow on PVC than on natural substrata. The experimental controls were not

given enough time to see any growth of macroalgae such as *A. spicifera*, but it is possible that given the time, we would see the invasive species growing on the PVC.

Our results are important for showing that the more plastic pollution such as PVC in the marine environment could easily result in the increase of nonnative species that have the potential become invasive and decrease the native biodiversity of marine environments. Other plastics could potentially be even more harmful to native species such as Fotopoulou and Karapanagioti (2015) found, so future studies could analyze the settlement of species on different types of plastics. In addition, plastic debris is slow moving and degrades slowly, providing an opportunity for a lot of settlement (Barnes 2002), so it would be interesting to look into effect of different velocities on the settlement of organisms on plastics. Other factors that can decrease the fitness of native species such as climate change also play a role in the spread of invasion (Occhipinti-Amrogi 2006), and should be taken into account along with pollution.

Our results show that the more plastic pollution in the marine environment, the more likely non-indigenous species are to grow and possibly become invasive. Though PVC may not be harmful enough to prohibit settlement in Cantamar beach, it is possible that different areas will experience different reactions to such plastics. To protect the marine environment from issues such as the spread of non-indigenous species, stricter regulations on the manufacturing of plastics should be created internationally.

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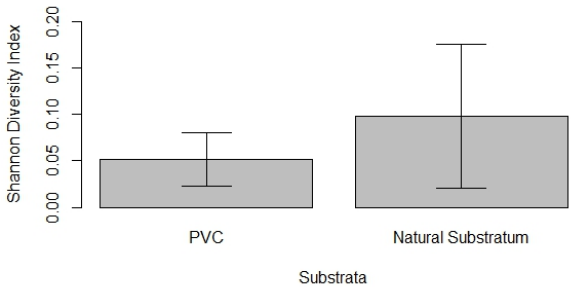
Figure 1. a. Mean Shannon Diversity Index of experimentally placed PVC pipe and scrubbed rock natural substratum. The PVC and rocks were of similar surface area. Fourteen replicates were placed randomly around Cantamar beach below the tidal line. The pairs were removed after five days of settlement and the species were identified and counted b. Mean Shannon Diversity Index of pre-settled macro-invertebrates from 1000cm^2 plots of PVC pipe and natural substrata. The PVC plots were measured using a transect and the natural substrata were measured with a quadrat of equal area. Measurements were taken every ten meters along two PVC pipes, resulting in seven replicates. These were paired with natural substrata at the same depths near the pipes. The percent cover of each algal species was used to find the diversity index c. Mean Simpson's Diversity Index of pre-settled macroalgae from 1000cm^2 plots of PCV pipe and natural substrata d. Proportion of non-indigenous algal species on the pre-settled PVC pipes and natural substrata. The percent cover of each algal species was measured and the proportions taken from this percentage.

Table 1. Species list of every settled macro-invertebrate and macroalgae and surrounding fishes with 0.5m of both the pre-settled and experimental PVC and natural substrata; each species was identified and listed as native (N), nonindigenous (NIS), or cryptogenic (C)

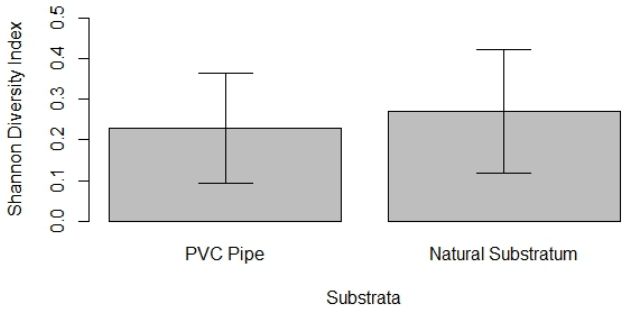
Location	Species	Status
Pre-settled PVC Pipe		
	Naticidae sp.	N
	Axoclinus carminalis	N
	Segastes rectifaenum	N
	Tetraclita stalactifera	N
	Phataria unifasciali	N
	Canthigaster punctatissma	N
	Columbellidae sp.	N
	Siboglinidae sp.	C
	Tetraclita stalactifera	N
	Cutleria hancockii	N
	Algas coralinas incrustante	N
	Ulva spp.	N
	Ralfsia spp	N
	Acanthophora spicifera	NIS
Pre-settled Natural Substratum		
	Tetraclita stalactifera	N
	Columbellidae sp.	N
	Siboglinidae sp.	C
	Segastes rectifaenum	N
	Bunodosoma californica	N
	Porites panamesis	N
	Algas coralinas incrustantes	N
	Dictyota spp.	N
	Amphiroa annulate	N
	Padina spp.	N
	Gelidium pusillum	N
	Pocillopora elegans	N
Experimental PVC Pipe		
	Tetraclita stalactifera	N
	Chthamalus anisopoma	N
	Gelidium decompositum/johnstonii	N
	Ophicoma alexandri	N

	Eurythoe complanata	N
	Phialoba steinbecki	N
	Diadora inaequalis	N
	Enchiridium punctatum	N
	Chiton virgulatus	N
Experimental Natural Substratum		
	Pleurobranchus areolatus	N
	Chiton virgulatus	N
	Chthamalus anisopoma	N
	Siboglinidae sp.	N
	Gelidium pusillum	N
	Phyllactis bradleyi	N

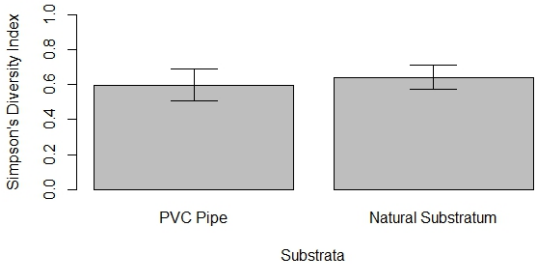
a.


Shannon Diversity Index
0.00
0.05
0.10
0.15
0.20
PVC
Natural Substratum
Substrata

b.


Shannon Diversity Index
0.0
0.1
0.2
0.3
0.4
0.5
PVC Pipe
Natural Substratum
Substrata

c.


Simpson's Diversity Index
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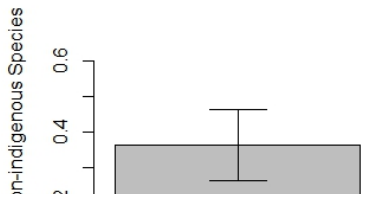
0.2
0.4
0.6
0.8
1.0
PVC Pipe
Natural Substratum
Substrata

d.


n-indigenous Species
0.4
0.6

The Effect of Nitrogen High Fertilizer on different locations of Mangrove Forests Bacterial Community Growth in Baja California Sur

Running head: Effect of Fertilizer on Mangrove Bacterial Growth

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The Effect of Nitrogen High Fertilizer on different locations of Mangrove Forests Bacterial Community Growth in Baja California Sur

Abstract

Mangrove forests are complex ecosystems that are economically and ecologically important. Many Mangroves are a nursery ground for fish, and a home to a variety of species. Part of Mangrove forests ability to thrive comes from the diverse bacterial community it contains. Many species of bacteria live in the soil and break down plant deposition. The bacteria recycle materials and create free nutrients. Changes in the bacterial community can be detrimental to the ecosystem due to their important roles. Bacteria might be changed by anthropogenic impacts on the ecosystem, such as developments, and agricultural or aquaculture runoff. However, some locations of Mangroves may be more resistant to manmade impacts because of genetic diversity between different locations. Bacteria species and concentrations vary from locations of mangroves around the world. Some species of bacteria may be more resistant to chemical changes in the environment. In this study we hypothesized that the bacterial communities collected near aquaculture will have no negative impact on growth rate with the introduction of fertilizer to the growing environment, and bacterial communities that have not previously had the influence of aquaculture runoff on the ecosystem will experience a negative impact on growth rate with the introduction of fertilizer. This was tested by culturing bacteria from three sites of mangroves, one site that has aquaculture runoff, and measuring time of first bacterial colonization. We found that there was significantly slower first colonization time for the fertilizer treatment with one site that was not previously under the influence of aquaculture runoff, but the other site did not have significant difference in growth time. Additionally, the site that had aquaculture runoff did not have a significant difference in growth time. This work helps

to convey the importance of the effect of aquaculture and agriculture on bacteria communities in Mangroves.

Resumen

Los manglares son ecosistemas complejos económica y ecológicamente importantes. Parte del éxito de los manglares es la diversidad bacteriana que poseen. Las bacterias viven en el sedimento y descomponen las deposiciones de los mangles. Reciclando y volviendo biodisponibles los nutrientes. Las comunidades bacterianas pueden ser alteradas por causas antropogénicas, como los desarrollos urbanos, y los desechos agrícolas o acuícolas. Sin embargo, algunos manglares pueden ser más resistentes a los impactos causados por el hombre debido a la diversidad genética entre las distintas locaciones. Algunas especies de bacterias pueden ser más resistentes a los cambios químicos en el medio ambiente. En este estudio planteamos la hipótesis de que las comunidades bacterianas recolectadas cerca de una granja de camarones no se verán afectadas negativamente respecto a la tasa de crecimiento con la introducción de un fertilizante rico en N en el medio de cultivo, mientras que las comunidades bacterianas que anteriormente no tuvieron la influencia de los desechos de la acuicultura experimentarán un impacto negativo en la tasa de crecimiento con la introducción del fertilizante. Esto se probó cultivando bacterias del sustrato de tres sitios diferentes de manglares, un sitio con desechos de acuicultura y dos sitios lejos de granjas acuícolas, mientras se midió el tiempo de la primera aparición de colonias. Descubrimos el primer tiempo de colonización de uno de los sitios alejados de la granja fue significativamente más lento para el tratamiento del fertilizante, sin embargo el otro sitio no tuvo una diferencia significativa en el tiempo de crecimiento aun estando lejos de la granja. El sitio expuesto a los desechos acuícolas no tuvo una diferencia significativa en el tiempo de crecimiento. Este trabajo ayuda a difundir la

importancia del efecto de la acuicultura y la agricultura en las comunidades de bacterias en los manglares.

Keywords

Microbiology, Aquaculture, Agriculture, Pollution, Nutrient variability

Introduction

Mangrove forests are self-sustaining systems that offer vital services to the ecosystem. These forests are located in tropical and subtropical regions, and have environments that vary greatly due to tidal influx and changing seasons (Behera et al. 2014). There are 60 species of Mangrove trees that have been discovered, eight of which live in the Americas (Houlgin 2000). They are among one of the most carbon fixing ecosystems, and with increasing carbon being released into the atmosphere Mangrove forests can help diminish anthropogenic effects globally. Mangrove forests also offer nurseries to many species of fish and invertebrates. Additionally, they slow down costal erosion, and can diminish the damaging impacts of hurricanes and tsunamis. The ecosystems are economically valuable, as they offer a site for many fisheries, the production of crabs and shrimp for human consumption are common in these forests globally (Anneboina & Kumar 2017). On average the forests are worth 37,500 USD per hectare. In Mexico specifically, fish and Blue Crab that come from Mangrove forests are worth 19 million USD per year (Dalton, 2008). Mexico contains 3.7% of the worlds Mangroves, which is about 770,000 hectares (Adame et al. 2018).

Currently Mangrove forests are considered to be under threat of endangerment and extinction worldwide due to manmade interactions such as costal development, agricultural development, aquaculture development, and aquaculture or agricultural runoff. Mangroves in

Brazil have been greatly reduced by human waste runoff, which causes in imbalance in bacterial communities. The introduction of human waste causes an increase in pathogenic bacteria which can affect the productivity of fisheries due to health hazards (Grisi et al. 2010). In the past three decades 4% of total Mangrove area in Brazil has been lost (Ferreria & Lacerda 2016). It is estimated that 2.1% of Mangrove area is lost globally each year (Valiela et al. 2001).

Due to the high level of plant deposition, Mangrove forests contain a diverse bacterial community that help to promote plant growth in the Mangroves (Pupin & Nahas 2013). The very top layer of sediment contains aerobic bacteria, while anaerobic bacteria lives deeper in the sediment. The bacteria offer a variety of services such as fixing nutrients in the soil, degrading phytotoxic compounds, and generating new soil. Certain bacteria can also produce plant hormones in order to promote plant growth (Gonzalez & Bashan 2000). The bacterial communities contain a large variety of different bacteria, many of which offer different benefits to the forest. In the Mangroves located in India, the bacterial community was found to have cellulolytic, amylolytic, pectinolytic, and proteolytic activity (Matondkar et al. 1981), and 44 phyla of bacteria were found in the sediment using a metagenome sequence (Basak et al. 2016). Mangroves might be subjected to the rhizosphere effect which is the high activity and proliferation of microorganisms due to favorable environments, there is increasing richness of bacteria from sediment found close to the sea to sediment found in areas with more vegetation because vegetated areas have a more favorable environment (Rocha et al. 2016). It is expected that there is a variation in diversity between locations of different Mangroves around the world, and Mangroves in the same location due to different environmental preferences within bacteria.

There may also be a large range of diversity because of bacteria's high mutation rate, which is due to their very short generation time, and ability in some bacteria to pass DNA within

their same species and among different species. These high mutation rates can give lineage to new species of bacteria which increase bacterial diversity, this has been seen through metagenome sequencing of sediment in mangroves (Andreote et al. 2012). Bacteria constantly mutating can also create some bacteria populations that are more resistant to influxes in the environment then others (Larsson 2018).

Application of chemical fertilizers and manure has been found to alter bacterial communities (Liu et al. 2017) and increase antibiotic resistance in bacteria (Munir & Xagoraraki 2010). Additionally, aquaculture and agriculture runoff can carry new bacteria which can affect and alter bacterial communities (Walker 1990). Disruptions in Mangroves bacterial community can have a negative impact on the ecosystem as a whole. With increasing agricultural and aquaculture development in and around Mangrove trees, bacterial disruptions become an increasing concern. It was hypothesized that among three Mangrove locations located near La Paz, Baja California Sur that the location closet to a source of aquaculture runoff would contain bacteria that would have no negative change with the introduction of fertilizer to the growing environment because of increased exposure creating bacteria more resistant to high levels of Nitrogen and Phosphorus, and variation in bacterial communities between different Mangrove sites. While the sites that have not been introduced to aquaculture would have a negative change in growth of bacteria with the introduction of fertilizer.

Methods

The top layer of sediment in three different locations of Mangroves located near La Paz, Baja California Sur was collected. The sediment was diluted using standard serial dilution methods, and the bacteria contained in the sediment were grown on nutrient gelatin. Half the nutrient gelatin contained fertilizer in order to test the effect of fertilizer on bacterial

communities from different locations of mangroves. The bacteria were then watched, and first colonization was recorded in each petri dish.

Collection

Three sediment samples were collected from Balandra (site 1), UABCS Marine Rehabilitation center in Pichilingue (site 2), and Mangroves at Playa Solitaria (site 3) which is located downstream of a shrimp farm. The samples were collected by taking a core of the sediment 15 cm down near Rhizophora mangle. Collection areas within the site were haphazardly chosen, we walked for about 5 minutes each time from an accessible area in the Mangroves and then stopped and took the sample where the Rhizophora Mangle met the water. The sediment samples were kept in sealed containers at room temperature until dilutions were completed.

Dilution

The sediment samples were diluted using standard serial dilution methods. 95 ml of purified water was added to 10 grams of sediment, the solution was mixed for several minutes. one ml of sediment solution was pipetted out and placed in a test tube that contained 9 ml of purified water, this new solution was then mixed. From the new sediment solution one ml was removed and pipetted into 9 ml of purified water. This process was repeated two more times to obtain five serial dilutions.

Nutrient Gelatin Preparation

Nutrient gelatin was prepared by boiling beef meat and bones for two hours to make a concentrated beef stock. The stock was then strained to remove any pieces. 30 cups of purified water was then boiled, and 30 tablespoons of unflavored gelatin, 30 tablespoons of sugar, and 30 tablespoons of concentrated beef stock were added. This solution was mixed for 5 minutes to allow everything to dissolve. 5 tablespoons of fertilizer were then added to 15 cups of solution in

order to obtain a fertilizer level of 5.36 ppt. The fertilizer used was Miracle·Grow® which contained 24% total Nitrogen, and 8% Potassium, this fertilizer was chosen due to its high concentration of Nitrogen. Each 10 petri dishes were labelled with their corresponding treatment, Nitrogen or control, and their corresponding site, 1,2, or 3. There were 60 total petri dishes. The two solutions were then poured into the labelled petri dishes. The petri dishes were covered with plastic cling wrap and put into a refrigerator to set for 13 hours.

Plating

Once the nutrient gelatin in the petri dishes had set, 60 µl of the corresponding serial dilution was pipetted onto the dish using a micropipette and spread throughout the dish using a sterilized spoon. The spoon was sterilized using alcohol that was lit on fire to kill any bacteria, the spoon was then allowed to cool to prevent killing the bacteria that were plated. The petri dishes were recovered with plastic cling wrap and placed in a 19°C to prevent the gelatin from liquefying. All the dishes were placed on a table under tinfoil to keep light from affecting bacterial growth.

Growth

The dishes were numbered 1-30 for the control group and 1-30 for the Nitrogen group. Every two hours the petri dishes were observed, and the first bacterial growth was noted. Observation started at 6:00 pm 7/18/2018 and ended 8:00 pm 7/20/2018. First growth was identified as the first visible bacterial colony. The petri dishes were observed until bacteria growth had been observed on all plates.

Statistical Analysis

A survival analysis was used in the program R to determine if there were significant differences in time taken for first colonization of bacteria to appear on each plate. Specifically, the packages in R used were Survminer and Survival. The two treatments were compared against each other for each site. A cox regression was then used to graph the survivability rate against

time, which in this case survivability rate will be defined as time until first bacterial colonization from time of plating.

Results

The Cox regression proved that there was no significant difference between the control and Nitrogen treatment for the sites 1 and 3 as seen in the P values, 0.62 and 0.18 respectively (Figure 1 and 3). Although it was not significant, on average the bacteria under the Nitrogen treatment at site 1 had a longer time period until first visible colonization where compared to its control, while the Nitrogen treatment at site 3 had a shorter time period until first visible colonization when compared to its control. For site 2 there was a significant difference between treatments, with a P value of 0.007888. The Nitrogen treatment at site 2 took a significantly longer amount of time until first colonization was visible when compared to its control treatment (Figure 2).

Discussion

The results for Site 2 and 3 support the biological hypothesis, that the bacterial communities of Mangroves living near aquaculture are more resistant to the pollutants and so, there would be no significant difference seen between treatments in site 3. While the bacterial community at site 2 had not previously been introduced to aquaculture, so it was expected that site 2 would have a significantly longer time until first colonization when subjected to the Nitrogen treatment. However, the results of site 1 did not support the hypothesis and are conflicting to the results seen at site 2 because site 1 had not been previously introduced to aquaculture runoff.

In this study we explored the impact of eutrophication due to Nitrogen enrichment in the bacterial communities of Mangroves in three different sites in La Paz B.C.S, taking into consideration that anthropogenic activities are the major cause of wetlands deterioration, including Mangrove forests (Valiela et al. 2001). In previous decades the major threats to mangrove forests where agriculture, salt pan development and war time use of chemicals. However, in recent times mariculture and aquaculture have had a heavy ambient impact, shrimp farm development specifically is one of the largest threats to Mangroves (Anh et al. 2010). Some of the problems with aquaculture, especially with pond aquaculture, is that there is the release of toxins into the water, the development of acid sulphate soils, reduced water quality, the release of antibiotics, and the introduction of excess nutrients. Aquaculture can promote eutrophication, introduce pathogenic bacteria to new areas, and change the biochemistry of the nutrients which affects the coastal water quality (Chavez-Crooker & Obreque-Contreras 2010; Bala 2011). Waste waters can contain nutrients such as Nitrogen and Phosphorus; high levels of nutrients in the water can reduce the available amount of oxygen, which can result in hypoxia and anoxia of bacteria (Valiela et al. 2001; Alongi 2002; Anh et al. 2010; Chavez-Crooker & Obreque-Contreras 2010). The contaminated soil near shrimp farms and other aquaculture farms can also be enriched with reservoirs of viruses associated with organic detritus (Chávez-Crooker & Obreque-Contreras 2010). Virus contamination in the water is caused by the use of large amounts of pesticides, and antibiotics, to increase economic productivity. Some of the pesticides used contain toxic compounds, which is particularly concerning when speaking of Mangrove ecosystems, because of the fragility of the bacterial communities that live in the sediment, and the importance of bacteria to survival of the ecosystem as a whole (Marcial et al. 2008; Anh et al.

2010). Mangrove forests are a highly productive niche that support a detritus-based food web, by recycling nutrients and making them bioavailable (Bhattacharyya et al. 2015).

Is important to take into consideration the effects on the bacterial communities that the aquaculture runoff might have. The composition of marine bacterial groups is sensitive and they are not immediately resilient to many disturbances. Also, the changes in bacteria composition are associated with changes in the ecosystem process rates, which can further negatively impact the productivity of the bacterial community creating a negative feedback loop (Allison & Martiny 2008; Gilbert et al. 2011). Although the nutrient enrichment delivered by shrimp farms is not constant during the whole year, temporary variability of nutrient disposition can alter the growth and communities of bacteria, which can affect the turnover rate of dissolved organic matter and organic nutrients available for use by other organisms in the ecosystem (Pinhassi et al. 2006).

Thus, the impact of shrimp farms on the mangroves were tested, specifically the impact of Nitrogen enrichment on the environment. The results from site 2 (Pichilingue) suggest that the impact of the Nitrogen enrichment slow bacterial production, due to the increased Nitrogen concentrations, causing the bacteria to grow slower (Fig 2.). The increase of dissolved Nitrogen and Phosphorus can alter the biogeochemistry and the nutrient structure, promoting harmful algal blooms (Bala 2011). The anoxic conditions caused by the nutrient pollution can have an adverse effect in the growth of the mangroves, as low available oxygen facilitates the microbial conversion of sulphate (abundant in salt water) to sulphides which are toxic to many plants (Reef et al. 2010). In regular conditions the degradation of the organic matter occurs via sulphate reduction, and therefore reversing the conversion of sulphate to sulphides, and lowering the toxicity of the soil. (Reef et al. 2010).

Even though many species of mangroves are sensitive to changes in the nutrient availability, Mangrove forests can assimilate to an excess of nutrients, depending on the type and frequency of the discharge, tidal range, watery dimensions, climate, plankton productivity and abundance (Alongi 2002; Reef et al. 2010).

Results for site 1 (Balandra beach) show that there is no difference between treatments, this could possibly suggest that there is an input of enriched water that make the bacterial communities resistant to high concentrations of Nitrogen. The reasons for this are not clear as Balandra beach is not near any aquaculture. However, there is high human activity, as Balandra is one of the most popular beaches in La Paz, and in this time of the year activity is even higher due to summer vacations. Human interaction might modify the bacterial communities' ability to respond to chemical stressors.

There is a strong possibility that the bacterial communities at the three different sites vary, genetically and in species composition, this is because the nutrient addition to a marine system can affect bacterial genetic diversity and activity (Schäfer et al. 2011). Which would help to explain the difference between the reactions of the bacterial communities to the treatments. Additionally, the nature and availability of organic and inorganic nutrients play a role in the energetic activation and inactivation of bacterial species in coastal eutrophic areas. Therefore, nutrients might affect the taxonomic structure of natural bacterial communities temporally and spatially (Lebaron et al. 2000). Also, it is possible that environmental conditions such as temperature and salinity are similar between sites 1 and 3 which could explain their similar response to the Nitrogen treatment, environmental factors such as these should be tested in any follow up experiments.

The reaction of bacterial communities in Mangrove forests to anthropogenic effects depends on a complex series of factors. Bacterial communities are sensitive and prone to changes in structure due to nutrient pollution pressures, it is important to keep in mind said effects on the future of Mangrove forests. Further studies need to be done in order to understand the complexity of bacterial relationships with Mangroves and the rate at which communities of bacteria are able to change. It is also important to understand what changes in the bacterial community represent to the Mangrove ecosystem as a whole.

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Figure 1. Depicts a Cox regression graphed using the program R, depicting the survival rate of bacteria from site 1 with both the control and the Nitrogen treatment. The y-axis shows the survivability rate which in this case is defined as time of first visible colonization of bacteria growth from the time of plating. The x-axis is defined as time in hours.

Figure 2. Depicts a Cox regression graphed using the program R, depicting the survival rate of bacteria from site 2 with both the control and the Nitrogen treatment. The y-axis shows the survivability rate which in this case is defined as time of first visible colonization of bacteria growth from the time of plating. The x-axis is defined as time in hours.

Figure 3. Depicts a Cox regression graphed using the program R, depicting the survival rate of bacteria from site 3 with both the control and the Nitrogen treatment. The y-axis shows the survivability rate which in this case is defined as time of first visible colonization of bacteria growth from the time of plating. The x-axis is defined as time in hours.

Figure 4. A map showing the collection location at the Mangroves in Balandra.

Figure 5. A map showing the collection location at the Mangroves in the UABCS marine animal rehabilitation center

Figure 6. A map showing the collection location at the Mangroves in Solitaria beach.

Figure 1.

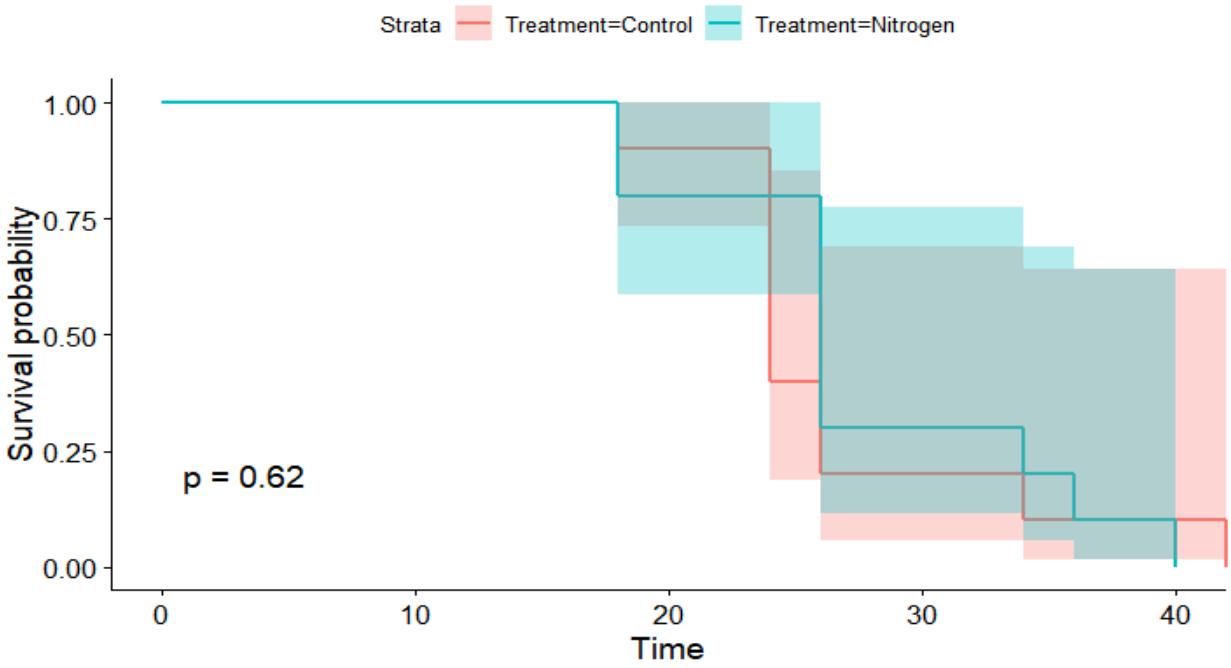

Figure 2.

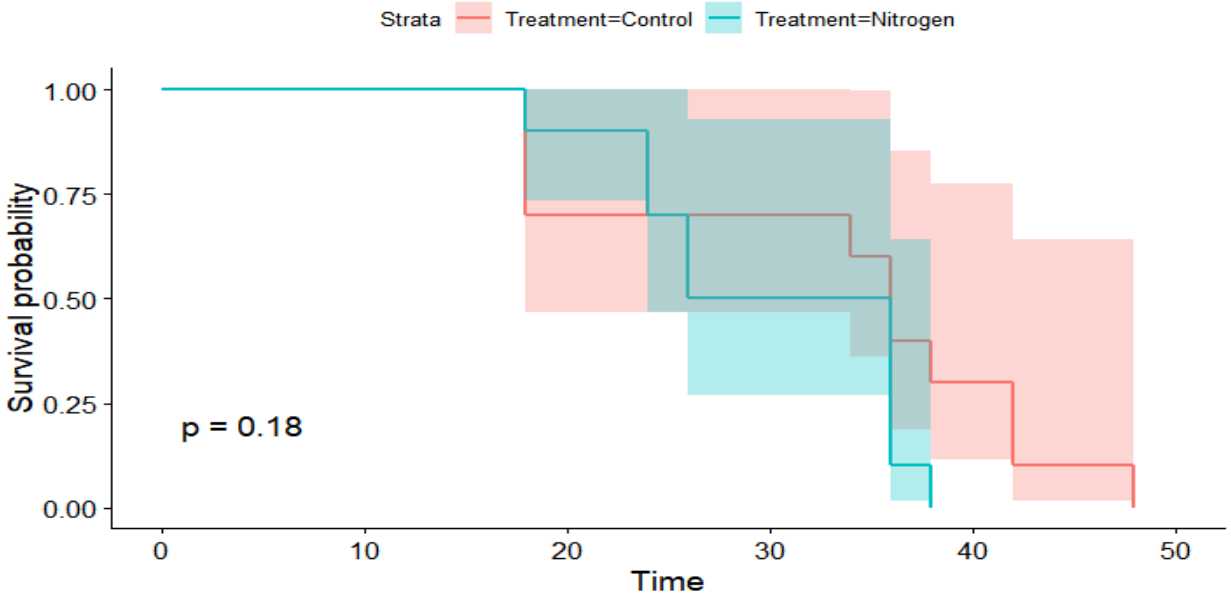

Figure 3.

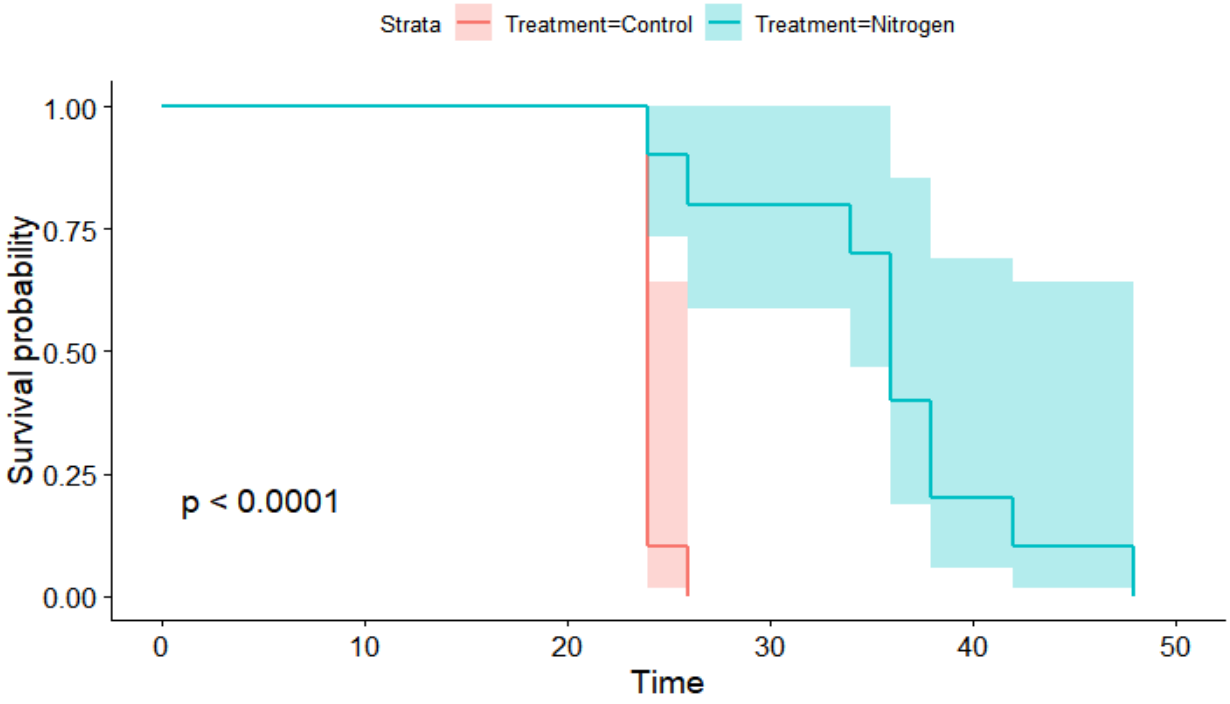

Figure 4.

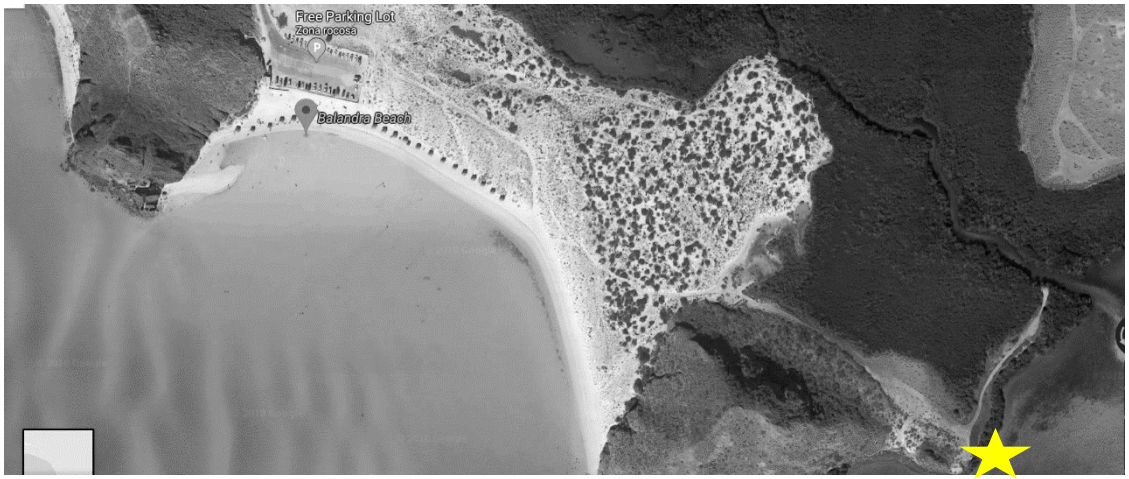

Figure 5.

Figure 6.

Cytochrome oxidase I (COI) barcode identification of sushi species in the capital of Baja California Sur

Key Words: La Paz, seafood mislabeling, DNA barcoding, crab, tuna

Palabras Clave: La Paz, mal etiquetado de mariscos, AND codigo genetico, cangrejo, atún

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ABSTRACT

Seafood mislabeling continues to be a growing concern globally as the popularity of seafood continues to increase. In the state of Baja California Sur, where the fisheries industry encompasses a majority of economic activity and seafood consumption is relatively high, the ramifications of mislabeling are potentially severe. The excessive number of sushi restaurants in the tourist town of La Paz, BCS and the importance of seafood to the area prompted the question of whether mislabeling was taking place within local sushi as had been found in other studies. The high incidence of mislabeling within sushi globally led to the hypothesis that there would be evidence of mislabeling within sushi restaurants of La Paz. To test this, all fish nigiri options were purchased from five different sushi restaurants in the area that varied in price range. This produced 24 samples from which small biopsies were obtained using sterile methods; genomic DNA extraction and a polymerized chain reaction (PCR) proceeded in the standard manner to isolate and amplify the cytochrome oxidase I (COI) region. True species of samples were determined by comparison of the COI region to sequences stored in GenBank using the Basic Alignment Search Tool (BLAST). Identified species were also run through the Food and Drug Administration Seafood List and the International Union for Conservation of Nature Red List of threatened species list to determine mislabeling verdict and conservation status. Results found that a majority (84.6%) of samples were mislabeled and that tuna and crab followed trends from other studies in terms of mislabeling. Ultimately, sample size prevented any conclusive statements, but consumers should be aware of mislabeling occurring in the area and further studies should be done to verify severity. Detrimental effects of mislabeling to the area, ecologically and socially, prompt the need for better regulation of seafood.

RESUMEN

El aumento en el mal etiquetado de los mariscos es una preocupación a nivel mundial causada por la creciente demanda de productos del mar, sus implicaciones son graves. Baja California Sur sustenta su economía en actividades pesqueras. El alto consumo de mariscos deriva en la presencia excesiva de restaurantes de sushi incitarón la pregunta de este trabajo. La alta incidencia de sushi mal etiquetado globalmente llevó a la hipótesis de que habría evidencia de un etiquetado incorrecto en los restaurantes de sushi locales. Para probar esto, se visitaron 5 restaurantes que varía en el rango de precios con venta de nigiris de pescado en sus menús. Esto produjo 24 muestras de las cuales se obtuvieron pequeñas biopsias usando métodos estériles; la extracción genómica de ADN y una reacción en cadena de la polimerasa (PCR) procedieron de manera estándar para aislar y amplificar la región del citocromo oxidasa I (COI). Las especies se determinaron mediante la comparación de la región COI con las secuencias almacenadas en GenBank mediante la herramienta de búsqueda de alineación básica (BLAST). Las especies identificadas también se revisaron en la lista de mariscos de la FDA y la lista roja de especies amenazadas de la UICN para determinar el veredicto y el estado de conservación. Los resultados encontraron que la mayoría (84,6%) de las muestras fueron etiquetadas incorrectamente y que el atún y el cangrejo siguieron las tendencias de otros estudios en términos de etiquetado indebido. El tamaño de la muestra previno cualquier declaración concluyente, pero los consumidores deben ser conscientes del etiquetado que ocurre en el área. Se recomienda un estudio más profundo para verificar la gravedad del problema. Los efectos nocivos del mal etiquetado en el área, ecológica y socialmente, incitan a la necesidad de una mejor regulación de los mariscos.

INTRODUCTION

Fish are considered an important part of the diet for many people; approximately 17% of the protein consumption worldwide comes from fish (Christiansen, et al., 2018). For several decades, the global fish supply for human consumption was above the population count making it a viable food choice. However, in 2009 the population exceeded the supply, but the demand continued to increase over time. Measures indicated that in 2015 demand exceeded 20 kilograms per capita (FAO, 2016). In developing regions, fish consumption continues to increase steadily, (26.8 kg in 2013), leading to a greater reliance on imports as national production declines simultaneously. In contrast, the consumption in low-income countries is estimated to be between 3.5 and 7.6 kg and limited to local availability.

This increase in demand for seafood products worldwide has resulted in a global increase in vigilance against seafood mislabeling. Mislabeling occurs when fish are sold as something other than their true species name or what the country deems a suitable market label, wherever such regulations exist (De la O Burrola, 2015). Mislabeling can occur at any point in the supply chain, from the fisherman to the retailer, and in all types of seafood, including fresh, frozen, processed and ready to eat products. It can be a result of bad identification, use of a common name or loss of information. In some cases, it is used to introduce illegal catches into legal trade (Willete, et al., 2017).

The mislabeling of species has political, social and ecological implications. In the first case, the management of these resources is important due to the present state of fisheries; currently 30% of fish stocks are overexploited, 57.4% are fully exploited and 7.6% come from collapsed or recovering populations (FAO, 2012). Mislabeling makes it hard to enforce regulations already

attempting to deal with these issues. The social problem lies in the lack of information on the part of the consumer and the risk they unknowingly assume. Previous examples of this include the sale of pufferfish as monkfish (Cohen et al. 2009) or the equally dangerous substitution of oilfish for cod (Ling et al. 2008). Both resulted in serious health complications for the consumers. Laws such as Washington D.C.'s Consumer Protection Procedures Act (CPPA) try to prevent these issues by giving consumers more power to hold retailers accountable (Stern et al., 2017). Ecologically, mislabeling leads to an underestimation of fisheries. For example, sharks targeted for their fins can be sourced from legal fisheries, by-catch, and illegal, unreported and unregulated (IUU) fisheries. Unfortunately, the number of sharks caught as undeclared by-catch and in IUU fisheries probably far outweighs those from legal fisheries (Willete et al., 2017). This can ultimately lead to over-exploitation of the sharks and eventually a collapse of populations.

For this reason and several others, seafood mislabeling has gained popularity as a study topic in recent years. Between 2010 and 2012, Oceana conducted a study sampling 21 states in the United States. Results showed that one third of the samples were mislabeled, with the most commonly erroneous products being Snapper (87%) and tuna (59%). Additionally, the study found the percentage of mislabeling taking place in supermarkets (18%), restaurants (38%) and sushi (74%). Similarly, in 2017 UCLA conducted one of the most complete studies in which they sampled 26 sushi restaurants and three luxury markets over four years evaluating 9 of the most common fish in sushi. Much like other recent studies (Cawthorn et al., 2011, Vandamme et al., 2016, Staffen et al., 2017), they did this through the extraction of genomic DNA. They found that 47% of the products were mislabeled and that halibut, red snapper, yellowfin tuna and yellowtail had the highest mislabeling rate with more than 77% of samples incorrectly labeled. In general all the restaurants visited had at least one case of mislabeling. Despite the increase in regulatory

measures and media attention, they found that mislabeling of fish was still prevalent (Willette et al., 2017).

As that study and many others have pointed out, DNA extractions have become the easiest way to identify these mislabeled items, producing reliable evidence when taxonomic characteristics are absent (Carvalho, 2014). Although the method itself has been standardized, the gene used for identification has not been consistent. In more recent studies, sequences of the cytochrome oxidase I (COI) region from mitochondrial DNA have been used to produce reliable results (Hebert et al., 2003).

This study aims to utilize this more reliable method at a local scale within Mexico. Specifically, we will be looking into the mislabeling of seafood in the state of Baja California Sur (BCS). BCS alone contains 19.2% of the country's total coastline, so it is no surprise that the primary economic activity here is within the fishing industry and that consumption of seafood is up to 7 kg per capita for its inhabitants (De la O Burrola, 2015). The state's capital, La Paz, is a popular tourist location and has consequently seen the number of sushi restaurants increase among the various other restaurants in the area offering seafood. This high number of sushi restaurants, (more than 30), along with the state's dependence on seafood and its proximity to the source led us to question whether there was evidence of mislabeling taking place within the local sushi. The high numbers of mislabeling found in both the Oceana and UCLA studies of sushi prompted us to hypothesize that there would be mislabeling evidence within sushi restaurants of La Paz, BCS.

METHODS

In order to determine whether mislabeling was taking place, sushi restaurants of La Paz had to be compiled, collection sites determined, samples obtained, and sample species verified.

Because the fillets used for nigiri lack morphological traits, species identification of each sample was done with molecular analysis.

Sampling

With over 30 restaurants in town selling sushi exclusively and several others listing it on their menu, the restaurants to be sampled had to be narrowed down due to time constraints on the study. For this reason, only those restaurants with nigiri on their menu were included in the list of possible sampling sites. Nigiri was selected as the menu item of choice because it consists solely of a slice of raw fish over a ball of rice. This made it less likely to be contaminated during dish preparation and it kept the cost low as well. However, wherever nigiri options were limited, a roll with distinct fish was also purchased.

Once a list of all restaurants selling nigiri was compiled, restaurants were ordered by price from the cheapest to the most expensive. Restaurants with nigiri priced from 45-65 pesos (2.25-3.25 USD) were put into the intermediate category. Those with nigiri priced at <45 pesos (2.25 USD) were considered economic and those with prices >65 pesos (3.25 USD) were considered expensive. Restaurants with limited nigiri options were then removed from each category until only two remained in each.

Sampling was carried out over one day (July 15th, 2018). At each restaurant, all available fish nigiri options were purchased, excluding the squid and shrimp whenever it was offered. At one restaurant, Wok and Sushi, a rainbow roll was also purchased due to the limited number of nigiri choices (only two). In total, 28 samples were collected from five different sushi restaurants. One of the economic restaurants was excluded because of inaccessibility during collecting time. Once samples returned to the lab, only 24 proceeded to DNA extraction while

the other four were thrown out due to lack of information (fish label at moment of sale, price, etc.).

DNA Extraction, PCR, and Gel Electrophoresis

Molecular analysis began with the extraction of genomic DNA from small biopsies (200–500 mg) through digestion with proteinase-K at 55° C for 2 h, and a standard LiCL extraction protocol (Aljanabi S, 1997). Obtained DNAs and primers, COI_F: TCAACCAACCACAAAGACATTGGCAC and COI_R: TAGACTTCTGGGTGGCCAAAGAATCA (Pank et al. 2001), were used to amplify 670-bp DNA fragments corresponding to the mitochondrial cytochrome oxidase I (COI) gene region; following Shivji et al. (2005). PCR reactions were performed in 25mL containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1M of each primer, 1 unit of Platinum® Taq DNA polymerase (Invitrogen) and 1mL of template DNA. PCR amplifications were carried out in a 96-well applied biosystems SimpliAmp Thermal Cycler, programed to perform an initial denaturation at 95° C for 2 min; followed by 35 cycles of DNA denaturation at 95°C for 60s, primer annealing at 65°C for 60s and extension at 72°C for 60s, followed by a final extension at 72°C for 5min. PCR products of expected size were detected through 1% agarose Ethidium Bromide gel electrophoresis and UV trans illumination. Only 13 of the 24 samples were detected. These 13 products were cleaned with ExoSAP-IT (Affymetrix, Santa Clara, CA). Each product was then sequenced in the 5’ direction using Big Dye (Life Technologies) cycle sequencing protocol (Macrogen Inc., Seoul, Korea).

DNA Analysis

COI sequences were then run through the Basic Alignment Search Tool (BLAST) from the Nation Center for Biotechnology Information (NCBI) website in order to compare them to

those found in the GenBank database. To find the most similar sequences in the database, the MegaBlast choice was selected. BLAST results returned with >98% match were considered significantly homologous to the sample run. If multiple results met this qualification, highest total score determined the species match. BLAST species results were then used to verify labeling of samples in accordance with the FDA Seafood List of acceptable label names (FDA, 2017). Identified species were also run through the International Union for Conservation of Nature (IUCN) Red List of Threatened Species List in order to determine their conservation status (IUCN, 2018).

RESULTS

The analysis of the identified sushi sequences using the FDA Seafood List of acceptable market names showed that a majority (84.6%) of the menu items were incorrectly labeled (Table 1). Also, among all the identified species, three cases of repeated species were witnessed; Alaska pollock (*Gadus chalcogrammus)* comprised 23% of our samples while wahoo (*Acanthocybium solandri*) and greater amberjack (*Seriola dumerili*) each made up 15.4% of the samples (Table 1). The remaining 46% of the identified species were only seen once (Figure 1).

When analyzing samples in terms of the species label at purchase and the true identities of samples within each label, only the tuna and crab had multiple samples to review. If the Bluefin tuna and yellowfin tuna samples were combined with the other vaguely labeled tuna samples into one general tuna category, we found that 80% of samples were mislabeled (Table 2). This tuna group contained 38.5% of the total samples (Table 2). Additionally, 60% of these tuna samples were identified as not being in the *Thunnus* family at all (Table 2). Similarly, of the four samples falling under the crab label, results confirmed that all were mislabeled; 75% of

these samples were actually Alaska Pollock (*Gadus chalcogrammus*), (Table 2). These samples sold as crab made up 30.8% of our total samples (Table 2).

If samples were categorized by the restaurant of origin, results showed that 50% of the restaurants had mislabeling in all samples purchased (Figure 1). Of the restaurants that sold correctly labeled samples, Jiro had 66.6% mislabeling of menu items identified while Wok had 75% (Figure 1). These restaurants with some correct labeling were the sources for 53.8% of our samples; the remaining 46.2% were sold by the restaurants with only mislabeled samples (Figure 1).

In terms of the IUCN statutes noted, less than 40% of the samples were deemed either near threatened or vulnerable (Figure 2). Samples were 1.75 times more likely to be considered of least concern than they were to be in the vulnerable status (Figure 2). However, samples were almost 4 times more likely to be of a vulnerable status than the lower status of near threatened (Figure 2). Overall though, there were only 1.17 times more samples in the least concern status than all other categories combined (Figure 2).

DISCUSSION

It is estimated that the highest percentage of mislabeled seafood corresponds to those purchased from sushi restaurants. Studies have found 32% of sushi mislabeled in the UK (Lowenstein, 2009) while Los Angeles had 47% of samples incorrectly labeled (Willette, 2017). Results of this study found the greatest proportion of mislabeling yet with almost 85% of the products incorrectly labeled; this result is even higher than that found by Warner in 2012 (76%).

Additionally, the same study found that tuna is one of the seafood products with the highest rate of mislabeling (Warner, 2012). Our results followed this trend as a significant proportion

(80%) of the samples that were analyzed were incorrectly labeled. However, an interesting result came from the Tokyo tuna nigiri. This sample was correctly labeled, but we found that it was actually yellowfin tuna (*T. albacores)* being sold generically as tuna. Yellowfin is one of the most sought after tunas worldwide (Essington, 2002), so we found the lack of specification at Tokyo peculiar. On the other hand, Odayaka and Wok sushi, the most expensive and the cheapest restaurant respectively, both had mislabeling of their tuna items. We found evidence that Odayaka did specify their types of tuna sold, Bluefin and yellowfin tuna, but that these were not the true species. The yellowfin was identified as wahoo (*A. solandri*) while the Bluefin was bigeye tuna (*T. obesus*). Wahoo has been recognized for its high mercury content (Adams, 2010), while bigeye tuna has the highest content of fat and heavy metals among tuna types (Lowenstein, 2009). Mislabeling in this scenario then compromises the health of the consumers; individuals may be attempting to make smart food choices, but are ultimately duped at the point of sale. An additional concern lies within the sale of the bigeye tuna sample. This species is classified as vulnerable due to its exploitation globally, but it is also vital to commercial fisheries around the world. Collette et al (2011) reported landings increasing gradually from 808 tonnes in 1950 to an average of approximately 400,000-450,000 tonnes from 1996- 2006 (FAO, 2009). The continued sale of this species to consumers, as witnessed in this study, maintains this aforementioned strain on the global populations.

At Wok sushi, two samples were taken from different products, nigiri and a roll, but both were identified as Greater amberjack (*S. dumerili*)**.** This species is considered of least concern as populations have been deemed stable. It does have minor commercial importance; Greater amberjack is mainly caught by means of purse seines, fixed nets, hooks and lines (Di Natale, 2011).

Samples were likely sourced locally, which would justify the low price of $35 pesos, and it supports the idea of the fisheries industry importance to the area.

Crab represented a good portion of the samples obtained for this study (31%). Vartak (2017) ensures that 100% of crab sold in sushi restaurants is not crab. Under this assumption, we proceeded to perform DNA extraction knowing that only fish primers were available for the crab sample. The success in amplification of all samples already supported the conclusions of Vartak. From returned sequences, we found that a majority (75%) of samples were from *Gadus chalcogrammus* (Alaska Pollock). Samples were obtained from three different restaurants, Odayaka, Tokyo and Wok, which also supported the idea that imitation crab is generally sold in all sushi despite restaurant. Alaska Pollock is a vulnerable species native to the Atlantic (Sobel, J. 1996.); this implies that species were imported to replace the crab. This observation is important because of the high demand of sushi places on this species, not only locally but globally as well. This can have considerable implications for the species as the sushi industry continues to gain popularity. There is some hope for imitation crab; the sample from Jiro sushi was identified as Tilapia guineensis (Tilapia). This may indicate that the pressure on the more vulnerable Alaska Pollock may not be as high.

The salmon sample from Tokyo sushi was one of the two correctly identified samples. Salmon has increased in popularity among seafood in the last century. As a result, aquaculture for these species has increased for human consumption (WCMC, 1996). This is may be due to studies stating that it is the best source for omega-3; approximately 115 g of salmon provide 2 g of this fatty acid, which is considered vital for the cardiovascular system (Kris-Etherton, 2003). Additionally, the characteristics of the salmon fillet make it difficult to replace it with other products. The increase in its aquaculture and its distinct appearance may be the reason that the

likelihood of a correctly labeled product is higher. This may indicate that improvements to aquaculture of other in-demand species may be a viable solution to help reduce exploitation and mislabeling**.**

Overall, the selection of sushi restaurants sampled was made based on the availability of nigiris on their menus and the costs of the products. This means that restaurants were not chosen randomly and that further studies could change this in order to improve the conclusiveness of their results. Among our sampled restaurants, we found that all sold poorly labeled products; however, the limited size of our samples prevents us from saying that 100% of the products offered by Odayaka and Jiro restaurants were in fact mislabeled. Additionally, our study suggests that there is no relationship between the price of products and the certainty of receiving the products offered at the time sale. The difference may lay in the variety of products offered by an expensive restaurant, some have up to 15 different nigiri options, versus the smaller variety, one to two options, an economic restaurant can offer. These conclusions however would likely require further analysis of the data which our study did not have time for.

Overall, these study results emphasize the severity of the seafood mislabeling issue; this issue has made its way into localities where access to fresh seafood products is relatively easy and where the fishing industry is a main element in the economy. If mislabeling is taking place within even these regions, then the severity of the issue has in fact not improved and may even continue to worse. Serious thought and study must be done in order to find a global solution to mislabeling in order reduce exploitation as well.

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Table 1. Summary of the menu items purchased, the purchase location and cost of items, the BLAST identified species of menu items, verdict on mislabeling, and the conservation status of the identified species according to the IUCN Red List.

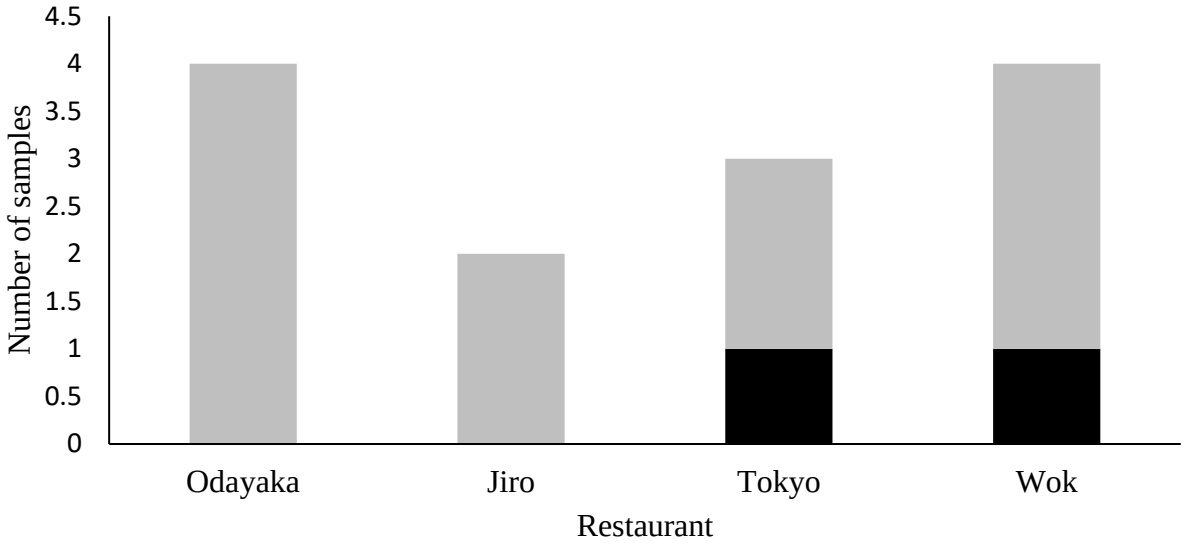
Restaurant	Cost	Menu item	BLAST species result	Mislabeled	IUCN status
Odayaka	$95	Bluefin Tuna Nigiri	*Thunnus obesus*	YES	Vulnerable
Odayaka	$60	Crab Nigiri	*Gadus chalcogrammus*	YES	Vulnerable
Odayaka	$60	Yellowfin Tuna Nigiri	*Acanthocybium solandri*	YES	Least concern
Odayaka	$90	Hamachi Nigiri	*Caranx caninus*	YES	Least concern
Jiro	$60	Crab Nigiri	*Tilapia guineensis*	YES	Least concern
Jiro	$70	Roe Nigiri	*Mallotus villosus*	YES	Not evaluated
Tokyo	$112	Crab Roll	*Gadus chalcogrammus*	YES	Vulnerable
Tokyo	$112	Yellowtail Roll	*Acanthocybium solandri*	YES	Least concern
Tokyo	$56	Tuna Nigiri	*Thunnus albacares*	NO	Near threatened
Wok	$120	Salmon Roll	*Salmo salar*	NO	Least concern
Wok	$120	Tuna Roll	*Seriola dumerili*	YES	Least concern
Wok	$120	Crab Roll	*Gadus chalcogrammus*	YES	Vulnerable
Wok	$35	Tuna Nigiri	*Seriola dumerili*	YES	Least concern

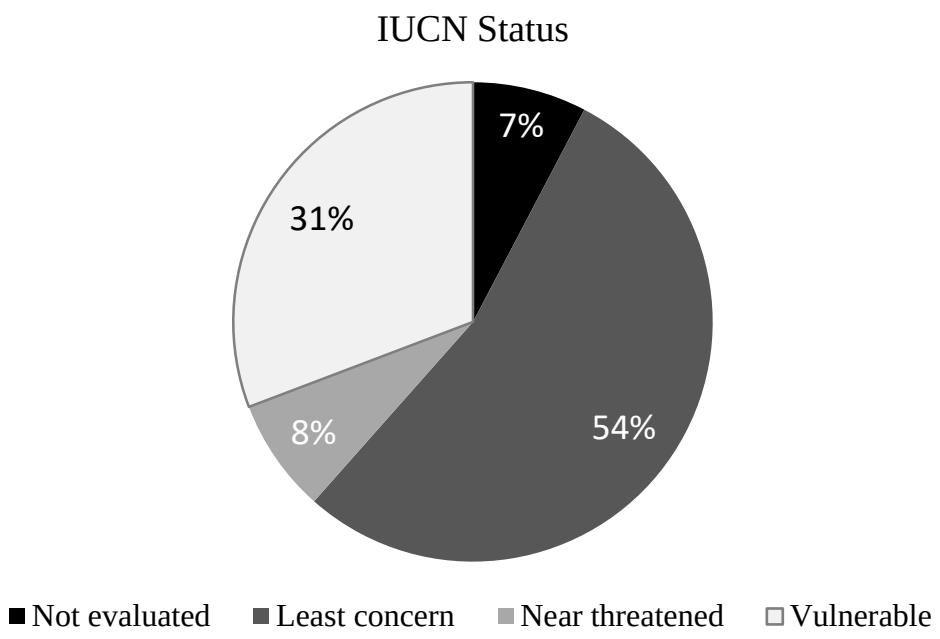
Table 2. Summary of the various label species encountered at time of sale along with the true identity of the samples under that label and their common name. All bolded species were correctly labeled.

Labeled species	Real species	Common name
Yellowfin Tuna	*Acanthocybium solandri*	Wahoo
Bluefin Tuna	*Thunnus obesus*	Bigeye Tuna
Tuna	***Thunnus albacares***	**Yellowfin Tuna**
	Seriola dumerili	Greater amberjack
	Seriola dumerili	Greater amberjack
Salmon	***Salmo salar***	**Atlantic salmon**
Hamachi	*Caranx caninus*	Pacific crevalle jack
Crab	*Gadus chalcogrammus*	Alaska Pollock
	Gadus chalcogrammus	Alaska Pollock
	Gadus chalcogrammus	Alaska Pollock
	Tilapia guineensis	Tilapia
Roe	*Mallotus villosus*	Capelin
Yellowtail	*Acanthocybium solandri*	Wahoo

Figure 1. Number of samples mislabeled and correclty labeled for each restaurant. Correctly labeled samples represented in black while grey represents mislabeled samples.

Figure 2. Percentages of sushi samples with a given IUCN status.


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Number of samples
Odayaka
Jiro
Tokyo
Wok
Restaurant


IUCN Status
7%
31%
8%
54%
Not evaluated
Least concern
Near threatened
Vulnerable

Population density and aggression in Mexican Fiddler crabs

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Key words: *Uca crenulata*, waving, posturing, sexual dimorphism, feeding, disturbance

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Population density and aggression in Mexican Fiddler crabs

Abstract

Mexican Fiddler crabs (*Uca crenulata*) live along the coasts of the Sea of Cortez in mangrove forests and on tidal flats. Each individual creates a burrow in the substratum, and occasionally these burrows are located in close proximity to those of their neighbors. Because Fiddler crabs can only feed at low tide during the day, the opportunity to feed is limited when the crabs encounter rivals or other disturbances. Considering the close proximity and limited feeding window, I hypothesized that as population density increased, the frequency of aggressive behaviors would increase as well. In this study, I examined the frequency of aggressive behaviors in response to population density of *Uca crenulata* by observing the frequency of waving, posturing, fighting, and other aggressive behaviors in quadrats containing the burrows of *Uca crenulata*. Ultimately, the results yielded no definitive correlation between aggression and population density, but studies to help us understand the behaviors of Fiddler crabs are immensely valuable; because the crabs' preferred habitat is the mangrove forest, the gathered data can be used for future conservation efforts.

Resumen

Los cangrejos violinistas mexicanos (*Uca crenulata*) viven a lo largo de las costas del Mar de Cortés en los manglares y en las planicies de marea. Cada individuo crea una madriguera en el substrato, y ocasionalmente estas madrigueras se ubican muy cerca de las de sus vecinos. Debido a que los cangrejos violinistas solo pueden alimentarse durante la marea, en el día, la oportunidad de alimentarse es limitada cuando el cangrejo se compite con sus rivales. Teniendo en cuenta la proximidad y la ventana de alimentación limitada, formulé la hipótesis de que a

medida que aumentaba la densidad en la población, la frecuencia de comportamientos agresivos también aumentaría. En este estudio, examiné la frecuencia de los comportamientos agresivos en respuesta a la densidad de población de *Uca crenulata* al observar: la frecuencia con la que agitan las tenazas, posturas, peleas y otros comportamientos agresivos, en cuadrantes sobre las madrigueras de *Uca crenulata*. En última instancia, los resultados no arrojaron una correlación definitiva entre la agresión y la densidad de población, pero los estudios que nos ayudan a comprender el comportamiento de los cangrejos violinistas son inmensamente valiosos; Debido a que el hábitat preferido de los cangrejos son los manglares, los datos recopilados pueden usarse para futuros esfuerzos de conservación.

Introduction

Large populations of Mexican Fiddler crabs (*Uca crenulata*) create their burrows on the sandy beaches of Baja California Sur. It has been observed that the location and quality of the burrow is important to the female's choice in mate, and she will pass over unsatisfactory burrows (Backwell & Passmore 1996). Further, Fiddler crabs exhibit sexual dimorphism in which the male is asymmetrical with one enlarged claw which they use to fight for access to territory containing important resources, like food or females (Swanson, 2013). It has also been noted that females choose males with the largest and strongest claw as their mate, and that raising and waving the claw is interpreted by other males as aggression (Murai & Backwell, 2006). Fiddler crabs are diurnal and can only feed while the tide is low, therefore the time available for nutrient consumption is limited (Salmon, 1984). Because of the limitations placed on feeding, any time spent displaying aggressive behaviors to one's neighbors would equal the loss of important calories. In this study, I will examine the effect of population density of Fiddler crabs measured

as the number of burrows per 0.5-meter quadrat on the frequency of aggressive behaviors displayed by individuals. As we all know from personal experience, living in close proximity with others can be stressful, therefore I believe that as the density of burrows increases, the frequency of aggressive behaviors will increase as well.

Methods

I observed a large population of *Uca crenulata* at Balandra in Baja California Sur during low tide for four consecutive days. Observations began at 7:00am. A 0.5-meter quadrat was randomly placed on the beach by throwing a small stone over my shoulder, then placing the quadrat with one corner touching the stone in such a way that the quadrat was to the right of the stone and closest to the water line. For each quadrat, I sat and observed from one meter away, then waited for a two-minute adjustment period so that the crabs could become comfortable with my presence. During this time, I counted each of the burrows within the quadrat to calculate the population density. Following the adjustment period, I observed the behaviors of individuals whose burrows were located inside the meter plot for 15 minutes and tallied any aggressive behaviors such as waving, posturing, fighting, or "other". After the 15 minute observation period elapsed, a new meter plot was randomly chosen, and the process repeated for a total of four hours on each observation day. This resulted in a total of 46 quadrats observed.

"Waving" was defined as the presentation and subsequent wagging of one or both claws either vertically or horizontally. "Posturing" was defined as the presentation of one or both claws with no further movement. "Fighting" was defined as two individuals touching claws or other parts of the body for longer than a second. "Other" was defined as any other behaviors that resulted one individual retreating out of the area, including but not limited to; chasing, charging, and sneaker activity. Acts of aggression were only tallied for the aggressor. Any retaliation on

behalf of the victim or victims was not counted. For example, a situation in which two crabs were waving was only counted as one act of aggression.

Because the goal of this study is to examine the relationships between individuals of *U. crenulata*, randomly chosen plots that contained two or less crabs were discarded, as it was impossible to examine the relationships between crabs that did not exist. Finally, this study was completed on a very popular beach and occasionally human activity disturbed the crabs, forcing them back into their burrows. A "disturbance" was defined as any human action which caused *U. crenulata* individuals to cease activity, such as noise, footsteps, and thrown debris like beach balls or garbage. Plots disturbed more than six times in three minutes were observed for an additional three minutes. Data from plots that were disturbed any more than nine times total were discarded, and a new plot was chosen instead. No animals were harmed during this study. U. crenulata individuals were not handled in any way, and I took care to rest the PVC quadrat gently on top of the sand so as not to disturb the burrows.

Results

The data were analyzed with Microsoft Excel using linear regression. Waving behavior exhibited a positive relationship to population density, with a slope of 0.1016 (Fig. 1, df = 44, p-value = 0.3244). Posturing behavior also displayed a positive relationship to population density, with a slope of 0.0288 (Fig. 2, df = 44, p-value = 0.0222). Additionally, fighting behaviors also exhibited a positive relationship to population density, with a slope of 0.0429 (Fig. 3, df = 44, p-value = 0.0592). Finally, other aggressive behaviors also displayed a positive relationship to population density, with a slope of 0.0433 (Fig. 4, df = 44, p-value = 0.0553). Overall, the crabs' acts of aggression were positively correlated to the population density.

Discussion

Although each of the aggressive behaviors were positively correlated with population density, only the posturing behavior was significant. Because the posturing behavior results are significant, I reject the null hypothesis and accept the alternative hypothesis, meaning that the crabs' posturing behavior is in fact correlated with population density. The results of waving behavior were not significant, thus I accept the null hypothesis and reject the alternative hypothesis, meaning that the crabs' waving behavior is not correlated with population density. The results of fighting behavior were not significant, therefore I accept the null hypothesis and reject the alternative hypothesis, meaning that the crabs' fighting behavior is not correlated with population density. Finally, the results of other aggressive behaviors were not significant, thus I accept the null hypothesis and reject the alternative hypothesis, meaning that the crabs' other aggressive behaviors are not correlated with population density. Because only one of the behaviors I observed turned out to be significant while the others remained insignificant, I must conclude that overall the frequency of aggressive behaviors is not correlated with population density of *U. crenulata*. However, the results of the fighting behaviors and other aggressive behaviors were very nearly significant, thus further sampling will be necessary to accurately determine the presence or absence of overall correlation between aggression and population density.

I encountered difficulty when the activity of human beachgoers disturbed the crabs. Occasional disturbance of *U. crenulata* by humans was expected, and I believe it is important to observe individuals under disturbance as their day to day lives are certainly affected by human leisure activity on the beach. However, too much disturbance forced crabs into their burrows, simply rendering observations of behavior impossible. I discarded data from several quadrats

that were heavily disturbed because the crabs' natural behavior and interactions could not be observed. In order to avoid such disturbance on the part of humans, further observations at Balandra should be completed between the hours of 7:00am and 10:00am on weekdays, when human presence and subsequent disturbance at the beach is minimal. It may also be beneficial for future studies to observe *U. crenulata* in several more locations to avoid extrapolating the relatively small population of Balandra to represent the species as a whole.

One possible explanation for the results I obtained is the fact that the time available for the crabs to feed is limited. Because of this, it could be that the crab's burrows are spaced in such a way that minimizes conflicts with one's neighbors, thus the individuals can spend more time feeding and less time in conflict with others. This is supported with data collected by Koga and Ikeda (2010) that males transplanted far from their home burrow more likely to win territory fights against the resident, suggesting that individuals are more likely to be involved in conflict when they are far away from their home burrows. Using this logic, individuals that stayed near their home burrows such as the ones I observed in my study would be less likely to be involved in disputes with neighboring crabs. Another possible explanation for the results I obtained is simply that conflict is energetically expensive. Posturing, which involves the presentation of one or both claws with no further movement, is the least energetically expensive of the four behaviors observed, therefore it makes sense that posturing would be used more frequently than any other aggressive behavior.

Future studies might benefit from recording the sexes of crabs involved in conflicts, as this could imply the type of sexual selection which drives their evolution and sexually dimorphic appearance. Should the majority of conflicts involve only males, then we may infer that male-male competition is the driver for the species' appearance and evolution. Likewise, if conflicts

involve an almost equal number of males and females, we may infer that female preference is the main diver for the species' appearance and evolution. The data from the study of Murai and Backwell (2006) suggest female preference to be the main driver of *U. crenulata's* sexual dimorphism, yet their observations of male-male competition suggest that both intra- and inter-sexual selection had occurred.

The results of this study are important mainly because of the implications of *U. crenulata's* preferred habitat. In addition to living on open tidal flats, *U. crenulata* often makes burrows under mangrove roots. Though *U. crenulata* is not an endangered species, mangrove forests are susceptible to rapid deforestation due to waterfront development for tourism and shrimp farming (Paez-Osuna, 2003). Therefore, the continued reduction of mangrove habitats could soon push *U. crenulata* onto the endangered species list. *U. crenulata* has also been suggested to be essential to the decomposition of mangrove leaves and other debris by providing an ideal environment for microbial colonization and chemical reactions (Bertics and Ziebis 2009). Additionally, human disturbance in *U. crenulata* habitats further limits the time available for individuals to feed, which could result in death from starvation and ultimately a reduction in the size and genetic diversity of *U. crenulata* populations. In general, understanding whether or not population density affects the frequency of aggressive behaviors in *U. crenulata* could assist in future conservation efforts, for example it may help determine the minimum area of land that should be dedicated to wildlife protection and conservation maintain a stable and genetically diverse population, or it may dictate the maximum number of individuals that should be released into a region while allowing individuals to maximize food intake.

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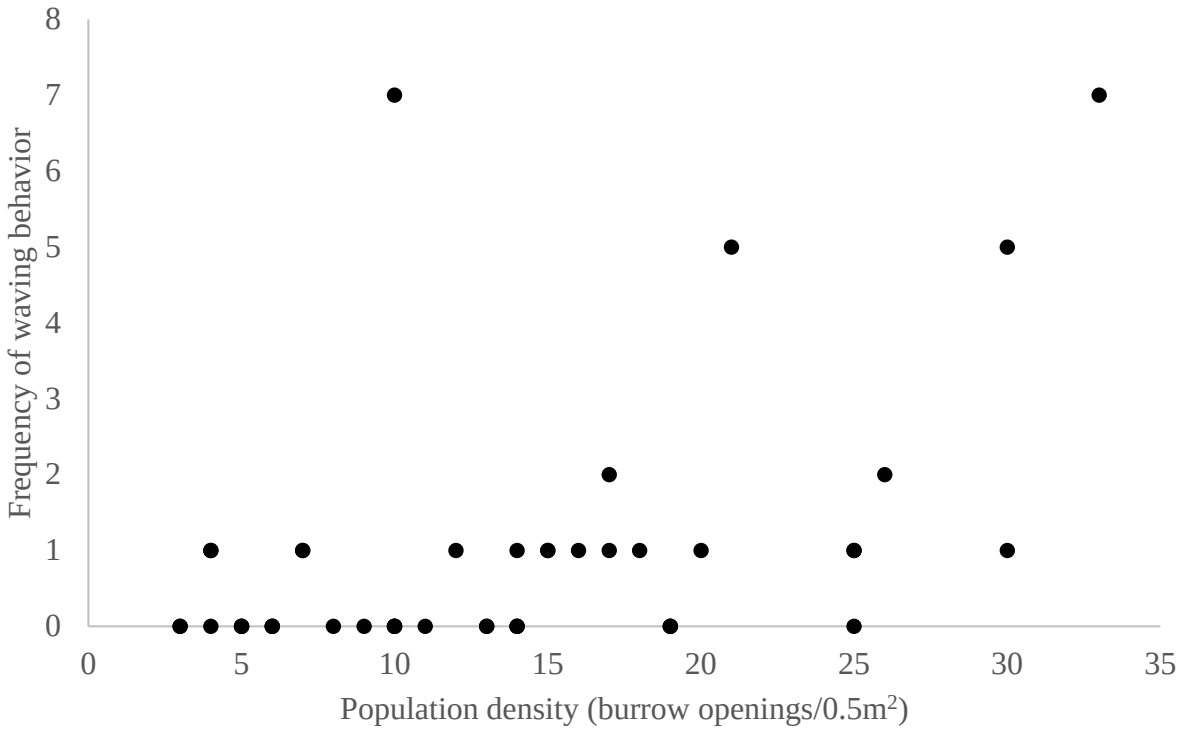
Figure Captions

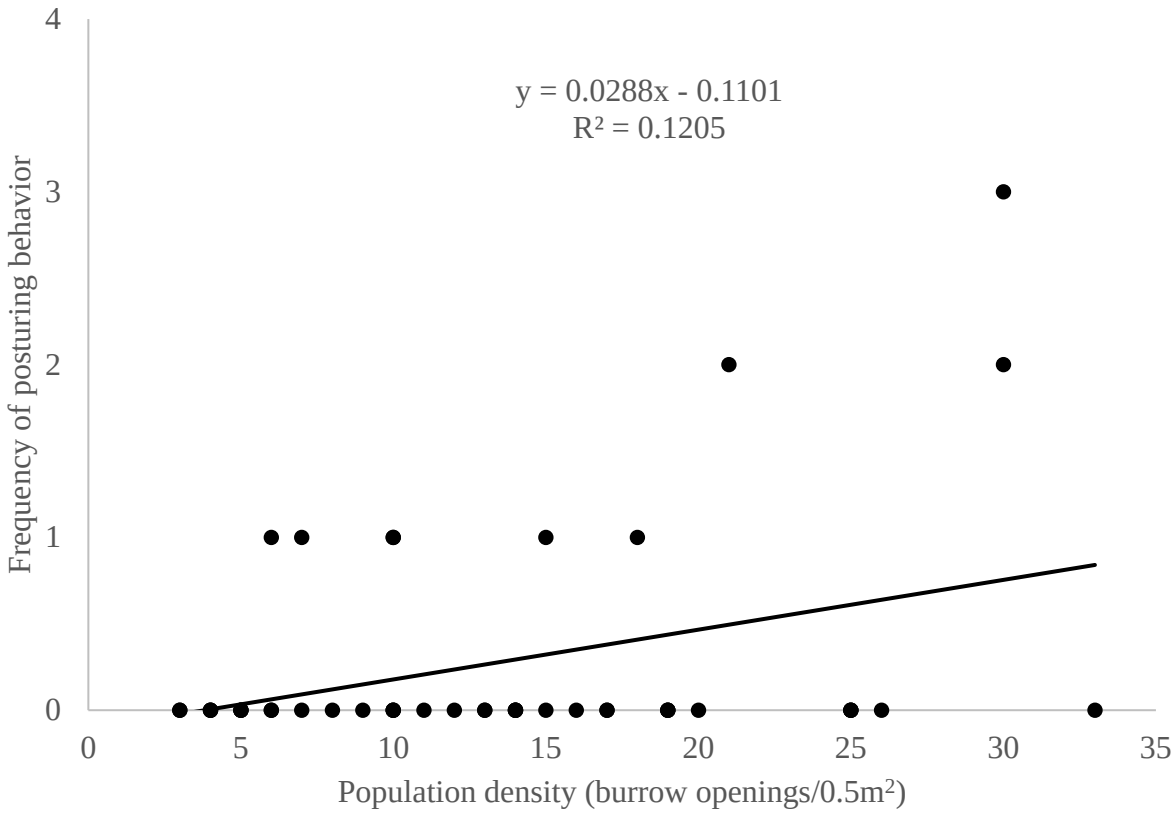
Figure 1. The population density for each quadrat is displayed with the observed frequency of waving behavior.

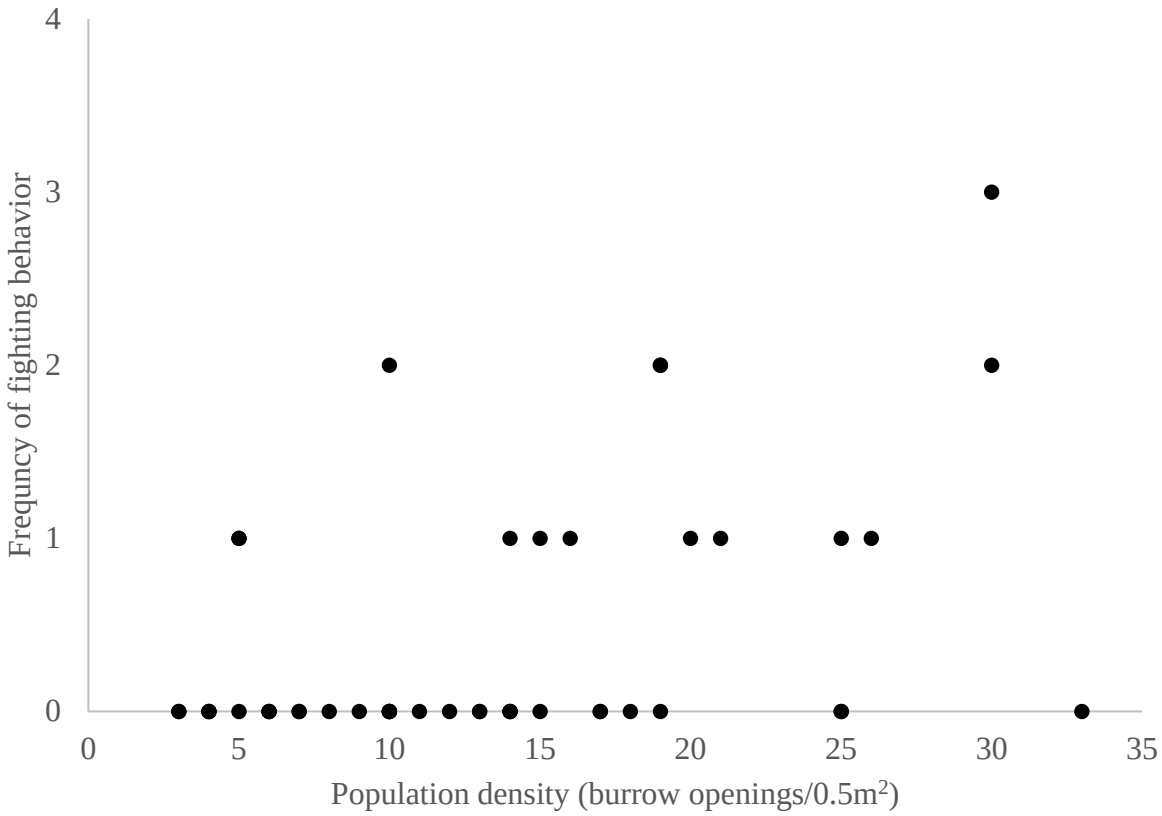
Figure 2. The population density for each quadrat is displayed with the observed frequency of posturing behavior.

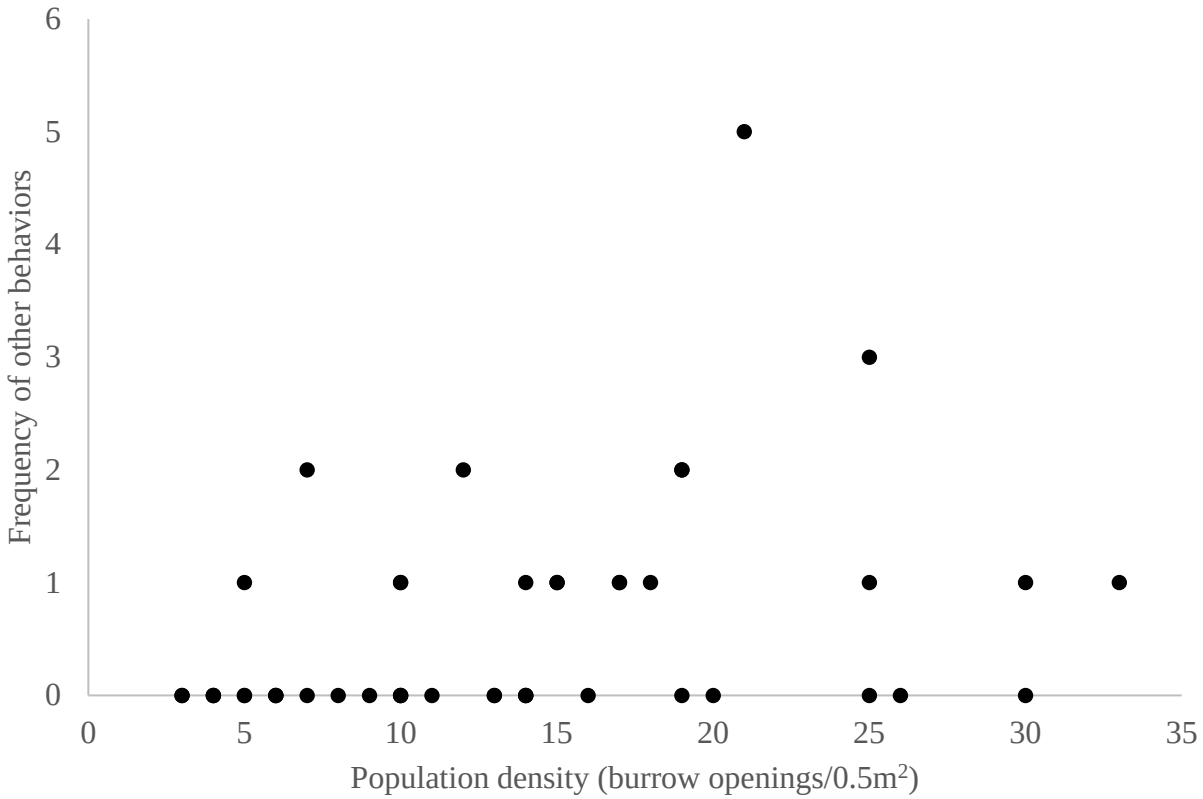
Figure 3. The population density for each quadrat is displayed with the observed frequency of fighting behavior.

Figure 4. The population density for each quadrat is displayed with the observed frequency of other aggressive behaviors.


Frequency of waving behavior
0
1
2
3
4
5
6
7
8
0
5
10
15
20
25
30
35
Population density (burrow openings/0.5m2)


y = 0.0288x - 0.1101
R² = 0.1205
Frequency of posturing behavior
Population density (burrow openings/0.5m²)
0
1
2
3
4
0
5
10
15
20
25
30
35


4
3
2
1
0
Frequncy of fighting behavior
0
5
10
15
20
25
30
35
Population density (burrow openings/0.5m2)


Frequency of other behaviors
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35
Population density (burrow openings/0.5m2)

Pomacentrids and invertebrates associated with *Diadema mexicanum* (Echinodermata: Diadematidae), in the Bay of La Paz, Baja California Sur, Mexico

Benthic Species Associated with *Diadema*

Keywords: Mysid, new distribution, ecological interaction, taxonomy, sea urchin.

Palabras clave: Mísido, nueva distribución, interacciones ecológicas, taxonomía, erizo de mar.

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Pomacentrids and invertebrates associated with *Diadema mexicanum* (Lamarck, 1816) (Echinodermata: Diadematidae), in the Bay of La Paz, Baja California Sur, Mexico

Abstract

The symbiotic relationships that form between two species can be imperative to the survival of one or both organisms. Some of these relationships are rarely studied and much is still to be learned from them. Many species of fish and invertebrates have been known to use sea urchins, such as *Diadema antillarum,* as shelter or a nursery for their offspring. We set out to determine what organisms use *D. mexicanum* as shelter in the Bay of La Paz, Baja California Sur, Mexico. As our collection of these organism was in the middle of the spawning season of *Abudefduf troschelii* we hypothesized that we would find the greatest proportion of the samples we collected to be *A. troschelii* larvae. While snorkeling at four separate locations throughout the Bay of La Paz we collected samples of the organisms living in and around the spines of *D. mexicanum.* Using a microscope and a species identification key it was determined that all the samples we collected were the same species, *Mysidium pumae,* a mysid that has been found within the Mazatlán harbor, in the southeast Gulf of California, Mexico. The presence of this species in the Bay of La Paz has not been reported and no studies have been done involving the interaction between *D. mexicanum* and *M. pumae.* With this information about their presence, further studies could be made into the interaction between these two species, specifically focusing on whether it is truly a symbiotic relationship.

Key words: Mysid, new distribution, ecological interactions, taxonomy, sea urchins.

Resumen

Las relaciones simbióticas que se forman entre dos especies pueden ser imperativas para la supervivencia de uno o ambos organismos. Algunas de estas relaciones rara vez se estudian y aún queda mucho por aprender de ellas. Se sabe que muchas especies de peces e invertebrados usan erizos de mar, como *D. antillarum* refugio o guarderías para sus crías. Por ello, nos propusimos determinar qué organismos usan a *D. mexicanum* como refugio en la Bahía de La Paz, Baja California Sur, México. Como nuestra toma de muestras es llevo a cabo durante la temporada de desove de *Abudefduf troschelii*, supusimos que encontraríamos una mayor proporción de larvas de esta especie. Mientras se snorqueleaba en cuatro lugares diferentes a lo largo de la Bahía de La Paz, se recolectaron muestras de los organismos que viven en y alrededor de las espinas de *D. mexicanum*. Usando un microscopio y una clave de identificación de especies, se determinó que todas las muestras que recogimos eran de la misma especie, *Mysidum pumae*, un mísido que se ha encontrado solamente en el puerto de Mazatlán, en el sureste del Golfo de California, México. La presencia de esta especie no ha sido reportada en la Bahía de La Paz y no se han realizado estudios que involucren la interacción entre *D. mexicanum* y *M. pumae*. Con esta información acerca de su presencia alrededor de *D. mexicanum*, se podrían realizar más estudios sobre la interacción entre estas dos especies, enfocándose en si realmente se trata de una relación simbiótica

Palabras clave: Mísido, nueva distribución, interacciones ecológicas, taxonomía, erizo de mar.

Introduction

Some species of fish and crustaceans form symbiotic relationships with a variety of echinoderm hosts, such as predation, parasitism, mutualism and commensalism. However, the ecological relationships of most decapod-echinoderm symbioses remain poorly studied (Hayes,

2007). However, most of the symbiotic relationships between crustacean echinoderm and echinoderm fish are still poorly studied, although interspecific relationships are important for understanding the ecology and behavior of organisms, there is not much information about these interactions, especially within the genus *Diadema*.

This genus of echinoderms is composed of eight species, *Diadema mexicanum* being the species of interest for the present study, due to its fundamental role in the structuring of the benthic community (Espino et al., 2006), since it is still unknown what is the ecological relationship that occurs between *D. mexicanum* and certain organisms of the benthic community. This species is characterized for being distributed along the eastern Pacific coast spanning from southern California to Peru, including the Gulf of California and all costal islands (Alvarado et al. 2015), and for living in shallow rocky and rocky-sandy bottom habitats. Normally, *D. mexicanum* looks for shelter in caves and crevices to protect itself from predators (Casañas et al., 1998, Herrera et al., 2000, Tuya et al., 2004c), besides presenting a marked nocturnal activity, with an accentuated fidelity to the refuge (Tuya et al., 2004a, 2004b). Levitan and Genovese (1989) suggest that this nocturnal behavior can be a mechanism to substantially avoid the predation to which it is subjected during the day. During these nocturnal movements, this sea urchin is associated with other invertebrate species, representing possible refuge for fish and invertebrates, mainly for larvae belonging to the family Pomacentridae, especially of the genus *Abudefduf* (Fishelson, 1970; Townsend & Bologna, 2007; Hernández 2008).

One of the main interactions described for this species is the competition with other herbivores for resources, being mainly fish and some invertibrates (Beníte Villalobos & Valencia-Méndez, 2015). Olivares-González (1986) and Alvarado et al. (2015) had also described a parasitic relationship between the gastropod *Echineulima mittrei* and *D. mexicanum* in the Gulf of Mexico.

In addition, several authors have reported the association of decapod crustaceans to certain species of echinoderms, such as *D. mexicanum* (Chace, 1969, Críales, 1984, Bruce, 1986, Berggren & Svane, 1989, Marin & Anker, 2009).

Other associations described for members of this genus have been reported by Hartney and Grorud (2002), which suggest that sea urchin spines are used by small fish to evade predators, and that the use of this structure increases survival of juvenile fish. Other studies have pointed out that swarms of mysids (*Mysidium* sp.) are associated with *Diadema*, especially with the species *D. antillarum*, to protect against the predation of fish (Twining et al., 2000). This association has only been reported for Atlantic resident organisms, especially in the Caribbean Sea, and not much is known about these interactions in the organisms D. mexicanum and *M. pumae*, residents of the Gulf of California.

Therefore, the objective of this study is to determine which species are associated or congregate around the sea urchin *D. mexicanum* and their abundance. Because it has been observed that this species is distributed in the rocky areas near the nests of *A. troschelii*, it is expected to find a greater abundance of larvae belonging to this species.

Methods

Between 16 July 2018 and 19 July 2018, we conducted a field survey at four separate locations throughout La Paz Bay, Baja California Sur, Mexico, Cantamar, Calerita, Balandra, and San Juan De La Costa (Figure 1). The bay of La Paz is an important body of water since it is one of the largest and deepest in the Gulf of California and is located from 24 ° 07 'to 24 ° 21' N and 11 ° 17 'at 110 ° 49' (Obeso-Nieblas et al., 2004). It communicates with the Gulf of California

through three entrances: Boca Norte, the San José canal at the south end and the San Lorenzo canal (Obeso-Nieblas et al., 2004). In the bay, three characteristic wind patterns can be identified: northwesterly winds, north winds and southwest winds (Jiménez-Illescas et al., 1997). The winds, during the months of November to May, blow in the morning of the northwest and after the twilight they change to winds of the south. During the rest of the year the southeast and southwest winds are the predominant ones, while the calms are frequent during spring and summer (Obeso-Nieblas, 2004).

Samples were taken of the organisms that could be seen congregating in and around the spines *D. mexicanum,* which could be found in the rocky areas of the subtidal regions. These collections were done to determine what organisms use the spines of *D. mexicanum* as protection or a nursery for their offspring.

Sampling was done using snorkeling gear and a fine mesh hand net. Samples were collected and stored in plastic containers for transportation. A count of how many sea urchins with or without congregating species was made through visual assessment.

Samples were then taken to the Pichilingue lab the same day to be individually labeled and examined by microscope and then documented via photography by a mobile phone (12 Megapixel). Once visual examinations were complete, the photos were compared to a species key to determine the exact species of the organism. Using Ortiz 2016 as a species identification key we paid close attention to several unique characteristic of *M. Pumae*, such as the lanceolate appendix masculina, the male pleopod 4, the telson, and the uropod.

After determining the species, each sample container was counted for how many organisms were caught and what species were found and documented. Additionally, a generalized

linear model was conducted to determine if there was a correlation between the presences of organisms on or around the *D. mexicanum* and each of the collection sites. An Akaike Information Criteria (AIC) test was used to determine whether collection site was an important factor when determining the presence of organisms on or around *D. mexicanum.*

Results

For all the collection sites there was no significance to the presence of the organisms around *D. mexicanum* spines and the location. The AIC test results showed that the model with the site included has the best results, therefore site is an important factor in the presence of mysids near urchins (Table 1).

Of the urchins that were observed at each location, 21.1% of the urchins in Cantamar had organisms in and around their spines. 0.0% of the urchins found in Balandra, and 50.0% of the urchins found in San Juan De La Costa had organism in or around their spines. No *D. mexicanum* were found at the collection site in Calerita (Table 2).

After careful examination of the 867 organism which were caught from around the *D. Mexicanum* and determined that they were all *Mysidium pumae*.

The shape of the appendix masculina is longer than it is wide, tapering to a rounded end. Each of the two appendix masculina has two distinct tufts of setae on them, one at the distal end and another larger one running along the proximal edge.

The mandibular palp of the organism has a swollen second article, which when compared to other known Mysida in this region, which do not have a swollen second article.

Male Pleopod 4 consists of four long narrow articles and reaches to the end of the telson. The peduncle of this structure is shorter than the combined length of the articles. Though setae could be seen at the end of the pleopod 4 we were unable to get a picture clear enough to count them.

The uropod though it has fine setae it lacks stiffer, more stout setae. They are narrow and tapered, with a clearly distinct statocysts on each uropod. The endopod of the uropod is about half the length of the exopod.

The telson is tapered with a rounded tip and is longer than it is wide. Between 50 and 60 short, sharp setae line the edge of the telson from the rounded tip to roughly halfway up both sides.

Discussion

The only and most abundant species found associated with the spines of sea urchins was *Mysidium pumae*, this association was observed in two of the four sites studied (Cantamar and San Juan de la Costa). This interaction may be due to the fact that the organisms belonging to the genus *Mysidium* are located at the bottom of the seabed near structurally complex three-dimensional substrates. This coincides with the sites where they were found (rocky substratum), including the spines of *D. mexicanum* (Twining et al., 2000). This behavior has also been observed with organisms belonging to the same genus in the Caribbean Sea, in which swarms of *M. gracile* associate in search of protection against predation with *D. antillarum* (Twining et al., 2000), which would indicate that *M. pumae* uses the spines of *D. mexicanum* for the same purpose. There is a history of this protective behavior reported for *D. antillarum*, which not only provides protection to mysids, but also to decapods, fish and shrimp of the genus *Cinetorhynchus* (Miller et al., 2007).

Because the samplings were only conducted during the day, the behavior mentioned by other authors was not observed, which suggests that the mysids only swarm near the sea urchins or in certain places during the day and disperse during the night, which it could indicate that this organism uses some kind of return behavior to re-join in discrete schools after nighttime dispersion (Twining et al. 2000, Townsend & Bologna, 2007).

The difference in the presence of mysids between the sites may be due to the size of the sea urchins (Townsend & Bologna 2007). It was found that the diameter of *D. antillarum* influences the presence of swarms of mysids because these organisms were observed only on individual and small urchins (90-120 mm). Because this was not one of the objectives of our experiment, it could not be corroborated if there was the same relationship with the specimens in our study and would be interesting for further studies.

The relationship between *D. mexicanum* and *M. pumae* could be essentially for protection, but this aggregate behavior can also be due to food reasons. Like the vast majority of crustaceans (Ruppert & Barnes, 1969), *M. pumae* feeds on organic matter particle. Therefore, it is possible that they are feeding on fecal material expelled by the sea urchin. It should be noted that the fecal matter of the echinoids constitutes an organic source of quality nutrients, since they defecate 75% of the biomass they ingest (Mamelona & Pelletier, 2005).

It should be noted that this could be one of the first records of *M. pumae* in the Bay of La Paz. This could be due to the mesoscale eddies, since they affect the vertical and horizontal distribution of phytoplankton and zooplankton (Contreras-Catala et al., 2012; Sánchez-Velasco et al., 2013), as is the case of the mysids. Some authors have presented evidence that larvae of coastal species could be transported by mesoscale eddies between the peninsular and continental coast of

the southern Gulf of California (Contreras-Catala et al., 2012; Sánchez-Velasco et al., 2013), so that the presence of *M. pumae* in the Bay of La Paz could be due to these eddies generated by the baroclinic instability caused by the Mexican Coastal current and the topography of the area (Zamudio et al., 2008).

Due to the fact that *D. mexicanum* is a mobile organism it may not prove to be a good nursery for fish larvae. This could be why no larvae of *A. troschelii* were found within and around the spines of the urchins. Another factor that could have influenced the *A. troschelii* travels to feed on algae diurnally as observed by Fishelson (1970), it could be that their larvae could have the same habits, which could explain why we did not find them in our samples.

Now that we know what species are associated with *D. mexicanum* in the Bay of La Paz, it would be interesting to take a closer look at the interactions between the two species. Further studies could be done on the behavioral interaction between *D. mexicanum* and *M. pumae*, for example to determine if the relationship is truly commensal in nature, or if the urchin benefits from the presence of the mysids. Potential symbiotic interactions could be that the mysids could be cleaning the urchins or removing parasitic microorganisms that the sea urchins couldn't do themselves.

Although this interaction has been studied in the Caribbean, due to the importance of *D. antillarum*, the ecological interaction between *D. mex*icanum and *M. pumae* has been very little or nothing studied in the Bay of La Paz, so this work could provide important information for future studies on the ecological interactions of this sea urchin with the fauna that makes up the benthic communities.

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Tables and Figures

Figure 1. Map of the study area within the Bay of La Paz, Baja California Sur, Mexico. Diamonds indicate each of the four collection sites (Torres-Alfaro et al. 2012).

Table 1. This table shows the results of the Akaike Information Criteria Analysis to determine if "Site" is an important factor in calculating the distribution of Mysids.

	DF	AIC
Site	4	234.7531
Intercept Only	0	310.5299

Table 2. This table shows total number of *D. Mexicanum* that were recorded at each site and the number of urchins that had M. *pumae* in and around the spines of.

Site	Total Urchins	With Mysids	Total Mysids Caught
Cantamar	204	43	672
Calerita	0	0	0
Balandra	7	0	0
San Juan De La Costa	12	6	195
Total	216	49	867


E.U.A.
México
24° 40'
Bahía de la Paz
Isla
Espíritu
Santo
San Juan de
la Costa
Calerita
Balandra
Cantamar
Ensenada
de la Paz
24° 00'
110° 10'

Figure 1.

Levels of Coral Bleaching in Coral Friendly Sunscreen Compared to Normal Sunscreen

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Keywords: Coral, Bleaching, Reef, Sunscreen, Coral, Friendly

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Abstract

Corals around the world are threatened by coral bleaching. This phenomenon is when, in response to a stressor, coral expels its symbiotic algae that resides within it thus losing all of its color. Coral bleaching has been occurring at increasing rates recently due to a number of factors including global warming, ocean acidification, and man-made chemicals (Hughes 2017; Downs 2015; Knowlton 2010; Danovaro 2008). Due to this decline, there has been a similar decline in marine species that rely on coral reefs (Jones 2004). Sunscreen is a major way in which harmful chemicals are able to enter our reefs. Chemicals in sunscreen can bleach corals at extremely low concentrations and often reefs are subjected to concentrations of sunscreen exceeding these levels. Recently many people have been turning to so called “coral safe” sunscreens in order to minimize their impacts on coral. These sunscreens do not contain the chemicals that are believed to be the most hazardous to corals, however their effectiveness at protecting reefs has been largely untested. In this experiment I tested whether coral friendly sunscreen is actually effective at protecting reefs. I believed that coral friendly sunscreen would cause less bleaching than normal sunscreens at all concentrations but would still cause some bleaching at higher concentrations. To test this I made two concentrations of both types, 100µL/L and 300µL/L, and subjected coral polyps to them once a day over the course of five days. I found that coral subjected to the normal sunscreens were more bleached than those subjected to the coral friendly variety. Coral friendly sunscreen appears to be successful in protecting reefs, however it is unknown whether or not they would be as effective if the coral was constantly subjected to the solutions.

RESUMEN

Los corales de todo el mundo están amenazados por el blanqueamiento. Este fenómeno sucede en respuesta a un factor estresante, el coral expulsa sus algas simbióticas que residen dentro de el, perdiendo así todo su color. Este fenómeno es cada vez más frecuente, debido a una serie de factores que incluyen el calentamiento global, la acidificación de los océanos y los productos químicos fabricados por el hombre. El uso de bloqueador solar es uno de los principals factores que influyen en la afectacion de arrecifes a causa de los productos químicos nocivos que contiene. Los productos químicos de los bloqueadores solares, pueden causar blanqueamiento a los corales en concentraciones extremadamente bajas. Recientemente, muchas personas han recurrido a los llamados protectores solares "anti-blanqueamiento" para minimizar el impacto en los arrecifes de coral. Estos bloqueadores solares no contienen los productos químicos que se cree, son los más peligrosos para los corales, sin embargo, su efectividad para proteger los arrecifes no se ha probado en gran medida. En este experimento, puse a prueba si los bloqueadores que no afectan a los corales, son realmente inofensivos. Creo que los bloqueadores solares que no afectan corales cuasaran menos blanqueamiento en comparacion a los bloqueadores solares convencionales, en cualquier concentracion, sin embargo, aun podrian causar afectacion en concentraciones muy altas. Para hacer esto, realize dos concentraciones: 100µL/L y 300µL/L, para ser aplicadas a los pólipos de coral una vez al día, durante cinco días. Descubri que los corales sometidos a los protectores solares convencionales presentaban mas blanqueamiento que los sometidos a bloqueador solar anti-balnqueamiento. Los protectores solares anti-blanqueamiento parecen tener éxito en la protección de los arrecifes, sin embargo, se desconoce si serían o no tan efectivos si el coral fuera constantemente sometido a las soluciones.

Ethics

To minimize damage to coral each replicate was only tested on a single polyp of coral. Also if a polyp was bleached it was no longer subjected to the solution in order to minimize damage to the coral.

Introduction

Coral reefs are one of the most productive and diverse habitats in the world (Knowlton 2010). It is estimated that anywhere between 500,000 and 10 million species of organisms inhabit reefs worldwide; meaning reefs might account for 35% of marine species (Knowlton 2010). Even though they harbor hundreds of thousands of species, they are incredibly rare, coral reefs only cover approximately 0.2% of the earth's surface, which is only 5% of the area covered by rainforests (Knowlton 2010). The reason so many species inhabit coral reefs is due to their usefulness as shelter and as nurseries. The stony corals that make up much of our planets reefs are exceptionally good at these due to their defined structure and ability to produce oxygen. Stony corals are able to produce three times as much oxygen as they use up aiding in the productivity of their habitats (Wilkinson 1983). However, these stony corals are not as diverse as the organisms they support; there are fewer than 1000 species of stony corals worldwide (Knowlton 2010).

Unfortunately, coral reefs around the world have been declining at increasing rates due to a phenomenon known as coral bleaching (Coles 2003). Coral bleaching occurs when certain stressors cause the coral to expel the symbiotic algae that lives in the coral (Hayley & Clayton 2009). This alga aids the coral by providing nutrients that the coral is unable to acquire due to the nutrient poor environments it lives in (Muscatine & Porter 1977). Coral has evolved an obligate mutualism with this algae (Zandonella 2016); therefore, without this algae the coral can't gain all

the resources necessary to survive and slowly dies. Dead coral is unable to maintain its structure and degrades losing any ability of it to serve as nurseries for fish, or as shelter for any of the thousands of species that inhabit reefs. Meaning the degradation of coral reefs could lead to the extinction of thousands of species due to habitat loss (Veron et al. 2009). These bleaching events can be cause by a number of human caused factors, such as global warming, ocean acidification, or chemicals added to the water from sources like sunscreen.

Most common brands of sunscreen contain oxybenzone and octinoxate which, even in exceptionally low concentrations, can be potentially hazardous to coral (Downs 2015). Sunscreen might be harmful in concentrations as low as 10μL/L. In many places where sunscreen are common, such as tourist locations, concentrations of sunscreen might be many times higher than it needs to be to harm coral. These chemicals cause many problems in corals, from decreasing viral resistance, to ossification, to deforming motile coral larvae into a sessile state effectively killing them (Downs 2015, Knowlton 2010). Sunscreen are thought to have such adverse effects on tropical reefs that Hawaii has recently passed a bill banning sunscreens containing oxybenzone and octinoxate in favor of "coral friendly" sunscreens. These sunscreens don't contain many of the chemicals blamed for bleaching, however there are very few studies actually testing how effective these sunscreens are at protecting reefs.

In order to compare normal sunscreen brands with coral safe brands I subjected coral polyps to two concentrations (100 μL/L and 300 μL/L) of each brand once per day. I had two brands of normal sunscreen, Banana Boat and Renewal, and one brand of coral friendly sunscreen, Badger. I hypothesized that on average, coral friendly sunscreen would cause less bleaching at both concentrations due to the lack of oxybenzone and octinoxate. However, even though they lack the ingredients that traditionally are blamed for bleaching, it is likely that there might still be

certain chemicals in coral friendly sunscreen that could cause harm to coral. Therefore, at higher concentrations, coral friendly sunscreen will cause some harm to coral.

Methods

To test the effectiveness of coral friendly sunscreen at protecting reefs, different concentrations of two normal sunscreens and one coral friendly sunscreen were tested. Coral polyps were subjected to them over the course of a week. Four replicates of each solution were tested on polyps of *Pocillopora elegans* at the reefs of Cantamar resort near La Paz in the Gulf of California, Mexico.

Two solutions were made for each brand of sunscreen using seawater and appropriate amounts of the sunscreen: 100µL/L and 300µL/L solutions were made. Using a micropipette, 50 µL and 150 µL of sunscreen was added to flasks containing 500mL of sunscreen. The flasks were then swirled until the sunscreen was fully mixed into the seawater, then the solution was poured into 500mL water bottles and shook to ensure the sunscreen was homogenous with the seawater.

Once the solutions were made, zip ties were placed around 28 coral polyps were marked for each solution. Each polyp was assigned a concentration, including the control, and brand; frou polyps for each of the nine brand-concentrations treatments. The location of the polyps were not randomly chosen, replicates of the same solution were kept relatively near each other; this was done to help keep track of the location of every replicate. The level of bleaching on the coral was rated on a scale of 0-3, 0 fully bleached, 1 mostly bleached, 2 low levels of bleaching, and 3 no bleaching on coral. The bottles containing the solutions were allowed to rest in the water for ten minutes to ensure they were the same temperature of the surrounding water. The polyps were then subjected to their solution using a 3mL pipette, the pipette was placed as close to the polyp as

possible before administering the solution so that it was not diluted by the surrounding water before it reached the polyp. Solutions were re-administered every 24 hours and data were collected, making sure that the same individual measured the levels of bleaching due to the subjectivity of the technique.

Data were compared between coral safe and normal sunscreens by averaging all the day five data across each brand-concentration solution. Using R, the data was analyzed to determine if bleaching levels were affected by brand, concentration, or both.

An issue I ran into was that at first first coral was marked by gently tying caution tape around the polyps, however the caution tape floated and therefore would not stay attached for extended periods of time. To remedy this, I switched the caution tape out for zip ties, again making sure they were not attached tightly to avoid any unnecessary damage to coral.

Results

The results show that on average, Badger sunscreen had less coral bleaching than either Renewal or Banana Boat. At 100µL/L there was not a significant difference between Badger and Renewal; however, the coral subjected to Banana Boat was 46 percent more bleached than those subjected to Badger. At 100µL/L Banana Boat was significantly different from Renewal normal sunscreen causing 30 percent more bleaching (Fig. 1). The results were even more dramatic at 300 µL/L. Again, Banana Boat caused the most bleaching of all the solutions causing 66 percent more bleaching than Badger. In this case Renewal also caused more bleaching than Badger, having 44 percent more bleaching. There was no significant difference in the bleaching of Renewal and Badger (Fig. 1).

There were also some differences in bleaching events between concentrations of solutions. Banana Boat was the most extreme example with the higher concentration causing almost twice as much bleaching as its lower counterpart. Renewal also had some significant difference between the bleaching of the high and low concentrations. Coral polyps that were subjected to the high concentration of Renewal had 40 percent more bleaching than those subjected to the low concentrations.

Out of the six solutions there were three that were not significantly different from the control. Those being both the Badger solutions and Renewal 100µL/L. Banana Boat 300µL/L had the solution with the most bleaching, 60 percent more than the control. The solution that was closest to the control while still having a significant amount of bleaching was Banana Boat 100µL/L with 30 percent more bleaching.

Discussion

The results of this study supported our first hypothesis that normal brands of sunscreen would on average lead to more bleaching than the coral safe brand. Every one of the solutions with significant levels of bleaching were from normal sunscreen brands. While, the coral safe brand accounted for two of the three solutions that were not significantly different from the control (Fig. 1). It appears that Badger did not contribute as much to the bleaching of the polyps when compared to the normal brands. The lack of chemicals such as oxybenzone and octinoxate is likely what helped prevent any sort of bleaching. Sunscreen always causes bleaching within 4 days (Danovaro 2008) however, corals subjected to Badger over five days had almost no signs of bleaching meaning the chemicals the used to replace things like oxybenzone are likely not harmful to corals.

Both of the sunscreen concentrations used in this study should have been more than enough to damage corals, and even cause full bleaching in a few days (Danovaro 2008) however, even though the concentration should have easily been able to bleach the coral within a matter of days, there was no significant difference between the Renewal 100µL/L and the control. This is likely due to the fact that corals were only subjected to solutions once per day; corals have a fairly high regenerative ability (Diaz-Pulido 2009). So they were likely able to recover between treatments before the stressors caused bleaching. It could also be possible that some factors, like decreased viral resistance were unable to have an effect in the short time span of our experiment.

Our hypothesis that higher concentrations of coral friendly sunscreen would also cause some level of bleaching was not shown in our data. Neither of the solutions for Badger sunscreen caused any significant level of bleaching when compared to our controls. Once again our data seems to lead to the conclusion that Badger had no significant effect on coral or its symbiotic organism. The lack of bleaching could be due to the corals regenerative ability; however, it is notable that in Renewal, there was no significant bleaching in the lower concentrations, but there was in the higher solution (Fig. 1). This could show that Badger is effective at coral protection at more extreme concentrations due to its lack of damaging chemicals. It is also possible that Badger did cause some minor damage to the reefs, however not enough to cause the corals to be unable to recover before the next treatment.

Both of our normal sunscreen brands showed more damage in higher concentrations (Fig. 1). This is interesting due to the fact that either of the concentrations should have been enough to bleach coral. This might indicate that even if coral is only briefly subjected to theses chemicals, it could still cause severe damage if concentrations are high enough. So, coral near popular tourist

locations might be especially at risk since they are constantly being subjected to trace amounts of sunscreen, which might just need a small push to cause a bleaching event.

The damaging effects of oxybenzone on coral has been well documented in the past. Even in small concentrations oxybenzone can cause ossification, reduced viral resistance, increased rates of mutation in DNA, and deformation of coral larvae (Downs 2015; Danovaro 2008). Due to these effects, many consider oxybenzone to be the most harmful chemical to coral reefs that sunscreen contains. Therefore, it is not surprising that our sunscreen with the highest concentration of oxybenzone was the most damaging to reefs. Banana Boat caused more bleaching than any other solution (Fig. 1).

Subjecting coral to single doses of high concentrations of sunscreen once a day made our results less definitive than expected. Sunscreen concentration in tourism heavy reefs are consistently higher than the 10µL/L needed to harm corals, therefore it would likely be more effective to bathe coral polyps in their solutions in a controlled environment. This method could also allow researchers to see if there might be an interaction between sunscreen and temperature on the levels of bleaching.

Coral friendly sunscreens caused significantly less bleaching at both concentrations than normal sunscreens. Overall it seems that coral safe sunscreen might be an effective way of protecting reefs, however some tests might need to be ran to see if constant exposure to these sunscreens is harmful. Hawaii has already passed a ban on sunscreens containing harmful chemicals and if more governments would follow their lead I could be one step closer to protecting these habitats.

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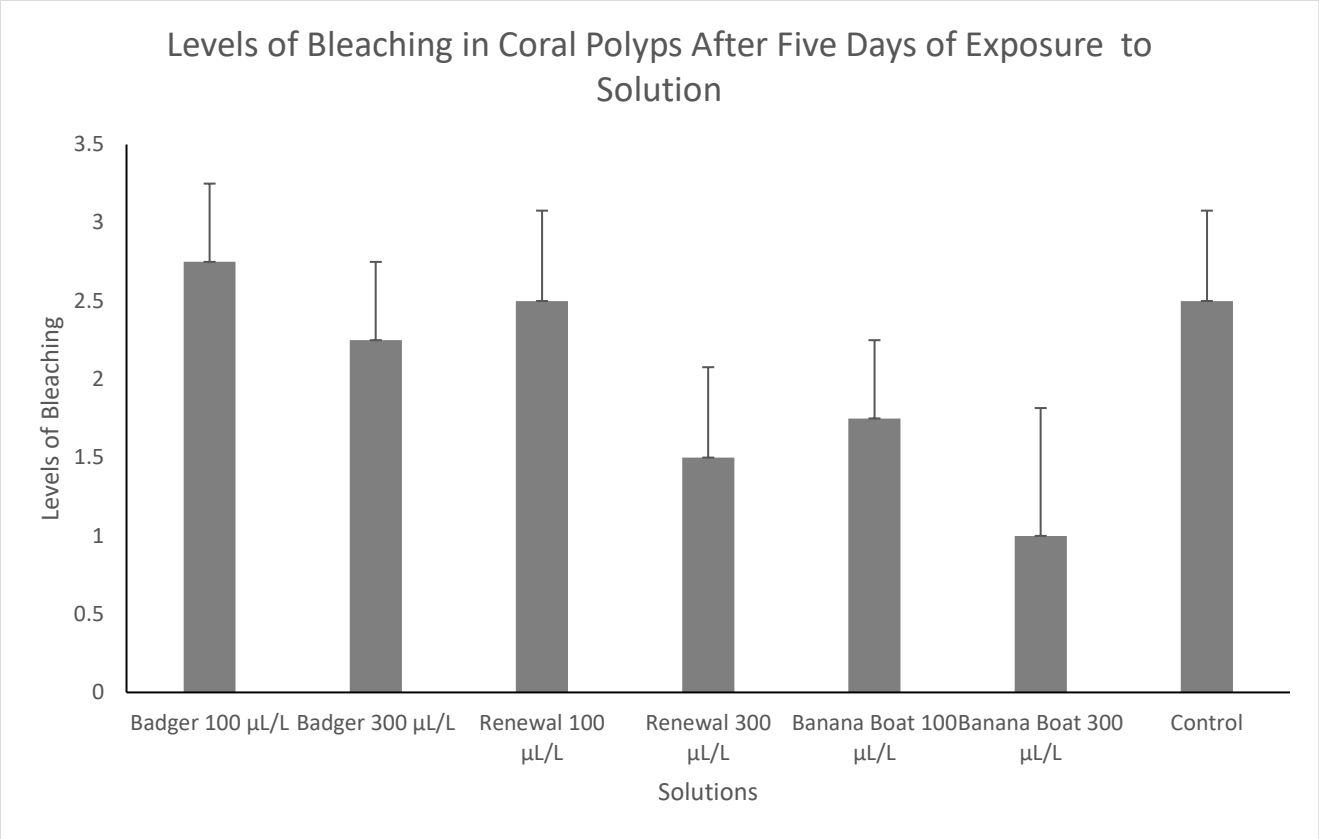
Figure 1. Average coral bleaching of individual polyps of *Pocillopora elegans* subjected to different brands and concentrations after five days. Bleaching was rated on a scale of 0-3 with 3 being no bleaching and 0 being fully bleached. Four replicates were placed for each solution and subjected to their solutions using a 3mL syringe over five days. Error bars represent standard error.


Levels of Bleaching in Coral Polyps After Five Days of Exposure to Solution
Levels of Bleaching
3.5
3
2.5
2
1.5
1
0.5
0
Badger 100 µL/L
Badger 300 µL/L
Renewal 100 µL/L
Renewal 300 µL/L
Banana Boat 100 µL/L
Banana Boat 300 µL/L
Control
Solutions

Effect of Nutrients on Bioluminescent Activity

Effect of Nutrients on Bioluminescent Activity

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Key Words: runoff, nitrogen, phosphorus, fertilizer, toxicity assessment, plankton, Gulf of California

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Abstract

Runoff caused from anthropogenic effects creates toxic water conditions by over-loading oceans and lakes with heavy metals and excess nutrients. These conditions can harm the bioluminescent organisms, including algae, bacteria and zooplankton, who are necessary for maintaining the health of the ecosystem. Heavy metals can decrease bioluminescence, and nutrients such as phosphorus and nitrogen are seen to change cell size and density. Death of these organisms can create bottom-up effects, creating problems for the entire ecosystem. Their importance and versatile use both in and out of water make it necessary for us to understand the implications of runoff. Bioluminescent organisms create a large tourist attraction, and contribute to local economy, farming, waste treatment and toxic assay. Their use as a toxic assay make it easy to evaluate the impacts of heavy metals and toxic compounds on the organisms. However, not much research has been conducted on the impacts of nutrient offloading. Specifically, we wanted to know more about these implications in La Paz, Mexico due the abundance of bioluminescence and importance to the economy. We asked: how does bioluminescent activity respond to different nutrients? We hypothesized that the bioluminescent activity would be affected in the same way by both nitrogen and phosphorus. Bioluminescence was collected at Cantamar Beach in La Paz, Mexico and added to either nitrogen, phosphorus, or plain sea-water treatments. After 48 hours, the amount of activity was counted for each treatment. We found no significant difference, which supported our hypothesis that nitrogen and phosphorus affect bioluminescence in the same way (ANOVA: $F_{(2, 29)} = 0.122$, $p = 0.886$). However, both treatments had no difference in bioluminescent activity as the control. From this, we can conclude the fertilizers used do not have an effect on bioluminescent activity in this area. Future studies should aim to isolate each nutrient, and investigate the effects in different concentrations.

Abstract (Spanish)

La escorrentía causada por efectos antropogénicos crea condiciones tóxicas de agua al sobrecargar los océanos y lagos con metales pesados y exceso de nutrientes. Estas condiciones pueden dañar a los organismos bioluminiscentes, incluyendo algas, bacterias y zooplancton, los cuales son necesarios para mantener la salud del ecosistema. Los metales pesados pueden disminuir la bioluminiscencia, mientras que los nutrientes como fósforo y nitrógeno pueden cambiar el tamaño y la densidad celular. La muerte de estos organismos puede crear efectos ascendentes, creando problemas en todo el ecosistema. La importancia y uso versátil de los organismos bioluminiscentes nos permite comprender las implicaciones que conlleva la escorrentía. La bioluminiscencia resulta atractiva para el turismo y contribuye en cierto sentido a la economía local, a la agricultura, al tratamiento de residuos y evaluaciones de toxicidad. Su uso en la valoración de toxicidad mejora la interpretación de los impactos de los metales pesados y compuestos tóxicos en el ambiente marino. Sin embargo, son pocas las investigaciones acerca de los impactos generados por la descarga de nutrientes. Específicamente, queremos conocer más sobre las implicaciones que tiene la bioluminiscencia en La Paz, Baja California Sur debido a la presencia abundante en sus playas y la importancia que genera en la economía. La pregunta a responder es: ¿Cómo las actividades bioluminiscentes responden a nutrientes diferentes? Nuestra hipótesis responde a que las actividades bioluminiscentes han sido afectadas de la misma manera por el nitrógeno y el fósforo. Los organismos bioluminiscentes fueron recolectados en la playa del Hotel Cantamar en La Paz, México y se agregó nitrógeno, fósforo, o agua de mar sin nutrientes adicionales. Después de 48 horas, la cantidad de actividades fueron contadas por cada tratamiento. No encontramos una diferencia significativa que respaldara nuestra hipótesis donde el nitrógeno y fósforo afectan bioluminiscencia en la misma manera (ANOVA: $F_{(2, 29)} = 0.122$, $p = 0.886$). Sin embargo, en ambos tratamientos no hubo diferencias significativas en la actividad bioluminiscente del control. A partir de esto, podemos concluir que los fertilizantes utilizados en el presente trabajo no tienen un efecto en las

actividades bioluminiscentes en el área. Recomendamos para futuros estudios aislar cada nutriente y llevar a cabo una investigación exhaustiva sobre los efectos de estos en diferentes concentraciones.

Introduction

Urban runoff and pollution can create toxic water conditions by contaminating lakes and oceans with heavy metals, excess nutrients, and harmful chemicals (Heimann et al. 2002). One of the largest contributors to this problem are farmers using pesticides for agriculture. Environmental management practices have been aiming to step away from these harmful chemicals. However, in tropical regions like Mexico, the demand for agrochemicals increases by 9% annually in coastal areas (Carvalho et al. 2002). It is still unclear the effects these chemicals and nutrient rich fertilizers are having on the marine ecosystems, trophic food-webs, fisheries and aquaculture activities (Carvalho et al. 2002).

Bioluminescent organisms can be used to better understand the impact of runoff on coastal ecosystems. Bioluminescence is a phenomenon used by a variety of organisms, usually as a defense mechanism, resulting in emission of light from a biochemical reaction (Kahlke & Umbers 2016). Algae, zooplankton, and bacteria are the three most prominent species to produce this spectacular event in coastal surface waters. Cultured dinoflagellates, an algal species, can be exposed to harsh conditions and the amount of bioluminescence emitted acts as an indicator for the toxicity of the waters. Higher toxicity will lead to an inhibition of bioluminescence (Lapota et al. 2007). Bacteria can also be used in a similar way. Cultures exposed to organic extracts will have inhibited bioluminescence if the extract is toxic (Bihari et al. 1989). With increasing harmful effects from anthropogenic activities and settlement, these bioluminescent assays can help us be more aware of the impacts we are having on marine and estuary environments (Heimann et al. 2002).

Most research conducted using bioluminescent toxic assay focuses on the impact of heavy metals, and not much research has been conducted on the impact of nutrient influx. Heavy metals decrease the

amount of bioluminescence emitted, resulting in their defense being less productive in polluted areas (Boyd 2010). This will also interfere with the attraction of prey and mates (Boyd 2010). The amount of light emitted will determine their ability to scare off predators. The higher the organism's capacity for bioluminescence, the lower the ingestion rates by other organisms including copepods (Esaias & Curl 1972). Point and non-point sources of nitrogen and phosphorus can cause toxic algal blooms, creating undesirable effects on the entire ecosystem (Graham et al. 2016). In studies done on growing algal cells, it was found that nitrogen to phosphorus ratios affect cell size, biovolume and cell density (Lim et al. 2009).

Understanding the impacts of toxic waters on bioluminescence can lead to healthier coastal waters, resulting in thriving marine ecosystems as well as better conditions for humans. These small bioluminescent organisms make up the primary producers of our oceans. Not only are they important to their ecosystem, but can provide many uses both in and out of water. Large shifts in their community can have dramatic impacts in the environment around them. Many bioluminescent species are the preferred food for larger organisms that humans fish or farm for food. Algae can also be used as food, supplements and biofuel (Graham et al. 2016). Similar to using algae for a toxic assay, the organisms can be used to clean waste water by removing pathogenic microorganisms (Graham et al. 2016). Bacteria and zooplankton play an equally important role in maintaining the health of marine and estuarine ecosystems. Besides the functional use of bioluminescence, they are also greatly appreciated for their beauty. Tourists will travel to remote locations just to witness this phenomenon for themselves. In this sense, bioluminescence can contribute to local economy. When we consider all of these factors, we can greatly appreciate the necessity to protect these bioluminescent species.

Our study focused on the impact of nitrogen and phosphorus on bioluminescent activity in Bahia de La Paz. To better understand the impacts of these nutrients, we chose to study the bioluminescence at Cantamar Beach in La Paz, Mexico. Because it is easy accessible and has an abundance of bioluminescent organisms, it made this site optimal for research. It is unknown whether runoff has an effect in this location, so we were curious to see if subjecting bioluminescence to nitrogen and phosphorus would have

an effect on their activity. We expect activity to respond in the same way when subjected to either nutrient. By doing so, we aim to better understand how anthropogenic activities can harm these important communities in our coastal waters.

Materials & Methods

The study was conducted in Bahia de La Paz because minimal research has been done on the bioluminescence in the bay, despite the high levels of industrial runoff and the local economy's dependence on marine life. Cantamar beach was chosen as the specific study site because the location was easily accessible and high concentrations of bioluminescence had been regularly observed in the water there.

Creating Nutrient Treatments

We created nutrient treatment solutions by mixing common garden fertilizers with seawater collected from Cantamar beach. The concentrated nitrogen solution was created by mixing seawater with Miracle-Gro fertilizer that contained 24% nitrogen by mass. 0.0268 grams of fertilizer were mixed with 504 mL of seawater to create a 911 μM stock solution. The concentrated phosphorus solution was created by mixing sea water with Magic Root fertilizer that contained 48% P_2O_2 by mass. 0.0482 grams of fertilizer were mixed with 500 mL of seawater to create a 985 μM stock solution. Thirty water bottles (10 per treatment) were filled with 42 mL of either concentrated stock nitrogen solution, concentrated stock phosphorus solution, or plain seawater. Later, after bioluminescence was collected, approximately 79 mL of additional seawater was added to each bottle. The final molarities of the nitrogen and phosphorus treatment solutions were approximately 316.3 μM and 341.9 μM, respectively.

Collecting and Rearing Bioluminescent Organisms

After dark, a plankton tow was used to collect bioluminescent organisms at Cantamar Beach to place in the treatment solutions. The tow was dragged by hand through the surface waters for approximately 3 meters, starting at the shoreline, and dragged back to shore again; creating a total 6 meters of collection that was consistent across all samples. About 79 mL of bioluminescence-containing seawater was collected in a cup at the end of the tow and poured into water bottles containing 42 mL of stock nutrient solutions. The bottles were trapped between two milk crates, held together by zip ties, and weighted with large rocks. The milk crates were tied to anchors to keep the solutions in the water and maintain the temperature of the solutions.

Data Processing

After 48 hours of being in treatments, the crates were pulled out of the ocean to test the water samples in the bottles for bioluminescent activity. The bottles were taken to a dark room, then randomly selected by being blindly pulled out of the crates. The contents were then poured into a clear plastic cup. The cup was handed to an observer in a dark closet, who did not know which treatment they were observing. The cups were stirred using a plastic zip-tie and the number of individual flashes seen was counted by the observer, then recorded.

The data were fitted using a linear model in R, then an ANOVA test was used to compare the mean number of flashes seen in response to each of the three treatment conditions. Before running the ANOVA test, a linear model was used to fit the data so they met the assumptions of the test. All data were run in R Studio (1.1.383).

Results

The mean number of flashes seen across all treatments was 1.433 ± 2.82 with no significant difference between the means of all treatment groups (ANOVA: $F_{(2, 29)} = 0.122$, $p = 0.886$). The samples from the phosphorus treatment group had the greatest range in number of flashes seen, with the highest number of flashes being 11, more than 1.5 times the maximum number of flashes seen in both the control and the nitrogen treatment groups (Figure 1). However, at 1.2 flashes the phosphorus treatment also had the lowest mean, followed by the control treatment at 1.3 flashes (Figure 1).

Discussion

As expected, there was no significant difference between the nitrogen and phosphorus treatments; however, neither nutrient treatment had an effect relative to the control, which was not expected (ANOVA: $F_{(2, 29)} = 0.122$, $p = 0.886$). This supported our hypothesis that there would not be a difference between the two nutrient treatments. Previous research by Lim et al. (2009) found high concentrations of phosphorus to contribute to a high volume of cells. Nitrogen is also known to contribute to large blooms of algae, such as the kind that create this bioluminescence (Graham et al. 2016). Due to the similar effects caused by an excess of both of these elements in the water, these results were expected. However, the similarity across all treatments was not expected. This could have been because of a time constraint that was not present in previous experiments done by Jakobsen et al. (2015) and Lim et al. (2009). Because the particular species that produce bioluminescence in this location could not be identified, further research could be done to test the effects of specific nutrients on specific species.

Both of the fertilizers that were used in this study contained nitrogen, phosphorus, and potassium, which could have affected the results of the treatment by interfering with each other. Dinoflagellates, one type of bioluminescent organism present in coastal waters, are known to take up excess phosphorus when it is available, but it is not known if this luxury uptake of phosphorus inhibits nitrogen uptake (Pleissner

& Eriksen 2012). It is known that nitrogen and phosphorus concentrations do have an interactive effect on growth rates. However, there has not been any research studying the individual effects of each of these nutrients (Lim et al. 2009). It has been found that high nitrogen-to-phosphorus ratios inhibit cell growth and the highest cell densities have been seen in high-phosphate conditions, but studying the effect of nitrogen or phosphorus in isolation could provide insight as to why this might be (Lim et al. 2009).

It would also be interesting to study the effects of different concentrations of the isolated nutrients. Lim et al. (2009) studied the interactive effects of nitrogen and phosphorus in different ratios and found that cell growth and density increased up to a certain concentration of phosphorus, but declined after a certain phosphorus concentration. Understanding if that maximum concentration was the same with or without nitrogen present could provide additional insight into the effects of each of the nutrients in isolation and the importance of keeping each of these nutrients below a certain concentration.

Future replications of this study might also ensure that there was significant oxygen input into the treatments and that there was sufficient mixing of the water. It is known that bioluminescence is caused by a reaction between luciferin and oxygen, catalyzed by the enzyme luciferase (Kahlke & Umbers 2016). While there was sufficient air in the bottles throughout the treatment process, the concentration of usable oxygen may have been depleted enough to become a limiting factor in the reactions. It has also been found that increased water movement is positively correlated with increased intensity of bioluminescence (Latz 2004). While stirring the cups to produce bioluminescence during data collection may have been enough to produce some light, constant mixing of the treatments may have been beneficial to the overall health of the bioluminescent individuals. Mixing allows nutrients to be distributed throughout the water and helps to increase the concentration of dissolved oxygen so that cells can respire normally and achieve maximum functionality. Due to the need for circulation to incorporate oxygen into the water, future researchers may also want to focus on the interactive effects between different nutrients and the presence of usable oxygen or flow.

Understanding the effects of different nutrients on the environment can be beneficial to scientists and citizens. Bioluminescent organisms make up the base of most coastal food webs and therefore support local ecosystems and the people who depend on them (Jakobsen et al. 2015). Knowing how to best protect the coastal waters allows the wildlife and industries that depend on them to remain healthy. In this particular location, the shrimp and fishing industries depend on the bioluminescent plankton to feed animals that are farmed for food. The phenomena of seeing bioluminescence in the waters also helps to feed the tourism industry. While these results indicate that the bioluminescence in Bahia de La Paz may not be at risk from excess nutrient input, it is important that we understand the impacts of each of the nutrients alone, as well as the effects of higher concentrations of these nutrients.

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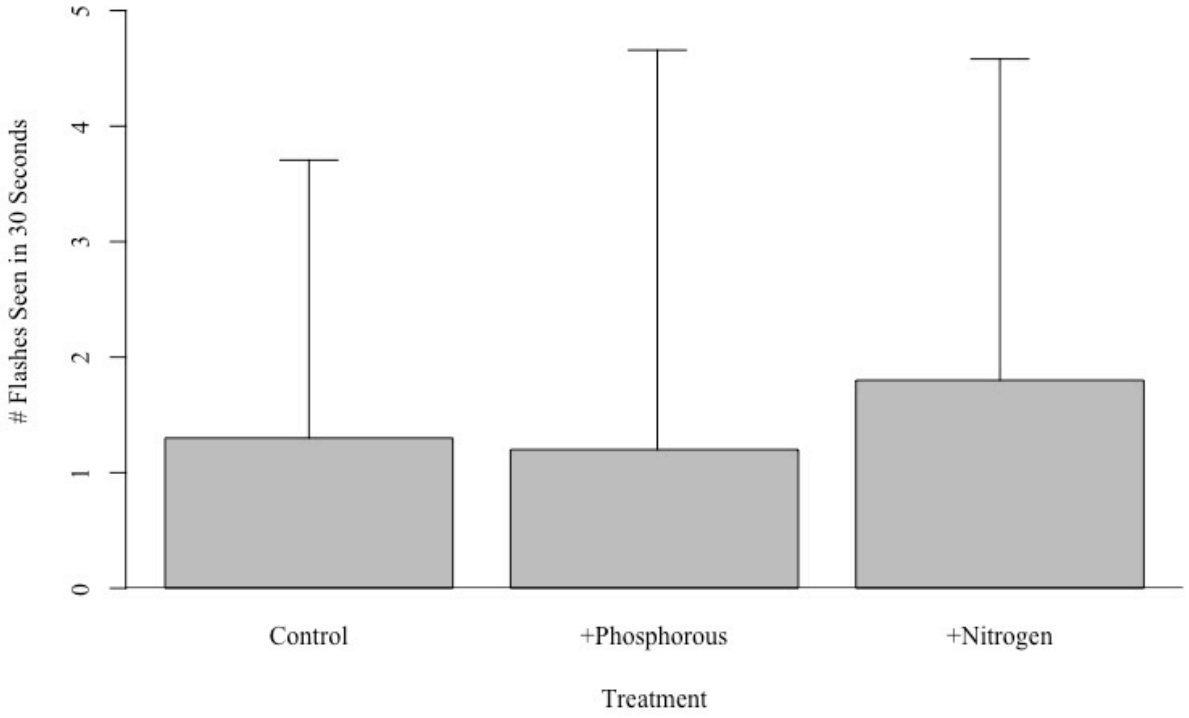
Figure Captions

Figure 1. Bar graph of the mean number of bioluminescent flashes seen in 10 replicates each of a normal seawater control treatment, normal seawater plus nitrogen, and normal seawater plus phosphorus. Flashes were counted in a 30-second period of stirring each of the samples. Error bars are to +1 standard deviation.

Figure 1.


Flashes Seen in 30 Seconds
0
1
2
3
4
5
Control
+Phosphorous
+Nitrogen
Treatment

Size in comparison to territory protection in the Cortez Damselfish (*Stegastes rectifraenum*) in the Gulf of California Mexico

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Size in comparison to territory protection in the Cortez Damselfish (*Stegastes rectifraenum*) in the Gulf of California Mexico

Abstract:

Observing behaviors of many different organisms is important as it allows us to observe those organisms natural behaviors and environment. This can be done in two ways, one being in a lab where we remove an organism from the natural environment and observer their behaviors in a controlled setting and the other being in the natural habitat. In the natural habitat, we are able to observe more real-life behaviors that an organism may do after it is acclimated to my presence. The Gulf of California, also called the Sea of Cortez, was analyzed in Pichilingue, Baja California Sur. In the Gulf of California, an endemic species called the Cortez damselfish (*Stegastes rectifraenum*) is found. The Cortez damselfish is a territorial herbivore that feeds on algae on rocky substratum. Both the male and females defend territories surrounding large rocks within the water column. Within the Sea of Cortez, Cantamar beach was used as the study site. At Cantamar beach it is common to see tourists and divers interact with the Cortez damselfish often. This would allow us to ask the question of how does the size of the Cortez damselfish effect how far it will allow an observer to get before it removes itself from the interaction? The methods that were used were snorkeling out to the point at Cantamar beach and observing damselfish for an hour by trying to go near them and observing how close I could get to the fish using a transect line. It was found that there was no correlation between the relative size of the fish and how close the observer could get to it. Further studies could be examined such as looking at the depth of the water column and algal growth and how close I could get to the Cortez damselfish.

Keywords: Cortez damselfish, Behavior, Pomacentridae, Gulf of California, Cantamar Beach.

Resumen

Observar el comportamiento de organismos diferentes es importante ya que nos permite entender comportamientos naturales. Esto se puede hacer de dos maneras, una en un laboratorio donde se observa un organismo y su comportamiento y el otro en el hábitat natural. En el hábitat natural, somos capaces de observar los comportamientos de manera más real, como los comportamientos que un organismo puede tener cuando se aclimata a mi presencia. El estudio fue realizado en Pichilingue, ubicado en El Golfo de California, también llamado el mar de Cortés, Baja California Sur, México. En el Golfo de California se encuentra una especie endémica llamada Jaqueta de Cortez (*Stegastes rectifraenum*). La Jaqueta de Cortez es un herbívoro territorial que se alimenta de algas en sustrato rocoso. Tanto los machos como las hembras defienden territorios que rodean grandes rocas dentro de la columna de agua. En el mar de Cortez se encuentra la playa de Cantamar. En la playa de Cantamar es común ver a muchos turistas y buceadores que interactúan con el Jaqueta de Cortez. Esto nos permitiría formular la pregunta siguiente pregunta: ¿Cómo influye el tamaño de la Jaqueta de Cortéz en la distancia que puede mantener el observador de ellas? El método utilizado fue esnorquelar en la playa de Cantamar y observar a la Jaqueta de Cortéz durante una hora tratando de acercarse a ellos, se midió la distancia entre el pez y el observador usando una línea de transecto. Se determinó que no existe una correlación entre el tamaño relativo del pez y la distancia del observador. Se recomienda realizar más estudios, utilizando otros parámetros como: la profundidad de la columna de agua, el crecimiento algal, y que tanto se puede acercar a la Jaqueta de Cortéz.

Palabras claves: Jaqueta de Cortez, comportamiento, Pomacentridae, Golfo de California, playa Cantamar.

Introduction:

Behavior is the way in which an organism interacts with the environment, its own species and other organisms. Many of those behaviors are important to how an organism survives within its environment. Observing behavior is important as it allows scientists to investigate their world and observe animals in their environment. These behaviors can determine how and if fish school or not with similar species or with other species. Behavior can be observed in many ways. It can be observed in a lab or in the field. In the lab, behavior is observed by removing an environment from its natural environment into a simulated natural environment with the controls and manipulations that are needed to understand their behavior. In the field, we observe behavior by snorkeling, diving and using boats to obtain data on marine animals. In other environments we go into their natural environment and observe. By observing them in their natural habitat, we are able to see the true behaviors they have after an acclimation period.

The Gulf of California is located within the Baja California Peninsula off the Eastern Pacific. The Gulf of California is also called the Sea of Cortez. It is home to a diverse amount of biodiversity over the spread of 760-mile-long and average of 95 miles wide gulf (The Editors of Encyclopedia Britannica 2013). Do to this span of area, it allows for many diverse ecosystems to form. Roughly 271 species are found in the reefs of the Gulf of California in Baja California Sur. Within those, only seventeen percent of the fish are endemic (Arreola 2002). Within those endemic species the Cortez damselfish is found.

The behavior of many fish can be looked at while they are nesting. Reproductive behaviors are understudied in the Gulf of California (Lobel P. S. 1978). In reproductive success, parental care is a part of how offspring succeed and thrive. Parental care is defined as “any

investment by the parent in an individual offspring that increases the offspring's chance of surviving and hence reproductive success, at the cost of the parent's ability to invest in other offspring". (Wisenden B. D. 1999). Many fish though, have one parent that takes care of the embryos. This is called alloparental care. In the case of the family Pomacentridae, the male will guard the nest before spawning and by doing so they are protecting future embryos. When they defend a nest, they are also showing a female they are able to protect her future offspring. For this family, the female only courts with the males and then she leaves after laying eggs. For female fish this is common. For males, many fish protect the nests as well. The Cortez damselfish is one of those fish that have the relationship that the female has no parental care and the male guards the nest.

For this study the Cortez damselfish (*Stegastes rectifraenum*) was of interest. The damselfish is within the family Pomacentridae and is one of many groups of marine fishes found in tropical and temperate waters. The Pomacentridae family has 340 species within it spread across the world (Moreno-Sánchez, Xchel G., et al. 2011 Referenced in Allen 1991 and Nelson 2006). This family of fishes are one of the most abundant fish to be found on rocky areas and reefs (Rosalía Aguilar-Medrano R. et al, 2015). This allows for a great study subject. The Cortez damselfish, *S. rectifraenum*, is also endemic to the Mexican Pacific and is found from Puerto Peiiasco to Bahia Magdelena. Both the female and male adult fish will defend feeding territories. (Thomson et al., 1979). This is interesting as other fish that have been understood, seem to have males defend territories and only during reproduction such as the Sergeant major (*Abudufdef troschelli*). These Cortez damselfish are herbivorous. Do to these fish being known to be herbivorous, they are able to regulate algal growth in these territories which may determine which algae grow on the substrate they are found in (Rosalía Aguilar-Medrano R. et al, 2015).

Also some studies say that “Cortez damselfish should be considered as an omnivorous species and mainly a benthic feeder.” (Moreno-Sánchez, Xchel G., et al. 2011) This allows for us to then look at their distribution. The distribution of the damselfish is between one and ten meters on rocky substrate (Rosalía Aguilar-Medrano R. et al, 2015). The damselfish is also extremely intolerant to much activity around its nest. It will within seconds remove another fish or an object from its territory (Rosalía Aguilar-Medrano R. et al, 2015). The damselfish has been recorded to be aggressively territorial (Moreno-Sánchez, Xchel G., et al. 2011). Also, population densities of the Cortez damselfish have reached about one adult/m^2*S. (pg 21) (Hoelzer, G. A. 1989).

Our study site was Cantamar Beach. This beach is located in Pichilingue, Baja California Sur. This beach is home to many different diverse species like the Cortez damselfish. They are also home to many algae and coral as well. Cantamar is an area of beach that has a large amount of human interaction. Snorkels are seen in the water along with children near the shoreline. Cantamar is heavily trafficked by tours and diving expeditions which affect the marine environment. This beach is remote but due to having a hotel on the beach is it going to be busy. The fish are used to having interactions with several species of fish as well observers, but they still seem to move away from me. I wondered how does the size of the Cortez damselfish effect how far it will allow an observer to get close to them? It was hypothesized that as the fish got larger in size it will remove itself from the area of the observer slower than a smaller fish. This would be due to territoriality increasing as size does in this species of fish.

Methods:

In this experiment, many techniques were used to investigate and analyzed these observations. Over the course of a two days, Cantamar beach was analyzed for data. Cantamar

beach was sampled on the 20th of July 2018 through the 21nd. Collections from each site at the beach were taken for a period of one hour. Estimation was used to allow for data to be collected over the course of the entire hour of observation time. Over the course of an hour, snorkeling was done to many areas of the beaches to analyze the whole area to the best of the time constraint. A transect was used to estimate the distance, that I the observer, could get to the Cortez Damselfish. As I approached the organism, I allowed an approximate 30 second acclimation time to allow the fish to realize I was there, and then I approached. As I approached, I stuck one of my arms out with the transect line and measured how close I could get with my best estimate on the line. When the fish began moving away I recorded my data on a dive slate to keep track of my data points over each site at the beach. A total of 45 samples were collected. After data collection was done, graphs were made using Excel and the R program to analyze the data. A T test was used to analyze the data.

Results:

A total of forty-five observations of relative size of the Cortez damselfish and how close the observer, I, could get to the organism was collected over a two-day period. On average a large damselfish would allow the researcher to get 0.55 meters close over a total of twenty-seven observations. With the large fish, there was a standard error of 0.083 meters (Figure 1). For the small damselfish, the average distance for closeness was 0.42 meters with a standard error of 0.071 meters (Figure 1). Using the T test, the T value was 1.22, the p value was 0.11 and the degrees of freedom were 41.61. This p value shows that the correlation between size of Cortez damselfish and how close an observer can get to the fish is insignificant.

Discussion:

There was no apparent correlation between the relative size of the Cortez damselfish and how close I, the observer, was able to approach the fish. This was done by analyzing a T test which gave us the value of T being 1.22. This also gave us a p value of 0.11. The alpha value in this case is 0.05. As the p value is higher than 0.05, the alpha, then the results of not statistically significant to allow us to say there is a correlation. The hypothesis stating there is no correlation or a negative correlation in supported. This may be due the sampling technique and how fast the observer approached toward the fish.

The area of study, Cantamar Beach, is remote from La Paz. La Paz is a central area for tours and tourism Along Pichilingue, near Cantamar, many differing species are found and allows people to migrate here and take tours here as well. Marketing of Cantamars services is also shown in La Paz which allows for more people to be interested in this area. This may be why fish allow people to go near them, but they are still natural animals that move away when provoked. Fish are well accustomed to observers being around. I tried to go to places around the point on Cantamar that allowed for both places with interaction points and those farther away from those areas. Cantamar beach is a great place to understand how fish interact with humans.

It was odd observing these damselfish, with other fish. They were territorial to other fish and sometimes to me. This was rare towards the observer in comparison to other damselfish in the area. S. rectifraenum are located in many rocky crevices for defense and refuge from other competitor fish (Moreno-Sánchez, Xchel G., et al. 2011). Their territorial behavior and feeding area can be approximated to 1.7m2 per fish (Montgomery, 1980 Referenced in Moreno-Sánchez, Xchel G., et al. 2011). This is a range that they defend vigorously from other competitor species

(Thomson et al., 2000). Similar behavior is reported for many other damselfish species (Hata & Kato, 2002). This is seen in the reproducing Abudufdef species, also known as the Panamic Sergeant major. This fish turns a dark blue in coloring and will chase the other Abudufdef males away from their nests. This is related as they are territorial but only when they are reproducing. The Cortez damselfish is a territorial species all year round with both sexes doing the same activities.

New studies could be produced from this study. Studies discussing how depth may affect the size of the Cortez damselfish are plausible. This would potentially help understand algal growth as well due to its growth in varying depths. Algae needs to grow near the surface but how far may it grow down closer to the rocky substratum. This would be the question posed in a future study. Studies understanding the diet of the Cortez damselfish have occurred already, but correlating depth has not been researched. This damselfish is known the herbivorous but also omnivorous (Moreno-Sánchez, Xchel G., et al. 2011). We could look at the contents of the stomach and see what is more prominent, animals or plants. It could then be looked at how far each of the stomach contents is found and where the fish are located, near the plants or near the animals. When we do this, we would use the same methods as before, we would use a transect to see where the fish are at. We would then analyze the species of algae around and also take water samples and study them under a microscope to see what plankton are in the water column.

Another study that could be produced is one that analyzes how their territorial behavior affects reproduction. These fish are intolerant to many disturbances in their habitat. I questioned how that would work for reproduction. I propose that we analyze their habits before, during and after reproduction events to see how their behavior changes over time in the sense of territoriality. I would do this by analyzing how close I could get to the fish and if they react

aggressively more during reproduction events. We could do this by using a transect as we have in this study along with the dive slate. Overall many studies could come out of this study.

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Figure Legend:

Figure 1: In this figure, the average means of both the small and large damselfishes' ability to let the observer, I, get close to them. The errors were relatively small. For the large damselfishes, the average was 0.55 meters and the standard error were 0.083 meters. For the small damselfishes, the average was 0.42 meters and the standard error were 0.071 meters.

Figures:

Figure 1

Algal growth, in *Sargassum sinicola*, and total algal density over a possible phosphorus gradient around Bahía de La Paz, Mexico

Keywords: limiting nutrients, macroalgae, phosphorus impact, thallus height, nutrient cycling

Word Count: 3324

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Abstract

Photosynthetic marine organisms require key nutrients, such as carbon, nitrogen, and phosphorus, to maximize growth. These nutrients are limited in marine environments and form natural gradients due to currents. Macroalgae use phosphorus in the form of phosphate, to maintain photosynthetic rates. We observed how *Sargassum sinicola* growth and total algal density were influenced across a phosphorus gradient. We knew there was a phosphorite mining compound in Bahía de La Paz, BCS, Mexico, which could be a source of extra phosphorus into the marine environment. As the phosphorus is moved along with ocean currents, it can create a gradient in which many species can take up phosphorus. We hypothesized that waters with increased phosphorus will have more algal growth, measured as thallus height and algal density. As the concentration of phosphorus diminishes along with the currents, we expected shorter thalli and less algal density. To test this hypothesis, we measured the thalli length (cm) and density (using quadrats), and phosphate concentrations at six locations around Bahía de La Paz. We used phosphate test kits to determine the phosphate concentrations at each location we sampled using phosphate as our phosphorus indicator. We found two concentrations of phosphate (0.25 and 0.3 mg/L) rather than the phosphate gradient we had expected, with San Juan de la Costa was the only site with 0.3 mg/L. Regardless, we did find that in the higher phosphate concentration, thalli height of *S. sinicola* (R^2 = 0.0302, P = 0.029) and total algal density (R^2 = 0.2573, P = 0.0021) were larger. These results are likely caused by the recycling of phosphorus due to upwelling, nitrogen concentrations in San Juan de la Costa that were not tested, or grazing. This indicates that increased phosphorus does allow higher algal growth.

Resumen

Los organismos marinos fotosintéticos requieren nutrientes clave, como el carbono, el nitrógeno y el fósforo, para maximizar el crecimiento. En los ambientes marinos estos nutrientes son limitados y forman gradientes naturales debido a las corrientes. El fósforo es utilizado por las macroalgas en forma de fosfato, para mantener tarifas fotosintéticas. Observamos cómo el crecimiento de *S. sinicola* y la densidad algal total fueron influenciados a través de un gradiente del fósforo. Sabíamos que había un complejo minero de

fosforita en la Bahía de la Paz, BCS, México, el cual podría ser una fuente de fósforo adicional en el medio marino. A medida que el fósforo se mueve junto con las corrientes oceánicas, puede crear un gradiente en el cual muchas especies pueden aprovechar el fósforo. Presumimos que las aguas con mayor concentración de fósforo tienen un mayor crecimiento, medido como altura del talo y densidad algal. Como la concentración de fósforo disminuye junto con las corrientes, esperábamos un talo más corto y menos densidad de algas. Para probar esta hipótesis, se midió la longitud del talo (cm), la densidad algal (utilizando cuadrantes), y las concentraciones de fosfato en seis localidades alrededor de la Bahía de La Paz. Se utilizó un kit de prueba para determinar las concentraciones de fosfatos en cada ubicación muestreada. Se encontraron dos concentraciones de fosfato (0,25 y 0,3 mg/L) en lugar del gradiente de fosfato que esperábamos. San Juan de la costa fue el único sitio con 0,3 mg/L. Independientemente, encontramos que para la concentración más alta de fosfatos, la altura del talo de *S. sinicola* (R^2 = 0,0302, p = 0,029) y la densidad de algas totales (R^2 = 0,2573, p = 0,0021) eran más grandes. Estos resultados son probablemente causados por el reciclaje del fósforo y su reutilización en la columna de agua, las concentraciones de nitrógeno en San Juan de la Costa que no fueron probadas, o el pastoreo. Esto indica que el aumento del fósforo permite un mayor crecimiento de las algas.

Introduction

While organisms require different nutrients to survive, there is a universal need for select compounds: carbon, nitrogen, and phosphorus (Redfield 1958). Concentrations of these nutrients vary throughout ecosystems, leading zone of nutrient limitation (Ptacnik *et al*. 2010). Seasonal and spatial variation can change which nutrient is most limiting; in spring, phosphorus and silicate are found to be limiting in coastal marine ecosystems, while summer leads to nitrogen limitations (Conley *et al*. 2009). While concentrations of nutrients can cause limitation, there are areas in which co-limitation dominates (Ptacnik *et al*. 2010). Inquiries over which nutrient, nitrogen or phosphorus, is most limiting are still being debated, however Lapointe (1986) found that for algal growth phosphorus was more limiting.

Phosphorus is a highly utilized element, being used in many components of biological processes, such as DNA, RNA, ATP, and energy transfers (Conley *et al*. 2009). Many forms of phosphorus can be used by marine organisms, including inorganic phosphate (Graham *et al*. 2016). Primary producers use phosphorus to maintain photosynthetic rates and form organic matter (Redfield 1958; Graham *et al*. 2016). The demand for growth regulates the internal phosphorus concentrations of many organisms (Auer and Canale 1982). Summer production is supported by recycling of phosphorus throughout the upwelling of costal ecosystems (Martínez-López *et al*. 2001; Conley *et al*. 2009). Nutrient cycling is heavily influenced by the proximity to land, allowing for terrestrial run-off from erosion and weathering (Schaffelke and Klumpp 1998). Increased human activity around the Gulf of California has increased pollution discharge into coastal waters (Patrón-Prado *et al*. 2010). Around Bahía de La Paz, phosphorite mining has been introducing sediments to the water column, allowing for large algal beds to bloom (Rodriguez-Meza et al. 2007; Patrón-Prado *et al*. 2010). The enrichment of sediments is due to the St. Gregory rock formation (Riley 1989), consisting of many marine sedimentary rocks (Galli-Olivier 1993).

Macroalgae are an important part of many ecosystems, such as temperate rocky intertidal and tropical reef communities (Schaffelke and Klumpp 1998). Macroalgae interact with many trophic levels, providing shelter and a food source (Lapointe 1986; Pacheco-Ruíz *et al*. 1998; Graham *et al*. 2016). Grazers, such as sea urchins, are known to eat large amounts of brown algae, altering the structure of macroalgal communities (Van Alstyne 1988). The biomass of algal communities change with phosphorus uptake and seasonal variation (Auer and Canale 1982; Rodríguez-Montesinos *et al*. 2008). In environments rich in nutrients, species of *Sargassum* are able to become nutrient-sufficient (Schaffelke and Klumpp 1998).

The distribution, seasonal variation, and light availability limit growth of *Sargassum* spp. (Rodríguez-Montesinos *et al*. 2008; Graham *et al*. 2016). *Sargassum sinicola*, living in the subtropical zone, has been found throughout the Gulf of California (Pacheco-Ruíz *et al*. 1998). The seasonal variation of the Gulf of California leads to changes in thallus size, measured from holdfast to the tip of the blade

(Rodríguez-Montesinos *et al*. 2008). *S. sinicola* has high rates of nutrient uptake, allowing for phosphorus and other compounds to be utilized, increasing growth (Patrón-Prado *et al*. 2010).

Geologic foundations created the shelf of Bahía de La Paz, allowing for formations of phosphorite (Galli-Olivier *et al*. 2006). The phosphorite mine on the coast of San Juan de la Costa is a possible source of phosphate into the marine environment (Patrón-Prado *et al*. 2010). We expected water currents to move the introduced phosphate along the coast, creating a phosphorus gradient. As the concentration of phosphorus diminishes due to currents, we expected the growth of algae to decrease. We sought to assess how algal growth, in *S. sinicola*, and total algal density were influenced by a possible phosphorus gradient around Bahía de La Paz. We hypothesized that in areas with increased amounts of phosphorus there will be larger algal growth, shown in thallus height of *S. sinicola*, and greater algal density.

Methods

Study Site

Observational snorkel surveys were done at six locations around Bahía de La Paz: San Juan de la Costa at the phosphorite mining compound, El Sauzoso, El Califín, Cantamar, Balandra, and Calerita (Figure 1). The locations were chosen for the proximity to the phosphorus mining plant in San Juan de la Costa, with two sites being indiscriminately chosen after leaving San Juan de la Costa. The random selection of El Sauzoso and El Califín were chosen for the availability and access from the road. Each location had semi-rocky substratum that algae would use for holdfast attachment. Algal reefs were found within thirty meters of the shore, where *S. sinicola* was present. Upon arrival at each location, observations were made looking for algae, including *S. sinicola*.

Data Collection and Analysis

Once the species had been identified at each location, water samples were collected over the area of study, in plastic bottles for a total of six samples. The bottles were rinsed three times, prior to samples

being collected. After collection, bottles were placed in a refrigerator until testing. Phosphate concentrations were used as an indicator of phosphorus concentration. To test the phosphate concentrations, Nutrafin phosphate test kits were used, following the ascorbic acid modified method. To assess the precision of the test kit and consistency of the drops, a water sample was collected the morning of testing at the Club Hotel Cantamar. The kit had a three-solution system, including antimony potassium tartrate and 10 % sulfuric acid, each labeled as PO_4 #1, 2, or 3. The glass test tube in the kit was rinsed three time with each water sample before being filled with 5 mL of sample, using a plastic pipette. Three drops of PO_4 #1 were added to the solution, then the cap was replaced and the solution was mixed well. Three drops of PO_4 #2 were then added to the solution, capped, and thoroughly mixed. The PO_4 #3 container was held at a 45° angle for approximately 3-5 seconds, allowing for a single drop of solution to enter the vial. The vial was then capped, mixed, and sat for 2 minutes. After the 2 minutes, the solution was then held to the color indicator chart, provided in the test kit, to assess the concentration of phosphate in the vial. Measurements of phosphorus were blind, and the individuals assessing the color of the solution were unaware of the site that was being measured. Values were assigned in triplet of the sample test. After the precision of the equipment were understood, samples were tested in random order in triplet. For values that differed over the three tests, we made two more measurements, with the most common concentration used for analysis.

S. sinicola thalli height were indiscriminately measured (cm) on 20 individuals at each location and a total of 120 specimen, using a tape measure. The total height of the thallus included holdfast, stipe, and blade. To minimize bias, thalli were measured in multiple depths and proximity to the shore. A quadrat (m^2) was constructed out of PVC pipe. The pipe had an internal diameter of 1.4 cm. At each site, we measured the density of all macroalgae in five quadrats, for a total of 30 quadrats. To minimize damage to the algae and other organisms, the quadrat was held above the substratum while density was counted. All organisms were handled with care during measuring. The average of all five quadrat measurements were taken to represent the total algal density of a m^2 area.

Data were analyzed using linear regression to test if there was a relationship between thallus height and phosphate concentration as well as between total algal density and phosphate concentration. Analyses were done in R Studio, version 1.1.442 (R Core Team. 2018).

Results

We measured only two phosphate concentrations at our six sites (0.25 and 0.3 mg/L), however, our results showed a significant relationship between algal height and phosphate concentration and between algal density and phosphate concentration. The higher concentration was only found in San Juan de la Costa at the phosphorite mining compound. Our results showed significant relationship between phosphate concentration and thallus height ($R^2 = 0.0302$, $P = 0.029$) (Figure 2). *S. sinicola* was found to be larger in locations with higher concentrations of phosphate (0.3 mg/L). We also found a significant relationship between phosphate concentration and total algal density ($R^2 = 0.2573$, $P = 0.0021$) (Figure 3). Total algal density was found to be greater in locations with higher concentrations of phosphate.

Discussion

Despite the small change in concentration after only 2 km, the thalli height of *S. sinicola* were smaller than those found at the phosphorite mining compound. These results do support our hypothesis that higher concentrations of phosphorus would lead to higher algal growth, seen in thallus height. Our results also showed that the area with higher concentrations of phosphorus did have greater overall algal density, supporting our second hypothesis.

Throughout all six locations we sampled, we only found two phosphate concentrations along the coast, which was contradictory to our prediction that there would be a phosphorus gradient flowing south of the phosphorite mining compound. It is likely that the currents in Bahía de La Paz are not influencing the dispersal of phosphorus from the phosphorite mining compound or that the mining compound has less impact of the concentrations of phosphorus in the coastal waters. While currents may not have enough influence to form a phosphorus gradient, it is possible that the summer winds from the southeast and

upwelling may impact the results (Martínez-López *et al*. 2001). The forms of phosphorus, phosphate and phosphorite, may have different signatures in the water column, so it is possible that our phosphate test kit missed some of the phosphorus that was in the water (Rodriguez-Meza *et al*. 2007). We sampled along the coast at each location, however Schaffelke and Klumpp (1998) found that the distance to land can have an impact on the cycling of nutrients, thereby changing what nutrients are available to the algae. To test how cycling is affecting the nutrient uptake of algae, we would like to sample algal density at multiple distances from shore.

These results suggest that algae, including *S. sinicola*, are able to utilize small increases in phosphorus and maintain growth rates. Lapointe (1986) found that phosphorus was most limiting, however Graham *et al*. (2016) and Ptacnik *et al*. (2010) suggest that co-limitation of these two elements can have greater effects. We would be curious to see what nutrient levels optimize *S. sinicola* growth, allowing for nutrient-sufficient status to be achieved (Schaffelke and Klumpp 1998). For this, we would be interesting in testing the nitrate levels in the water as well as finding out how much nitrogen is taken up by algae before being limited by another nutrient.

Throughout our study we noticed the density of algae decreased with distance from the phosphorite mining compound. These observations and our hypothesis were supported, showing that in higher phosphate concentrations, algal density increases. Our observations, while we tried to cover the full range of the algal beds, only contained small portions of the bed at each site. To assess the full size and density of the algal beds, biomass and chlorophyll content could be utilized, using satellite imagery. As the biomass of algal beds change with nutrient availably and seasonal variation (Auer and Canale 1982; Rodríguez-Montesinos *et al*. 2008), observations over the full year could help ascertain patterns of algal growth. Further examinations would assist in understanding how these reefs and beds are used by other organisms, either as shelter or a food source (Pacheco-Ruíz *et al*. 1998). Sea urchins are known to graze on brown algae (Van Alstyne 1988). During our snorkel surveys, we noticed few urchins near San Juan de la Costa, while locations on the other side of the bay have large populations of urchins. From

these observations, we would be interested in researching if the patterns we found are heavily influenced by grazers.

For future studies, we recommend examining which nutrient causes higher limitation in *S. sinicola*, nitrogen, phosphorus, or co-limitation of the two elements (Lapointe 1986; Ptacnik *et al*. 2010; Graham *et al*. 2016). This could be tested in the laboratory by adding solutions of nitrogen, phosphorus, and a mix of both to ambient seawater. We have seen that *S. sinicola* does respond to increased phosphorus concentrations, however it would be interesting to see how the algae is affected with increased nitrogen. These studies would help us determine the effects of co-limitation of nitrogen and phosphorus on *S. sinicola*, as well as confirming the need for phosphorus. The results of further studies would indicate what nutrients are limiting to *S. sinicola* and help explain the uptake and allocation of phosphorus in macroalgae.

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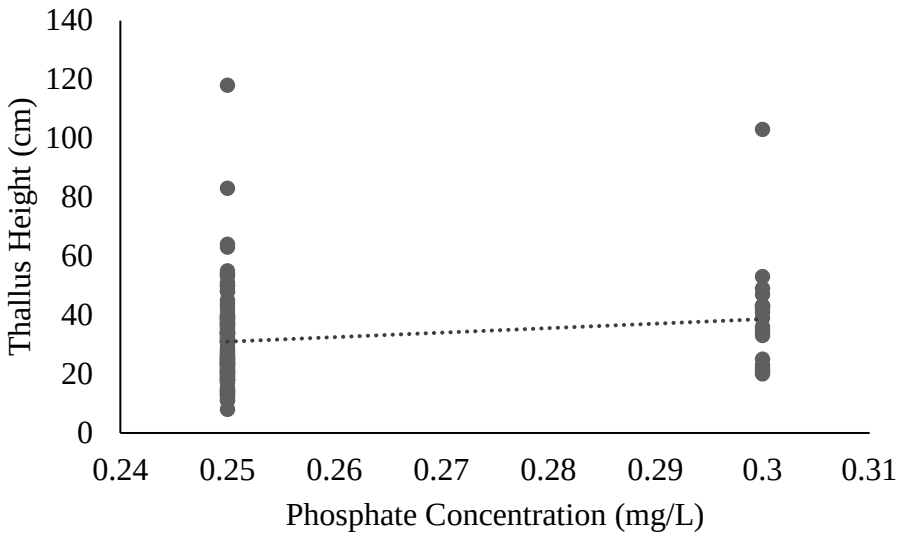
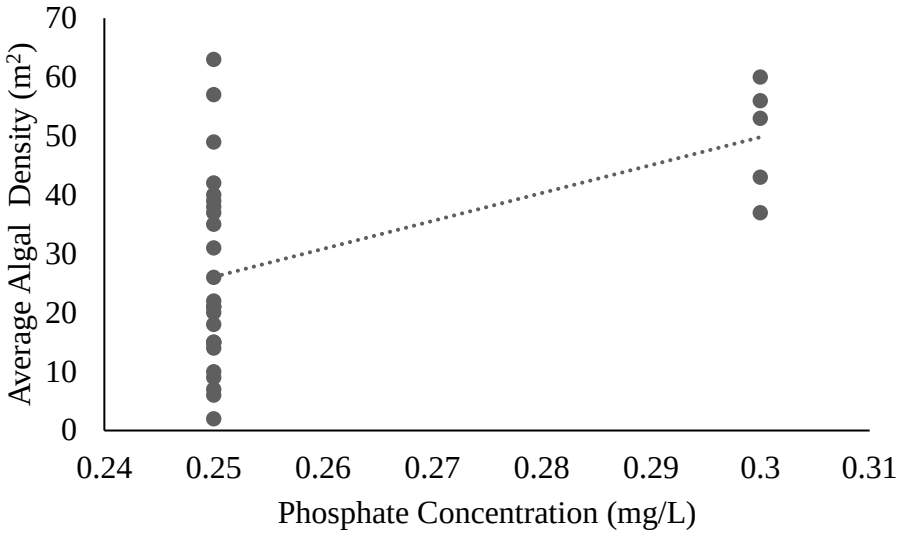
Figure 1. Map of locations sampled. The numbers represent the locations sampled: 1. San Juan de la Costa phosphorite mining compound, 2. El Sauzoso, 3. El Califín, 4. Cantamar, 5. Balandra, 6. Calerita.

Figure 2. A scatterplot of thallus height of *Sargassum sinicola*, given the phosphate concentration. Data was analyzed in R using a linear regression, $R^2 = 0.0302$, $P = 0.029$.

Figure 3. A scatterplot of total algal density, given the phosphate concentration. Data was analyzed in R using a linear regression, $R^2 = 0.2573$, $P = 0.0021$.


Isla Espirito Santo
1
2
3
4
5
6
El Cajete
El Quelele
La Paz
El Centenario


140
120
100
80
60
40
20
0
Thallus Height (cm)
0.24
0.25
0.26
0.27
0.28
0.29
0.3
0.31
Phosphate Concentration (mg/L)


70
60
50
40
30
20
10
0
Average Algal Density (m2)
0.24
0.25
0.26
0.27
0.28
0.29
0.3
0.31
Phosphate Concentration (mg/L)

The effects of climate change on bioluminescent activity: a look at the effects of temperature on bioluminescent activity in the bay of La Paz.

Key words: temperature, physiology, tolerance, enzymes, dinoflagellates, bacteria, zooplankton

Word count: 2,769

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Abstract

As climate change increases global temperatures, it becomes more important to understand the effects of increasing temperatures on marine environments and organisms. Increasing temperatures change physical ocean processes and ocean chemistry and create environments that no long sustain organisms. Increasing temperatures also impact the physiology of organisms by increasing the rates of biochemical reactions, namely those involving enzymes. Bioluminescent organisms generate their own light to aid in survival through an energetically costly enzymatic reaction involving the enzyme luciferase and the substrate luciferin in the presence of oxygen. In this study, I wanted to look at the effects of temperature on bioluminescent activity, and I hypothesized that as temperature increased so would activity. To test my question, I collected surface water bioluminescent organisms from the Bay of La Paz in Baja California Sur, on two separate nights, and either kept them at ambient temperature or put them in a warm water bath for 10 minutes. Samples were poured over a mesh net table, and I counted the flashes of bioluminescence on the mesh net as I poured. I repeated these steps 10 times for a total of 10 trials. My data were analyzed in R to look for differences in bioluminescent activity between the ambient and warm treatments. I found no significant differences in activity between the two treatments (t_9= -1.83, p= 0.051), but I did notice that bioluminescence at warm temperatures were dimmer than those at ambient temperatures, and there were less flashes observed the second night of sampling. Overall, I concluded that there was no effect of temperature on bioluminescent activity in the Bay of La Paz, and more research is necessary to fully understand the effects of climate change on bioluminescent organisms.

Key words: temperature, physiology, bacteria, dinoflagellates, zooplankton, tolerance, enzymes

Resumen

A medida que el cambio climático aumenta las temperaturas globales, se vuelve más importante entender los efectos de las temperaturas crecientes en los ambientes y organismos marinos. El aumento de las temperaturas cambia los procesos físicos del océano, tales como las corrientes y los patrones de la reactivación, que pueden ser perjudiciales para los organismos que dependen de las corrientes para la dispersión y el crecimiento de los nutrientes. El aumento de las temperaturas también impacta la fisiología de los organismos aumentando las tasas de reacciones bioquímicas, a saber, las que involucran a las enzimas. Los organismos bioluminiscentes generan su propia luz para ayudar en la supervivencia a través de una reacción enzimática energéticamente costosa que implica la enzima luciferase y el substrato lucifern en presencia de oxígeno. En mi estudio quise mirar los efectos de la temperatura en la actividad bioluminiscente, porque la temperatura podría potencialmente aumentar las tasas de las reacciones enzimáticas que tienen lugar para producir luz. Presumimos que como la temperatura aumentó, la actividad bioluminiscente también. Para probar mi pregunta, recogí organismos bioluminiscentes de agua superficial de Cantamar Playa Pichilingue, Baja California Sur en dos noches separadas y las guardó a temperatura ambiente o las puso en un baño de agua caliente por 10 minutos. Las muestras fueron vertidas sobre una red del acoplamiento y conté los destellos de la bioluminiscencia en la red de la malla mientras que vertí. Repetí estos pasos 10 veces para un total de 10 ensayos. Mis datos fueron analizados en R usando una prueba t emparejada de dos muestras para buscar diferencias en la actividad bioluminiscente entre los tratamientos ambientales y tibios. No encontré diferencias significativas en la actividad entre los dos tratamientos, pero noté que la bioluminiscencia a temperaturas cálidas era más tenue que aquellas a temperaturas ambiente, y hubo menos destellos observados la segunda noche de

muestreo. En general, llegué a la conclusión de que no había efecto de la temperatura en la actividad bioluminiscente en Cantamar Playa.

Palabras clave: cambio climático, fisiología, bacterias, dinoflagelados, zooplancton, tolerancia

Introduction

As global climate change steadily increases oceanic temperatures, it is becoming exceedingly important to understand the impact these increases have on marine ecosystems. Climate change is brought on by increases in greenhouse gas concentrations in the atmosphere that lead to increases in global temperatures and has many consequences for ocean environments, both physically and chemically (Doney et al. 2011). Physical repercussions to changes in temperature include changes ocean circulation and the prevention of upwelling, both processes important for the dispersal of organisms and nutrient availability (Doney et al. 2011; Harley et al. 2006). Changes in dispersal of organisms and nutrients can ultimately lead to the death of species that rely on currents to take them to locations where they can thrive, and species that rely on upwelling to bring nutrients from deep water to the ocean's surface (Doney et al. 2011; Harley et al. 2006). The chemical impacts of climate change on the ocean include decreasing the amount of dissolved oxygen available for organisms, as well as overall decreases in pH due to higher concentrations of CO_2 being transferred from the atmosphere to the ocean (Doney et al. 2011; Harley et al. 2006). Depleting oxygen concentrations can suffocate marine organisms and shifts in pH can also kill organisms and prevent the formation of calcium carbonate shells that many species rely on (Doney et al. 2011; Harley et al. 2006). While changing temperatures have major impacts on marine ecosystems, they can also have significant impacts on marine organisms.

Many marine species are only adapted to survive in specific ranges of temperature and increasing temperatures can expose these species to conditions outside of their range of tolerance. On a fundamental level, increases in temperature increase molecular motion and kinetic energy within a system (Doney et al. 2011; Wilmer & Stone 2005). Increases in temperature can increase the rates of biochemical reactions, commonly those involving enzymes, and be detrimental to the physiological processes that sustain organisms (Doney et al. 2011). These increases in the rates of biochemical reactions and can affect rates of cellular diffusion, enzymatic reactions, oxygen consumption, and can even change the viscosity of cellular membranes (Wilmer & Stone 2005). While many organisms can adapt to temporary shifts in temperature, prolonged exposure to temperatures outside of their range of tolerance can lead to death, as it is a major energy expenditure to maintain these increases in biochemical rates (Doney et al. 2011; Wilmer & Stone 2005). Increases in temperature may be especially problematic for organisms that rely on more complex enzymatic reactions, such as bioluminescence.

Bioluminescent organisms are organisms that produce their own light through internal processes to aid in survival. Light can be used to aid in dispersion, some species of bacteria generate light to attract consumers, so they can propagate in their guts (Zarubin et al. 2012; Nealson & Hastings 1987). Some species use bioluminescence to aid in prey defense by startling and scaring away predators or attracting larger predators to eat the predator of concern (Valiadi & Iglesias-Rodriguez 2013). Light can also be used to aid in prey capture, many species use light to attract prey close to them for an easy snack (Valiadi & Iglesias-Rodriguez 2013). Bioluminescent organisms also play an important role in ocean ecosystems. Bioluminescent organisms might provide light to aid in the survival of organism that classically lack light producing organisms (Kahlke & Umbers 2016). Many bioluminescent species are also primary

producers and an important source of food and energy in the oceans (Valiadi & Iglesias-Rodriguez 2013; Lapota et al. 1988; Nealson & Hastings 1987). Because of their role in oceanic ecosystems, it is important to understand how bioluminescent organisms function and how they might be impacted by climate change.

Generating light is important for bioluminescent organisms to thrive, but it is a sensitive process. Light is generated through an energetically costly reaction involving the enzyme luciferase and the substrate luciferin in the presence of oxygen (Valiadi & Iglesias-Rodriguez 2013). It is possible that in the face of rising temperatures, this enzymatic reaction may be thrown out of balance, creating real problems for the organisms that rely on their own light, but few studies have been done to confirm whether this is true. In this study, I wanted to look at whether temperature effects the bioluminescent activity of surface water bioluminescent organisms. Because increasing temperatures increase molecular interactions and would therefor increase the amount of interactions between luciferase and luciferin, I hypothesized that there would be an increase in the bioluminescent activity as temperatures increase.

Methods and Materials

I collected bioluminescent organisms from the surface waters in La Paz bay, at Cantamar beach in Pichilingue, Baja California Sur. I chose this location because bioluminescent activity was consistent on a near-nightly bases, and there was easy access to the beach for me to collect my samples. Once I collected my samples they were exposed to two different temperature treatments: either an ambient treatment or a warm treatment. After being exposed to the different treatments, I measured and analyzed bioluminescent activity to determine whether there was a difference in activity levels at lower temperatures and activity levels at higher temperatures.

I started collecting my organisms at night, to ensure that the organisms were luminescing for my study. To ensure I got measurable amounts of bioluminescence to carry out my experiment, I collected seawater containing specimens in two 10 L jugs. Once I collected my samples, I brought them up to the beach where they were exposed to one of the two temperature treatments. The jug given the “ambient” treatment was just left out and exposed to air temperatures. For the jug given the “warm” treatment, I brought a cooler down to the beach and filled it with hot water, the 10 L jug was then submerged in the hot water bath to raise the temperature by at least 5° C. Both samples were exposed to their respective treatments for 10 minutes. To randomize my treatments, I numbered the jugs and used a random number generator in excel to determine which jug was exposed to either treatment. This was repeated five times on two separate nights for a total of 10 trials. After the samples were exposed to their treatments, I was able to start collecting data.

I collected my data using a square mesh table held in place by PVC pipes. The samples were slowly poured from the jugs over the mesh to capture and illuminate the bioluminescent organisms. As samples were being poured, I counted flashes from the cells that were luminescing on the mesh net as metrics of bioluminescent activity. In between samples, the mesh net was rinsed so that residual bioluminescence from previous trials weren’t counted towards new trials. These data were then compiled for analysis.

I analyzed my data using a two-sample pairwise t-test in R to look for any differences in bioluminescent activity between the ambient treatment and the warm-water treatments in my study (R Studio Team 2016).

Results

I found no significant differences between bioluminescent activity at ambient temperature and bioluminescent activity at the warm temperature (t_9= -1.83, p=0.051) (Figure 1). However, the bioluminescent activity observed in the warm water treatment was 24.3% greater than that observed from the ambient treatment. I also observed a couple of patterns during my data collection. When looking at the bioluminescence from either treatment, the bioluminescence from the ambient treatment appeared to be brighter, although there was no true way to measure this difference in the context of my experiment. There were also a couple of differences in the measurements that I took between the two nights of collection. On the first night, all my measurements counted at around 100+ flashes per sample. On the second night I counted less than 30 flashes per sample.

Discussion

There are a handful of reasons that my study might not have found significant differences in bioluminescent activity between the ambient and warm water treatments. A previous study of bioluminescence in the Gulf of California found that the presence of bioluminescence is correlated with cooler water temperatures, and another study conducted on bioluminescent fungi found that at increased temperatures bioluminescence ceased for certain species (Lieberman et al. 1987; Weitz et al. 2001). Because I did not identify the species of bioluminescence in my study, it is impossible to know if the warm water treatments were pushing the limits of tolerance for these organisms and therefore lowering the number of bioluminescent organisms observed. The idea that I was pushing the edge of the tolerance zone for my organisms was also supported by the observation that bioluminescence in my warm-water treatments seemed to be dimmer than

those I observed in my ambient treatments, however, this might also be indictive of there being a more accurate way of measuring bioluminescent activity. Past studies measuring the impacts of different variables on bioluminescent organisms have used instruments to measure light intensity (Ramesh et al. 2014; Weitz et al. 2001, Lapota et al. 1988). Perhaps the intensity of light is more impacted by temperature changes than the abundance of light.

The differences in the numbers of bioluminescent organisms I observed between nights may have been related to the relative differences in the position of the moon between nights. One study that looked at the effects of light on bioluminescent marine bacteria found that exposure to natural light inhibited growth and light production, and it is known that bioluminescence ceases during the day either due to photoinhibition or circadian rhythms adopted by organisms (Ramesh et al. 2014; Valiadi & Iglesias-Rodriguez 2013). If the species collected in my samples ceased luminescing in response to photoinhibition, it is possible that the increasing brightness of the moon over the course my data collection resulted in reduced bioluminescent abundance my second night of collection.

While my data provides baseline ideas for the impacts temperature might have on bioluminescent organisms out at Cantamar beach, there is still further research that should be done to look at the overall impacts of climate change on these organisms. Research identifying the bioluminescent organisms present in this study would help to further understand the ranges of temperature tolerance and the impacts of ambient light on these organisms. As previously mentioned, climate change decreases oceanic dissolved oxygen and decreases pH, and the enzymatic reactions that produce bioluminescence require oxygen and might be pH sensitive (Valiadi & Iglesias-Rodriguez 2013; Harley et al. 2006; Weitz et al. 2001). A lab study looking at the impacts of these different variables on bioluminescent organisms could provide a more

comprehensive understanding of the overall effects of climate change on these organisms. Bioluminescence is an important survival mechanism for many organisms so it's increasingly important to understand the impacts all these variables might have on bioluminescent activity. From this study alone, I can only conclude that there are no significant effects of temperature on bioluminescence in La Paz bay, and more research is necessary to fully understand the impacts of temperature and climate change on bioluminescent organisms.

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Figure Legends

Figure 1. A bar graph comparing the differences in average bioluminescent activity between two temperature treatments (ambient and warm). The error bars represent SE, and a t-test found no significant differences in bioluminescent activity between treatments (t_9= -1.83, p= 0.051).

Figures

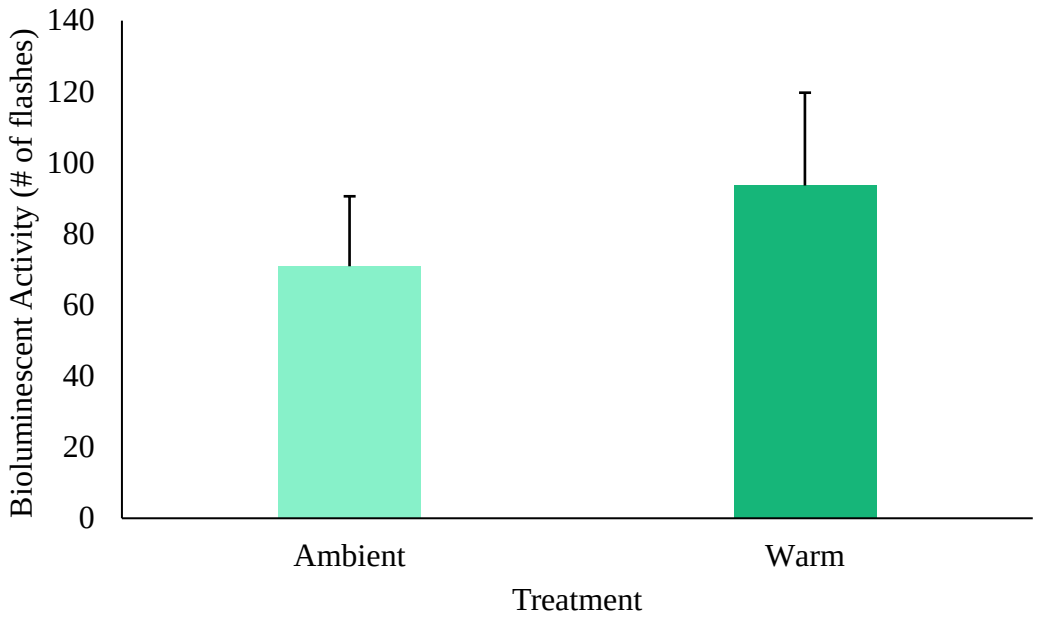

Figure 1

Salinity Preference of Sea Stars *Pharia pyramidata* and *Phataria unifascialis*

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Impact Statement: As global temperatures rise, oceanic salinities will change, which will affect many sea creatures such as sea stars.

Running Head: Salinity Preference of Sea Stars

Keywords: Osmoconformer, stenohaline, Gulf of California, water vascular system, climate change, acclimatization

Word Count: 3207

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Abstract

Invertebrates respond to stimuli in a variety of ways, one of those is by withdrawal of its body away from stimuli. One of the behavioral mechanisms used by sea stars, to avoid conditions they cannot tolerate, is movement. Sea stars have a water vascular system which they use as a hydraulic system for locomotion. Sea stars move by transporting water through their arms and ampullae to propel their bodies in a single direction. *Pharia pyramidata* and *Phataria unifascialis,* two common sea stars in the Gulf of California, are both osmoconformers and stenohaline organisms. Stenohaline organisms like *P. pyramidata* and *P. unifascialis* will avoid salinity levels they cannot tolerate through behavioral mechanisms such as movement. We wanted to test the hypothesis that sea stars will move towards ambient oceanic salinity, because they have acclimated to living there. For this experiment, three salinity solutions, 34ppt, 36ppt, and 38ppt, were created. The salinity treatments were then used to make a three-tiered gradient that sea stars were subjected to. Sea stars were observed in each salinity treatment as well as each level of the gradient. The data showed that no sea stars were located in the ambient level after the gradient, which does not support the hypothesis that sea stars would move towards ambient salinity. The results presented no statistically significant evidence that salinity affects the direction of movement by *P.pyramidata* and *P. unifascialis*. However, these results and those of proposed future studies can provide more information about the responses to stimuli in invertebrates.

Resumen

Los invertebrados responden a los estímulos de diferentes maneras, una de ellas es retirarse de algún estimulo irritante. Uno de los mecanismos de comportamiento, utilizados por las estrellas de mar para evitar condiciones que no pueden tolerar, es el movimiento. Las estrellas de mar tienen un sistema vascular acuífero que utilizan como sistema hidráulico para realizar movimientos. Las estrellas de mar se mueven por el transporte de agua a través de sus brazos y las ámpulas, para impulsar su cuerpo a cualquier dirección. *Pharia pyramidata* y *Phataria unifascialis*, son dos esteroideos que habitan en el Golfo de California, ambos organismos son osmoconformadores y estenohalinos. Organismos estenohalinos como *Pharia pyramidata* y *Phataria unifascialis*, evitaran los niveles de salinidad que no pueden tolerar, a través de mecanismos conductuales como el movimiento. Nosotros queríamos probar la hipótesis de que las estrellas de mar se moverán hacía un nivel de salinidad que se encuentre en su ambiente, ya que están aclimatadas a esas condiciones. Para ello, creamos tres soluciones diferentes de salinidad, 34 ppt, 36 ppt y 38 ppt. Las soluciones fueron utilizadas para hacer un gradiente con tres niveles de salinidad al que las estrellas fueron sometidas. Las estrellas de mar fueron observadas en cada tratamiento de salinidad, así como en cada nivel del gradiente. Los datos mostraron que no se localizaron estrellas de mar en el nivel ambiental después del gradiente, lo que no respalda la hipótesis de que las estrellas de mar se muevan hacia la salinidad ambiental. Los resultados no presentaron evidencia estadísticamente significativa respecto a que la salinidad afecta la dirección del movimiento de *P.pyramidata* y *P. unifascialis*. Sin embargo, estos resultados y los de los estudios futuros propuestos pueden proporcionar más información sobre las respuestas a los estímulos en los invertebrados.

Introduction

In past studies, it was confirmed that invertebrates respond to stimuli in a variety of ways, one of those is by withdrawal of its body away from stimuli (Peeke, Herz, & Wyers, 1973). In coastal systems, salinity is a very important environmental determinant in the performance of organisms. Most sea stars are considered osmoconformers and stenohaline organisms, so their capacity to regulate osmolarity and ionization is very limited (Held & Harley, 2009). Sea stars' body-wall tissues are highly permeable to water and salt which means that their coelomic fluid is generally isosmotic to the seawater where they reside. Therefore, sea stars will avoid salinity levels they cannot tolerate through physiological and behavioral mechanisms (Diehl, 1986).

Sea stars use a hydraulic system called the water vascular system for locomotion. Part of this water vascular system are small tubular feet on each of the sea stars arms which have suckers at the tips to help the sea star adhere to the substratum. The relaxation of longitudinal muscles moves fluid from the ampullae to the podia to extend the tube feet which briefly adhere to the substratum. Then the contraction of the postural muscle, near the tube feet, moves the body and therefore, performs the locomotion. Contraction of longitudinal muscles by the tube feet move the sea star close to substratum to anchor its tube feet. This allows sea stars to acquire prey faster (Hennebert et al., 2010).

The sea stars' nervous system also influences movement. Sea stars do not have brains, but they have a ring around the mouth and radial nerves in each arm, which lead to smaller nerves found in each tubular foot. This nervous system allows the sea stars to move, but only in one direction. This system is very important for essential activities such as predation for feeding and

activities of the intermediary metabolic system. Feeding response indicates chemosensory and neuromuscular activity, which could be affected by factors such as salinity (Hopkins, 1926).

The Gulf of California has significant diversity of echinoderms and Asteroidea is one of the classes located in the Gulf that has considerable species diversity and richness. Two of the most common sea stars in the Gulf of California are *Pharia pyramidata* and *Phataria unifascialis*, which are located near the shore and easily accessible (Solís-Marín et al., 2005). Sea stars from the Gulf of California are acclimated to living in oceanic salinity that varies between 34 to 36 parts per thousand (Beron-Vera & Ripa, 2002). Therefore, organisms like the *P. pyramidata* and *P. unifascialis* are acclimated to that salinity.

For our study, we chose *P. pyramidata* and *P. unifascialis*, because they are common species in the Gulf of California and are easily accessible and located close to shore. In addition, we chose these species because they are stenohaline, so we believed that they would respond to small differences in salinity. Our aim in this study was to identify the salinity preferences of *P. pyramidata* and *P. unifascialis* by observations of their movement in different salinity treatments. We hypothesized that sea stars would prefer to move towards ambient oceanic salinity, because they had acclimatized to living there.

Methods

Location

The sea stars in this study were taken from Cantamar beach, located in La Paz Bay, Baja California Sur Lat 24°15’07’’ Long 110°15’’(Fig.1). La Paz Bay has two patterns of wind that

occur in two different seasons. Wind blows from south and southeast in spring and summer but blows from north and northeast in fall and winter. La Paz Bay is classified as semi-arid which means that evaporation and radiation rates are very high (Shirasago-Germán, et al., 2007).

Data Collection

In July salinity preference of sea stars was tested at Cantamar in Baja California Sur. Two species of sea star, *Phataria unifascialis* and *Pharia pyramidata* were collected from Cantamar to be tested in various salinity solutions and observed for movement. The sea stars were then placed in one of three levels of a salinity gradient to determine if there was a preference for one salinity. Sea stars were also placed in salinity solutions to observe their movements and compare to movements in the gradient.

Five tanks were used in this experiment. Tank one had a continuous salinity of 34 parts per thousand and was labeled the low salinity treatment. Tank two had a continuous salinity of 36 parts per thousand, which is the ambient salinity of the Gulf of California. Tank three had a salinity of 38 parts per thousand and was labeled the high salinity treatment. A fourth tank, which was marked with 11-centimeter intervals, was used to create a gradient of the three salinity treatments. Tank five was a holding tank for sea stars that weren't being observed and contained sea water from the Gulf of California.

In the gradient tank the high salinity treatment filled the bottom up to the first 11-centimeter line. Then saran wrap was placed over the bottom layer, and the ambient treatment was slowly added to avoid mixing. After the second layer was filled to the 22-centimeter line, the saran wrap was slowly pulled out of the tank and a new piece of saran wrap was placed over the ambient layer. Finally, the low salinity treatment was slowly added over the saran wrap, and

when it was filled to the 33-centimeter line on the tank the saran wrap was carefully pulled out. The observations for this experiment occurred over a three-day period, and every day the gradient was re-made.

After the gradient was created, three sea stars of each species were collected. Once collected, the sea stars were kept in the fifth tank, which was full of sea water from the location in which they were collected, until they were placed in a treatment for observation. To observe a sea star in the salinity gradient the sea star was first placed on a plastic board. Once the sea star had attached itself to the board, it was carefully lowered into the salinity level that was observed that day. Sea stars in each treatment were observed for twenty-five minutes and then returned to the fifth tank. After a sea star was removed from the gradient, the gradient would be re-tested to make sure it hadn't mixed.

Sea stars in the gradient were observed to see if they would move from one salinity to another. Sea stars in consistent salinity solutions were observed for their movement patterns to compare to those in the gradient. On the first day, sea stars were observed in the high salinity tank, and were lowered into the bottom level of the gradient. On day two, sea stars were observed in the low salinity solution, and were placed in the top level of the gradient. On day three, sea stars were observed in the ambient salinity treatment, and were lowered into the middle level of the gradient. At least two sea stars of each species were observed in each treatment.

Statistical Analysis

Data were visualized using a mosaic plot which shows proportions of overlap between variables. Then three Chi Squared (χ^2) Contingency tests were performed in RStudio to

determine if there was a relationship between species and salinity preference in the gradient tank. Four more χ^2 contingency tests were performed to determine if there was a difference in the level of movement in different salinity treatments. Because there was a small sample size for all tests, a simulated p-value based on 2000 replicates was calculated, and therefore there are no degrees of freedom for the p-values.

Ethics statement

Because sea stars are important to the ecosystems in which they reside, researchers were careful not to harm any organisms during this experiment. When collecting and transporting sea stars, dive gloves were used to protect the sea stars from potential harm, and sea stars were gently removed from the substratum. After removal, sea stars were placed in a transportation tank that held sea water from the location where they resided. Sea stars were only placed in salinity treatments for observation for twenty-five minutes, and then returned to their transportation tank. After observation, sea stars were slowly reintroduced to their original habitat. Researchers were careful to ensure that the same sea stars were not used for observation on consecutive days, to reduce the stress of the organisms.

Results

None of the χ^2 values were statistically significant. A larger proportion of sea stars were found in the low salinity layer of the gradient versus high salinity (Fig.2). None of the sea stars placed in the gradient were found in ambient salinity after the observation period (Fig.2). An

equal proportion of *P. unifascialis* and *P.pyramidata* were found in the low salinity layer of the gradient after the observation period (Fig.2). However, there were a larger portion of *P. unifascialis* found in the high salinity layer of the gradient compared to *P. pyramidata* (Fig.2). The test for a relationship between species and salinity preference had a χ^2 value of 0.24444 (p= 1). The test for a relationship between *P. unifascialis* and salinity preference had a χ^2 value of 0.66667 (p= 0.6787). The test for a relationship between *P. pyramidata* and salinity preference, had a χ^2 value of 1.8 (p= 0.3668).

In the salinity treatment tanks, a larger proportion of sea stars showed movement from their original location compared to no movement (Fig.3). Sea stars placed in low and ambient salinity treatments showed an equal proportion of sea stars that showed movement, while a small proportion of sea stars placed in high salinity showed movement (Fig.3). A larger proportion of sea stars placed in high salinity showed no movement compared to a smaller proportion of sea stars that were placed in ambient that showed no movement (Fig.3). All sea stars placed in the low salinity treatment moved from their original location (Fig.3). The test for a relationship in the salinity treatment sea stars were placed in and whether they moved or not had a χ^2 value of 3.254 (p= 0.3958). The χ^2 contingency test for a relationship between ambient salinity and movement of sea stars had a χ^2 value of 1.0 (p= 0.6202). The χ^2 contingency test for a relationship between high salinity and movement of sea stars had a χ^2 value of 0.33333 (p= 1.0). The χ^2 contingency test for a relationship between low salinity and movement of sea stars had a χ^2 value of 3.0 (p= 0.2634).

Discussion

The data collected support the hypothesis that salinity does not affect the direction of sea stars movement. Data were visualized in mosaic plots which show the proportions of individuals in each location after the observation period. The mosaic plots showed that no sea stars were located in the ambient level after the gradient, which does not support the hypothesis that sea stars would move towards ambient salinity. However, the results showed no significant difference in the salinity preference of species or between species. Despite what we know about sea stars being highly affected by salinity, little is known about the affect of salinity on movement. However, salinity does show an effect on the development of sea stars, because they were unable to grow to adult form in low salinities (Casties et al., 2015).

It is important to test the effects of salinity on sea stars due to changing oceanic salinity levels. Global warming is causing an increase in the salinity of sub-tropical waters and a decrease in the salinity of high-latitudinal waters (Durack & Wijffels, 2010). An increase in the salinity of ocean waters also increases salt concentrations in the coelomic fluid of sea stars because they are osmoconformers. Additionally, global warming is rising surface temperatures of ocean waters which can also affect the health of sea stars (Xie, et al., 2010). Because sea stars are osmoconforming and ectothermic organisms, salinity and temperature are two of the largest factors that contribute to their health.

This study was conducted outside and therefore, temperature was a variable we were unable to control. Sea stars are easily affected by temperature because they are ectotherms, and therefore rely solely on their environment for the regulation of their body temperature. If the water they are in rises in temperature, they are unable to lower their body temperatures. A lethal

temperature for sea stars is approximately 35° C. When the sea stars body temperature reaches 35°C, its arms reach temperatures of approximately 39°C (Pincebourde et al., 2013). On the days of data collection, air temperatures in Cantamar reached 37°C, 38°C, and 39°C (The Weather Company, 2018). While the water temperature in the tanks most likely did not reach air temperatures, it was within a range that would reduce movement as the water approached lethal temperatures. In future studies, temperature should be controlled so that results are not skewed by the effects of temperature.

Unlike *P. pyramidata* and *P. unifascialis*, some species of sea stars such as, *Asterias rubens* are euryhaline organisms (Casties et al., 2015). Euryhaline organisms are able to tolerate a larger range of salinity changes and would likely not be affected by small salinity differences. Therefore, it would be interesting to conduct this study with different species of sea stars. It would also be interesting to test the movement of sea stars in larger salinity gradients. For our study we only used gradients of two parts per thousand but the results showed no effect of salinity on movement, so testing larger salinity differences might have more effect on sea stars' movement. Another interesting addition to this study would be to acclimate sea stars in different salinities and test their salinity preference after acclimation.

The results of future studies would give more information on salinity tolerance of different sea star species and enable the protection of sea stars for future salinity and temperature changes due to global warming. These results have also provided more information about the responses to stimuli in invertebrates.

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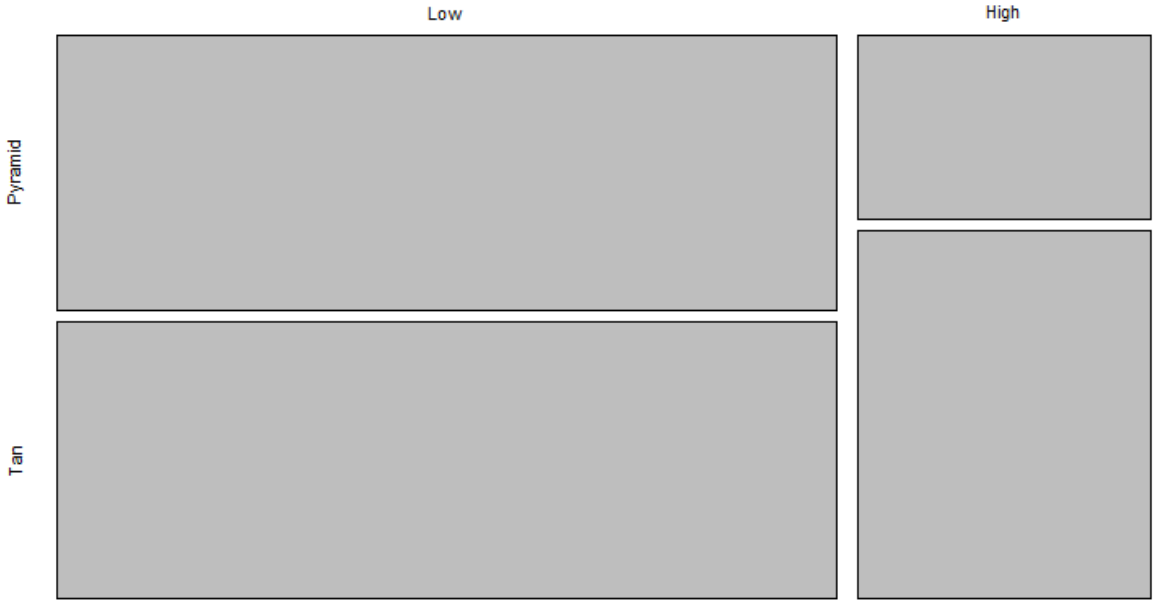
Fig.1 The study site location of Cantamar beach, La Paz, Baja California Sur, Mexico.

Fig.2 A mosaic plot that shows the proportions, adding to one, of *Phataria unifascialis* (Tan) and *Pharia pyramidata* (Pyramid) and their final locations in the gradient tank; high salinity and low salinity. See results for description.

Fig.3 A mosaic plot that shows the proportions, adding to one, of movement from original location or no movement for both *Phataria unifascialis* and *Pharia pyramidata* in different salinity treatment tanks. Treatment tanks were; low salinity, ambient salinity, and high salinity. See results for description.


Baja California Sur, Mexico
Gulf of California
Pacific Ocean
Cantamar


Low
High
Pyramid
Tan


Movement
No Movement
High
Ambient
Low

Effects of Mangrove Proximity on Microalgae Community Diversity

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Article Impact Statement:

There was no effect of study site, the proximity of mangroves or sample day upon the diversity of the sampled microalgae communities.

Key words: microalgae, communities, mangroves, cyanobacteria, diatoms

Effects of Mangrove Proximity on Microalgae Community Diversity

Abstract

Microalgae, such as cyanobacteria, can form mutualistic relationships with mangroves. This relationship allows the mangrove to receive nutrients while the cyanobacteria is provided food by the mangrove. I speculate that this close relationship between cyanobacteria and mangroves may affect microalgae community diversity and I predict that microalgae community structure will be different between mangrove and non-mangrove sites. I tested this prediction by doing surface plankton tows at four sites near Pichilingue in Baja California Sur, Mexico. The two mangrove locations were the Universidad Autonoma de Baja California Sur and a site at Balandra. The two non-mangrove locations were the beach at the Cantamar and a sandy beach at Balandra. I took surface tows at these four sites for three consecutive days. I then concentrated my samples and looked at them under a microscope to determine the genus richness and abundance of the microalgae community. I then calculated community diversity using the Shannon Wiener Diversity Index every day for each site. A blocked, nested ANOVA test showed the effect of location on the Shannon Wiener Diversity to be insignificant ($F_{(0.05(2),1,)}$=0.511, p=0.502). There were also some general, if statistically insignificant, patterns for Shannon Wiener Diversity as well as microalgae genera composition. Although my hypothesis that location would affect the Shannon Wiener Diversity Index was not supported it is possible that dominance of diatoms in my samples may have biased the Shannon Wiener Diversity Index to reflect mainly diatom diversity because the smaller microalgae may have been filtered out in the sample concentration process. Due to the continued removal of mangroves for human development now is the time for new studies to discover if mangrove presence or absence affects nearby microalgae communities.

Resumen

Las microalgas y cianobacterias pueden formar relaciones de mutualismo en conjunto con los manglares. Esta relación permite que el manglar reciba nutrientes mientras que el manglar proporciona alimento a las cianobacterias. Yo especulo que dicha relación puede influir en la diversidad y predecir si las diferencias en la estructura de la comunidad de microalgas seran diferentes entre los sitios de manglares y no manglares. Probé esta predicción haciendo arrastres de plancton superficial durante tres dias consecutivos en cuatro sitios distintos: Unidad Academica UABCS de Pichilingue, dos sitios en Balandra y Cantamar. Luego concentré mis muestras y las miré al microscopio para determinar la riqueza y abundancia del género de la comunidad de microalgas. Calculé la diversidad de la comunidad utilizando el índice de diversidad de Shannon Wiener para cada sitio. Una prueba de ANOVA bloqueada y anidada mostró que el efecto de la ubicación en la diversidad de Shannon Wiener fue insignificante ($F_{(0.05\,(2),\,1)}$ = 0.511, p = 0.502). También hubo algunos patrones generales, aunque estadísticamente insignificantes para el indice Shannon Wiener así como la composición de géneros de microalgas. Sin embargo, la hipotesis de que la ubicación afectaría al Índice de Diversidad Shannon Wiener no fue apoyada. Es posible que el dominio de diatomeas en mis muestras haya sesgado el Índice de Diversidad Shannon Wiener para reflejar principalmente la diversidad de diatomeas ya que las microalgas pudieron haber sido filtradas en el proceso de concentración de mis muestras. Para futuros estudios, descubrir si la presencia o ausencia de

manglares afecta a las comunidades de microalgas cercanas seria un gran paso para la creacion de propuestas que eviten que los manglares sean destruidos para dar paso a desarrollos y asentamientos humanos.

Introduction

Mircoalgae can be defined as “photosynthetic, oxygen producing aquatic bacteria or protists” that are only visible under the microscope (Graham *et al.* 2016). Microalgae can be found in communities which are formed when two or more species are coexisting in the same place at the same time. Microalgae communities can be very different even within a defined area (Painting *et al.* 1993). Mircoalgae communities can include diatoms, euglenoids, cryptomonads, dinoflagellates, stramenopiles as well as tiny red or green algae (Graham *et al.* 2016). Things that can influence microalgae community structure include sunlight availability, nutrient availability, grazing, and sinking (Lewis 1978). Furthermore, episodic succession due to changes in nutrient availability can temporarily change community structure by increasing relative abundances of species who are adapted to nutrient scarcity (Lewis 1978). Disturbances to the environment can also affect community diversity (Figuerido & Giani 2001).

Some microalgae, like cyanobacteria, are known for their symbiotic relationships. For example, cyanobacteria can form mutualistic relationships with mangroves wherein the cyanobacteria make nutrients available to the plant and the plants provide the cyanobacteria with food (Bashan & Holguin 2002). This mutualism can take place on the mangroves’ roots above and below the water, as well as on mangrove trunks and leaves (Alvarenga *et al.* 2015). This relationship between cyanobacteria and mangroves helps to maintain the entire mangrove forest (Alvarenga *et al.* 2015). In fact, this symbiosis can be used to help restore mangrove forests if the mangrove seedlings are given cyanobacteria (Bashan & Holguin 2002).

Due to this close symbiosis with cyanobacteria I am curious if microalgal community diversity near mangroves is different than diversity of microalgal communities not in proximity to mangroves. In

fact, previous studies have found different cyanobacteria species in the plankton nearby mangrove forests (Alvarenga *et al.* 2015). Even if I am unable to detect the cyanobacteria, they may outcompete some of the other microalgae which would result in a change in the community structure. Therefore, I hypothesize that microalgal community diversity will be different between mangrove and non-mangrove (hereafter open) locations.

To test my hypothesis, I plan to do plankton tows to collect microalgae at four locations: Open Balandra, Mangrove Balandra, Cantamar, and the mangroves at the Pichilingue lab of Universidad Autonoma de Baja California Sur (UABCS). I will do the tows at the four locations for three consecutive days. I will take the microalgae samples to the lab and determine the genus of the first 100 cells. I will also keep track of relative abundance for the first 100 cells. However, if I find cyanobacteria I will only identify them to the level of phylum which is just Cyanobacteria. I will calculate the Shannon Wiener Diversity Index per location per day and will eventually run an Analysis of Variance (ANOVA) test in R to determine if the mangrove versus open sites have different community structures.

Methods

Overall, I did three surface plankton tows at each site for three consecutive days at Balandra, the UABCS mangroves and Cantamar. I then concentrated the samples using a coffee filter and a 500-mL beaker. I then put a drop of the concentrated sample onto a slide and identified the microalgae to the level of genus or to phylum if I saw any cyanobacteria. I also counted the abundance of each genus up to the first 100 cells. Using genus richness and abundance I calculated the Shannon Weiner Diversity Index for every site for each day. Additionally, an ethics statement for this study is not applicable due to the fact that I was only looking at microalgae.

The four sites I sampled at were a mangrove location at Balandra, an open location at Balandra, the mangroves at the UABCS Pichilingue lab, and an open site at the Cantamar resort (Fig. 1). The

Balandra mangrove location was fairly isolated and was a slightly rocky area. The open Balandra location was at a sandy beach close to where many of the beachgoers were but not so close as to interfere with someone's swimming activities. The UABCS mangroves were at a sandy beach behind the lab buildings. The beach had a lot of the macroalgae, *Ulva* spp., on the shore and in the water. The Cantamar resort was a sandy beach with no mangroves. Cantamar also had few human visitors to the beach at the time of data collection although there were some waves caused by morning boat traffic.

I made a plankton tow with <1mm size mesh. I did three surface tows per site. Tows were completed in the morning for Balandra (both mangroves and open sites), the UABCS mangroves, and Cantamar on 7/17/18, 7/18 /18 and 7/19/18. Each tow lasted for 1 minute in about 1m of seawater. In all I took approximately 600mL of sample from the site though the amount of total water I walked through is unknown. The end of the tow had no holes in it so after collecting my samples I had to concentrate them.

To concentrate my samples, I put a coffee filter over a 500-mL beaker and gently poured 200mL from my sample into the beaker. Using a dropper, I then took up the last drops on the top of the coffee filter and put one drop onto a slide. I put the slide under a light microscope and looked for aggregations of microalgae at total of 40x magnification. If I saw what I thought was microalgae I increased the total magnification to 100x. I identified the algae's genus at 100x using plankton guides from Louisiana Universities Marine Consortium (LUMCON) and University of British Columbia (UBS). If I was unsure of the genus I would put the total magnification at 400x. This was especially critical for telling the difference between the smaller microalgae and marine debris. However, I would not attempt to do this if the microscope stage was too high due to the fact that the lens would bump into my slide and potentially damage the microscope.

For cyanobacteria I planned to identify them solely at the level of phylum which was just Cyanobacteria. However, I was also aware that I may not be able to positively identify cyanobacteria as

they may be smaller than the resolution of the microscope at 400x. Some things that I thought were cyanobacteria but were too small to positively identify were marked as unknown.

I only counted the first 100 individual cells and noted their corresponding genus in order to balance efficiency with precision. After this I used the Shannon Wiener Diversity Index to estimate diversity of each microalgae community at every site, every day, for three consecutive days.

I analyzed my data using a nested, blocked ANOVA test in the statistical program R. I used 0.05 as my critical alpha value. I first tested my *a priori* hypothesis concerning microalgae diversity between mangrove and open locations. I then tested other hypothesis including the effects of the site or sampling day on community diversity. I also examined the effects of site, location and sampling day on the number of microalgae genera.

Results

Overall the ANOVA tests showed no significance. The ANOVA illustrated that mangrove and open locations had no significant effect on the Shannon Wiener Diversity Index ($F_{(0.05(2),1}$,=0.511, p=0.502). Later ANOVA tests showed that site had no significant effect on Shannon Wiener Diversity ($F_{(0.05(2),3}$,= 0.298, p=0.826) nor did the sampling day ($F_{(0.05(2),2}$,= 2.736, p=0.116). Additional ANOVA testing revealed that neither location ($F_{(0.05(2),1}$,=0.947, p=0.353), site ($F_{(0.05(2),3}$,=0.907,p=0.491), or day ($F_{(0.05(2),2}$,=2.072, p=0.207) had any significant effect on the number of microalgae genera.

However, there were still some patterns in the Shannon Wiener Diversity Index. Mangrove locations had a higher Shannon Wiener Index by the third day of sampling compared to the open locations (Fig. 2). Even so this pattern proved to be insignificant ($F_{(0.05(2),1}$,=0.511, p=0.502). An additional pattern was that both the UABCS site and the open Balandra site had similar Shannon Wiener Indices (Fig. 2). Another pattern was that the Cantamar site had the lowest Shannon Wiener Index for both July 18th and July 19th (Fig. 2).

There were also some patterns in microalgae genera composition. Overall, most identified genera were diatoms (Figs. 3-6). The diatom *Leptocylindrus* spp. was always present on any given day for at least one site (Figs. 3-6). Additionally, the dinoflagellate, *Heterocapsa* spp. was detected on both July 18th and July 19th for at least two sites (Figs. 3-6). *Chaetoceros* spp., a diatom, was found at all sites on July 18th and July 19th (Figs. 3-6). Cantamar had the least number of total identified microalgae genera while the mangrove site at Balandra had the most total genera (Table 1).

Discussion

The *a priori* hypothesis that microalgal community diversity will be different between mangrove and open locations was not supported ($F_{(0.05(2),1}$,=0.511, p=0.502). This may be because the dominance of diatoms in my samples may have biased the Shannon Wiener Diversity Index to reflect mainly diatom diversity. So, due to the fact that I collected similar microalgae genera from different sites the diatom communities may have not been actually very diverse. In addition, since more than 900 diatom genera are generalists (Vanormelingen 2008) this could mean that genera composition can stay similar for extended periods of time regardless of location. This similarity of diatom genera for any given sample may also explain why my *post hoc* hypotheses showed no significance on the effect of site and sample day on the Shannon Wiener Diversity Index. The diatom dominance upon the Shannon Wiener Diversity Index could also be a reason for the lack of significance of location, site and sampling day on microalgae genera.

There were a few caveats to my experiment. One was that I mostly found diatoms. This may have been due the concentration process when I filtered my sample through a coffee filter This may have inadvertently left behind the larger microalgae like diatoms and dinoflagellates and filtered out the smaller microalgae. Another possible reason for the lack of finding smaller microalgae were the mistakes in microalgae identification because the microscope could not always zoom in far enough for me to tell the difference between debris and tiny green or red algae. In my uncertainty I left the unidentified pieces as unknown rather than struggle to identify something I couldn't see well enough to positively identify. A

second caveat was that I did not control for tide levels so in my effort to stay in approximately 1m deep water I could not always get near the mangroves at the mangrove sites when the tide was low. The effort to keep a constant depth rather than a constant distance from the shore may have affected the composition of my microalgae samples. However, the constant depth helped me to continuously collect microalgae from the upper euphotic zone rather than a mix of upper euphotic and benthic microalgae (Estrada *et al.* 2016). A third caveat was that July 17th and July 18th were overcast while July 19th was a sunny day. This may have affected genera composition at the surface since motile genera can swim up to get more sunlight or swim down to avoid overexposure to sunlight (Graham *et al.* 2016).

Though I did not find any differences between community diversity it is still possible that mangroves could be important for microalgal communities. For instance, a future experiment between mangrove and open locations could look at microalgae community diversity before and after mangrove removal since the symbiotic cyanobacteria, as nitrogen fixers, could affect nutrient availability to nearby microalgae (Bashan & Holguin 2002). If the microalgae community changed due to nutrient changes as an effect of mangrove removal this would have interesting implications on mangrove destruction. For example, a decrease in microalgae diversity in conjunction with a decrease a mangrove cover would imply that the mangrove ecosystem is not just an important source of organic matter for coastal zones (Alvarenga *et al.* 2015) but also plays a role in microalgae community structure.

In conclusion my hypothesis that mangrove microalgae community diversity and open microalgae community diversity would be different was not supported. However, due to the dominance of diatoms in my sample this may be a more of a reflection on the diatom community rather than on the microalgae community as a whole. This may be because in my effort to concentrate my sample I may have inadvertently filtered out the smallest members of the microalgae community. Additionally, now is the time for future studies to discover if mangrove presence or absence affects nearby microalgae communities because mangroves continue to be removed to make way for human developments like aquaculture (Alvarenga *et al.* 2015).

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Tables

Table 1. Total number of identified genera for each site for both mangrove and open locations.

Location Type	Site	Total Number of Genera
Mangroves	UABCS	10
	Balandra	15
Open	Cantamar	8
	Balandra	14

Figure Captions

Figure 1. Locations of the four sample sites in Pichilingue, Baja California Sur, Mexico. The red line represents 5.55 km. Image from Google Earth.

Figure 2. Shannon Wiener Diversity Index per day per site. The UABCS and Balandra Mangrove sites were the mangrove locations. Cantamar and Balandra Open were the sites without mangroves.

Figure 3. Proportion of microalgae abundance for each microalgae genus and overall type for the UABCS mangrove site. The unknown microalga are most likely cyanobacteria.

Figure 4. Proportion of microalgae abundance for each microalgae genus and overall type for the Balandra mangrove site. Green represents green microalgae. The unknown microalga are most likely cyanobacteria.

Figure 5. Proportion of microalgae abundance for each microalgae genus and overall type for the Cantamar open site

Figure 6. Proportion of microalgae abundance for each microalgae genus and overall type for the Balandra open site. Green represents green microalgae.


UABCS Open Site
Cantamar Open Site
Balandra Open Site
Balandra Mangrove Site
N

Figure 1.

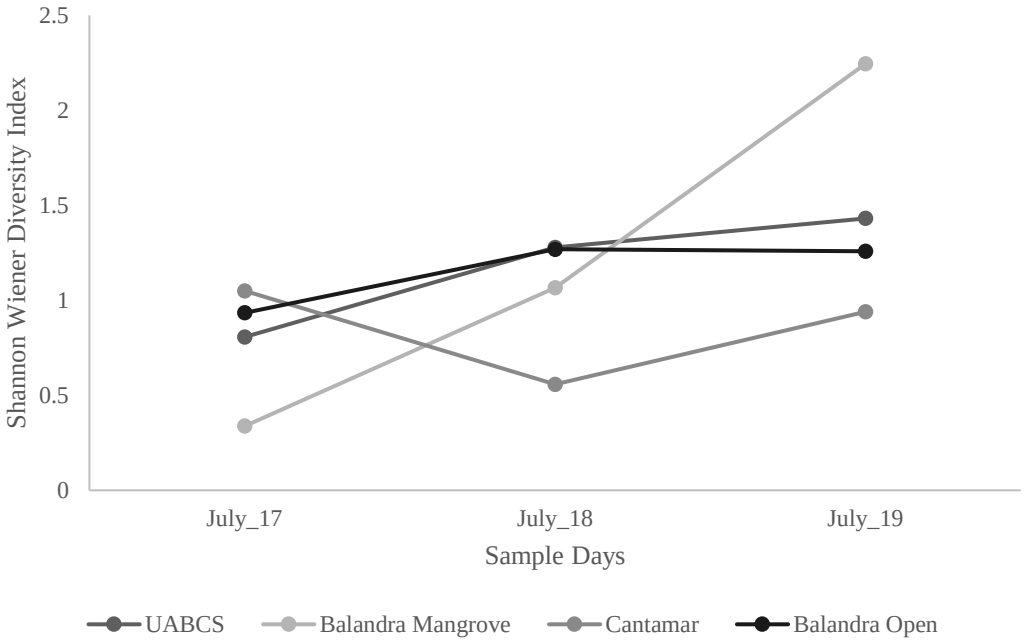
2.5
2
1.5
1
0.5
0
Shannon Wiener Diversity Index
July_17
July_18
July_19
Sample Days
UABCS
Balandra Mangrove
Cantamar
Balandra Open

Figure 2.

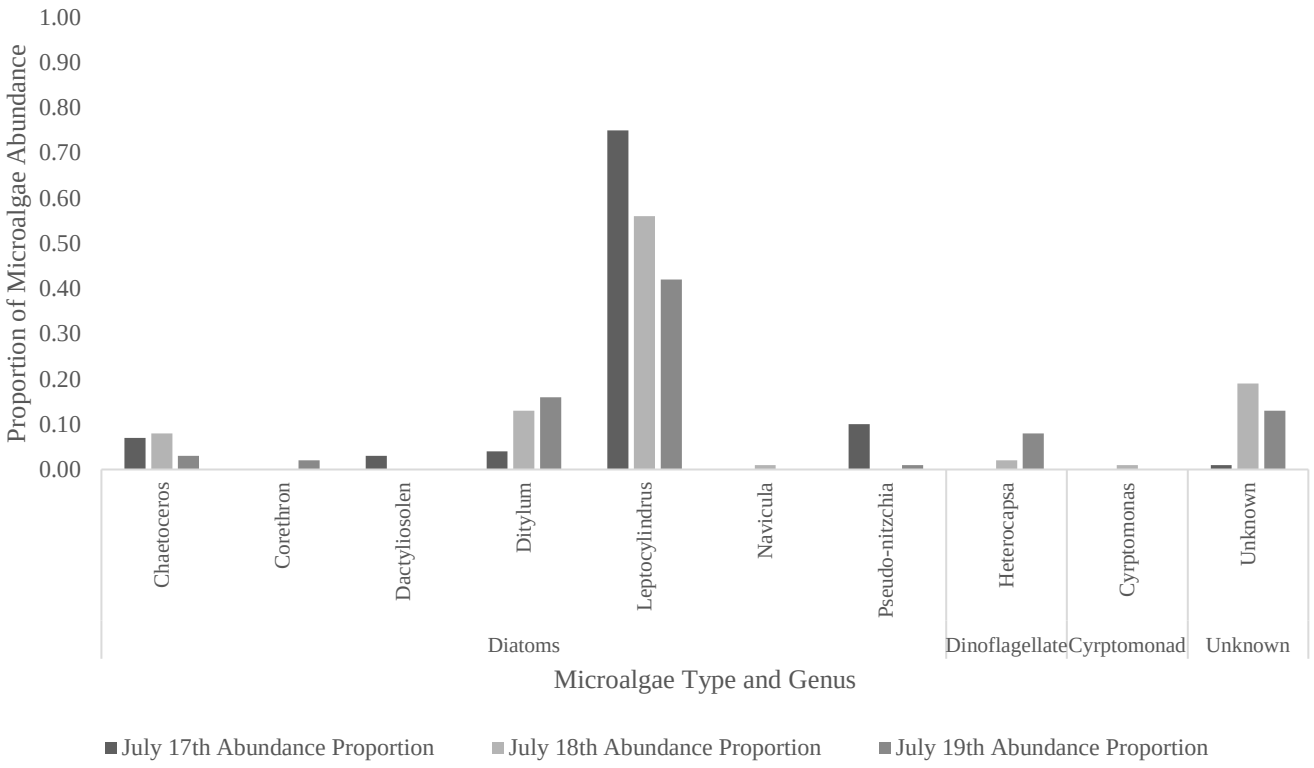
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Proportion of Microalgae Abundance
Chaetoceros
Corethron
Dactyliosolen
Ditylum
Leptocylindrus
Navicula
Pseudo-nitzchia
Heterocapsa
Cyrptomonas
Unknown
Diatoms
Dinoflagellate
Cyrptomonad
Unknown
Microalgae Type and Genus
July 17th Abundance Proportion
July 18th Abundance Proportion
July 19th Abundance Proportion

Figure 3.

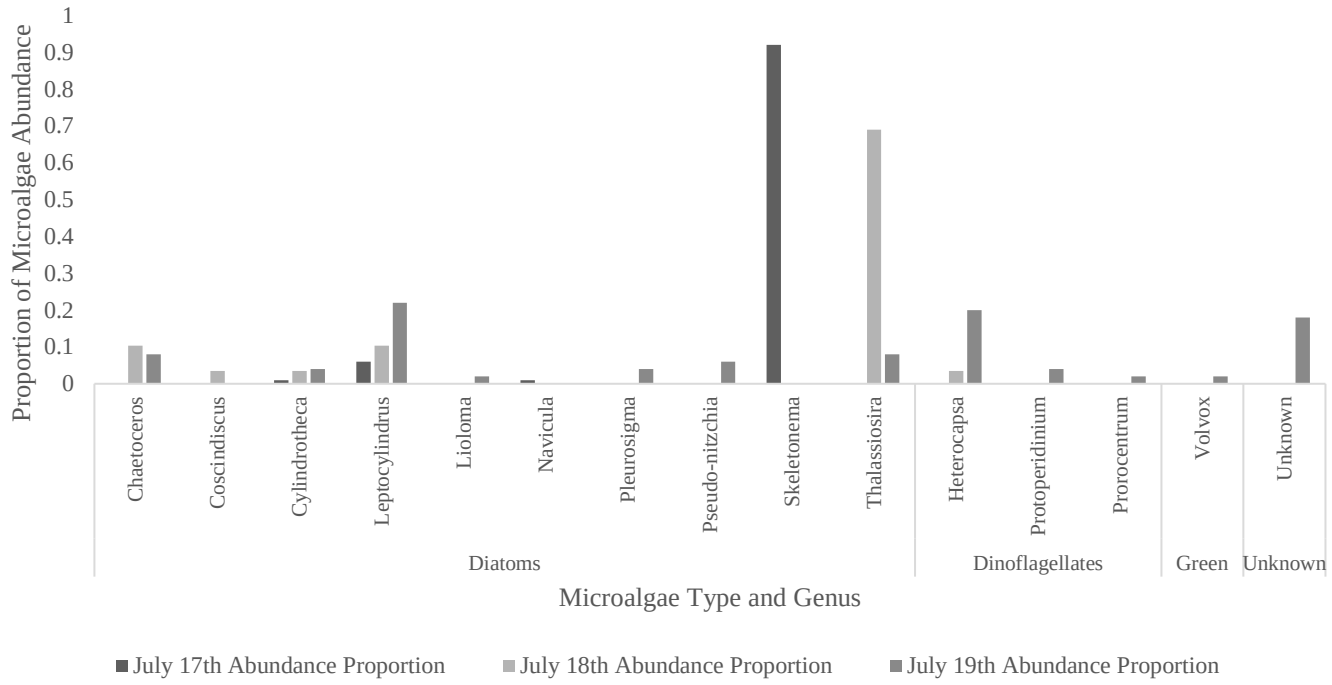
Proportion of Microalgae Abundance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Chaetoceros
Coscindiscus
Cylindrotheca
Leptocylindrus
Lioloma
Navicula
Pleurosigma
Pseudo-nitzchia
Skeletonema
Thalassiosira
Heterocapsa
Protoperidinium
Prorocentrum
Volvox
Unknown
Diatoms
Dinoflagellates
Green
Unknown
Microalgae Type and Genus
July 17th Abundance Proportion
July 18th Abundance Proportion
July 19th Abundance Proportion

Figure 4.

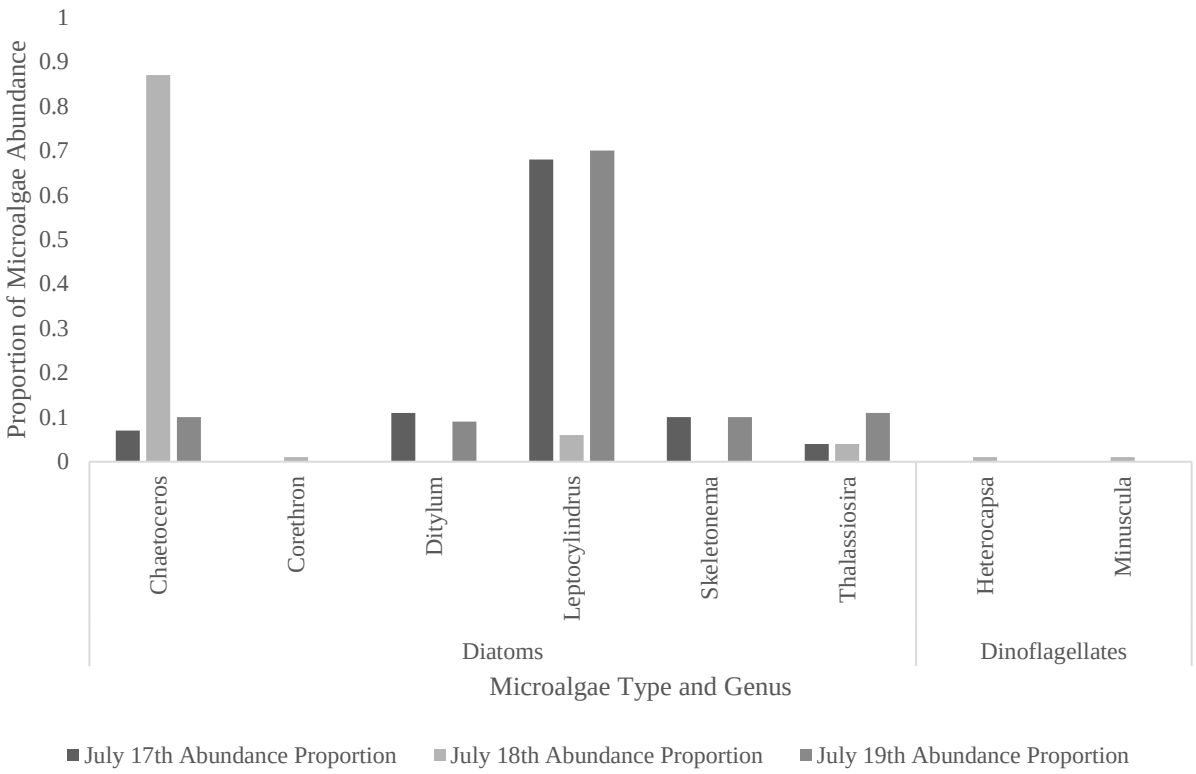
Proportion of Microalgae Abundance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Chaetoceros
Corethron
Ditylum
Leptocylindrus
Skeletonema
Thalassiosira
Heterocapsa
Minuscula
Diatoms
Dinoflagellates
Microalgae Type and Genus
July 17th Abundance Proportion
July 18th Abundance Proportion
July 19th Abundance Proportion

Figure 5.

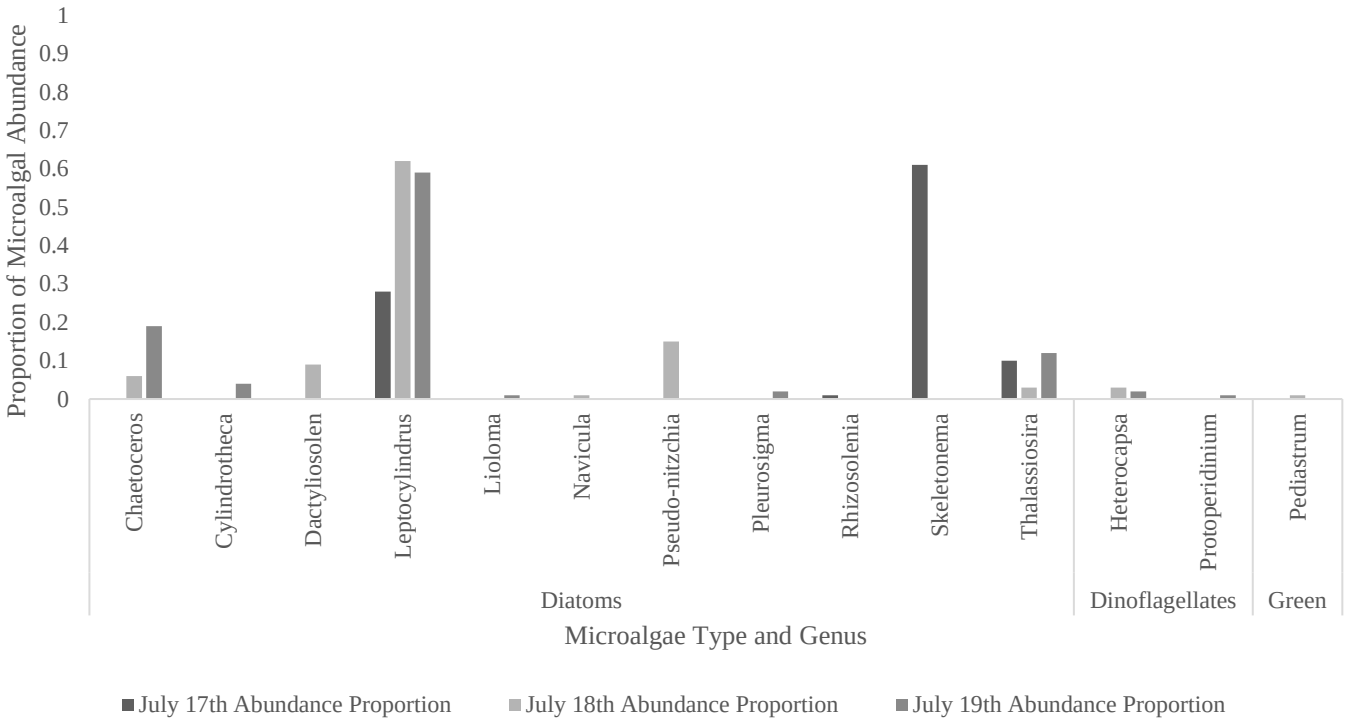
Proportion of Microalgal Abundance
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Chaetoceros
Cylindrotheca
Dactyliosolen
Leptocylindrus
Lioloma
Navicula
Pseudo-nitzchia
Pleurosigma
Rhizosolenia
Skeletonema
Thalassiosira
Heterocapsa
Protoperidinium
Pediastrum
Diatoms
Dinoflagellates
Green
Microalgae Type and Genus
July 17th Abundance Proportion
July 18th Abundance Proportion
July 19th Abundance Proportion

Figure 6.