

Independent Research Projects

Tropical Marine Biology Class
Summer 2016, La Paz, México

Western Washington University
Universidad Autónoma de Baja California Sur

Title	pp
Use of avian diversity surveys as an indicator of the effects of proximal development on arid mangroves near La Paz, Baja California Sur, Mexico.....	3
Oyster shell density in relation to pH in mangroves.....	23
The mobulas' response to human-influenced acoustic disturbance in the Gulf of California.....	37
How coral volume of <i>Pocillopora elegans</i> affects fish diversity in the Eastern Tropical Pacific.....	54
Snorkeling causes a temporary decrease in fish abundance and species composition in Baja California Sur.....	73
Phylogenetic analysis of cetacean communities of the Gulf of California and Eastern Pacific.....	101
Loggerhead turtle (<i>Caretta caretta</i>) mortality at Golfo de Ulloa, BCS, its relationship with productivity and sea-surface temperature.....	118
Selection on the Major Histocompatibility Complex (MHC) of killer whales (<i>Orcinus orca</i>) in the Gulf of California.....	130
Phylogenetic diversity of sharks at three locations in Mexico.....	151
The effects of human activity on the diversity of species inhabiting the prop roots of mangrove fringes in La Paz, Mexico.....	168

Summer 2016 Class



Students:

Sebastián Álvarez Costes
Siobhán Daly
Clarissa Felling
Lindsey Hanson
Angelica Kahler
Juhi La Fuente
Kayla Litterel
Hugo Sánchez Gómez
Jacqui Silva
Rachel Wachtendonk

Carlee Bock
Oliver Dev
Rachel Flanders
Jenna Hymas
Jesse Katz
Mara Landa Garza
Nathan Rouche
Emily Schultz
Helena Varela
Acy Wood

Faculty:

Alejandro Acevedo-Gutiérrez
Sergio Francisco Flores Ramírez

Deborah Donovan
Benjamin Miner

TA:

Jorge Guzmán

**Use of avian diversity surveys as an indicator of the effects of
proximal development on arid mangroves near
La Paz, Baja California Sur, Mexico**

Nathan Roueche

rouechn@wwu.edu

Biology Department, Western Washington University, 516 High St., Bellingham WA 98225, US

Universidad Autonoma de Baja California Sur, La Paz, Baja California Sur, Mexico

Acknowledgements

I would like to thank A. Acevedo-Gutierrez, S.F. Flores-Ramirez, D. Donovan and B. Miner for their support of this study, both conceptually and logistically. I would like to express my gratitude to Western Washington University and the Universidad Autonoma de Baja California Sur for arranging the opportunity for me to enact this study. I would also like to thank my classmates and colleagues from both institutions. You have all made this endeavor possible and worthwhile.

Manuscript word count: 3716

Use of avian diversity surveys as an indicator of the effects of proximal development on arid mangroves near La Paz, Baja California Sur, Mexico

Abstract

The near-total alteration of the planet's surface by mankind has led to a tremendous loss of the planet's biodiversity. Perhaps of greatest concern to ourselves as a species is the subsequent disruption of our planet's biogeochemical processes, which is a driving component of climate change. Many ecosystems have been shown to be more effective than others at the removal and storage, or sequestration, of atmospheric carbon. The most effective of these systems is broadly described as mangrove systems, and has been the subject of a tremendous amount of research and management policy. Recent studies have shown that the capacity of mangroves to remove and store atmospheric carbon varies with levels of human impacts, not only within stands but proximally and at basin-scales as well. Accurate assessment of the performance abilities of regional mangrove systems is key to effective management decisions, but isotope tracing and analysis techniques are complex, expensive, and impractical considering the global extent of mangrove distribution. Other research has demonstrated that the level at which mangrove systems function biogeochemically correlates positively to the diversity of animal communities within the mangrove stand. In other coastal ecotones, avian communities have been utilized to effectively demonstrate trends in system health. The purpose of this study

was to demonstrate a relationship between proximal and basin-scale anthropogenic landscape alteration and the diversity of avian communities within mangrove systems north of La Paz, Baja California Sur, Mexico. Point-count survey protocols were used to collect diversity index scores for avian communities. GIS technologies and raster imagery were used to categorize sites based on physical levels of human impacts on the adjacent landscape. The results of this study demonstrated no relationship between anthropogenic alterations and avian community diversity in the study area.

Resumen

La alteración casi total de la superficie del planeta por la humanidad ha dado lugar a una tremenda pérdida de la biodiversidad del planeta. Tal vez la mayor preocupación para los humanos como especie es el deterioro de los procesos biogeoquímicos de nuestro planeta, que es un componente motriz del cambio climático. Algunas ecosistemas han demostrado ser más eficaces que otros en la remoción y almacenamiento, o el secuestro, del carbono atmosférico. La más eficaz de todos se describen en términos generales como las sistemas de manglares, y ellos han sido el objeto de una enorme cantidad de investigación y política de gestión. Estudios recientes han demostrado que la capacidad de los manglares para absorber y almacenar carbono de la atmósfera varía dependiendo de los niveles de los impactos humanos, no sólo dentro de gradas, pero proximal y en la cuenca escalas también. La evaluación precisa de las capacidades de rendimiento de los sistemas de manglares regionales es clave para las decisiones de gestión eficaces. Sin embargo, las técnicas de rastreo y análisis de isótopos son complejos, caros y poco práctico teniendo la extensión global y la distribución de manglares. Otras investigaciones han demostrado que el nivel de la función biogeoquímica en una sistema de manglares se

correlaciona positivamente con la diversidad de las comunidades de animales dentro del manglar. En otros ecotonos costeros, comunidades de aves se han utilizado para demostrar de manera efectiva las tendencias en la salud del sistema. El propósito de este estudio fue demostrar una relación entre proximal y a escala de cuenca alteración del paisaje antropogénico y la diversidad de las comunidades de aves dentro de los sistemas de manglares al norte de La Paz, Baja California Sur, México. Encuestas de punto-conteo se utilizaron para recoger índice de diversidad de calificaciones para las comunidades de aves. Las tecnologías SIG y las imágenes raster se utilizaron para clasificar los sitios basados en los niveles físicos de los impactos humanos sobre el paisaje adyacente. Los resultados de este estudio demostraron que no había relación entre las alteraciones antropogénicas y diversidad de la comunidad aviar en la zona de estudio.

Keywords

Bioindicator, remote sensing, ecotone, carbon sequestration, fragmentation, basin-scale impacts

Introduction

While many organisms alter their environment in order to maximize their fitness, few have accomplished this task as broadly or as rapidly as the human species (Vitousek et al 1997). Mankind has nearly reshaped the entirety of the globe, predominantly through the transformation of land cover and the irreversible loss of biodiversity (Vitousek et al 1997). Although the impact of these anthropogenic changes are now experienced by near every process on earth, the change

to our planet's biogeochemical processes is likely the most looming for our own species (Vitousek et al 1997; Blanco-Libreros & Estrada-Urrea 2015). The effects of this biogeochemical alteration is largely acknowledged as the cause of increased levels of atmospheric and oceanic carbon, and referred to by the blanket term "Climate Change".

The concept of climate change incorporates a plethora of anthropogenic and biotic factors. Disruptions and alterations to our planet's natural capacity to absorb and sequester atmospheric carbon is one major focus of recent and ongoing research, particularly in regards to "blue carbon". Blue carbon is used to describe carbon that is sequestered from the atmosphere via marine processes (Marchio et al 2016), either via ocean-surface gas exchange or by accretion into sediments by coastal plant communities. The latter of these processes is conducted largely by saltmarsh grass communities or mangrove forests (Marchio et al 2016). Research currently shows mangrove-dominated ecosystems as the most effective biome for the removal and storage of atmospheric carbon, being as much as ten times more effective as coastal saltmarsh or northern peatlands (Ezcurra et al 2016).

Research has demonstrated that mangrove forests are not uniform in their ability to sequester carbon, with close correlation between this biogeochemical function and the level of anthropogenic impacts on the mangrove ecosystem (Twilley & Rivera-Monroy 2005; Lovelock et al 2013). Globally, mangrove systems are threatened via encroaching urbanization and development as well as stand removal as a result of the timber and aquaculture industries (Blanco-Libreros & Estrada-Urrea 2015). While total removal has obvious drastic and instantaneous effects on the natural processes performed by mangroves, what more commonly occurs is the fragmentation of larger systems into several smaller isolated mangrove stands. This

fragmentation has also been shown to have severe negative impacts not only on the stand composition, but on biogeochemical processes and the diversity of all organisms comprising the mangrove community (Vovides et al 2011).

Global concern over the rapid loss of mangrove systems has resulted in largely successful efforts to prevent the eradication of this vulnerable biome, and in many cases to replant and rehabilitate damaged systems (DeIVecchia et al 2015). While important, these restored systems have demonstrated a diminished capacity for carbon sequestration when compared to pristine mangrove systems (Vovides et al 2011). Recent research on mangrove systems in the state of Florida has discovered that the carbon sequestration abilities of a mangrove system are not only impacted by direct anthropogenic disturbance, but by surface alteration outside of the mangrove stand but within the associated hydrological basin (Marchio et al 2016). This reduced capacity was demonstrated not only in rates of organic accretion but in sediment storage capacity as well. Coastal development has long been the greatest threat to mangrove systems, and this discovery regarding the effects of proximal anthropogenic change on the ecological health of mangrove systems adds an even more complex facet to the preservation and management of mangrove forests.

Additionally, research has demonstrated links between the biogeochemical performance of mangrove systems and the quality of habitat provided to organisms within the stand community (Genthner et al 2013). This relationship was demonstrated in mangroves within the Gulf of Mexico, although the relative uniformity of mangrove botanical composition can allow the assumption that community interactions are approximately similar in mangrove systems across the globe. Other mangrove research conducted in the Gulf of Mexico (Florida) has

demonstrated a decrease in avian populations associated with mangrove systems, with the decline being particularly precipitous in mangrove-dependent species (Lloyd & Doyle 2011). Observations from studies on mangrove-dominated community trends have led to the use of animal species found within mangroves as bio-indicators of system health, with many of these studies returning mixed results (Mohd-Azlan & Lawes 2011; Blanco-Libreros & Estrada-Urrea 2015).

In the eastern Pacific basin, mangroves extend as far north as northwest Mexico on the Baja California peninsula, with stands consisting of highly isolated patches existing within arid lagoons and coves (Whitmore et al 2005). These stands have experienced a dramatic decline in recent decades, as levels of waterfront development on the Baja California peninsula rose sharply with an increasing local population and emerging tourism industry (Morzaria-Luna et al 2014). Formal protection for mangroves and stand rehabilitation efforts have been put in place (Vovides et al 2011), but in many cases development continues to encroach (Holguin et al 2006).

The goal of my study was to determine whether the effects of proximal anthropogenic disturbance on mangrove systems would correlate with diversity index scores of avian communities within discrete mangrove stands. Organism communities within mangroves have been demonstrated to serve as effective bio-indicators in certain cases (Blanco-Libreros & Estrada-Urrea 2015), and breeding bird communities have been shown to serve as accurate and accessible bio-indicators in other habitat types (Weber & Blank 2008). If this correlation were found to exist, the use of avian communities as bio-indicators of system health would both greatly decrease the time and funding required to assess mangrove health, while simultaneously expanding the global extent for which mangrove monitoring data is available.

Methods

Mangroves within the area of study were assigned to one of two groups, high disturbance and low disturbance, based on basin-scale anthropogenic alterations of the surface. Basin-scale disturbance scores were assigned via geospatial analysis via remote sensing. Avifauna diversity scores were calculated via point-observation field methods incorporating visual and auditory identification of species and individuals. Avifauna surveys were conducted at each location twice to account for both sunrise and sunset levels of activity. Surveys were conducted on the mangrove patch perimeter due to the small size of the stands sampled as well as the impenetrable nature and limited visibility within mangrove stands. Diversity scores were compiled for each of the two disturbance level groups, and a comparison was conducted via Welch's t-test.

The area of study consisted of the seven mangrove stands north of the city of La Paz and South of Laguna de Balandra in Baja California Sur, Mexico (Figure 1). Sites were identified using geospatial data delineating 2015 mangrove stands within Mexico accessed via the Comision Nacional Para el Conocimiento y Usa de la Biodiversidad (CONABIO) database. Mangrove stands were assigned a numeric ranking to serve as an identifier, with Site 1 being the first mangrove stand south of Laguna de Balandra and Site 7 being the first mangrove stand north of the city of La Paz. Observations were taken over four days in July, 2016.

Mangrove sites were categorized into High Impact and Low Impact disturbance sites based on their linear proximity to anthropogenic surface alteration. Greater weight was assigned to development and landscape alteration within 0.5 km from the mangrove stand, with development beyond 2.0 km receiving the lowest weight. Regardless of linear proximity, anthropogenic alteration outside of the most adjacent hydrological basin was not considered

during the categorizing mangrove sites under observation. All spatial analysis was conducted using ArcMap 10.3 and data accessed via CONABIO.

Avian diversity index scores were calculated via point-count survey protocols. Each location was selected randomly, and surveyed in the morning and evening. Evening surveys took place in a window bound by 1.5 hour before sunset and 0.5 hour after sunset. Morning surveys took place between 0.5 hour before sunrise and 1.5 hour after sunrise. Each point-count consisted of 10 minutes of simultaneous visual and auditory observation. Sites 3 through 7 were accessed via road, while Site 1 and Site 2 were accessed via kayak. Species and individuals were identified and tallied in the field as much as possible. Digital recordings were also made via smartphone for the duration of each observation in order to assist in proper identification of individuals only observed via their vocalization. All counts were conducted by the same observer in order to maintain sampling consistency between sites. Species richness and abundance were used to calculate Shannon-Wiener diversity index scores for each observation. Diversity scores were compiled according to the associated disturbance levels of the mangroves from which they were collected. Sites were then analyzed with a Welch's t-test, with the level of disturbance as the predictor parameter and avian diversity index scores as the response parameter. All statistical analysis was conducted via R software packages. At no point in this study were any organisms handled, harassed, harmed or manipulated.

Results

Categorization of mangrove stands based on proximal anthropogenic landscape alteration placed 5 locations into the “High” impact category and 2 into the “Low” impact category (Table 1). Point-count observations of birds resulted in 29 positively identified species, with 15 unidentified species incorporated into the survey results. Inclusion of these unidentified vocalizations into the Shannon-Weiner diversity index allowed for the use of 44 species in our analysis. Diversity index scores ranged from 1.52 to 2.35. The results of the Welch’s t-test did not show significant variation in avian diversity scores between “High” and “Low” anthropogenic impact categories.

Spatial analysis determined that the two northernmost locations had less anthropogenic disturbance within their hydrological basins than the 5 sites south of the BCS ferry terminal. The northern sites (1 and 2) were categorized as Low Impact, and sites 3 through 7 categorized as high impact. Over the majority of the hydrological basin, development was seen to be very minimal at all sites excepting 6 and 7, where a golf course and shrimp farm were proximal to the mangroves. Site 3 was adjacent to the Universidad Autonoma de Baja California Sur Pichilingue Marine Sciences Laboratory, as well as a large area of disturbed earth whose purpose was undetermined. Sites 4 and 5 were immediately adjacent to the coastal highway out of La Paz, and consequently had a large amount of alteration due to the road abutment and blasted hillsides. Site 1 contained a small permanent fishing encampment, but together with Site 2 the northern sites contained no more anthropogenic impact within their basins than the remnants of an old jeep trail.

Point-count observation data ranged from a low value of 1.51 (Site 6, evening survey) to a high value of 2.35 (Site 7, evening survey). The mean diversity score was 1.84, with SD = 0.27. Only 13 of the 14 planned point-count observations was conducted. We were unable to complete the evening observation at Site 3 due to scheduling conflicts and limited available timeframe for the collection of field data. The mean avian diversity score for the High Impact locations was 1.81 with SD = 0.32 (N=9). The avian diversity score for the Low Impact category was 1.89 with SD = 0.08 (N = 4). The Welch's t-test returned insignificant variation between impact categories ($t = 0.588$, $df = 8.47$, $p\text{-value} = 0.572$).

Discussion

The results of this study did not support our original hypothesis that avian community diversity in arid mangrove stands would be indicative of proximal anthropogenic disturbance levels. Diversity scores demonstrated less variance within the Low Impact category, however High Impact sites accounted for both the 4 lowest and 4 highest individual survey diversity scores out of the 13 surveys conducted. This overall trend remained true with the removal of the unidentified vocalizations from calculating the Shannon-Weiner diversity scores.

Several factors were noted during the study as having potential influences on the data that may have served to muddle the hypothesized relationship between avian diversity and proximal anthropogenic disturbance on the mangrove stands under observation. The spatial analysis revealed little to no human development farther away from the coastline than 0.5 km. With the majority of development in the area north of La Paz being directly on the waterfront, the effects

of basin-scale landscape alterations may not be experienced by these particular mangroves to a measurable degree. Additionally, what development was present in the basins associated with the research area was largely in support of the highway following the coast out of La Paz. While generally very proximal to the mangrove stands, the true impact of this landscape alteration may be more significant in regards to facilitating human access into the mangroves rather than disrupting any hydrological processes. Ultimately, the study sites producing both the highest and lowest diversity scores were separated by less than 1.0 km of highly developed beach, roadway and commercially developed property.

One observation of note during the conduction of this study was the influence of lagoons on the avian diversity recorded during surveys. All of the mangrove sites surveyed in this study existed in an inlet or cove to some capacity; this trait was uniform throughout the sites. Additionally, sites 1, 2, 3, and 7 contained lagoons. These lagoons were bodies of water isolated from the main cove but connected by a channel. Through this channel, the lagoons experienced tidal fluctuation. What was seemingly remarkable about these lagoons was that they lagged behind the tidal cycle due to the forced constriction of flow in the connecting channel. This pattern was observed most clearly during the Site 7 evening survey. Long-legged wading birds were abundant and actively feeding in the lagoon. Despite the high tide on the adjacent beach, the lagoon mudflats were still exposed and birds were actively feeding, perhaps as a concentrated gathering due to the opportunity. This occurrence resulted in the highest diversity score (2.35) despite Site 7 experiencing the highest degree of exposure to proximal anthropogenic impacts of all sites surveyed.

Another possible factor that might have prevented this study from demonstrating the expected relationship between avian diversity and proximal human landscape alteration was the uniformity of the surrounding terrestrial habitat matrix. All study sites were encompassed by arid desert dominated by low brush and large succulents, typical of the southern Baja California peninsula. The majority of the passerine species recorded during field observations are more typically associated with arid environments, with only one (*Dendroica petechial bryanti*) being considered as mangrove dependent. This lack of mangrove-dependent avian species is typical of the region, and likely result of the highly fragmented natural distribution of mangroves in the Gulf of California (Holguin et al 2005). Research on avian communities associated with mangroves in northern Australia found that avifaunal assemblages were more representative of variation and health of the surrounding habitat matrix, rather than of the mangrove stand in which they were observed (Mohd-Azlan & Lawes 2011). This was found to be particularly true for highly fragmented mangrove systems.

This study demonstrated a conundrum that seems to be a common and vexing theme throughout the onslaught of recent research on mangrove systems: that mangroves demonstrate remarkable variation in system dynamics between global regions and ecotypes. This is particularly concerning given that the majority of recent mangrove monitoring programs have relied heavily on remote sensing in the formation of policy, particularly LIDAR and LANDSAT imagery (Myint et al 2014; Lucas et al 2014). This illustrates the need for field testing of mangrove monitoring and assessment protocols in a variety of global mangrove-dominated systems. The findings of this experiment demonstrated that avian community diversity are not altered by proximal anthropogenic alterations of the landscape. This suggests that faunal

community diversity associated with highly fragmented, arid systems such as those typical of northwest Mexico might not be appropriate to use as bio-indicators of mangrove system health.

Literature Cited

Blanco-Libreros JP and Estrada-Urrea EA. 2015. Mangroves on the Edge: Anthrome-Dependent Fragmentation Influences Ecological Condition (Turbo, Colombia, Southern Caribbean). *Diversity* **7**: 206-228.

DelVecchia AG, Bruno JF, Benninger L, Alperin M, Banerjee O, Morales JD. 2014. Organic carbon inventories in natural and restored Ecuadorian mangrove forests. *Peer J. Electronic journal*.

Ezcurra P, Ezcurra E, Garcillan PP, Costa MT, Aburto-Oropeza O. 2016. Coastal landforms and accumulation of mangrove peat increase carbon sequestration and storage. *PNAS* **113**: 4404-4409.

Genthner, FJ, Lewis MA, Nestlerode JA, Elonen CM Chancy CA, Teague A, Harwell MC, Moffett MF, Hill BH. 2013. Relationships among habitat quality and measured condition variables in Gulf of Mexico mangroves. *Wetlands Ecology Management* **21**: 173–191.

Holguin G, Gonzalez-Zamorano P, de-Bashan LE, Mendoza R, Amador E & Bashan Y. 2005. Mangrove health in an arid environment encroached by urban development – a case study. *Science of the Total Environment* **363**: 260-274.

- Lloyd and Doyle. 2011. Abundance and population trends of mangrove landbirds in southwest Florida. *Journal of Field Ornithology* **82**: 132-139.
- Lovelock CE, Adame MF, Bennion V, Hayes M, O'Mara J, Reef R and Santini NS. 2013. Contemporary Rates of Carbon Sequestration Through Vertical Accretion of Sediments in Mangrove Forests and Saltmarshes of South East Queensland, Australia. *Estuaries and Coasts* **37**: 763-771.
- Lucas R et al. 2014. Contribution of L-band to systematic global mangrove monitoring. *Marine and Freshwater Research* **65**: 589-603.
- Marchio DA, Savarase M, Bovard B, and Mitsch WJ. 2016. Carbon Sequestration and Sedimentation in Mangrove Swamps Influenced by Hydrogeomorphic Conditions and Urbanization in Southwest Florida. *Forests* **7**: 116.
- Mohd-Azlan, J and Lawes MJ. 2011. The effect of the surrounding landscape matrix on mangrove bird community assembly in north Australia. *Biological Conservation* **144**: 2134-2141.
- Morzaria-Luna, HM, Castillo-Lopez A, Banemann GD Turk-Boyer P. 2014. Conservation strategies for coastal wetlands in the Gulf of California, Mexico. *Wetlands Ecology Management* **22**: 267-288.
- Myint SW, Franklin J, Buenemann M, Giri CP. 2014. Examining Change Detection Approaches for Tropical Mangrove Monitoring. *Photogrammetric Engineering and Remote Sensing* **80**: 983-993.

- Twilley RR and Rivera-Monroy VH. 2005. Developing Performance Measures of Mangrove Wetlands Using Simulation Models of Hydrology, Nutrient Biogeochemistry, and Community Dynamics. *Journal of Coastal Dynamics*. **40**: 79-93.
- Vovides, AG, Bashan Y, Lopez-Portillo JA, and Guevara R. 2011. Nitrogen Fixation in Preserved, Reforested, Naturally Regenerated and Impaired Mangroves as an Indicator of Functional Restoration in Mangroves in an Arid Region of Mexico. *Restoration Ecology* **19**: 236–244.
- Weber TC and Blank PJ. 2008. Validation of a Conservation Network on the Eastern Shore of Maryland, USA, Using Breeding Birds as Bio-Indicators. *Environmental Management*. **41**: 538-550.
- Whitmore, R.C., et al. 2005. The ecological importance of mangroves in Baja California Sur: conservation implication for an endangered ecosystem. Pages 298-333 in J.E. Cartron, G. Ceballos, and R.S. Felger, editors. *Biodiversity, Ecosystems, and Conservation in Northern Mexico*.
- Vitousek, P.M., H.A. Mooney, J. Lubchenco, and J.M. Melillo. 1997. Human domination of earth's ecosystems. *Science*. **227**: 494-499.

Tables

Table 1. Avian diversity index scores and proximal anthropogenic impact categorizations (H=High Impact, L=Low Impact) by survey site (AM: sunrise survey, PM: sunset survey).

Site:	1	2	3	4	5	6	7
Impact:	L	L	H	H	H	H	H
AM:	1.961	1.885	1.882	2.035	1.532	1.558	2.303
PM:	1.946	1.768	NA	1.642	1.542	1.519	2.352

Figure Legend

Figure 1. Study Sites 1-7, shown in relation to the city of La Paz within the state of Baja California Sur, Mexico (Mangroves shown in black enhanced visibility, size not to scale).

Figure 2. Results from Welch's t-test, showing lack of significant variation in avian community diversity scores (y axis) between High Impact and Low Impact (x axis) locations (error bars = 1 SE).

Figures

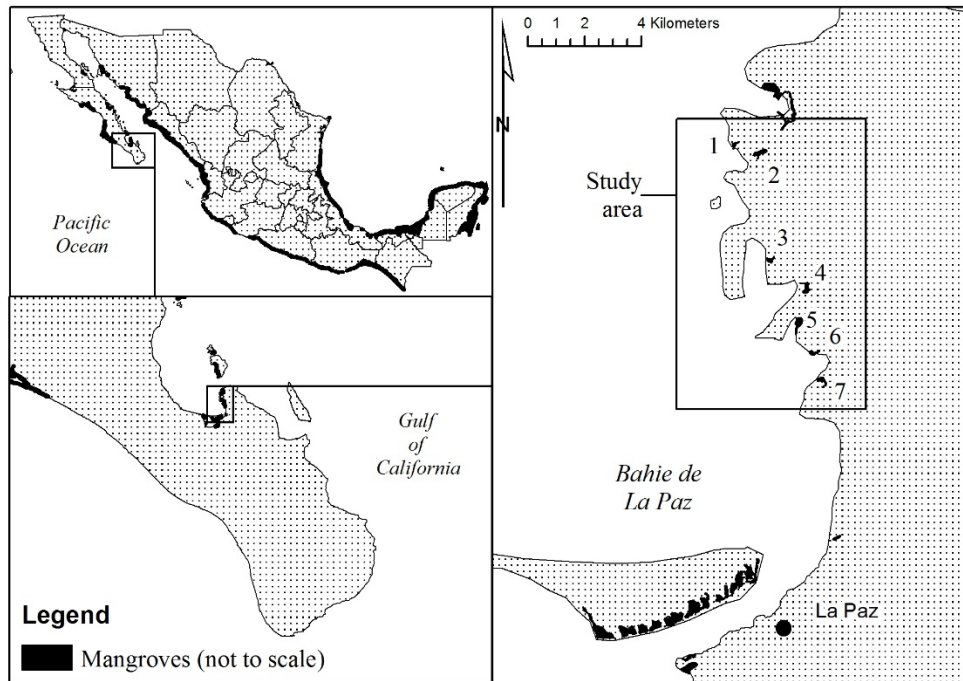


Figure 1

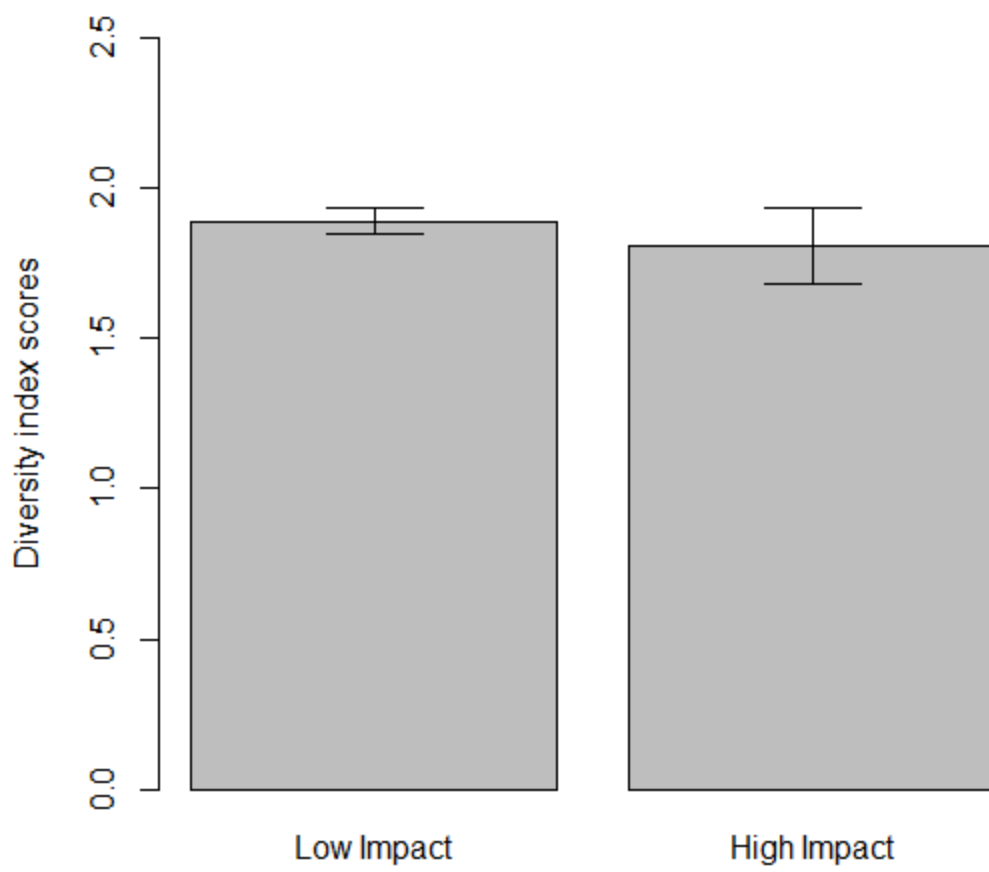


Figure 2

OYSTER SHELL DENSITY IN RELATION TO pH IN MANGROVES

Hymas, Jenna¹ and Silva, Jacqui¹

¹Western Washington University

516 High St, Bellingham, WA 98002

hymasj@wwu.edu, silvaj8@wwu.edu

Word count: 2463

Acknowledgements:

We'd like to acknowledge B. Miner for help with statistical analysis, innovative methods and transportation. A. Acevedo-Gutierrez for collection equipment and transportation. J. Guzman Loera for lab access. S. Francisco Flores Ramirez for testing equipment. Club Hotel Cantamar for equipment rental. Western Washington University and Universidad Autónoma de Baja California Sur for the opportunity to conduct this research. H. Sanchez for translation.

Keywords: Balandra, ocean acidification, La Paz, tannins, surface area, dissolution

OYSTER SHELL DENSITY IN RELATION TO pH IN MANGROVES

ABSTRACT

Mass mortality events of invertebrates have been observed globally as a result of decreasing pH due to ocean acidification. Many water parameters influence the shell growth of calcareous organisms, and such factors like water acidity has been widely studied due to its dissolving abilities. Ocean acidification has been associated with deformed or retarded growth and death among organisms such as molluscs, who commonly use calcium carbonate for the construction of shells. Though many are unable to survive acidic conditions, some bivalves have been found to be specially equipped to tolerate such environments. Diverse invertebrate populations are known to inhabit mangroves, which naturally acidify surrounding waters. Among the populations are calcareous organisms who are tolerant to acidic conditions. It could therefore be suggested that a gradient of shell densities exists between areas of low and high pH. This study aimed to determine if there was a difference in oyster shell density along a mangrove as a result of acidic waters. Oysters were collected at Balandra mangrove near La Paz, Mexico, along with pH measurements at two different sites. While there was a significant difference in the pH of the two sites, there was no significant difference between the shell densities. The interior shell densities were found to be 28% larger than the exterior, suggesting that the interior organisms' shells are not affected by the acidity of the water. The knowledge obtained from this experiment would establish baseline data that could be used in future evaluations of the health of Balandra mangrove, especially as ocean acidification becomes more prevalent.

ABSTRACTO

Mortalidades masivas de invertebrados han sido observadas en todo el mundo como resultado de un decremento en el pH debido a la acidificación de los océanos. Muchos parámetros relacionados con el agua influyen el crecimiento de los organismos calcáreos. Tales factores, como la acidificación del agua, han sido ampliamente estudiados debido a sus propiedades disolventes. La acidificación de los océanos ha sido asociada con deformaciones o retrasos en el crecimiento, e incluso la muerte en diferentes organismos como los moluscos, los cuales, utilizan carbonato de calcio para la construcción de sus conchas. A pesar de que existen muchas especies que no son capaces de sobrevivir a tales condiciones, se sabe que existen algunos bivalvos que pueden tolerar tales ambientes. Diversas poblaciones de invertebrados son conocidas por habitar en zonas de manglar, los cuales acidifican naturalmente las aguas adyacentes. Se ha sugerido que existe un gradiente de pequeñas densidades de bivalvos entre las áreas que presentan un bajo y alto pH. Este estudio tiene como objetivo el determinar si existe tal diferenciación en la densidad de las conchas a lo largo de los manglares debido a la acidificación de las aguas aledañas. Diferentes conchas fueron colectadas en el manglar de Balandra, próximo a la ciudad de La Paz, México, considerando el gradiente de pH. Se encontró que existen diferencias significativas en el pH de los dos sitios. Asimismo, el interior de la densidad de las conchas no resultó ser afectado por la acidificación del agua. El conocimiento obtenido a partir de este experimento puede establecer una línea base de datos que podrían ser usados en evaluaciones futuras respecto al estado de salud del manglar de Balandra, especialmente a medida que la acidificación de los océanos se vuelve mas relevante.

INTRODUCTION

Ocean acidification is an anthropogenic event described as the increase in ocean acidity as a result of excess carbon dioxide absorption from the atmosphere. Sudden decreases in pH can be detrimental for organisms that utilize calcareous shells. Acidic water can dissolve the shells of calcareous organisms and make it more difficult to obtain calcium carbonate for shell growth (Orr et al. 2005). This has been shown to cause shell deformation, slowed development, and death (Orr et al. 2005). Calcareous larvae are especially sensitive to acidic waters and often cannot survive beyond optimal ranges (Kurihara 2008). This was demonstrated by the near oyster industry collapse in Washington State, when juvenile oyster larvae failed to propagate from 2005 to 2009 due to a decreased pH of the area (Higley 2008). Though acidifying waters have been a cause for alarm, areas of low pH naturally occur around mangrove forests.

Mangrove trees are specialized for living in marine conditions and produce tannins to prevent damage from microbial activity in the water (Kimura et al. 1988). These tannins leech into the water through their roots and fallen leaves, acidifying the water (Maie et al. 2008). Mangrove forests act as nurseries for young fish and protect organisms from predators and harsh oceanic conditions (Jara et al. 2009; Beck, 1998). They are known to harbor diverse populations of invertebrates, including calcareous organisms, despite the low pH levels (Cantera et al. 1983). These organisms utilize different methods to survive acidic conditions, such as corbiculid bivalves which were found to secrete protective shell layers as a response to dissolution, allowing it to maintain shell integrity (Isaji 1995).

The mangroves of Balandra Bay in La Paz, Baja California Sur, Mexico consist of three species of mangroves the red mangrove, the white mangrove and the black mangrove. These

three species are stratified along the water's edge with red closest to the water and black the furthest away in the salt marshes. The red mangroves use prop roots, which extend from the trunk in every direction, to help support the tree. Since the red mangroves grow in an area that is mostly submerged in water where the ocean meets the land, they have adapted to use these aerial prop roots that they can also obtain oxygen and expel other gasses.

With mangroves being a direct influence on water acidity, it could be assumed that an increase in pH would be observed as the proximity to the mangrove increases. Therefore, it could be suggested that there exists an increasing gradient of shell density with corresponding increasing pH. Based on these assumptions, we hypothesized that the interior of the mangrove forest will have a lower pH and shell density compared to the exterior. The results of this study would establish baseline data for our study site, Balandra Beach. As ocean acidification becomes more prevalent, these data provide a basis for the assessment of the health of the area.

MATERIALS AND METHODS

Site Selection

Data were collected in the mangrove forest of Balandra Beach, La Paz, Baja California Sur, Mexico. The south east coastline of Balandra Beach contains fragmented mangrove forests in a sheltered bay. We focused on the forest immediately east of the public beach, which spans approximately 183 km². The study site was selected based on the maturity of the establishment, proximity to rocky coastline, large size, and access to center of the mangrove (Figure 1).

Water Parameter Measurements

Water parameters were measured every 200 m starting from the interior of the mangrove and moving westward along the south eastern coastline of Balandra Beach. Seven sample sites were measured and marked with flagging tape for future water parameter measurement.

Temperature, pH, and salinity were measured at each site during ebb and once again during flood tide of the same day for two days. Temperature and pH measurements were obtained using a portable pH tester (0.1 pH resolution and ± 0.1 pH accuracy) and salinity was measured with a handheld refractometer ($\pm 0.05\%$ accuracy).

Organism Sample Collection

Mangrove oysters of unknown species (*Crassostrea sp*) were collected based on their range throughout the site and presence of calcareous shells. Eight organisms were collected from randomized areas along a 20 m transect within the interior and exterior of the mangrove.

Organisms were placed into a labelled zip-closure bag filled with ambient seawater. Collected organisms were then placed into a cooler for transport.

Organisms were boiled in water to allow for the top valve to be removed for measurement. Any adhered organisms (e.g. tube worms, barnacles) were removed from the valve then the dry mass of each valve was measured using an analytical balance. Corresponding volumes were measured with water-displacement methods using a 10 mL graduated cylinder. The density for each shell was then calculated. Surface area of each valve was determined through ImageJ imaging software.

Data Analysis

Analysis of variance (ANOVA) test were conducted in the statistical program R. A two-way ANOVA was ran against pH and site and tide to determine relationships, if any, between the sites. A one-way ANOVA was ran to test the significance of pH and shell density of the interior and exterior of the mangrove. A final one-way ANOVA was ran against the shell surface area of the interior and exterior of the organisms.

RESULTS

The pH was analyzed with a two-way ANOVA and was found to be significant across all seven sites. The exterior of the mangrove (site seven) was more basic, with a pH of 8.1 compared to the inside of the mangrove (site one) at 7.9. We analyzed pH during the ebb and flood tides with a two-way ANOVA and found no significant values (Table 1).

Average densities of shells collected between both sites were found to be insignificant ($p > 0.97$). The interior shells had an average density 28% greater than the exterior shells (interior: mean=3394.3 mg/mL, standard error (SE)= 454.0 mg/mL, exterior: mean=2431.1 mg/mL, SE=124.8 mg/mL). The average surface areas of each site were also insignificant ($p > 0.99$). The average surface area of the interior shells was 41% greater than the exterior shells (interior: mean=904.9 mm², standard error (SE)= 156.1 mm², exterior: mean=535.1 mm², SE=35.6 mm²).

DISCUSSION

There was a significant effect of site on pH, indicating that acidity of the water decreases with decreasing proximity to Balandra mangrove. This supports the first part of our hypothesis stating that the interior of the Balandra mangrove were expected to have a lower pH

compared to the exterior. The pH measurements were taken during the ebb and flood tides to accounting for fluctuations and ensure accuracy. It was determined that tide had no effect on the pH of the water, suggesting that the acidic water is transported via other methods (Maie et al. 2008).

Although the pH across the seven sites was significant, we reject our hypothesis due to the average densities of the interior shells being considerably larger than those of the exterior. The average densities of the interior and exterior shells directly opposed our hypothesis, and we are confident that pH has no effect on the density of the calcareous shells in the mangroves. This suggests that pH is not a limiting factor in the development of the observed *Crassostrea* spp.

The average surface area of the interior shells were found to be significantly larger than those of the exterior, again directly opposing our hypothesis. We believe this to be a result of favorable living conditions on the interior of the mangrove, which provides protection from predators and harsh oceanic conditions (Chavez et al. 2001). The exterior shells were collected off the rocky substrate outside the mangrove entrance, which can be subject to desiccation, silting, and destruction from wave surge (Chavez et al. 2001). This differences between size and the location of growth of the oysters found at the Balandra mangroves suggest that pH is not affecting the oysters as we hypothesized. Further experimentation would be beneficial to see if these results are similar with other mangroves, either locally or on a global scale. If these results are replicated at multiple sites, we would be able to further test the calcareous *Crassostrea* oyster to see how it adapts to acidic water.

Studies of ocean acidification and the effects on benthic macrofauna communities is a necessity as increased carbon dioxide enters the earth atmosphere and the oceans. Bivalves have

been found to show signs of stunted growth at pH levels of 6.7, with death occurring around 6.0 pH (Marshall et al. 2008). Even more detrimental effects have been found in oceanic copepods which cannot tolerate acidification levels greater than 0.2 pH changes (Seibel and Walsh 2008). In the paper by Isaji (1995) it was discovered that the adaptations to acidic environments of the corbiculid *Geloina erosa* have been able to prevent the dissolution of their shells via organic sheets produced by the mantle. This may be a biotic factor contributing to our results. Due to ocean acidification being anthropogenically driven, the forecasting of this event is highly unpredictable and the fate of the *Crassostrea* oyster is unknown (Hoffman et al. 2011). Continued research is necessary to understand the limitations of the calcareous organisms within the Balandra mangroves.

In conclusion pH plays a valuable role in the mangrove communities within in Balandras mangroves. This study can benefit the Balandra mangroves by starting to piece together a baseline to assess the health of the mangroves now and in the future, for monitoring carbon dioxide increases and ocean acidification.

LITERATURE CITED

- Beck MW. 1998. Comparison of the measurement and effects of habitat structure on gastropods in rocky intertidal and mangrove habitats. *Marine Ecology Progress Series*, 169, 165-178.
- Chávez-Villalba J, López-Tapia M, Mazón-Suástegui J, Robles-Mungaray M. 2005. Growth of the oyster *Crassostrea corteziensis* (Hertlein, 1951) in Sonora, Mexico. *Aquaculture research*, **36**(14), 1337-1344.

- Higley R. 2010. Ocean acidification: the evil twin of climate change. Seminar presented at The Marine Science and Technology Center's Earth Week Science Seminar, Redondo, Washington. Retrieved from:
<https://media.highline.edu:8443/ess/echo/presentation/60ffa752-5809-4a83-9b4e-4874989e68b1>.
- Hofmann GE, Smith JE, Johnson KS, Send U, Levin LA, Micheli F, Matson PG. 2011. High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PloS one*, **6**(12), e28983.
- Isaji S. 1995. Defensive strategies against shell dissolution in bivalves inhabiting acidic environments: The Case of Galoina (Corbiculidae) in. *Veliger*, **38**(3), 235-246.
- Kimura M, Wada H. 1989. Tannins in mangrove tree roots and their role in the root environment. *Soil Science and Plant Nutrition*, **35**(1), 101-108.
- Kurihara, H. 2008. Effects of CO₂-driven ocean acidification on the early developmental stages of invertebrates.
- Maie N, Pisani O, Jaffé R. 2008. Mangrove tannins in aquatic ecosystems: Their fate and possible influence on dissolved organic carbon and nitrogen cycling. *Limnology and Oceanography* **53**.1:60.
- Marshall DJ, Santos JH, Leung KM, Chak WH. 2008. Correlations between gastropod shell dissolution and water chemical properties in a tropical estuary. *Marine Environmental Research*, **66**(4), 422-429.

Orr JC, Fabry VJ, Aumont O, Bopp L., Doney SC, Feely RA, Key RM. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437(7059), 681-686.

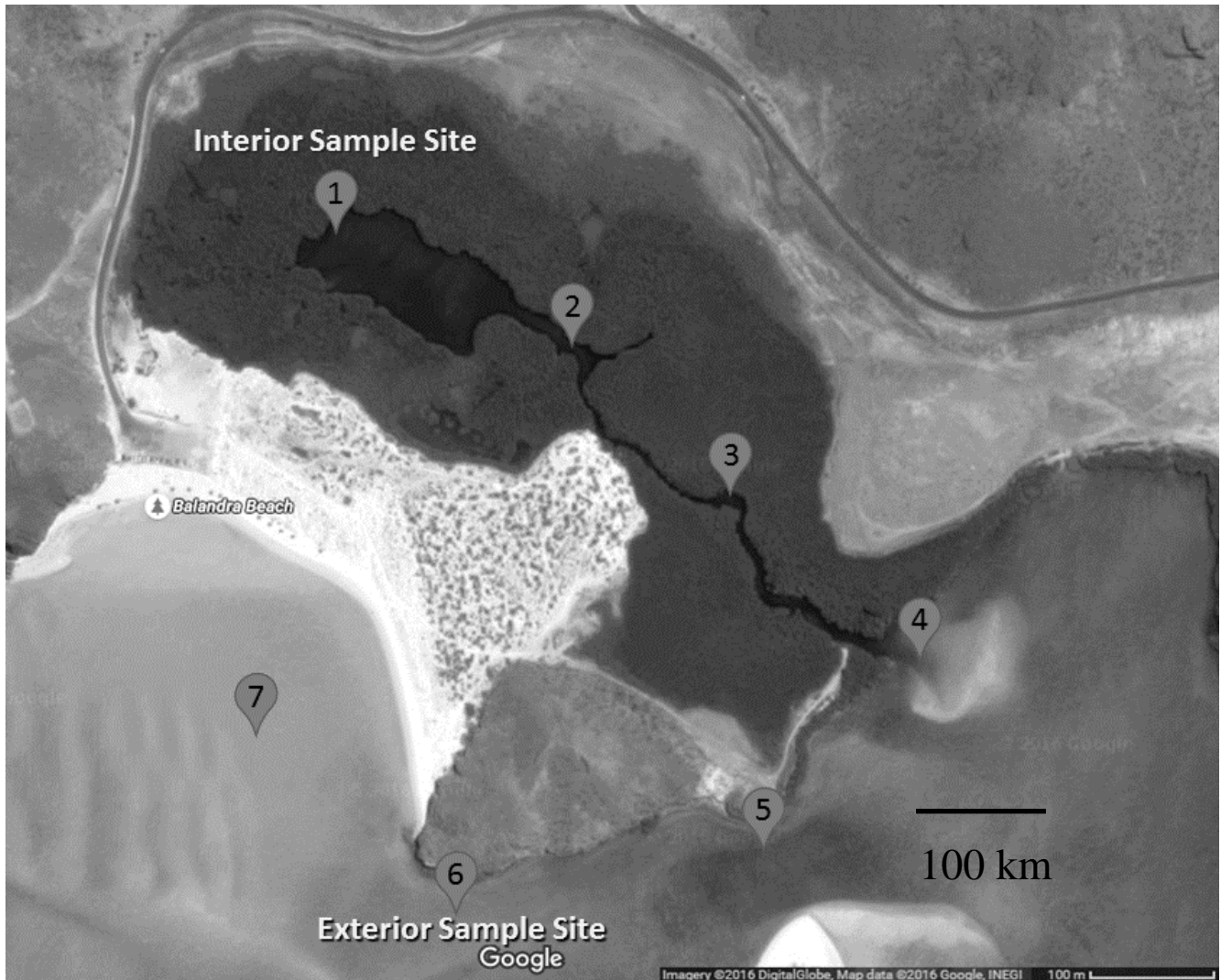
Seibel BA, Walsh PJ. 2003. Biological impacts of deep-sea carbon dioxide injection inferred from indices of physiological performance. *Journal of Experimental Biology*, **206**(4), 641-650.

Table 1. ANOVA (2-way) of pH at each site; site: interior, exterior; tide: ebb, flood.

	D.F	Sum of squares	Mean of squares	F Value	P (>F)
Site	1	0.150089	0.150089	35.8295	3.539e-06
Tide	1	0.003214	0.003214	0.7673	0.3897
Site:Tide	1	0.007232	0.007232	1.7265	0.2013

Figure 1. Sampling locations within Balandra Beach, La Paz, Mexico. Scale bar represents 100 km

Figure 1.



The Mobulas response to human influenced acoustic disturbance in the Gulf of California

Oliver Dev* & Jesse Katz*

**Western Washington University, 516 High St., Bellingham, WA 98225, USA, Universidad Autónoma de Baja California Sur, Carretera al Sur Km 5.5, 23080 La Paz, B.C.S, MX*

Keywords: elasmobranchs, anthropogenic sounds, jump, behavior, *Mobula* spp.

Word Count: 3404

Oliver Dev

7324 Puget Beach Rd NE, Olympia, WA, 98516, USA

devr@wwu.edu

ACKNOWLEDGMENTS

We would like to thank Alejandro Acevedo Gutierrez and Ben Miner for helping guide us through this project. We would also like to thank all of our classmates for supporting us and giving us helpful advice.

The Mobulas response to human influenced acoustic disturbance in the Gulf of California

ABSTRACT

Water possesses certain characteristics that make it an excellent medium for sound to travel through. Because of this, sound is an integral component of marine ecosystems that support a wide range of organisms. In times of low visibility, many of these organisms rely on sound to communicate, find food, and find mates. In the past few decades, an increase in coastal urbanization has led to an increase in anthropogenic acoustic influence within these delicate marine ecosystems. Organisms that are unable to surmount the increasing pressure caused by noise pollution can end up displaying erratic behavior; even going as far as intentionally harming themselves. Acoustic research is relatively new and there are still gaps in our knowledge on how certain species react to sound. Recently, elasmobranchs have become more prominent subjects in these acoustic studies. We present here an observational analysis into the reaction of an understudied genus of *Mobula* in response to the presence of motorized watercraft noise in a relatively busy bay in Baja California Sur, México. By counting the number of jumps and boats present over the course of 4 days we were able to find that *Mobula* spp. jumped more frequently in the presence of boats. Due to the nature of our data, we were also able to find that Mobulas jumped the most in the morning and the least in the afternoon. Furthermore, we were also able to find that *Mobula* jumping frequency increased as the day went on when boats were present. These results show that *Mobula* spp. could be jumping as an escape mechanism from excessive boat noise, but there could be other contributing factors. Understanding how *Mobula* spp. is affected by anthropogenic noise could help in their conservation, but more research needs to be done to fully understand how excessive noise affects them.

RESUMEN

La agua posee ciertas características que la hacen un medio excelente entre que el sonido puede viajar. Por eso, el sonido es un componente integral de las sistemas marinas que apoyan una amplia gama de organismos. Durante tiempos de visibilidad bajos, muchos de estos organismos dependen en el sonido para comunicar, encontrar comida, y encontrar un compañero. En las décadas pasadas, un aumento en la urbanización costera ha resultado en un aumento en la influencia acústica antropogénica en estas ecosistemas marinas delicadas. Los organismos que no pueden superar al aumento de la presión causado por la contaminación acústica pueden mostrar un comportamiento errático; a veces incluso hacerse daños. La investigación acústica es relativamente nueva y todavía hay muchos brechos en nuestro conocimiento de la manera que algunos especies reaccionan al sonido. Recientemente, los elasmobranquios se han convertidos a sujetos prominentes en estas estudios acústicas. Aquí presentamos un análisis observacional sobre los reacciones de un género *Mobula* poco estudiado en respuesta a la presencia del ruido de barcos de motor en una bahía relativamente ocupada en Baja California Sur, México. En contando los números de saltos y barcos presentes durante el curso de cuatro días hemos sido capaces de encontrar que *Mobula* spp. salté más frecuentemente en la presencia de los barcos. Debido a la naturaleza de nuestros datos, también hemos sido capaces de encontrar que las *Mobulas* saltaron más en la mañana y menos en la tarde. Adicionalmente, encontramos que la frecuencia de las saltando de las *mobulas* disminuido durante todo el día cuando los barcos estaban presentes. Estos resultados muestran que *Mobula* spp. podría ser saltando para escapar del ruido excesivo de los barcos, pero podría ser más factores contribuyendo. La compression de como *Mobula* spp. es afectado del ruido antropogénico podría ayudar con su conservación, pero

más investigación necesita ser hecho para entender completamente como el ruido excesivo las afectan.

INTRODUCTION

The soundscape of the ocean is filled with a plethora of incredibly diverse sounds. Initially, humans had discounted all of these sounds because of our inability to register the majority of them. However, developments in hydrophone technology have allowed us to detect and visualize natural sounds from all over the ocean and we are just beginning to understand how our own sounds affect marine organisms.

A review on the physics of sound

Regardless of medium, sound follows the same basic physics. Creation of sound is due to an object vibrating and sending out waves causing the surrounding particles of a medium to vibrate. These waves contain points of compression, where particles are squashed together, and points of expansion, where particles are pulled apart (Bradley & Stern 2008). Places of compression are known as regions of high pressure and places of expansion are known as regions of low pressure. Sound behavior varies tremendously between air and water due to their differences in density and compressibility. For example, the speed that sound travels in water is much quicker (~1500 meters/sec) than in air (~340 meters/sec). Additionally, water possesses characteristics such as increases in pressure and decreases in temperature that can allow sound to span entire ocean basins via the SOFAR (Sound Fixing and Ranging) channel (Northrop & Colborn 1974). Many marine organisms depend on these characteristics of underwater sound for various reasons and the presence of human influenced acoustic disturbance can have detrimental

effects on their livelihoods (Goldbogen et al. 2013). Characterization of anthropogenic signals have fallen into two categories: impulse and continuous (Board 2003). Impulse signals are sounds that are brief but loud such as air-guns, explosions, sparkers, and sonar pings. Continuous signals are sounds that are amplitude modulated (such as drilling rigs and ship engines) and broadband (such as ship noise and sonar). It has been suggested that sonar, propellers, and engines can have detrimental effects on animals that depend on sound to communicate (Tyack et al. 2006).

Effects of anthropogenic sound on marine organisms

Anthropogenic sound in marine environments can affect organisms in different ways; two examples are fish and marine mammals. Schooling fish have been known to disperse and become stressed due to sounds and presence of boats (Whitfield & Becker 2014). These stresses can lead to changes in feeding and mating behavior which could theoretically lead to negative cascade effects in a complicated food web. On the other end of the spectrum, marine mammals such as migrating whales can become disoriented and move erratically because of painful or frightening sounds thus splitting up populations and causing some individuals to run aground and become stranded (Barlow & Gisiner 2006). Strikingly, little is known about how elasmobranchs (sharks and rays) respond to various noise disturbances.

Background and physiology of the elasmobranch

Elasmobranchs are a subclass of cartilaginous fish that are characterized by the presence of 5 to 7 gill clefts, lack of swim bladders, rigid dorsal fins, and small placoid scales (Oguri 1990). In order to detect noise, elasmobranchs possess inner ear labyrinths that contain sensory

maculae. These maculae are lined with hairs that pick up vibrations including splashing and the sounds of injured prey. Prior research has found that elasmobranchs are more sensitive to acoustic particle motions than they are to sound pressure (Lobel 2009). It has been shown that at loud levels, they can distinguish differences between particle motion sounds and acoustic pressure sounds (Lobel 2009). Furthermore, studies on rays have found that they are more sensitive to direct and nearby vibrational noise compared to pressure transduced sound from a distance (Lobel 2009).

Background on the Mobula ray

Mobula rays (*Mobula* spp.) are a relatively understudied group of zooplanktivorous elasmobranchs that are part of the family Mobulidae. Thus far, there are 9 described species of *Mobula* that are distributed circumglobally in tropical, subtropical, and temperate coastal waters. A characteristic of *Mobula* spp. is their ability to breach the surface and reach heights of up to two meters before belly-flopping back into the water. Unfortunately, researchers don't know why *Mobula* rays jump. Prior hypotheses have included feeding behavior, courting behavior, and even getting rid of parasites. The skittish nature of this animal has made it difficult to study in the wild, other than when they exit the water.

Hypotheses

Here, we design an experiment where we test to see if *Mobula* spp. shows a jump response in the presence of acoustic disturbance by way of motorboat. We hypothesize that the jump frequency of *Mobula* spp. will increase with the presence of boats. Furthermore, during the experimental design stages we found that we were able to pose the question of whether time of

day affected *Mobula* spp. jumping frequency. We hypothesize that jumping frequency will be at its peak during morning and evening periods. We can relate this to our first question in that we believe the increased presence of boats in the water during these periods would cause increased jump frequency.

METHODS

All observations were taken at Club Hotel Cantamar, Baja California Sur, México (hereby referred to as Cantamar). Cantamar is on the western coast of the Gulf of California, and overlooks a small harbor that is an extension of La Paz Bay. This harbor is next to a popular beach and the Port of Pichilingue, and is therefore a common waterway for various small motorized boats as well as large ferries. *Mobula* spp. can be observed jumping with varying regularity in both the shallower harbor and the deeper bay. We did an observational study of the harbor and bay, noting when *Mobula* spp. jumped and when there were boats or ferries present or absent in the area. Data was analyzed using a χ^2 goodness-of-fit and a Pearson's χ^2 contingency tests. Due to the observational nature of this study, no Mobulas were collected or interacted with.

Site description

Field observations and data collection were carried out over the course of 4 days between the 20th and 23rd of July, 2016. Our viewpoint was on the roof of the tallest building in Cantamar which provided us an ideal frame of reference of the surrounding bay. This frame of reference was relatively large and each person had to observe a side so as not to miss any jumps within our study area (Figure 1). Observations were taken for 6 hours per day, divided into 3 blocks per day totaling 24 hours. Blocks took place from 8-10 am, 1-3 pm, and 6-8 pm in order to maximize

viewing during the morning, afternoon, and evening. The study area was established such that it extended past the island to accommodate the arrival of boats, but not so far that we couldn't determine whether a jump was caused by a *Mobula* (Figure 1).

Quantifying boats and jumps

Presence of motorized watercraft (e.g. jet skis, fishing boats, etc.) was recorded when boats entered our area of study. Furthermore, boats were only considered present if its motor was on. Once boats left the boundaries of our study area they were considered absent. *Mobula* spp. jumps were only counted when the organism was visibly seen fully breaching the water. We used a data entry program in Excel to record number of jumps, boats present, boats absent, as well as time of occurrence. In order to get a more accurate representation of the data, we communicated the presence and absence of boats and jumps in our respective areas to allow the other person to record their own set of data.

Data analyses

At the end of the observational period, we took the averages of our combined data. This included averages for number of jumps, total time for each block, and when boats were present. We ran two separate χ^2 goodness-of-fit tests using the statistical program R. The first test was to determine if number of jumps was independent from boats present, and the second test was to determine if number of jumps was independent from time of day. Finally, to test whether number of jumps was independent from boat presence and time of day combined, we ran a Pearson's χ^2 contingency test. A critical alpha of 0.01 was used to determine significance between factors. For

graphical presentation, values for jump frequency and time were expressed as averages for each block.

RESULTS

Response to boats

There were differences between the presence of boats and the frequency of jumps witnessed (Figure 2). Using a chi-squared goodness-of-fit, we found that there was a statistically significant difference between jumps that occurred when boats were present versus when they were absent, $\chi^2(1, N=2954) = 75.7, p < .01$. Jump frequency increased by 111% when boats were present versus when they were absent.

Response to time of day

Additionally, we found differences between the time of day and the frequency of jumps witnessed (Figure 2). Using a chi-squared goodness-of-fit, we found that there was a statistically significant difference between jumps and time of day, $\chi^2(2, N=2954) = 181, p < .01$. Jump frequency decreased by 51% between the morning and the afternoon followed by a 71% increase between the afternoon and the evening.

Response to boats and time of day

Finally, there were also differences between the frequency of jumps throughout the day when boats were present versus when they were absent (Figure 2). Using a chi-squared contingency test, we found that the jumping frequency of *Mobula* spp. was significantly different between morning, afternoon, and evening blocks when boats were present versus when they

were absent, $\chi^2(2, N=2954) = 251, p < .01$. We found that throughout the day relative jump frequency of *Mobula* spp. changed in the presence of boats. When boats were present jump frequencies increased throughout the day compared to when boats were absent. In the morning, jump frequency increased by 13%, in the afternoon jump frequency increased by 148%, and in the evening jump frequency increased by 407%.

DISCUSSION

Mobula spp. jumped more in the presence of boats, and we think this could be due to the sound produced by the motors or engines. There are several reasons why this could be the case. As mentioned earlier, marine animals can have negative reactions to high levels of anthropogenic sound (D'Spain et al. 2006). Specifically, increasing boat noise has been shown to cause some fish to swim away from the noise at increasing speeds, or exhibit general avoidance behavior (Jacobsen et al. 2014; Whitfield & Becker 2014). In addition, stingrays and eagle rays have been observed to respond to nearby vibrational noise such as the close sound of a motor boat (Lobel 2009). Since rays show the same avoiding behavior as other some other fishes when exposed to high levels of sound, *Mobula* spp. could be jumping in order to avoid the sound from motor boats.

Mobula spp. also jumped more in the morning and evening, and least in the afternoon, without regard to presence of boats. One potential explanation for this trend could be that *Mobula* spp. are less likely to jump in higher temperatures, such as the afternoon. Also, *Mobula* spp. jumped more as the day went on when there were boats present, and less as the day went on when there were no boats present. This trend could be explained by the fact that *Mobula* spp.

jumped more in the morning when fishing boats were leaving for the day, and also in the evening when there were more recreational boats out and the fishing boats were coming in.

While there are a variety of studies on general *Mobula* spp. biology, there is limited research on why they jump. Although we have reasons to believe that sound is causing the *Mobulas* to jump when there are boats around, there are many factors we didn't take into consideration that have been addressed in other studies. For example, the jumping could have something to do with feeding. Two Gulf of California species, *M. japonica* and *M. thurstoni*, feed on a species of zooplankton *Nyctiphanes simplex* (Sampson et al. 2010). *N. simplex* has observed swelling to the surface during the day, which draws a variety of predators including species of *Mobula* such as *M. japonica*, *M. thurstoni*, and *M. lucasana* (Gendron 1992). This could be one reason that schools of *Mobula* spp. often swim to the top of the water column and jump out of the water. This could also help explain why *Mobula* spp. tended to jump more in the morning.

This information on *Mobula* spp. response to boat noise is not only important for understanding why *Mobula* spp. jump, but also for *Mobula* spp. conservation. *Mobula* spp. are under varying levels of concern, including four species that are near threatened and one that is endangered, according to the IUCN Red List. One big reason for these classifications is that *Mobula* spp. are frequently caught as bycatch (incidental catches in fishing nets) due to the fact that they swim in large aggregations in semi-deep water near the coastline (Couturier 2012). So learning about how *Mobula* spp. respond to excess boat noise could be an important step towards protecting them, because that knowledge can be used to limit bycatch incidents. In addition,

because information on their behavior is lacking, any new information about how they respond to their environments and human influences would be helpful towards their conservation.

Further research should be done to figure out why *Mobula* spp. jump. If further researchers find similar results, it would be helpful to know whether jumping behavior around boats is different in various locations and across the nine *Mobula* species. And if *Mobula* spp. are heavily affected by anthropogenic sound, federal regulations should be enacted in various countries in order to help protect them. Regulations limiting boat traffic in certain areas could also help other marine animals such as fish and whales which are also negatively affected by anthropogenic sound. In addition, if *Mobula* spp. do avoid loud sounds, technology could be developed to drive Mobulas away from fishing nets.

We conclude that *Mobula* spp. could jump as an escape reaction to boat noise, but this could be in combination with other factors such as feeding and temperature. This information is crucial to *Mobula* spp. conservation, and because the genus is so common as bycatch, any hints on how to maintain populations would be helpful. In order to fully understand the effects of anthropogenic sound on *Mobula* spp., more research needs to be done, possibly focusing on the effect from species to species and over longer periods of time. Uncovering more results on this will help us understand how Mobulas respond to some human influences, and help protect them and other marine animals that react negatively to anthropogenic sound.

REFERENCES

- Barlow, J., & Gisiner, R. (2006). Mitigating, monitoring and assessing the effects of anthropogenic sound on beaked whales. *Journal of Cetacean Research and Management*, **7**(3), 239-249.
- Board, O. S. (2003). *Ocean noise and marine mammals*. National Academies Press.
- Bradley, D. L., & Stern, R. R. (2008). *Underwater sound and the marine mammal acoustic environment: A guide to fundamental principles*. Marine Mammal Commission.
- Courturier LIE, Marshall AD, Jaine FRA, Kashiwagi T, Pierce SJ, Townsend KA, Weeks SJ, Bennett MB, & Richardson AJ. 2012. *Journal of Fish Biology* **80**: 1075-1119.
- D'Spain GL, D'Amico A, & Fromm DM. 2006. Properties of the underwater sound fields during some well documented beaked whale mass stranding events. *Journal of Cetacean Research and Management* **7**(3): 223-238.
- Gendron D. 1992. Population structure of daytime surface swarms of *Nyctiphanes simplex* (Crustacea: Euphausiacea) in the Gulf of California, Mexico. *Marine Ecology Progress Series* **87**: 1-6.
- Goldbogen, J. A., et al. (2013). Blue whales respond to simulated mid-frequency military sonar. *Proceedings of the Royal Society of London B: Biological Sciences*, **280**(1765), 20130657.
- Jacobsen L, Baktoft H, Jepsen N, Aarestrup K, Berg S, Skov C. 2014. Effect of boat noise and angling on lake fish behaviour. *Journal of Fish Biology* **84** (6): 1768-1780.

- Lobel, P. S. (2009). Underwater acoustic ecology: boat noises and fish behavior. American Academy of Underwater Sciences.
- Northrop, J., & Colborn, J. G. (1974). Sofar channel axial sound speed and depth in the Atlantic Ocean. *Journal of Geophysical Research*, **79**(36), 5633-5641.
- Oguri, M. (1990). A review of selected physiological characteristics unique to elasmobranchs. Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries, NOAA Technical Report NMFS, **90**, 49-54.
- Sampson L, Galván-Magaña F, Silva-Dávila RD, Aguíñiga-García S, & O'sullivan JB. 2010. *Journal of the Marine Biological Association of the United Kingdom* **90**(5): 969-976.
- Tyack, P. L., Johnson, M., Soto, N. A., Sturlese, A., & Madsen, P. T. (2006). Extreme diving of beaked whales. *Journal of Experimental Biology*, **209**(21), 4238-4253.
- Whitfield, A.K., & Becker, A. (2014). Impacts of recreational motorboats on fishes: a review. *Marine pollution bulletin*, **83**(1), 24-31.

FIGURE CAPTIONS

Figure 1. Map of observation site. Black lines and gray shapes represent the study area boundaries. Dotted line splits area into two to allow for more accurate representation of jump frequency.

Figure 2. Number of jumps during the morning, afternoon, and evening, and when boats were present versus absent. The width represents total number of jumps.

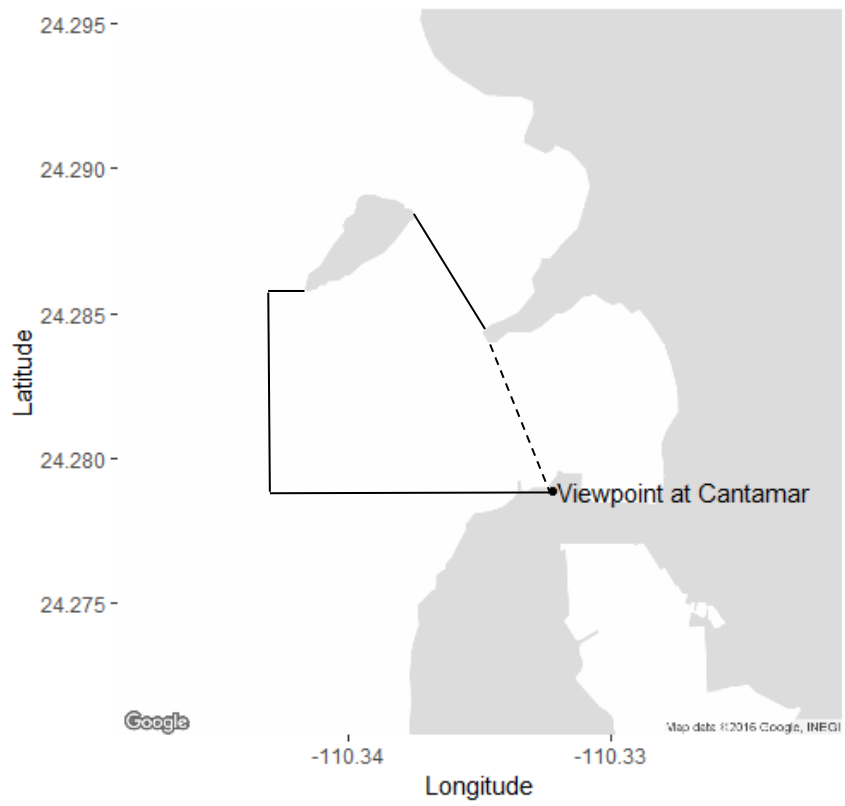


Figure 1.

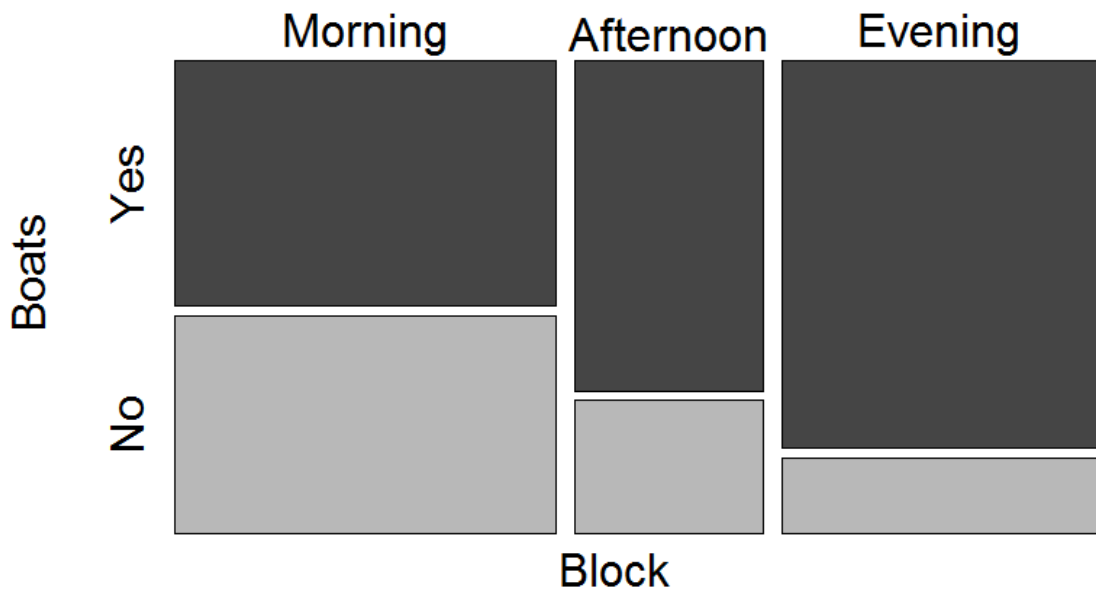


Figure 2.

**How coral volume of *Pocillopora elegans* affects fish diversity in the
Eastern Tropical Pacific**

Clarissa Felling, Kayla Litterell, Lindsey Hanson, and Siobhán Daly

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

Keywords: species richness and evenness, fish diversity, coral reef conservation, elegant coral, *Pocillopora elegans*, coral head volume, Baja California Sur

Palabras Clave: Arrecifes artificiales, coral elegante, *Pocillopora elegans*, Baja California Sur, volumen de coral, la diversidad de peces

How coral volume of *Pocillopora elegans* affects fish diversity in the Eastern Tropical Pacific

Abstract

Coral reefs have been appropriately named the “rainforest of the ocean” as they exhibit the widest range of biodiversity found in oceanic habitats. Unfortunately, the majority of the world's coral reefs are in a state of rapid decline. Elegant coral, also known as *Pocillopora elegans*, construct a large portion of the coral reefs found in La Paz, Baja California Sur, Mexico. Many studies have focused on percent coral cover none thus far have solely examined coral head volume with respect to species diversity. The main focus of our study was to make a connection between the biodiversity of reef fish with the volume of elegant coral heads for the purpose of coral reef conservation. Our hypothesis suggests that coral volume affects fish diversity. We predicted that as the volume of live elegant coral increased, the richness and evenness of fish species would increase independent of site location. We took species evenness and richness observations from 24 coral heads at two sites in Cantamar and one site in Calerita, La Paz, for a total of 72 data points. Our data analyses indicated that there was a significant correlation between coral volume and the diversity of fish surrounding the coral ($p=0.005$). Furthermore, the data supported our prediction that reef fish richness and evenness were independent of the study site. The positive correlation between reef size and fish diversity is increasingly important with conservation and preservation efforts for today's coral reefs as well as the design and establishment of artificial reefs in the future.

Los arrecifes de coral del mundo han sido denominados atinadamente como “los bosques tropicales del mar” ya que presentan el rango de biodiversidad más alto en los ambientes oceánicos. *Pocillopora elegans*, conocida comúnmente como coral elegante, constituye la mayor porción de los arrecifes coralinos que se encuentran en La Paz, Baja California Sur, México. Muchos estudios se han enfocado en estudiar el porcentaje de cobertura de los arrecifes de coral y no en la relación entre diversidad de especies y el volumen de cada cabeza de coral. El objetivo principal de este estudio fue encontrar si existe una relación entre la biodiversidad de peces con respecto a el tamaño de las cabezas de coral con el propósito de mejorar las estrategias de conservación, nuestra predicción es conforme se incrementa el volumen de las cabezas de coral la riqueza y abundancia de peces se incrementara en igual medida independientemente del sitio de muestreo. Tomamos muestras de abundancia y riqueza de especies en 24 cabezas de coral en dos sitios en Cantamar y uno en Calerita, La Paz, obteniendo un total de 72 puntos de muestreo. Nuestros análisis de datos indicaron que existió una correlación significativa entre el volumen de coral y la diversidad de especies. Además, nuestros datos reafirmaron nuestra hipótesis afirmando que la diversidad y abundancia serían independientes del sitio muestreado. La correlación positiva entre el tamaño del arrecife y la abundancia de peces refuerza la importancia de la conservación y esfuerzos para la preservación de los arrecifes coralinos que actualmente existen, así como la necesidad de diseñar y construir arrecifes artificiales en el futuro.

Introduction:

Coral reefs are one of the most diverse ecosystems in the world and are considered the “rainforests of the ocean” (Enoches et al. 2012). Elegant corals (*Pocillopora elegans*) are widespread throughout the Eastern Tropical Pacific and are vital to marine ecosystems due to their complex structures (Robinson & Thomson 1992). The complex structure of these corals supports a high diversity of fish by providing food, protection, as well as nesting grounds (Wagner et al. 2015). Elegant coral are rich in algae and polyps which provide fish with the necessary nutrients for survival (Horn 1989). In terms of protection, elegant coral have hole-like structures ranging in size that provide reef fish with crevices to protect them from potential predators, competitors, and currents (Ménard et al. 2012).

A positive correlation has previously been found between coral cover and branching with structural complexity (Graham & Nash 2012). A larger volume increases surface area which increases branching and structural complexity therefore allowing more space for reef fish to nest and hide. Having defendable nesting sites within coral heads increases the likelihood of reproductive success and offspring survival. All else being equal, as the coral volume increases, the access to these resources also increase which positively affects species abundance and richness (Wagner et al. 2015). While many studies have analyzed the relationship between species diversity and overall structural complexity, no studies thus far have solely examined coral head volume with respect to species diversity. Our study takes a small scale approach to isolate the importance of coral volume with reef fish diversity.

Our study serves ecological importance because coral reefs are indicative of ecosystem health. When coral is damaged due to water chemistry changes, tourism, or storms, their volume

and size decreases (Galzin et al. 2016; Mellin et al. 2016). Since a reef's structural complexity is positively related to reef fish diversity, density, and biomass, without a healthy population of coral, fish species diversity will suffer (Wagner et al. 2015). Our study investigates how a reduction in natural coral head volume could affect the diversity of reef fish in Baja California's tropical waters. This information could then weigh in on conservation decisions regarding the design of artificial reefs.

Given the importance of structural complexity within coral ecosystems, we hypothesized that the coral volume has an effect on fish diversity. We predicted that as the volume of live elegant coral increases, the richness and evenness of fish species will increase independent of site since coral ecosystems provide reef fish with protection, food, and nesting areas. To test this relationship, we measured species richness, evenness, and volume for 24 elegant coral heads at three coral reefs in La Paz, Baja California Sur for a total of 72 data points.

Methods:

We sampled three separate coral reefs in La Paz, Baja California Sur during the month of July 2016. Two of the coral reefs were located off-shore of Hotel Cantamar and the other coral reef was at Calerita beach. The first reef at Cantamar is located at $24^{\circ} 27'85.47''$ N and $110^{\circ} 33'40.45''$ W. The second reef at Cantamar is located at $24^{\circ} 28'41.57''$ N and $110^{\circ} 33'37.10''$ W. The third reef at Calerita beach is located $24^{\circ} 35'43.22''$ N and $110^{\circ} 28'35.73''$ W. We chose each site knowing that all are roughly the same size and have comparable species diversity. In terms of exposure to urbanization, the first site at Cantamar experiences more human disturbance

since the coral reef shares the coastline with a hotel. The second site at Cantamar is slightly more secluded but does have remanence of human debris, indicating it is not fully isolated from human disturbance. Calerita is a fringing reef and is considered our most “conserved” site since it is rarely visited by tourists and has minimal exposure to urbanization. The area we chose to sample began at the first sighting of coral and then continued along the coral reef. To minimize bias, we conducted a practice run to solidify a method that each pair carried out.

At each site, we randomly separated into pairs and collectively examined 24 coral heads. Coral heads were selected at random by first identifying five heads of coral within our occupied area and then consulting a random number generator to help dictate which head to observe. Once the coral head for observation was identified, we waited for the fish to resume a natural state. This was to reduce the effects of human disturbance we may have introduced while snorkeling to the coral head. Once the fish appeared to be settled, we assessed overall fish diversity as a “snapshot” in time. One person from each pair assessed species richness by counting the number of different species found within one meter of the coral; a method used by Roberts (1987). The second person from each group simultaneously assessed species evenness by totaling the number of individuals for each identified species within one meter of the coral. Once both people were confident in their numbers, one person recorded them on a dive slate while the other took any photos of undefined species. Each person was assigned the same job at every site to minimize human sampling error. Both species richness and evenness were used as variables in our overall assessment of species diversity.

After species richness and evenness were assessed, one partner took length and height measurements with a lead-free scientific measuring tape. When the coral head exceeded the arm-

span of one person, we would lay one end of the measuring tape on top of the coral and reel out until it reached the opposite end. Once the dimensions were recorded on the dive slate, each pair moved forward until they were out of range from the five previously selected coral heads to ensure no duplicate data was taken.

To calculate the overall species diversity for each coral head we employed species richness and evenness to estimate the Shannon-Wiener Index (Shannon & Weaver 1949). To calculate the volume of each coral head, we used the equation for an ellipsoid (Connell et al. 1997). We then ran a regression in a statistical analysis software to assess the relationship between species diversity and coral volume. We then ran a regression between species richness and coral volume and species evenness and coral volume. This was to see if our relationship between species diversity and coral volume was driven by either species richness, species evenness, or both. Once we plotted our regressions we noticed there was a large cluster of small coral volumes so we took the log of our volume to spread out the data in our graphs. To determine if the relationship between species diversity and coral volume varied across sites we ran a two-way analysis of variance.

Ethical statement:

Our study involved no handling of fishes or corals and only a brief disturbance of fishes while observing diversity and coral volume. Our procedures were approved by A. Acevedo-Gutiérrez, B. Miner, and S. Francisco Flores Ramírez of Western Washington University.

Results:

There was a significant relationship between species diversity and elegant coral head volume (Intercept: $t=11.898$, $p<2e-16$, Head Volume: $t=2.971$, $p=0.00406$; $SE=\pm 0.338$, $df=70$; $\alpha=0.05$, $n=72$). There was also a significant relationship between species richness and elegant coral head volume (Intercept: $t=19.835$, $p<2e-16$, Head Volume: $t=4.848$, $p=7.25e-06$; $SE=\pm 0.865$). Species evenness and coral head volume had a significant relationship as well (Intercept: $t=6.493$, $p=1.03e-8$, Head Volume: $t=4.040$, $p=0.000135$; $SE=\pm 12.34$). As the volume of elegant coral head increased species diversity also increased (Fig. 1). The volume of elegant coral increased along with species richness (Fig. 2) and species evenness (Fig. 3) as well.

There was no significant difference among sites (Table 1).

Discussion:

The data collected during our study supported our predictions that as the volume of live elegant coral increases, the richness and evenness of fish species would increase independent of site location (Table 1 and Fig. 1). We predicted a positive relationship between coral volume and species diversity due to the increased availability of resources for the fish. These resources include shelter, food, and nesting habitats (Bell & Galzin 1984). We visually observed that coral heads with a larger volume had a larger surface area and more crevices compared to smaller coral heads. An increase in the variability and abundance of holes can provide more protection and nesting grounds for a diversity of fish species (Ménard et al. 2012). Larger coral heads also provide fish with more food considering the positive relationship between algae growth, polyp

concentration, and coral volume (Horn 1989). Although our data does not directly explain which of these variables were responsible for the diversity, our results underline the importance of coral volume on fish diversity.

Our results were similar to other studies which analyzed the relationship between coral characteristics and fish abundance. For example, a study conducted by Wagner et al. (2015) found a positive correlation between fish abundance and coral patch size. He attributed his findings to an increased number of shelters or holes (Wagner et al. 2015). Similarly, a study by Ménard et al. (2012) found a positive relationship between shelter size and fish abundance. This positive relationship was explained by the obvious physical availability of space for more fish. For instance, a small shelter would physically exclude a large number fish while a large shelter could accommodate multiple fish (Ménard et al. 2012). We believe that the relationship between coral head volume and fish diversity was similar to the results found by Wagner et al. and Ménard et al. (2015, 2012). A reason for this relationship could be that as coral volume increases, so does surface area which increases the space availability allowing more fish to inhabit a single head of coral.

Territorial fish are an important explanation in this relationship. Ménard et al. (2012) found that the number of fish inhabiting one shelter was limited by the number of territorial fish. Cortez damselfish (*Microspathodon dorsalis*) were found at a vast majority of the observed coral heads, along with a fair quantity of tinsel squirrelfish (*Sagocentron suborbitalis*). These two species are both territorial and capable of defending multiple shelters at a time (Ménard et al. 2012). This relates to coral head volume, because smaller coral heads are more easily dominated by a single fish, thereby reducing the number of fish per coral head. Similarly, as the volume of

the coral head increases, we presume that the ability of a single fish to monopolize the whole coral head decreases allowing other fish to occupy the same head of coral. Therefore territorial fish are a viable explanation for the relationship between coral head volume and fish diversity.

Our data can be used in accordance with other studies to show that many coral characteristics can have significant interactions with species richness, evenness, and diversity. For example, Risk (1972) showed a positive correlation between fish species richness and structural complexity of the reef environment. Likewise, Luckhurst and Luckhurst (1978) found a positive relationship between structural complexity and fish abundance. Given that structural complexity has been positively correlated with an increase in live coral cover and the branching of coral, our study on coral volume is comparable to studies on structural complexity (Graham & Nash 2012). The relationship between coral volume, structural complexity and fish diversity further develops our understanding of coral reef ecosystems.

However, it is important to consider other factors that might affect fish diversity. Other studies have found no correlation between structural complexity and fish species richness but did find that fish species richness was positively correlated with increased coral diversity (Roberts 1987). While our study did not directly examine coral diversity, the positive relationship we found examining one coral species speaks to the importance of a single coral species on fish diversity. Even though, our results indicate that the volume of one species of coral has a significant effect on fish diversity, other species of coral should be further investigated to see if the positive correlation between coral volume and fish diversity is consistent among coral species. Given that elegant coral has a highly complex rocklike structure and was the most abundant coral in our study site we suspect that this relationship will not be consistent among

other coral species. We predict that with less complex coral or less abundant coral the volume will not correlate with fish diversity to the same degree, because the coral will not provide the fish with the same level of resources. The discrepancy between these studies sheds light on the complex network of variables that are at play in coral reefs and that multiple variables need to be considered to fully understand how to conserve these delicate ecosystems.

Our study can be used as a baseline for informative decisions regarding the design and implementation of artificial reefs to increase the fish diversity in the Gulf of California and help sustain fishing communities. One unique design would be to use biorock which is engineered rock that uses low-voltage currents to create a limestone covering. The electrolysis draws in free floating calcium carbonate which in turn, accelerates the growth of the attached coral. This rapid growth of the calcium carbonate structure allows the coral to expend more energy on reproduction and overall growth (Henderson 2001). This “low-cost, high reward” artificial reef is most optimal for our study since its size and shape can be manipulated to optimize reef diversity. It was noted in other studies that if we not only increase volume, but the surface area to volume ratio of artificial reefs then we can increase the fouling community thereby benefits herbivores (Dance 2011). With artificial reefs we can provide habitat and vital nutrients to both piscivores, with increases in fish evenness, and herbivores, with an increase in surface area. In fact, some studies regarding artificial reefs have found a positive correlation between population density with shelter availability in coral reef fishes (Almany 2004). Future studies could explore this further by engineering different sized rock and how fish diversity responds in threatened or damaged coral reefs. Constructing the most optimal version of artificial reefs could promote diversity around Baja California Sur and help keep marine protected areas safe from human disturbances.

Acknowledgments:

We thank A. Acevedo-Gutierrez for his help and support throughout this project. We thank B. Miner for his help with statistical analysis and guidance throughout this project. We thank D. Muñiz Nieto for her support with translating our abstract into Spanish. We thank Hotel Cantamar for the complimentary kayaks which were used to obtain data from a collection site.

Literature Cited:

- Almany GR. 2004. Differential effects of habitat complexity, predators and competitors on abundance of juvenile and adult coral reef fishes. *Oecologia*, 141(1): 105-113.
- Bell JD, Galzin R. 1984. Influence of live coral cover on coral-reef fish communities. *Marine Ecology Progress Series*, 15(3): 265-274.
- Connell JH, Hughes TP, Wallace CC. 1997. A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. *Ecological Monographs*. 67(4): 461-488.
- Dance MA, Patterson III WF, Addis DT. 2011. Fish Community and Trophic Structure at Artificial Reef Sites in the Northeastern Gulf of Mexico. *Bulletin of Marine Science*. 87(3): 301-324.
- Enoches IC, Manzello DP. 2012. Responses of Cryptofaunal Species Richness and Trophic Potential to Coral Reef Habitat Degradation. *MDPI, diversity*, 4: 94-104.

- Galzin R, Lecchini D, Lison de Loma T, Moritz C, Parravicini V, Siu G. 2016. Long term monitoring of coral and fish assemblages (1983–2014) in Tiahura reefs, Moorea, French Polynesia. *Cybium*, 40: 31-41.
- Graham NA, Nash KL. 2012. The importance of structural complexity in coral reef ecosystems. *Coral Reefs*, 32.2: 315-326.
- Henderson C. 2001. How to grow a living reef from iron and seawater: Weird Science: Biorock may help reverse the depletion of the world's coral, says caspar henderson. *Financial Times*.
- Horn MH. 1989. Biology of marine herbivorous fishes. *Oceanography Marine Biology*. 27: 167-272.
- Luckhurst BE, Luckhurst K. 1978. Analysis of the influence of substrate variables on coral reef fish communities. *Marine Biology*. 49(4): 317-323.
- Mellin C, et al. 2016. Humans and seasonal climate variability threaten large-bodied coral reef fish with small ranges. *Nature communications*. 7.
- Ménard A, Turgeon K, Roche DG, Binning SA, Kramer DL. 2012. Shelters and their use by fishes on fringing coral reefs. *PloS one*, 7(6): 2-12.
- Risk, M.J. 1973. Fish Diversity on Coral Reefs in the Virgin Islands. *Atoll Research Bulletin*. 153: 1-6.
- Roberts, C. M. 1987. Habitat complexity and coral reef fish diversity and abundance on Red Sea fringing reefs. *Marine Ecology Program Series*. 41: 1-8.

Robinson JA, Thomson DA. 1992. Status of Pulmo Coral Reefs in the Lower Gulf of California. Environmental Conservation. 19(3): 261-264.

Shannon CE, Weaver W. 1949. The mathematical theory of communication. University of Illinois Press, Urbana, 104-107.

Wagner EL, Roche DG, Binning SA, Wismer S, Bshary R. 2015. Temporal comparison and predictors of fish species abundance and richness on undisturbed coral reef patches. PeerJ, 3.

Tables:

Table 1. Two-way analysis of variance of the effects of site and coral head volume on species diversity (n=72 coral heads) with an asterisk indicating $p < 0.05$.

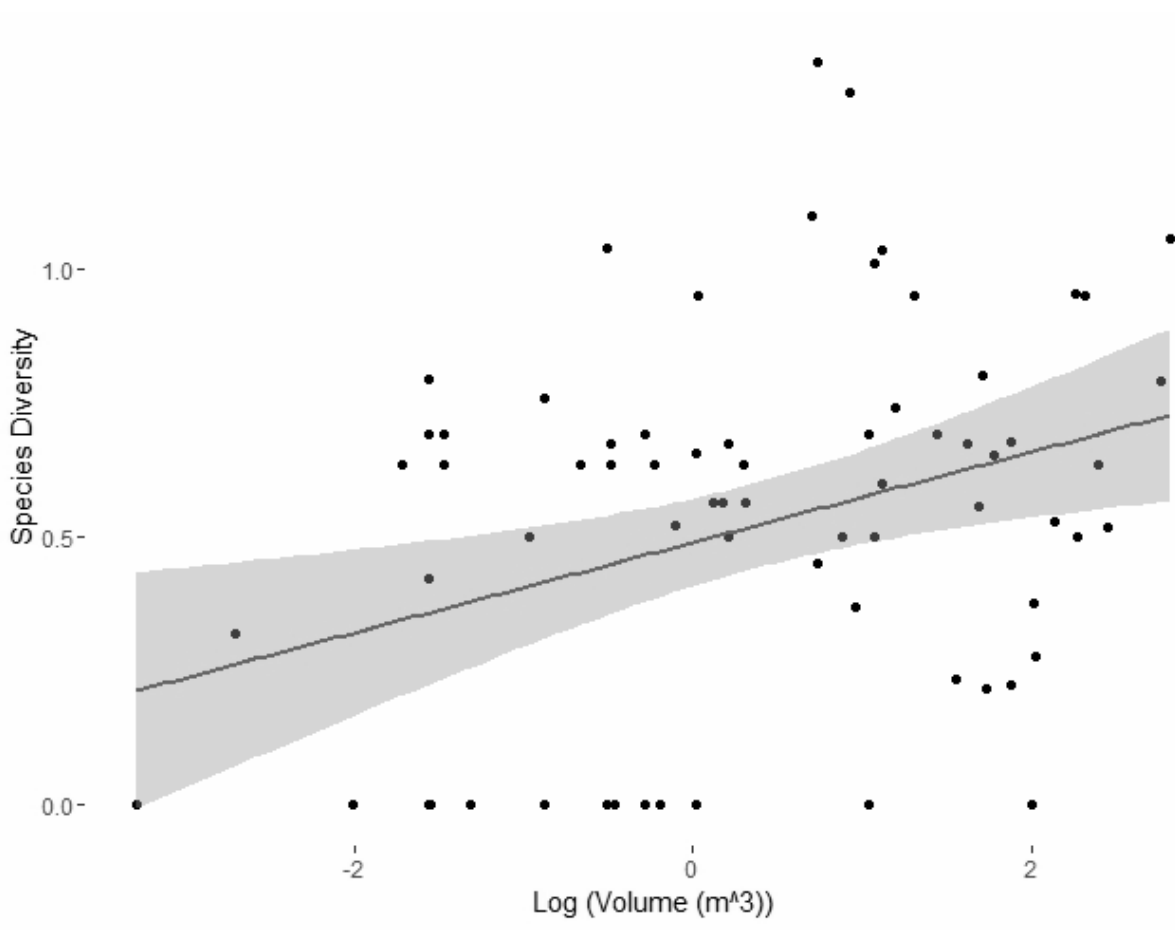
	df	SS	MS	<i>F</i>	<i>p</i>
Volume	1	1.011	1.011	8.554	0.00472*
Site	2	0.155	0.0776	0.656	0.522
Volume x Site	2	0.0594	0.0297	0.251	0.779
Residuals	66	7.801	0.118		

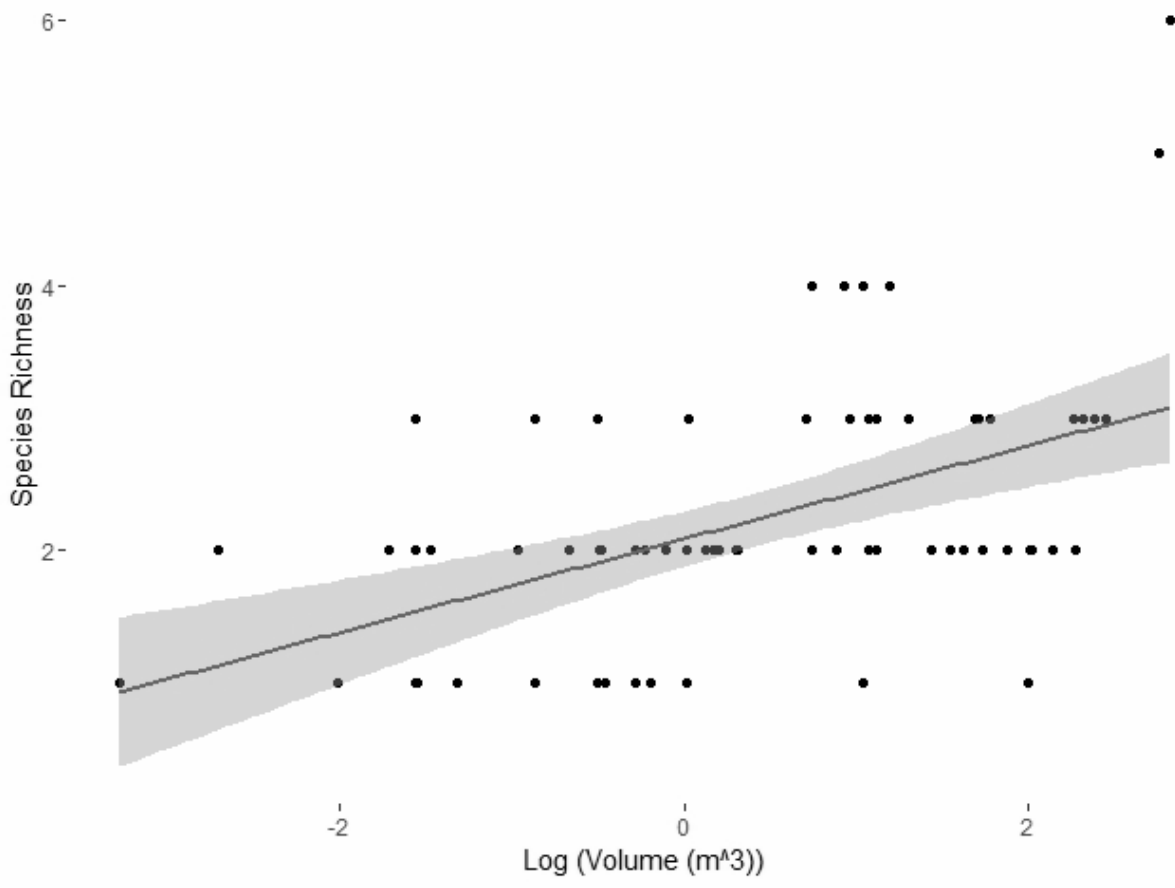
Figure legends:

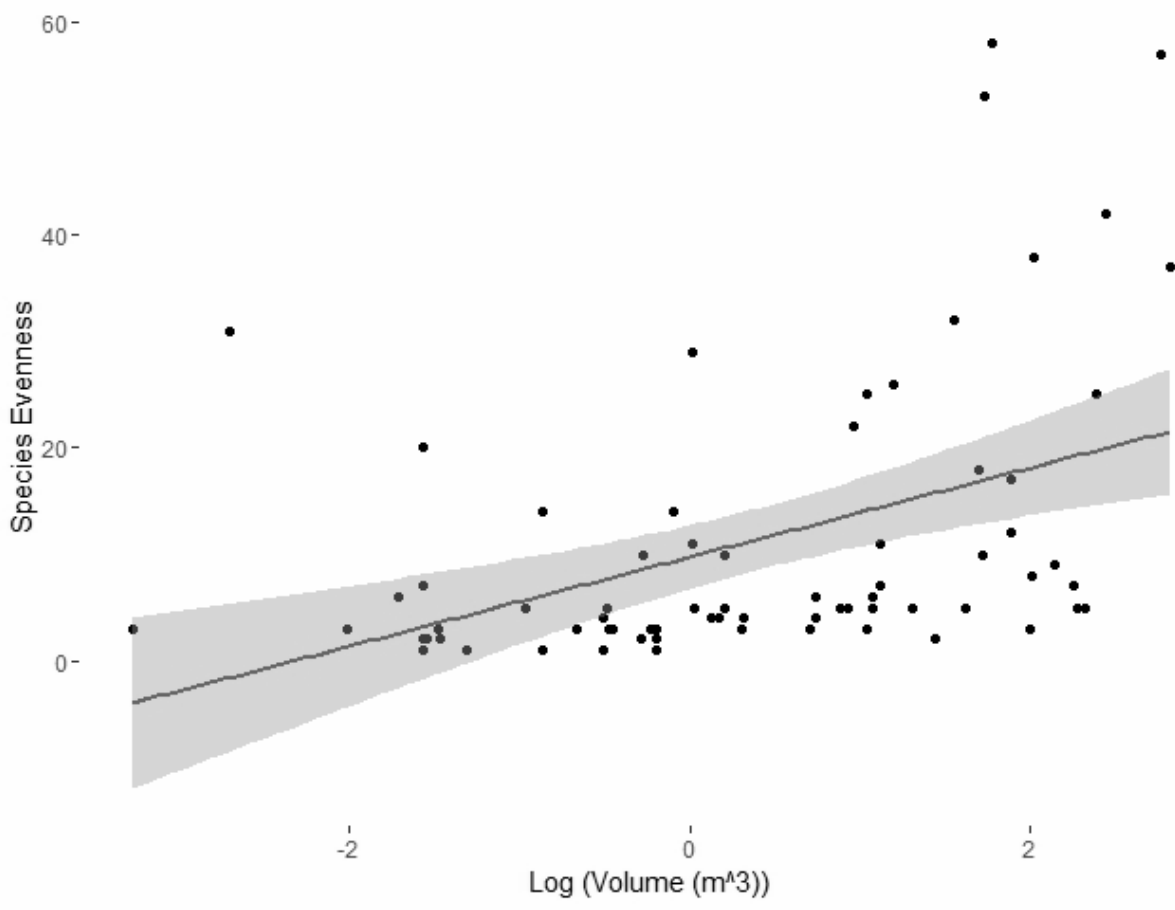
Figure 1. Regression of species diversity with elegant coral head volume with a line of best fit \pm SE (n=72).

Figure 2. Regression of species richness with elegant coral head volume with a line of best fit \pm SE (n=72).

Figure 3. Regression of species evenness and elegant coral head volume with a line of best fit \pm SE (n=72).







Snorkeling causes a temporary decrease in fish abundance and species composition in Baja California Sur

Rachel Flanders*

Angelica Kahler[‡]

Rachel Wachtendonk[‡]

*Environmental Science Department Western Washington University 516 High St. Bellingham, WA 98225

[‡]Biology Department Western Washington University 516 High St. Bellingham, WA 98225

Key Words: Disturbance, Marine conservation, Marine tourism, Prey adaptations, Reef communities

Acknowledgments

We would like to thank Benjamin Miner for his edits, and statistical guidance. Also, Alejandro Acevedo-Gutierrez and Sergio Francisco Flores Ramírez for their help in the methodology of this study. A thank you to Deborah Donovan for her support and guidance. Thank you to Western Washington University and Universidad Autónoma de Baja California Sur for funding and providing this amazing opportunity.

Word Count: 4362

SNORKELING CAUSES A TEMPORARY DECREASE IN FISH ABUNDANCE AND SPECIES COMPOSITION IN BAJA CALIFORNIA SUR

Abstract

Coral reefs suffer because of many human activities. In this paper we will focus on the effects snorkeling has on these delicate systems. Snorkeling is a popular form of marine tourism. Marine tourism has become one of the largest industries in the world, and is continuing to expand. It helps bolster local economies both directly and indirectly. Tourism also generates concern and empathy within travelers through first-hand experiences. This inspires everyday citizens to take part in future environmental preservation. Marine tourism can also be used as a platform for education regarding the importance of marine communities. There are also many negative effects associated with marine tourism. Coral reefs are increasingly susceptible to death as temperatures rise, and the negative effects of marine tourism only amplify coral destruction. Popular tourist activities such as snorkeling and self-contained breathing apparatus (SCUBA) diving have been linked to reef degradation. Our study took place on the Pichilingue bay, La Paz, México. The reef community here is unique in the sense that it is largely composed of endemic species which occupy similar niches. We decided to test the effect varying levels of snorkeling had on fish and species abundance. We believed with increasing levels of disturbance, time it took the reef community to recover would also increase. We found that the level of disturbance did not have a significant effect on fish or species abundance however there were significantly less fish and species throughout disturbance periods. We believe this is linked to individual fish personality, distinct investments in reef communities, or prey adaptations. Though we found snorkeling does not have long term effects on reef communities, we believe it is still imperative to practice safe snorkeling habits to ensure that the reefs can be enjoyed by generations to come.

Resumen

Los arrecifes de coral son uno de los ecosistemas más afectados por actividades humanas. En este estudio nos enfocamos en analizar los efectos que tiene la actividad de snorkel en estos delicados sistemas. La actividad de snorkel es muy popular en el turismo marino, el cual se ha convertido en una de las industrias más importantes en el mundo y aún sigue expandiéndose, creando empatía y conciencia en las personas, siendo una plataforma para la educación acerca de las comunidades marinas y además ayuda a las economías locales directa o indirectamente. Los corales han incrementado su mortalidad debido a los incrementos en la temperatura y a la actividad turística que ha incrementado en los últimos años considerablemente que a su vez a incrementado la destrucción incidental de estos ecosistemas. Este estudio fue realizado en la bahía de Pichilingue en La Paz, México. La comunidad arrecifal en esta localidad es única en el sentido de que está compuesta por mucha especies endémicas que ocupan nichos similares. Se analizó el efecto que diferentes intensidades de “snorkeleo” tienen sobre la diversidad y abundancia de peces en los arrecifes de coral, hipotetizando que con el incremento de la intensidad de la actividad aumentará el tiempo de recuperación de la comunidad de peces. Encontrándose que el nivel de intensidad de la actividad no tiene un efecto significativo en la abundancia de peces, sin embargo, hubo un descenso significativo en el número de especies y en la abundancia durante los periodos de actividad. Sugerimos que esto está relacionado con el comportamiento de cada uno de los peces y con las adaptaciones antipredatorias que estos puedan tener. A pesar de haber encontrado que la actividad humana no tiene un efecto significativo en comunidades arrecifales, creemos que es fundamental practicar estas actividades de manera sustentable para la conservación de estos ecosistemas.

Introduction

The tourism industry is one of the largest businesses in the world (Miller 1993). Marine tourism specifically is a major component to this industry (Hall 2001). When traveling abroad, especially to tropical destinations, it is common for travelers to snorkel or SCUBA dive. The urge to see organisms up close and first-hand is palpable during these getaways. However, putting humans in an unfamiliar environment can cause irreparable damage. There are certain species of fish that do not mind humans, however there are fish that race for shelter immediately. In order to entice these timid fish from their hiding places, snorkelers and SCUBA divers alike, must maintain patience and tranquility. It is important for snorkelers and SCUBA divers to understand the environmental implications these enjoyable watersports have on marine ecosystems. Without safe and intelligible practices, we cannot hope to continue our enjoyment of the ocean.

People of all ages, backgrounds and experience levels can enjoy marine tourism. Marine tourism activities include water sports, snorkeling, SCUBA diving, swimming and other activities. In particular, snorkeling allows participants to experience the marine environment first-hand in a way that is cost effective, easy and fun. This type of tourism has many impacts on not only the marine environment, but the surrounding areas as well.

Tourists who visit a coastal environment with the intent of engaging in marine tourism bring money and business to the area. In 1997, tourism brought in 423 billion U.S dollars, and it is predicted in 2020 to bring in five times that amount (Orams 1999). This money then goes into the local economy both directly, indirectly and through organizations, and businesses that are tied to and rely on the marine environment (Orams 1999). Tourism also helps local people find

new and sustainable ways to make a living. Fisherman may take tourists on snorkeling trips, or marine mammal tours. This then provides them with an income that is less harmful to the marine environment (Roberts & Hawkins 2000). This input of money boosts local economies and businesses. Locals of these popular tourist sites can encourage more travelers to visit the area as the community begins to expand.

While traveling tourists might gain interest and passion for the environment in which they are immersed in. Those who participate in snorkeling do so because they enjoy it and they have curiosity for the creatures they see. This interest and passion can turn into concern for the marine environment. Most people, after seeing the ocean's beauty first-hand, will spend money on activities that are helpful in the creation of preserves to protect the beauty seen on their trip (Wilson & Tisdell 2006). When people see picturesque organisms and pristine reefs, they feel inspired to protect these areas and connect with the organisms they are seeing. This will lead to further preservation in the future (Wilson & Tisdell 2006).

Another positive aspect that marine tourists can take away from visiting an area is knowledge. Many tourist activities involve an aspect of teaching. Visitors typically learn about the local area and the organisms they will find in the environment. The guides for most tourist activities share their local knowledge and tips for enjoying the sites and creatures. They also provide groups with warnings, and regulations about the area. This creates awareness and interest for the environment, which increases overall tourism to the area. However tourism has a number of negative effects on coastal environments as well.

Now more than ever natural ecosystems are in jeopardy of being destroyed. Current science suggests that as temperatures rise, coral reefs are increasingly susceptible to bleaching

and death. If tourism continues irresponsibly it may mean the end of coral reefs (Wilkinson et al 1999). Even water sports deemed “environmentally friendly”, like SCUBA diving, have been linked to deterioration of global reef systems (Davenport & Davenport 2006). This only reinforces the need for further research. The tourism industry needs to find a balance between oceanic immersion and enjoyment without the fear of irreparable damage.

Our study focuses on the temporary effects snorkeling has on reef communities, namely fish density as well as species composition. Marine tourism is one of the fastest growing industries and yet only recently, has meaningful scientific research emerged (Hall 2001). Without scientific basis regarding the harm related to tourist activities, like snorkeling, we cannot hope to educate vacation-goers on safe practices. Many studies have been published on the effects of snorkeling on reef communities. One such study focused on changes in fish abundance and species richness with the presence of snorkelers and SCUBA divers (Dearden et al. 2010). The study noted a significant drop in species richness during snorkeling events (Dearden et al. 2010). A study done regarding snorkelers on the Great Barrier Reef reported extensive damage to reefs when snorkelers were highly concentrated in one area and when their tours were self-guided (Plathong et al. 2008). The addition of interpretive signs explaining the dangers of snorkeling to reef systems acted as an enforcer rather than a deterrent of damage. This suggests, to reduce degradation of marine ecosystems, snorkeler behavior should be monitored in the water (Plathong et al. 2008). Though snorkeling can be an enjoyable way to pass time while vacationing, it does not ensure the safety of marine environments. Without further scientific examination regarding sustainable tourism, abuse will continue to harm delicate reef communities.

One such delicate coral community can be found in Baja California Sur, México. The rocky reefs found in the Gulf of California are home to roughly 271 species of fish. Seventeen percent of these fish are endemic (Arreola 2002). Many dominant species are found in great abundance. Some such species include sergeant majors, spotted sharpnose puffers, yellowfin surgeonfish, reef cornetfish, spotted grunts, and rainbow wrasses. Species composition changes with depth, as well as between sheltered and exposed reefs (Arreola 2002). The reef fish here have a large amount of niche overlap (Aburto-Oropeza 2001). This is due to the generalized nature of reef fish. Reef species are equal in their ability to make space for themselves (Arreola 2002). Lack of competitive advantages allow many species to coexist in the same area.

Marine substratum plays a large role in determining the diversity of species that inhabit the area (Aburto-Oropeza 2001). Rocky walls that are common in the Gulf of California can support a large amount of life. The presence of crevices in the walls relate to a large diversity of species (Aburto-Oropeza 2001). The diversity of life found in the Gulf of California is comparable to places such as the Galápagos Islands, Hawaii, and the Bahamas (Arreola 2002).

The aim of our study was to see if level of disturbance had an impact on the time it takes for a reef community to recover. We hypothesized that with an increasing level of disturbance, the time it takes for the reef community to recover would also increase.

Methods

We conducted an observational field study. We collected fish abundance and species composition data to see how snorkeling disturbance affects recovery time of reef communities.

We recorded underwater video footage and counted fish that passed over a two meter transect in front of the camera. We statistically analyzed our data using the program R.

We observed fish in two sites within Club Cantamar beach, which is within Pichilingue bay, La Paz, México (Fig. 1). Both sites are rocky with interspersed patches of elegant coral, and have steep downward slopes which end at mud flats. Site one is located at 24.279668° N and 110.332066° W. Site two is located at 24.279271° N and 110.333643° W. These two sites are 170 meters apart (Fig. 2), and consisted of separate reef communities. On day one we visited site two, which was chosen randomly. After this we alternated which site we surveyed each day. The whole process was conducted over four days, spanning from July 20th to 24th 2016. We surveyed twice a day, once at 9:30, and again at 15:30.

Once at a particular site, we placed a waterproof camera at the bottom of the rocky substratum, angling the camera up the rocky slope. A two meter polyvinyl chloride (PVC) pipe was placed in front of the camera to give a point of reference within which to count fish. This pipe ran perpendicular to the camera face. We then began recording for ten minutes, corresponding to our baseline interval. During all baseline intervals we left the area for ten minutes to record an undisturbed reef community. After this baseline recording, we returned and started our ten minute disturbance interval. We conducted our disturbances at three different levels of intensity. Level one consisted of all three observers floating at the surface observing fish behavior. Level two consisted of us floating at the surface and free diving down to the reef while maintaining a one to two meter distance from the reef. Level three consisted of us actively chasing, agitating, and harassing fish. After ten minutes, performing one of three disturbances, we stopped the recording and promptly began another ten minute interval which we termed

recovery. After these ten minutes were up, one individual, chosen at random, would stop the recording, pick up the PVC pipe and camera, and move them to another randomly chosen location within the site. The camera and PVC pipe were placed in the same configuration previously mentioned. The individual would then start a new baseline recording and leave the area. We recorded all three intensities at one site in the morning and all three intensities at that same site in the afternoon.

Data was sorted by site and each minute interval was averaged. We ran a three-way ANOVA test between time block, site, and disturbance level. From this we found that disturbance level had no effect therefore we ran a two-way ANOVA test between time block, and site. This was followed by a post hoc Tukey Test to compare fish counts amongst time blocks. These tests were repeated with species count data. All tests were run in the program R.

Results.

We found no significant variation in fish and species recovery times at different levels of disturbance. We did however find significant variations in fish and species abundances during disturbance intervals.

Fish abundance per minute ranged from zero to 75 individuals, and average number of fish was 2.5 fish. Species composition ranged from zero to seven different species, and average number of species was 1.3.

Regarding fish count, there was a significant three way interaction between site, time block and disturbance level. We discounted disturbance level because we found no significant variation in fish and species abundances with increasing intensity. This lack of significance was

due to high variation in fish count at site one as well as low fish counts at site two during the baseline intervals. Our data showed significance between time block and sites (Table 1). Site one had significantly more fish during the baseline than the disturbance interval. However, there were no significant trends between baseline and recovery intervals nor were there significant trends between disturbance and recovery intervals. We saw an intermediate recovery of number of fish (Fig. 3). Site two showed no significant difference for any relationships meaning there are no trends present (Table 2).

Regarding species count, there was a significant three way interaction between site, time block, and disturbance level. We discounted disturbance level due to high variation in species count at site one and low species counts at site two during the baseline interval. Site one showed a significant decrease of species between the baseline interval and disturbance, and there was a significant increase of species between disturbance and recovery (Table 3). There was no significant difference between the baseline and recovery intervals meaning there was a full recovery of species diversity (Fig. 3). Site two showed a significant increase between the disturbance and recovery intervals, but other comparisons were not significant (Table 4).

Discussion

Our study showed there was no significant effect of increasing intensity on recovery time of reef fish. This result could imply several different trends. One trend we considered was prey adaptations. Behavioral responses are a consequence of growth and are mediated by experience through interactions with the environment and its inhabitants (Fuiman & Magurran 1994). There are evolutionary predispositions to respond to experience (Fuiman & Magurran 1994). Reef fish encounter more predators as they mature and may be evolutionarily predisposed to respond to

predators by hiding. If the reef fish we were observing viewed us as potential predators, they would have hidden while we were in the area. Studies suggest that it is not advantageous to continue hiding once a threat is gone (Helfman 1989). We hypothesized fish began to recover immediately for this reason. A study regarding the adaptation of predator avoidance further supports our theory, stating prey will allocate energy to feeding, courtship, and territorial defense rather than allocate energy to avoiding predators (Helfman 1989). Helfman (1989) also states that prey's reactions to predators vary with the magnitude of threat displayed by predators. The reef fish may have noted the low magnitude threat we displayed because we were not physically harming fish. There was no blood in the water, nor were we physically touching or catching fish. The prey fish may have considered this less threatening and responded by hiding while we were present but recovering quickly once we left.

Åbjörnsson (2004) provided further support for the theory that predator avoidance behavior is genetically inherited. When the parental population is exposed to predation, regardless if the next generation lives in predator infested water, offspring will respond to predators. However, the same is not true for offspring of parents not raised in predator infested waters (Åbjörnsson et al. 2004). It is very likely the fish we were observing had these genetic inheritances and as large shadows swam overhead, fish responded through classic predator avoidance behaviors.

We also found a study on the effects of predator fishing on prey abundance. Willis and Anderson (2003) found a positive relationship between the two, meaning that as predators are removed, reef fish abundance increases. The two sites we examined are frequented by fishermen. Due to their presence and absence of prey our sites could be experiencing little predation;

therefore reef prey fish are not accustomed to hiding for extensive periods of time. This would suggest that fish might hide during a disturbance but only for a short amount of time before reemerging. This further supports why fish in our study made an immediate recovery after disturbances.

Fish abundance and species composition significantly dropped during disturbance times. This trend is supported through literature (Helfman 1989; Davenport & Davenport 2006; Dearden et al. 2010). Fish will hide while predators are in the area and it is likely the fish we were observing viewed us as predators. Once this threat is gone fish abundance began to recover. However, ten minutes after the disturbance, fish abundance had not fully recovered while species composition did make a full recovery. Literature suggests different species of fish will react differently to snorkelers (Table 5), but species made a full recovery which suggests they are reacting similarly. However, individual members within those returning species appear to be acting independently. Certain members of each species are returning while others continue to stay away. Studies have shown many organisms in the animal kingdom display distinctive personalities (Wolf & Weissing 2012). It is possible the reef fish we were observing share in this phenomenon. Fisheries have found certain fish are more likely to be caught in particular nets based on boldness and tepidness (Biro & Post 2008; Wilson et al. 2011). Boldness may increase the likelihood of certain fish to return to the reef after we have disturbed it while timid fish tend to stay in hiding.

Another possible suggestion is that certain fish have a personal investment in the reef community and so are returning to the reef sooner than those fish without such an investment. We encountered many damselfish during our observations which tend to be territorial (Fishelson

1970; Francini-Filho et al. 2012). Certain members of this species could have been returning to their nests, while other fish, with no nest in the area would have returned more slowly. Rainbow wrasses are territorial in their initial phases, but not in their terminal phases (Warner 1982). We saw wrasses in both phases while disturbing. Perhaps those wrasses in their initial phase were returning while those in their terminal phase were not. Amarillo snappers also frequented both our observation sites. This fish has been known to aggregate throughout the year with aggregations reaching maximum density during spawning times in winter (Sala et al. 2003). When it is not spawning season, snappers can be seen dodging in and out of crevices sporadically (Sala et al. 2003) and these actions may explain why certain members of the species were returning to the reef. Though this brief explanation does not encompass all the reef fish we encountered it seems that many reef fish act in accordance with one or multiple of the above trends.

Though the time it took reef fish to recover from snorkeling disturbances did not vary with increasing intensity of disturbance, it did appear the presence of snorkelers had a significant effect. Fish abundance as well as species composition decreased during snorkeling, regardless of intensity. It is tempting to say snorkeling may have no long term effects on reef communities however it is probable that, in areas continuously frequented by humans, this is not the case. Fishery-induced evolution is a term for the trend in which larger fish are caught by particular gear and so their genes are removed from the population (Biro & Post 2008). In this sense, fisheries select and remove larger individuals from the natural population. This leaves potential lasting evolutionary effects on fish populations (Biro & Post 2008). If snorkelers are consistently swimming in one area, and there is a disturbance effect on fish populations specific to certain

fish personalities, then it is possible non-territorial fish are being removed from reef communities. If this is true snorkeling will have long term effects on reef communities.

Snorkelers pose a threat to reef communities through decreased fish presence and degradation. Snorkeling and scuba diving alike have been linked to coral destruction (Davenport & Davenport 2006; Plathong et al. 2008). Corals are already at increased susceptibility to bleaching and death due to climate change (Wilkinson 1999; Hoegh-Guldberg 2007). If irresponsible snorkeling habits and marine tourism continue it will be too late to save vulnerable coral environments. Experiments like ours might lead people to believe they can snorkel as aggressively and plentifully as they like but this is not the case. Without considering additional research a reader will be unaware of the negative effects snorkelers and irresponsible travelers have on reef communities. It is the responsibility of the scientific community as a whole to keep the public informed of such dangers and how these dangers change with a changing climate. It is up to this generation to inform future generations of the threat we pose to coral communities. We must also ensure these threats are heeded. If we continue to ignore the warning signs it will be too late and generations to come will never experience the unique habitats coral reefs have to offer.

Literature Cited

- Aburto-Oropza, O., Balart, E. 2001. Community structure of reef fish in several habitats of a rocky reef in the Gulf of California. *Marine Ecology* **22**: 283-305
- Åbjörnsson, K., L.A. Hansson, and C. Brönmark. 2004. Responses of prey from habitats with different predator regimes: local adaptation and heritability. *Ecology: A Publication of the Ecological Society of America* **85**: 1859-1866.
- Arreola, J., Elorduy, J. (2002). Reef fish diversity in the region of La Paz, Baja California Sur, México. *Bulletin of Marine Science* **70**: 1-18.
- Biro, P.A. and J.R. Post. 2008. Rapid depletion of genotypes with fast growth and bold personalities traits from harvested fish populations. *Proceeding of the National Academy of Sciences* **105**: 2919-2922.
- Davenport, J., and J.L. Davenport. 2006. The impact of tourism and personal leisure transport on coastal environments: a review. *Estuarine, Coastal and Shelf Science* **67**: 280-292.
- Dearden, P., M. Theberge, and M Yasué. 2010. Using underwater cameras to assess the effects of snorkeler and SCUBA diver presence on coral reef fish abundance, family richness and species composition. *Environ Monit Assess* **163**: 531-538.
- Fishelson, L. 1970. Behavior and ecology of a population of *Abudefduf saxatilis* (Pomacentridae, Teleostei) at Eilat (Red Sea). *Animal Behavior* **18** 225-237.

- Francini-Filho, R.B., E. O. C. Coni, C.M. Ferreira, A.C. Alves, L.S. Rodrigues, and G.M. Amado-Filho. 2012. Group nest clearing behavior by Sergeant Major *Abudefduf saxatilis* (Pisces: Pomacentridae). *Bulletin of Marine Science* **88**: 195-196.
- Fuiman, L.A. and A. E. Magurran. 1994. Development of predator defences in fishes. Review in *Fish Biology and Fisheries* **4**: 145-183.
- Hall, M.C. 2001. Trends in ocean and coastal tourism: the end of the last frontier? *Ocean and Coastal Management* **44**: 601-618.
- Helfman, G.S. 1989. Threat-sensitive predator avoidance in damselfish-trumpetfish interactions. *Behavioral Ecology and Sociobiology* **24**: 47-58.
- Hoegh-Guldberg, O. et al. 2007. Coral reefs under rapid climate change and ocean acidification. *Science* **318**: 1737-1742.
- Human, P. and Deloach. 2004. Reef fish identification: Baja to Panama. 1-343. New World Publications, Inc, Jacksonville, Florida.
- Miller, M.L. 1993. The rise of coastal and marine tourism. *Ocean & Coastal Management* **20**: 181-199.
- Orams, M. 1999. *Marine Tourism Development, Impacts and Management*. New York: Routledge.
- Plathong, S., G.J. Inglis, and M.E. Huber. 2008. Effects of Self-Guided Snorkeling Trails on Coral in Tropical Marine Parks. *Conservation Biology* **14**: 1821-1830.

- Roberts, C.M., & Hawkins, J.P. 2000. Fully-protected marine reserves: a guide. The University of York Printing Unit.
- Sala, E., O. Aburto-Oropeza, G. Paredes, G. Thompson. 2003. Spawning aggregations and reproductive behavior of reef fishes in the Gulf of California. *Bulletin of Marine Science* **72**: 103-121.
- Warner, R.R. 1982. Mating systems, sex change and sexual demography in the rainbow wrasse, *Thalassoma lucasanum*. *Copeia* **3**: 653-661.
- Wilkinson, C., O. Lindén, H. Cesar, G. Hodgson, J. Rubens and A.E. Strong. 1999. Ecological and Socioeconomic impacts of 1998 coral mortality in the Indian Ocean: an ENSO impact and a warning of future change? *Ambio* **28**: 188-196.
- Wilson, C., & Tisdell, C. 2006. Conservation and Economic Benefits of Wildlife-Based Marine Tourism: Sea Turtles and Whales as Case Studies. *Human Dimensions of Wildlife*, 49-58.
- Wolf, M. and F.J. Weissing 2012. Animal personalities: consequences for ecology and evolution. *Trends in Ecology and Evolution* **27**: 452-461.

Table 1: ANOVA table for site one fish counts.

	Degrees of Freedom	Sum Squared	Mean Squared	F-Value	Pf(>F)
Time	2	323	161.67	7.363	0.000771
Residuals	267	5862	21.96		

Table 2: ANOVA table for site two fish counts.

	Degrees of Freedom	Sum Squared	Mean Squared	F-Value	Pf(>F)
Time	2	31	115.52	0.647	0.524
Residuals	357	8568	24.00		

Table 3: ANOVA table for site one species counts.

	Degrees of Freedom	Sum Squared	Mean Squared	F-Value	Pf(>F)
Time	2	35.7	17.837	11.76	1.27E ⁻⁵
Residuals	267	404.9	1.517		

Table 4: ANOVA for site two species counts.

	Degrees of Freedom	Sum Squared	Mean Squared	F-Value	Pf(>F)
Time	2	8.84	4.419	5.207	0.0059
Residuals	357	302.98	0.849		

Table 5: Common reef fish we observed and their reactions to snorkelers (Humann & Deloach, 2004)

Fish Common Name	Reaction to Snorkelers
Panamic Sargent Major	Tend to ignore divers, territorial due to nest guarding
Scissortail Chromis	Tend to ignore divers but retreat when closely approached
Cortez Damselfish	Unafraid, will even nip divers
Amarillo Snapper	Somewhat wary, usually move away when approached
Spot tail Grunt	Appear unconcerned usually allow a slow, non-threatening approach
Graybar Grunt	Appear unconcerned, usually allow a slow non-threatening approach
Barred Pargo	Move away when approached
Yellowfin Surgeonfish	Wait, but somewhat curious, tend to approach divers when they appear disinterested
Goldrim Surgeonfish	Shy tend to avoid divers
King Angelfish	Tend to ignore divers but move away when approached
Cortez Angelfish	Relatively unconcerned and often appear curious

Azure Parrotfish	Wary, usually move away
Banded Wrasse	Wary, usually move away
Rainbow Wrasse	Somewhat wary, usually move away
Spotted Sharpnose Puffer	Seem curious and unafraid
Balloonfish	Somewhat wary
Panamic Green Morray	Curious
Reef Cornet Fish	Ignores divers, move away when approached

Figure 1: Map of study location

Figure 2: Map of site locations at Cantamar Beach

Figure 3: Fish abundance per time interval in minutes at both sites

Figure 4: Species composition per time interval in minutes at both sites

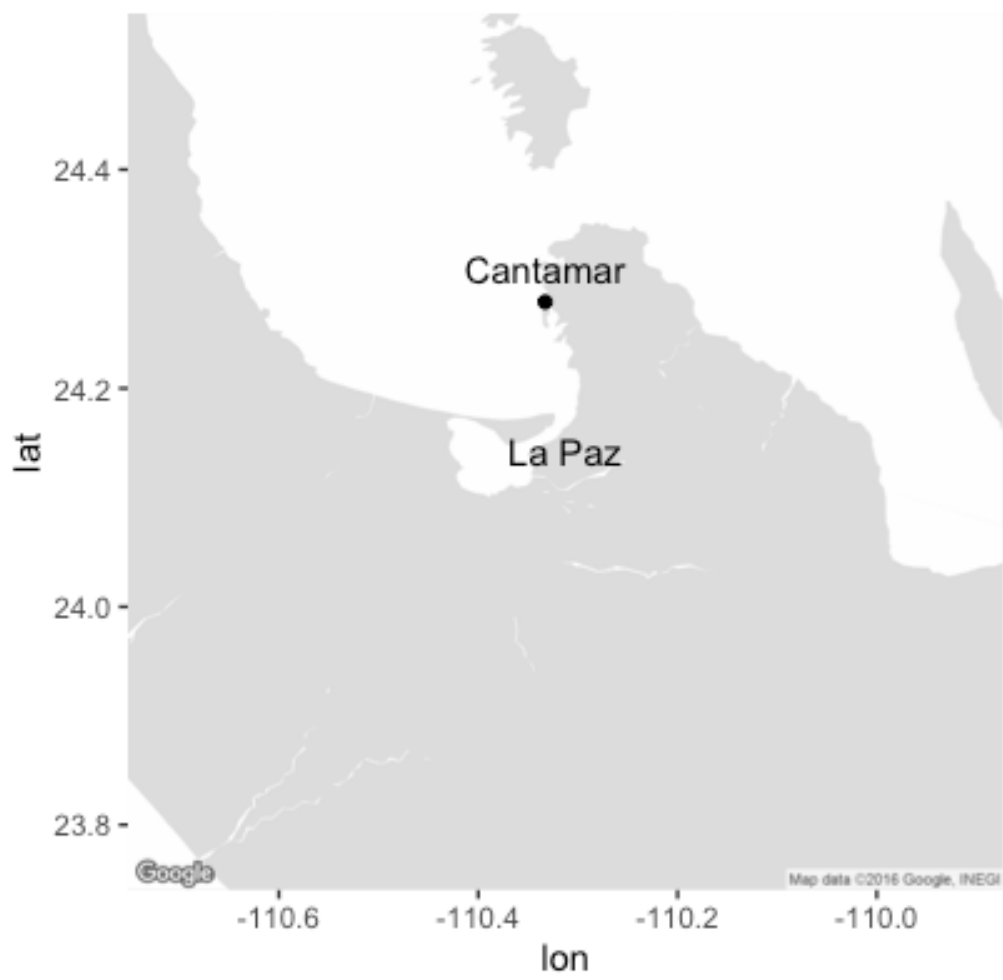


Fig 1

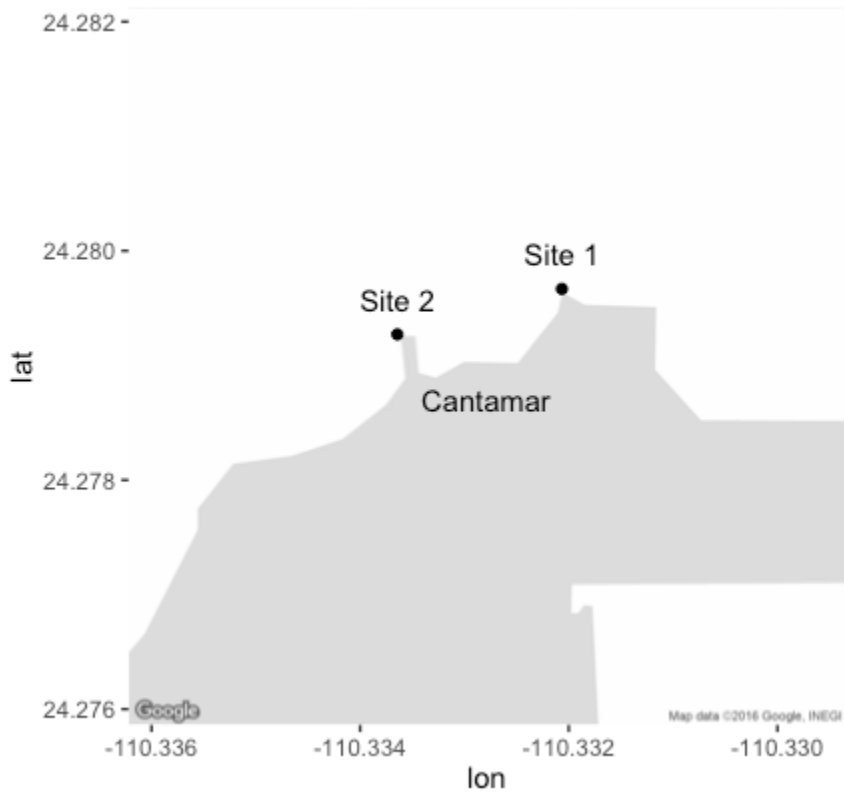


Fig 2

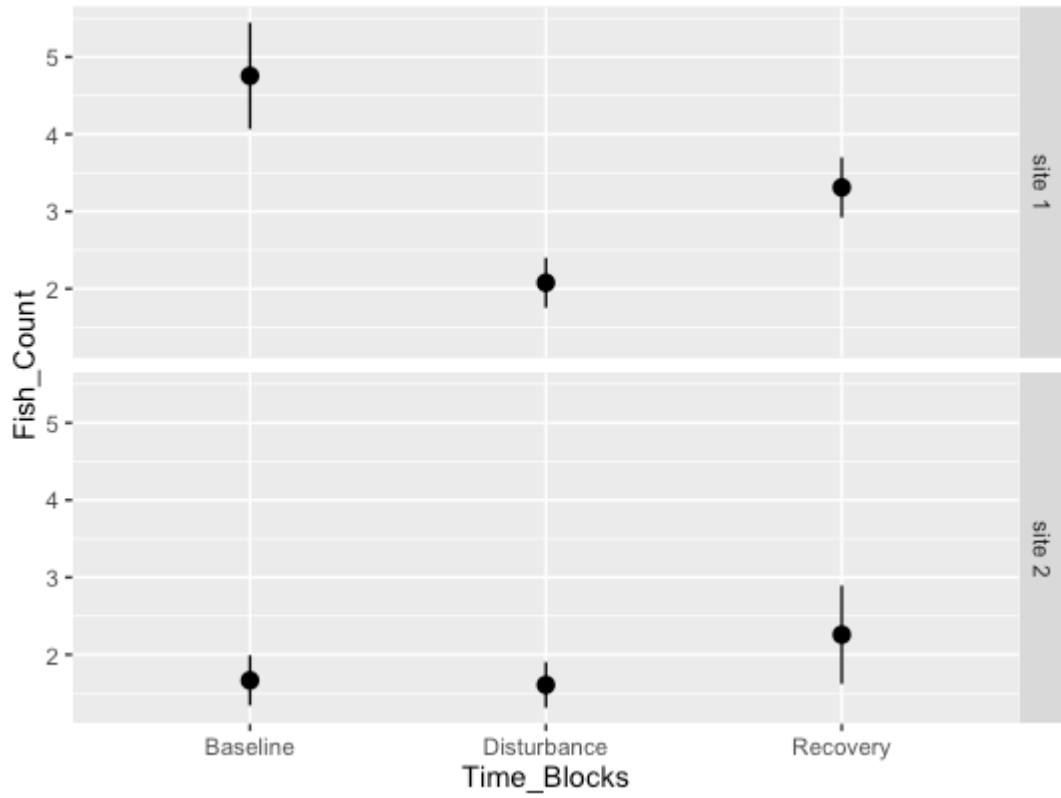


Fig 3

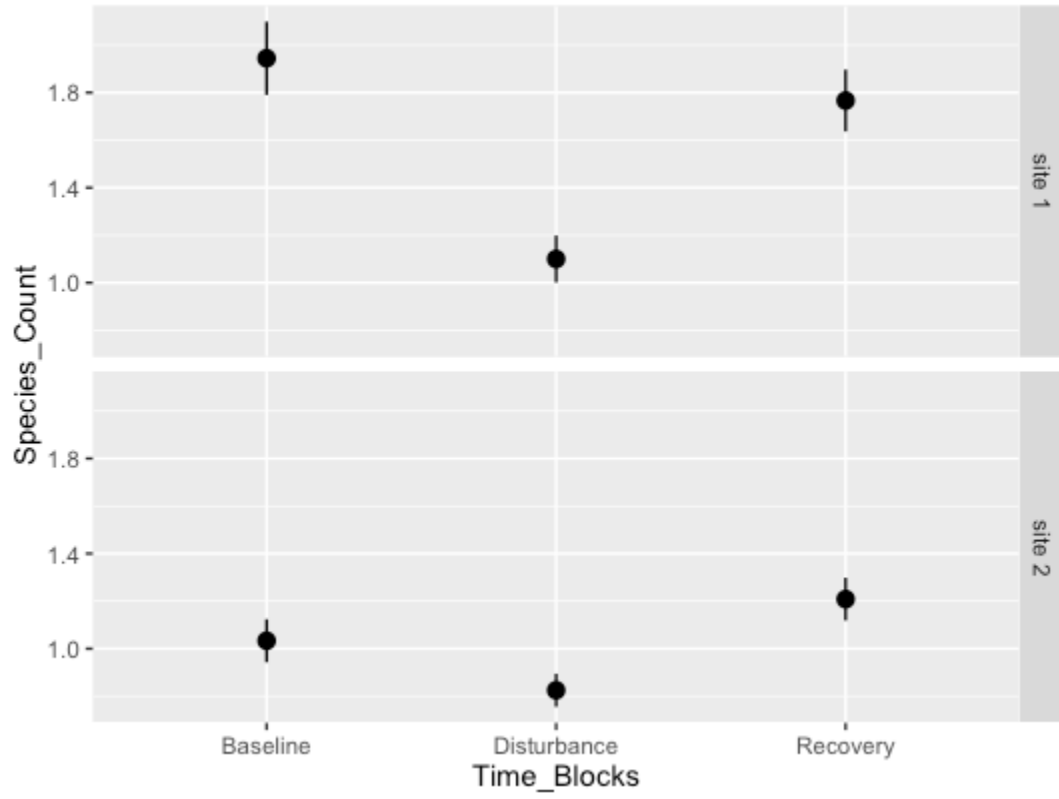


Fig 4

Phylogenetic Analysis of Cetacean Communities of the Gulf of California and Eastern Pacific.

Key words: Phylogenetic diversity, Taxonomic distinctness, Cetaceans, Eastern Pacific, Gulf of California, *Phocoena sinus*.

Word count: 3193

Mailing adress: Universidad Autónoma de Baja California Sur, Department of Marine and Coastal Sciences, Carretera al Sur Km 5.5, 23080 La Paz, B.C.S, México.

Emails: sebas.alco@gmail.com; maraclg96@gmail.com.

Acknowledgments:

This paper would not have been possible without the support and advice of Alejandro Acevedo-Gutiérrez, Benjamin Miner, Deborah Donovan and Sergio Francisco Flores-Ramírez who helped us in the problem statement of the study and who gave us useful recommendations for the writing of the manuscript.

Phylogenetic Analysis of Cetacean Communities of the Gulf of California and Eastern Pacific.

S. Álvarez-Costes and M. C. Landa-Garza

*Universidad Autónoma de Baja California Sur
Western Washington University*

ABSTRACT

Mexico is one of the countries with the highest density of cetaceans in the world, with eight of the thirteen existing families. The Gulf of California harbor porpoise or Vaquita marina is the only marine mammal endemic from Mexico and the smallest of the cetaceans. We analyzed the differences between the phylogenetic diversity of the Gulf of California and the Eastern Pacific and also obtained the taxonomic distinctness indexes from the two zones. Data were obtained from a 1993 report on cetacean species sightings and abundance, during a marine mammal survey in the Eastern Pacific and the Gulf of California, from the 28th of July to the 9th of November. Then we analyzed the data with the software R, obtaining three phylogenetic trees and the taxonomic indexes for the two areas. We also found the Vaquita extinction and recover simulation in the gulf, the Vaquita extinction was simulated eliminating the abundance obtained and analyzing the same indexes but only in the Gulf of California. Our results suggested that the Eastern Pacific region had the highest phylogenetic diversity, lowest value of taxonomic distinctness (Delta*), and the highest index for (Delta+). The indexes for the Gulf of California region were not affected by the vaquita marina extinction or recovery simulations. We expected more diversity for the Gulf of California, but due to the season of the survey we obtain low values. Despite the Vaquita loss or recovery simulations not affecting the indices for the gulf, it is important to conserve the organism because extinction would results in the loss of a complete family in the gulf.

Key words: Phylogenetic diversity, Taxonomic distinctness, Cetaceans, Eastern Pacific, Gulf of California, *Phocoena sinus*.

RESUMEN

México es uno de los países con mayor diversidad y densidad de cetáceos en el mundo, teniendo en su territorio ocho de las trece familias existentes. La Vaquita marina es el único mamífero marino endémico de México y es el cetáceo más pequeño existente, además es uno de los cetáceos más amenazados debido al enmalle incidental de estos organismos en las redes de pesca. En este estudio comparamos y analizamos la diversidad filogenética para la comunidad de cetáceos del Golfo de California y del Pacífico y además obtuvimos los índices de distintividad taxonómica para ambas zonas. Los datos fueron obtenidos del reporte de especies de cetáceos de un crucero llevado a cabo del 28 de julio al 6 de noviembre de 1993 y luego fueron analizados en el Software R obteniendo tres árboles filogenéticos y los índices de distintividad taxonómica para las dos zonas y para la simulación de la extinción y recuperación de la vaquita, la cual se llevó a cabo primero eliminando los datos de abundancia registrados y luego agregando 1000 organismos a la población. El área con la mayor diversidad filogenética fue el Pacífico está teniendo también el menor valor de Delta* y el mayor de Delta+, por su parte, la extinción y recuperación de la vaquita no afectaron los índices en el Golfo de California. Se esperaba tener mayor diversidad filogenética en el Golfo de California pero debido a la temporada en que se llevó a cabo el crucero se obtuvieron valores bajos. A pesar de que la simulada extinción y recuperación de la vaquita no tuvieron efectos significativos en los índices del Golfo, es importante recuperar la población de esta especie porque si se pierde, se perderá toda una familia y una línea evolutiva en el golfo.

Palabras clave: Diversidad filogenética, Distintividad taxonómica, Cetáceos, Pacífico Este, Golfo de California, *Phocoena sinus*.

INTRODUCTION

Phylogenetic diversity (PD) is often referred to as “evolutionary diversity” and is a generic term. PD is a biodiversity measure based on evolutionary relationships between species. It is commonly used by researchers and is extremely relevant for targets of conservation, mainly because it can be related to processes such as extinction, biotic invasion, ecosystem functioning, and even ecosystem services (Winter et al. 2012). Recent studies have suggested that phylogenetic diversity (the distinct evolutionary history in a community) also can be used as a proxy for ecological measures of functional diversity (the functional trait distinctiveness in a community) (Flynn et al. 2011).

It is important to monitor changes in biodiversity in space and time so there are measures based only on the number of species present, but taxonomic distinctness measure incorporates more information. This measure is a univariable index which calculates the average distance between all pairs of species in a community sample. So it can therefore be seen as a measure of pure taxonomic relatedness whereas mixes taxonomic relatedness with the evenness properties of the abundances distribution (Pienkowski et al. 1998; Clarke & Warwick 1999).

The Order Cetacea is divided in two sub-orders, the suborder Mysticeti (baleen whales) with three families: right whales (Balaenidae), rorquals (Balaeonopteridae) and the gray whale (Eschrichtidae). And the suborder Odontoceti (toothed whales) with 8 families: oceanic dolphins (Delphinidae), porpoises (Phocoenidae), beaked whales (Ziphiidae), dwarf sperm whales (Kogiidae), sperm whale (Physeteridae), narwhal and beluga (Monodontidae) and three other families of river dolphins (Salvadeo 2008).

Mexico is one of the countries with the highest density of cetaceans in the world, with eight of the thirteen existing families containing 39 of the 86 known species worldwide. In the Gulf of California lives 35% of all recorded species. This region is characterized by its intense fishing activity, tourism, aquaculture, and the presence of attractive cities and major ports (Guerrero-Ruiz et al. 2006).

The Gulf of California harbor porpoise “Vaquita marina” is the only marine mammal native to Mexico, and is the smallest of the cetaceans. It is robust, with long and concave pectoral fins; high, triangular and slightly falcate dorsal fin (CONABIO 2010). It is endemic to the Upper Gulf of California in Mexico and there is no sign of its presence in the South, for these reasons it is proposed that this was its original distribution (Gerrodette & Rojas-Bracho 2011). The Vaquita lives in a very small region, in the north of an imaginary line connecting Puertecitos in Baja California and Puerto Peñasco in Sonora. It is one of the world’s most endangered marine mammals, mainly because the by-catch mortality due to the fisheries activity in its distribution region. The totoaba illegal fishery is also a main cause for mortality because of China’s demand for swim bladders from this fish (Jaramillo-Legorreta et al. 2016).

One of the goals of this study is to compare the PD of two different cetacean communities, the Gulf of California community and the Eastern Pacific community. This allowed us to analyze how these communities differed in species richness and abundance, and to determine the taxonomic diversity of each. We also considered the Vaquita Marina conservation problem, which is a very important issue in the Gulf of California. We analyzed how the extinction or recovery of the vaquita affected the phylogenetic and taxonomic diversity in the Gulf of California. This revealed information about the importance that a single species has in a

community. We predicted the following two hypotheses (1) that the diversities of the Gulf and the Eastern Pacific will be similar and (2) that the extinction and recovery of Vaquita populations will decrease and increase respectively with the indexes and the diversity of the Gulf of California.

MATERIALS & METHODS

Data collection

Data were obtained from a report of cetacean sightings during a marine mammal survey in the Eastern Pacific ocean and the Gulf of California aboard the National Oceanographic and Atmospheric Administration (NOAA) ships “McArthur” and “David Starr Jordan” on 1993.

The abundance of each species sighted on that cruise were estimated for the Gulf of California and the Eastern Pacific by summing the school size of each sighting. The researchers then analyzed the coordinates for each sighting to determine the location of data collection for each school. Then all of the species abundances for the Gulf and the Eastern pacific were compiled into three taxonomic lists, one for each area and one for all the cetacean species sighted on the cruise.

Data analysis

The data was analyzed with the software R, specifically with the package “Vegan”. We made three separate phylogenetic trees, the first for all cetaceans measured on the study, the second for the cetaceans sighted in the Eastern Pacific, and the final for the cetaceans sighted at the Gulf of California. Then we obtained the taxonomic distinctness index (Delta*) to evaluate the taxonomic distances while considering the species richness and the abundance of each, it was

not necessary take into consideration the effort of sampling, the samples size, or whether or not it was a normal distribution. We also obtained the average taxonomic distinctness index (Delta+) to evaluate the richness and the taxonomic distance between the species, defined as a tree of Linnaean classification using absence and presence data. This would explain that the more species of different genus and families you have, the higher the index is going to be, so there is more diversity in an area with a high index (Juaristi 2014).

We simulated the Vaquita extinction by eliminating the abundance obtained and analyzing the same indices only in the Gulf of California. We analyzed the population increase by using the indices in the Gulf of California, adding 1000 vaquitas to the amount obtained before.

RESULTS

All the species sighted at both areas are shown in Table I. The area with more species sighted was the Eastern Pacific, with a total of 27, while the Gulf of California had 19 species. The most common species sighted in both areas were members of the suborder Odontoceti, or toothed whales, particularly members of family Delphinidae. The delphinid *Stenella longirostris* was exclusive to the Gulf of California, whereas for the Eastern Pacific the exclusive delphinids were *Lagenorhynchus obliquidens*, *Lissodelphis borealis*, *Feresa attenuate* and *Pseudorca crassidens*. For the family Phocoenidae, both areas have representatives, but only one for the Gulf of California, the endemic *Phocoena sinus* and two for the Eastern Pacific, *Phocoena phocoena* and *Phocoenoides dalii*. The only member of the family Physeteridae sighted at both sites was *Physeter microcephalus*, as well as *Kogia sinus* and *Kogia breviceps*, from the Kogiidae family. The family Ziphiidae had representatives at both areas, with *Ziphius cavirostris*

at the gulf, and *Mesoplodon densirostris*, *Berardius bairdii* and *Z. cavirostris* at the EP. For the suborder Mysticeti or baleen whales, there were only sighted members of the family Balaenopteridae at both the gulf and EP. Although, at the Eastern Pacific there were more species sighted, being exclusive for the area *Balaenoptera musculus* and *Balaenoptera borealis*. At both EP and the gulf they sighted *Balaenoptera acuturostrata*, *Balaenoptera edeni*, *Balaenoptera physalus* and *Megaptera novaeangliae*.

Table I. Cetacean species list of Gulf of California and Eastern Pacific.

Species #	Gulf of California	Eastern Pacific
1	<i>S. attenuata</i>	<i>S. attenuata</i>
2	<i>S. longirostris</i>	<i>S. coeruleoalba</i>
3	<i>S. coeruleoalba</i>	<i>S. bredanensis</i>
4	<i>S. bredanensis</i>	<i>D. delphis</i>
5	<i>D. delphis</i>	<i>D. capensis</i>
6	<i>D. capensis</i>	<i>T. truncatus</i>
7	<i>T. truncatus</i>	<i>L. borealis</i>
8	<i>G. griseus</i>	<i>F. attenuata</i>
9	<i>G. macrorhynchus</i>	<i>P. crassidens</i>
10	<i>O. orca</i>	<i>L. obliquidens</i>
11	<i>P. sinus</i>	<i>G. griseus</i>
12	<i>P. macrocephalus</i>	<i>G. macrorhynchus</i>
13	<i>K. simus</i>	<i>O. orca</i>
14	<i>K. breviceps</i>	<i>P. phocoena</i>
15	<i>Z. cavirostris</i>	<i>P. dalii</i>
16	<i>B. acutorostrata</i>	<i>P. macrocephalus</i>
17	<i>B. edeni</i>	<i>K. simus</i>
18	<i>B. physalus</i>	<i>K. breviceps</i>
19	<i>M. novaeangliae</i>	<i>M. densirostris</i>
20		<i>Z. cavirostris</i>
21		<i>B. bairdii</i>
22		<i>B. acutorostrata</i>
23		<i>B. edeni</i>
24		<i>B. borealis</i>
25		<i>B. musculus</i>
26		<i>B. physalus</i>
27		<i>M. novaeangliae</i>

The complete cetacean phylogeny of all species sighted (Figure 1) shows the division from a common ancestor between the two suborders of cetaceans. The phylogeny also shows that they divided into two large groups, with a total of 29 species. The odontocetes observed first diverged into 6 families: Ziphiidae, Kogiidae, Physteridae, Phocoenidae and Delphinidae. The Delphinidae was one the most diverse family in the study, with 14 different species in 11 genus. Meanwhile, the mysticetes observed were only members of the family Balaenopteridae, being 5 of the 6 species that were members of the genus Balaenoptera, with the last being the only species of the genus Megaptera.

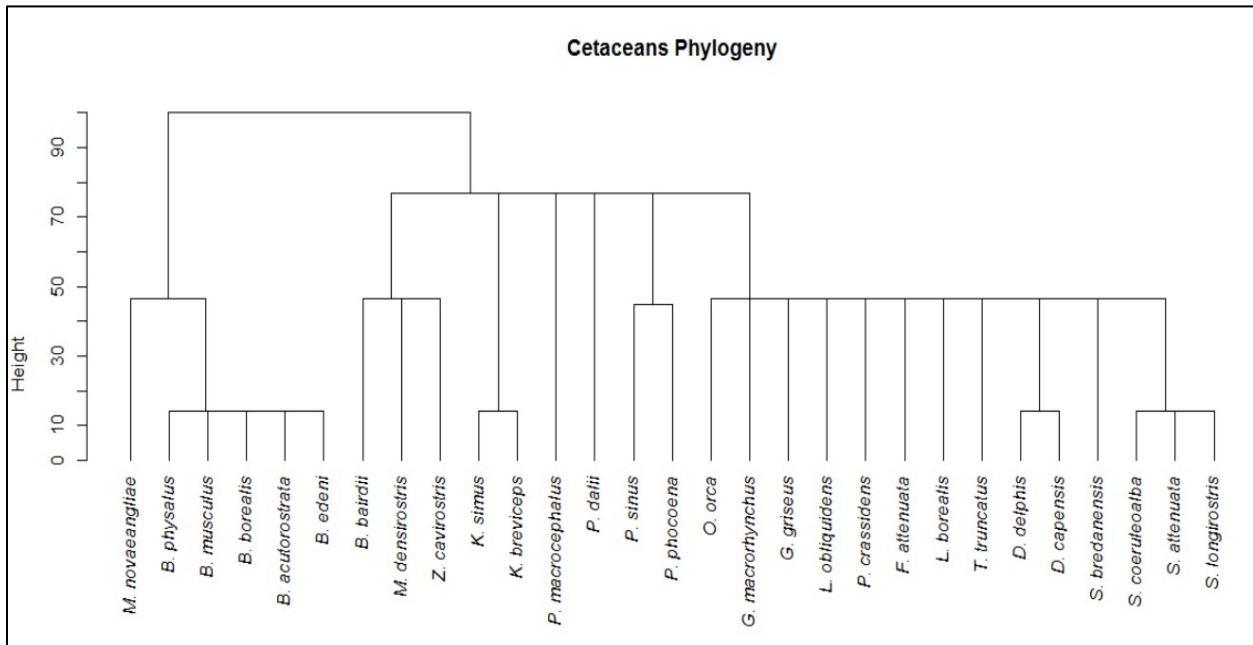


Figure 1. Phylogenetic tree of all cetaceans sampled in the Gulf of California and Eastern Pacific

The phylogenetic tree of the Gulf of California cetaceans (Figure 2) is very similar and have the same families as the complete tree of all the cetaceans sighted, although it is less diverse. For the family Delphinidae, which was the most diverse, there are 10 species, whereas

the families Ziphiidae and Phocoenidae have only one species. For the Mysticetes the family and the genus are the same but there are only three members of the genus Balaenoptera.

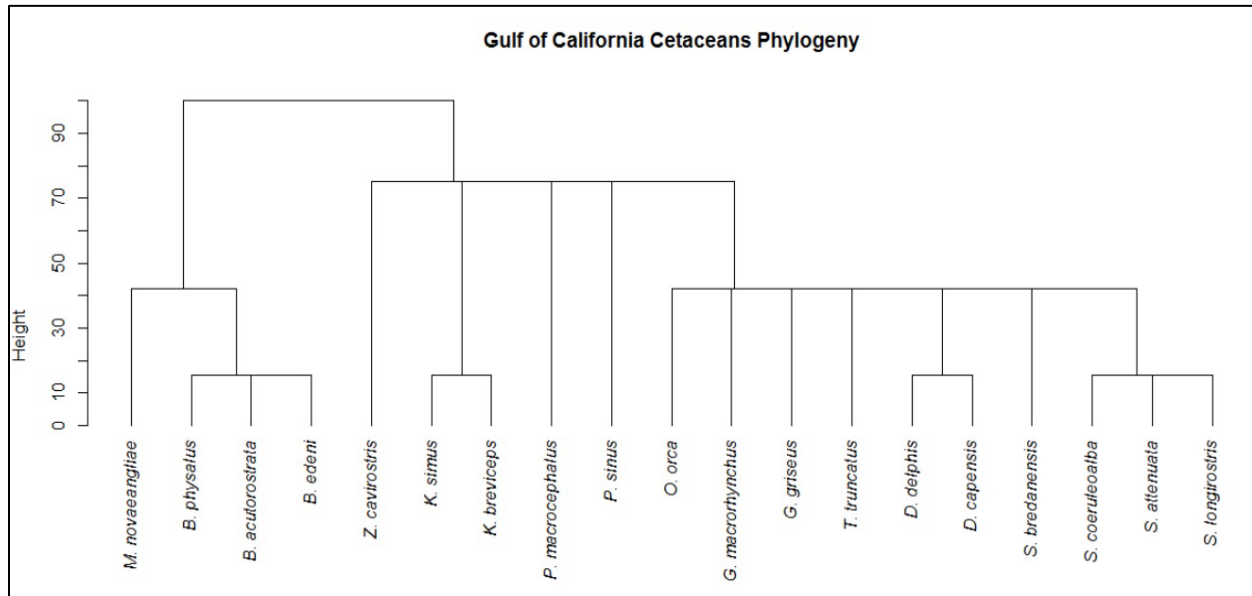


Figure 2. Phylogenetic tree of Gulf of California cetaceans.

The Eastern Pacific tree (Figure 3) suggests that there were more species in that area. For the family Delphinidae there were 13 species. The families Ziphiidae and Phocoenidae had more species than the gulf, with 3 and 2 respectively. For the Mysticetes the only change is that there are two more species in the genus Balaenoptera than in the Gulf of California.

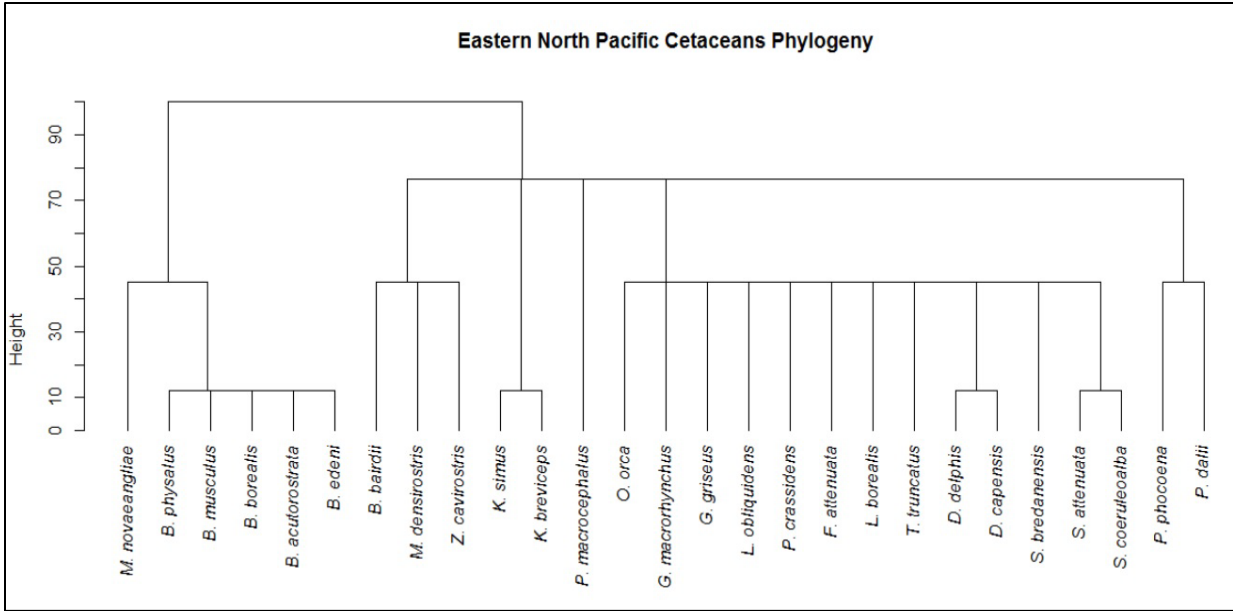


Figure 3. Phylogenetic tree of Eastern Pacific cetaceans.

When comparing the taxonomic distinctness indices (Delta*) (Figure 4) from the two areas and the simulated scenarios with Vaquita we found the lowest index value for the Eastern Pacific, and the highest values for the Gulf of California, but the differences with vaquita simulations were very small, so they are not significant.

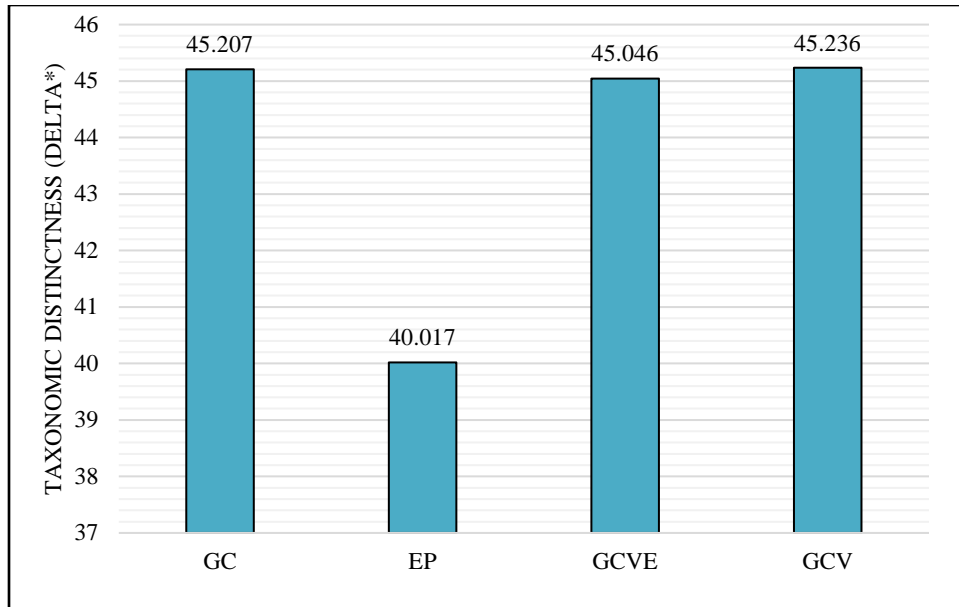


Figure 4. Taxonomic distinctness index (Delta*) for Gulf of California (GC), Eastern Pacific (P), simulated extinction of Vaquita in the Gulf (GCVE) and simulated increase of Vaquita population in the Gulf (GCV).

The average taxonomic distinctness indices (Delta+) (Figure 4) for the two geographic areas and the two simulated scenarios with Vaquita showed that the lowest index value was for the Gulf of California with the Vaquita population increased (GCV), and the highest value was for the Eastern Pacific. The second highest value was the Gulf of California. In both scenarios, extinction and Vaquita population recovery, the index decreased.

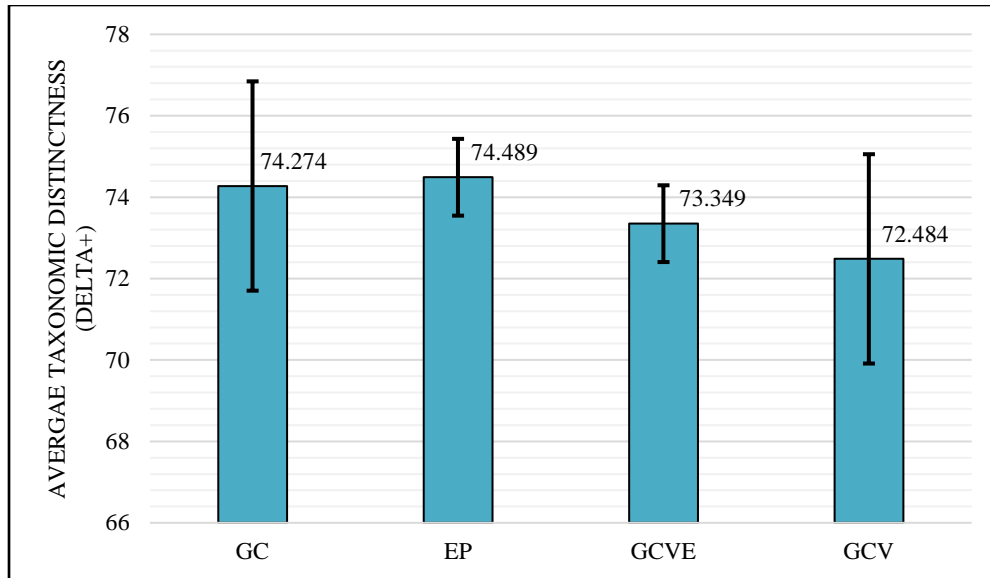


Figure 5. Average taxonomic distinctness index (Delta+) for Gulf of California (GC), Eastern Pacific (P), simulated extinction of Vaquita in the Gulf (GCVE) and simulated increase of Vaquita population in the Gulf (GCV).

DISCUSSION

The Gulf of California is known as an area with a very high diversity of marine mammals because the gulf is a temporal feeding and breeding area for some cetaceans such as blue, humpback, sperm, pilot, beaked whales and others. Also have different stocks of resident cetaceans that also use the area as feeding and breeding grounds, but remain in the Gulf all the year (Lluch-Cota et al. 2007). The low diversity of the Gulf of California as due to the season in which the study was conducted (July 28-November 6). Research done by Urbán-Ramirez et al. (2005) suggests that the highest diversity of cetaceans in the gulf was in winter because of the high primary productivity of this season causing migratory species to come to the gulf. This could explain why there were more species sighted from different genus on the Eastern Pacific as compared to the Gulf of California. As previously mentioned, the study was conducted between

July and November, missing the breeding season and migration of some of the baleen whales, such as the humpback and blue whale, as well as some of the odontocetes species that are more common in the gulf throughout winter. However it is known that the Eastern Pacific has the highest marine mammal diversity in México, having representatives of 11 families from the 12 that are in México. At the species level, this zone has 30% of the global diversity of marine mammals and 75% of marine mammals in Mexico. This diversity is related to the confluence between the cold water of the California current and the warm water of the north equatorial current, making it a highly productive zone, which allows the region to present species with affinities of cold, warm and tropical waters (Torres et al. 1995).

The average taxonomic distinctness index (Delta+) in Figure 5 evaluates the taxonomic distance between each pair of species, defined through a tree of Linnean classification. Each taxonomic hierarchical level receives a discrete and proportional value within a range of 100 units, depending on the number of levels used (Barjau 2012). As we can see the area with the highest taxonomic distinctness index was the Eastern Pacific, with 74.48. Whereas the Gulf of California received a slightly smaller index of 74.27. That means that the Eastern Pacific presented more richness and distance between each pair of Cetacean species.

The Vaquita is the most critically endangered marine small cetacean in the world, in the last years it has been very affected by gill nets for fish and shrimp causing very high rates of by-catch mortality rates (Gerrodette & Rojas-Bracho 2011). The population numbers are decreasing constantly despite the government effort to save the vaquita. The by-catch of vaquita is not the only threat that this species faces, there are other long term factors affecting this organism, such as the potential disturbance by trawling and construction in the Colorado River delta that could

affect vaquita behavior. Another possible disturbance to this population is the lack of freshwater input from the river (Rojas-Bracho et al. 2006). As our results show (Figures 4 and 5) the distinctness indices did not change drastically with vaquita extinction, possibly because the vaquita numbers are already very low, so the change in the numbers did not affect the indices that depend on their abundances. Similarly, the simulated recovery did not affect the indices, this may be because 1000 vaquitas is not a high abundance as compared to other delphinids species which dominate in the Gulf of California. Despite there being no significant differences between indices, it is important to continue to protect the vaquita population. If their population went extinct it would be a significant loss to the diversity in the Gulf of California because they are the only representative of the family Phocoenidae in this area.

LITERATURE CITED

- Barjau, E. 2012. Estructura comunitaria y diversidad taxonómica de los peces en la Bahía de La Paz y la Isla San José, Golfo de California. CIBNOR. 135 pp
- Clarke, K.R. and R.M. Warwick. 1999. The taxonomic distinctness measure of biodiversity: weighting of step lengths between hierarchical levels. *Marine Ecology Progress Series*.184:21-29.
- Flynn, D., N. Mirotchnick, M. Jain, M. Palmer & S. Naeem. 2011. Functional and phylogenetic diversity as predictors of biodiversity–ecosystem–function relationships. *Ecology*, 92(8): 1573–1581.
- Gerrodette, T & L. Rojas-Bracho. 2011. Estimating the success of protected areas for vaquita, *Phocoena sinus*. *Marine Mammal Science*. 27(2): E101-E125.
- Guerrero-Ruiz, M., J. Urbán-Ramírez y L. Rojas-Bracho. 2006. Las ballenas del Golfo de California. Instituto Nacional de Ecología. México. 524 p.

- Jaramillo-Legorreta, A., G. Cardenas-Hinojosa, E. Nieto-Garcia, L. Rojas-Bracho, J. Hoefb, J. Moorec, N. Tregenzad, J. Barlowc, L. Thomas, B. Taylor & Tim Gerrodette. 2016. Passive acoustic monitoring of the decline of Mexico's critically endangered vaquita. *Conservation biology*. 26 pp.
- Juaristi, D. 2014. Contribución al conocimiento de la diversidad taxonómica de los peces de fondos blandos en Laguna San Ignacio, Baja California Sur, México. UABCS. Thesis. 58 pp.
- Lluch-Cota S.E., Aragón-Noriega E.A., Arreguín-Sánchez F., Auriolles-Gamboa D., Bautista-Romero J.J., Brusca R.C., Cervantes-Duarte R., Cortés-Altamirano R., Del-Monte-Luna P., Esquivel-Herrera A., Fernández G., Hendrickx M.E., Hernández-Vázquez S., Herrera-Cervantes H., Kahru M., Lavín M., Lluch-Belda D., Lluch-Cota D.B., López-Martínez J., Marinote S.G., Nevárez-Martínez M.O., Ortega-García S., Palacios-Castro E., Parés-Sierra A., Ponce-Díaz G., Ramírez-Rodríguez M., Salinas-Zavala C.A., Schwartzlose R.A. & Sierra-Beltrán A.P. 2007. The Gulf of California: Review of ecosystem status and sustainability challenges. *Progress in Oceanography*. 73: 1–26.
- Pienkowski, M.W., A.R. Watkinson, K.R. Clarke and R.M. Warwick. 1998. A taxonomic distinctness index and its statistical properties. *Journal of Applied Ecology*. 35(4):523-531.
- Rojas-Bracho, L., R. Reeves & A. Jaramillo-Legorreta. 2006. Conservation of the vaquita *Phocoena sinus*. *Mammal Rev*. 36(3): 179-216.
- Salvadeo, C. 2008. Análisis de la comunidad de odontocetos y la relación con su ambiente, en el extremo sur-occidental del Golfo de California, México (2003-2006). CICIMAR-IPN. Master's thesis. 63 pp.
- Torres, A., Esquivel, C. & Ceballos, G. 1995. Diversidad y conservación de los mamíferos marinos de México. Centro de Ecología, UNAM. 43 pp.
- Urbán-Ramírez, J., L. Rojas-Bracho, M. Guerrero-Ruiz, A. Jaramillo-Legorreta y L. Findley. 2005. Cetacean diversity and conservation in the Gulf of California, 276-

297. In: Cartron, J.L.E., G. Ceballos & R.S. Felger. (Eds.) Biodiversity, ecosystems and conservation in northern Mexico. Oxford University Press

Winter, M., V. Devictor & O. Schweiger. 2012. Phylogenetic diversity and nature conservation: where are we?. *Trends in Ecology and Evolution*. 30: 1-6.

Loggerhead turtle (*Caretta caretta*) mortality at Golfo de Ulloa, BCS. Its Relation to Productivity and Sea Surface Temperature

Resumen

La caguama (*Caretta caretta*) es una especie altamente migratoria con un ciclo de vida complejo que se caracteriza por varios estadios previos al maduro que ocupan hábitats diversos, desde exclusivamente oceánicos hasta neríticos, y que al alcanzar la madurez realizan migraciones hacia las playas de anidación. La captura incidental o dirigida de tortugas marinas, alcanzaron niveles críticos, lo cual causó que colapsaran numerosas poblaciones de tortugas marinas colocándolas en Peligro de Extinción por la UINC. El Pacífico mexicano se considera una importante zona de alimentación, se localiza frente a las costas de la península de Baja California Sur, una zona de alta productividad y biodiversidad, lo que da al lugar a una alta concentración de alimento para las tortugas (una alta concentración de langostilla *Pleuroncodes planipes*, fuente principal de alimento de la tortuga caguama en esta región). Por lo tanto, se analizará la relación de la productividad proxy (mg/m³) compilando una base de datos de webside NOAA/GIOVANNI; con la variable de temperatura superficial del mar en C°, de igual manera compilando una base de datos de la misma website; seguido a esto se correlacionó con las altas tasas de varamientos de tortuga caguama en un período de 13 años (2003-2015). Los datos de temperatura (SST) mostraron un patrón consistente con la presencia de eventos de surgencia en el Golfo de Ulloa de diciembre a marzo. Sin embargo las concentraciones de clorofila son mayores de marzo a julio. Por lo tanto, parece que las condiciones para el desarrollo de fitoplancton se acumulan de enero a marzo. Estos resultados requieren un análisis detallado incluyendo el esfuerzo de pesca ejercido en la zona.

Palabras clave: Productividad, Temperatura superficial del mar, mortalidad, tortuga caguama.

Abstract

Loggerhead turtle (*Caretta caretta*) is known as a highly migratory species with a complex life cycle characterized by having several previous stages before adult age that live in diverse habitats, going from only oceanic to neritic, that will migrate to their nesting sites as soon they reach mature stage. Bycatch and overfishing of this sea turtle reached critic levels causing its populations collapse putting them into the endarged list by the UINC. Mexican Pacific is considered an important feeding area in front of Baja California Sur coast, is a high productivity area with also high biodiversity, what turns into a big food source for the loggerhead turtle (with abundant *Pleuroncodes planipes*, main food source for this species on the area). Therefore, the relation of proxy productivity levels (mg/m³) is going to be analyzed with an online data base from NOAA/GIOVANNI; considering sea surface temperature on C° also from the online database, followed by correlation of high rates of dead turtles stranding during a 13 year period (2003-2015). SST data showed a consistent pattern compared to surgency events on Ulloa Gulf from December to march season. However, chlorophil concentrations where higher from march to july. So far, conditions for fitoplancton development increase from January to March. This result suggests that further analisys had to be done including fishing effort reached in the studied area.

Keywords: Productivity, Sea surface temperature (SST), loggerhead, mortality.

Introduction

The North Pacific population of the loggerhead turtle (*Caretta caretta*) nests exclusively in the Japanese archipelago. When the young are born traverse across the Pacific Ocean to reach BCS, a migration of about 12,000 kilometers. These turtles, all juveniles, remain feeding near the coast of BCS, until they are ready to breed, approximately 35-50 years old and not before (Peckham *et al.* 2011). Unfortunately, the population of loggerhead presents a dramatic decline in recent years. Recently fewer than 2,000 individuals nest in the entire coast of Japan per year (Conant *et al.* 2009). In the 1960s, the catch (Nietschmann *et al.* 1995;.. Fleming *et al.* 2001) overfishing and threatened extinction of sea turtles (Caldwell 1963; O'Donnell 1974. Clifton *et al.* 1982), by the intense collection of their eggs and directed and incidental captures reached critical levels. Described collapse caused many sea turtle populations; prompting the Mexican government to completely prohibit his capture in 1990 (DOF 1990) and determining that the International Union for Conservation of Nature (IUCN) and the Convention on International Trade in Endangered Species (CITES English) listed to all species as endangered. In 1994 all species of sea turtles joined the Mexican Official Standard NOM-059-ECOL-1994 (DOF), species and sub-species of flora and terrestrial and aquatic fauna endangered, which establishes measures for their protection

(NOM-059-SEMARNAT-2010).

Also, the Mexican Pacific is considered an important feeding area, is located off the coast of Baja California Sur, particularly between Punta Eugenia and Bahía Magdalena lagoon complex in the so-called Gulf of Ulloa. According to results of aerial surveys and satellite tracking turtles that inhabit the Gulf of Ulloa spend long periods in coastal waters about 32 km from the coast of BCS. The Gulf of Ulloa is an area of high productivity and biodiversity, which gives the place a high concentration of food for turtles (a high concentration of *Pleuroncodes planipes*, main food source of the loggerhead in this region) and other commercially important species (sharks, rays, fish, clams, abalone, squid, lobster, shrimp, crabs, snails and crabs) which are used by artisanal fishermen in the area through networking and bottom longlines and surface (SAGARPA 2014; Ramirez-Cruz *et al.* 1991;. Aurioles-Gamboa 1995; Peckham and Nichols 2002).

Therefore, in this paper it is to analyze the relationship of the proxy productivity, based on the concentrations of chlorophyll per mg per cubic meter; with variable sea surface temperature in C °, in turn correlate ruling out possible relationships with high rates of strandings of loggerhead turtle (*Caretta caretta*) over a period of 13 years (2003-2015).

Methods

The proxy chlorophyll concentration (mg / m³) as productivity and sea surface temperatures (C °), is I analyzed a database with the average per month was obtained for 13 years 2003-2015. a program of NASA / NOAA using GIOVANNI to compile and standardize the database was used. tortuguero group of Californias B.C. He collaborated for stranded turtles frequencies per month in Playa San Lazaro during those years.

An analysis time series autocorrelation and cross to measure the similarity of two data series correlation was used become a function of lag relative to the other. The latter, to evaluate the strength with these variables has been correlated in the area, and evaluating the sudden changes in correlation.

Results

The temporal variation of sea surface temperature (red line) and productivity (using grams of chlorophyll per cubic meter as a proxy) in the Gulf of Ulloa (Fig. 1) was obtained .The notes that some time (months) after the temperature (SST) surface of the sea reaches its lowest values in the area, and productivity increases significantly.

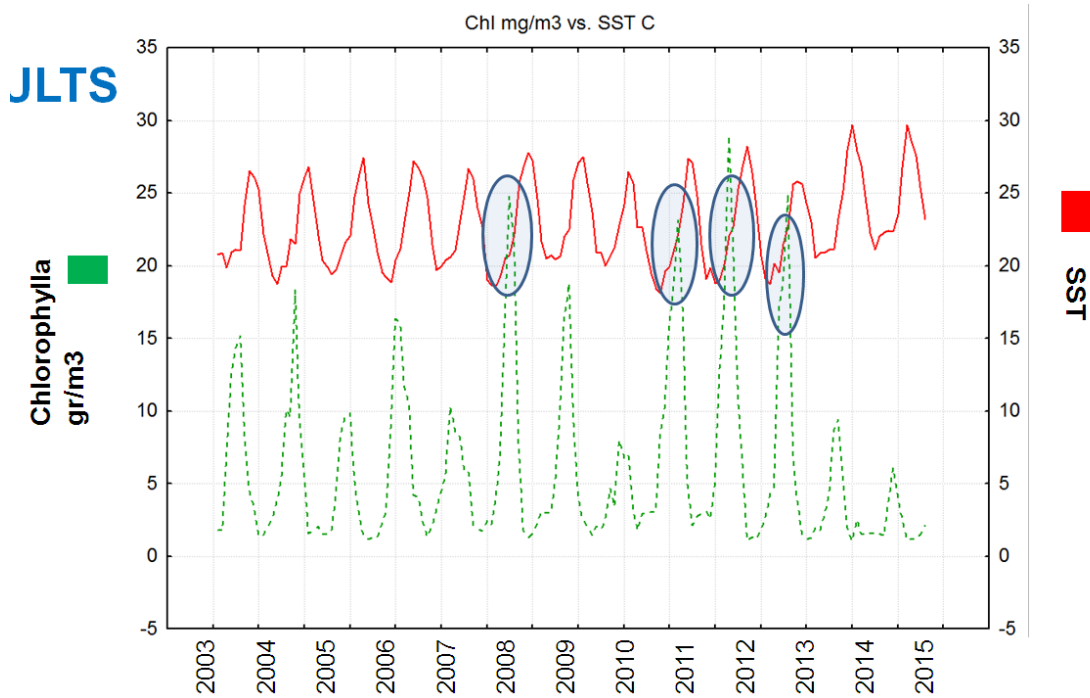


Fig. 1. Temporal variation of sea surface temperature (SST) and Productivity (Chlorophyll mg / m3).

Autocorrelation plots, a tool for finding repeating patterns, such as the presence of a periodic signal (seasonal) were obtained. On the left and on the right productivity of sea temperature in the Gulf of Ulloa. Both variables show a strong seasonal pattern delimited by periods of 6 months, this means that the area is six months predominant productive cold waters and six months of unproductive warmer waters (Fig. 2).

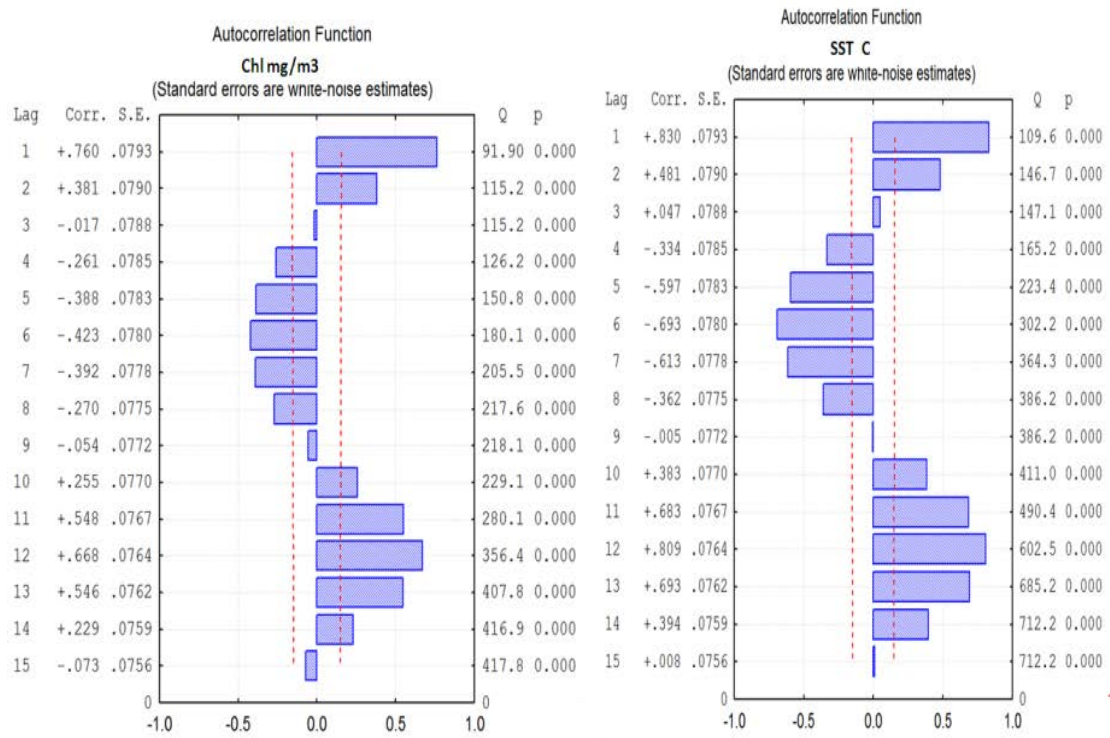


Fig. 2 autocorrelation of chlorophyll (left) and sea surface temperature (right).

Series of monthly time according to the mortality rate in strandings (pink), and productivity in milligrams of chlorophyll per cubic meter (Fig. 3) was obtained. Both series are offset. In general it can be perceived as *Caretta caretta* mortality increases as productivity in the Gulf of Ulloa aumentaa in different month.

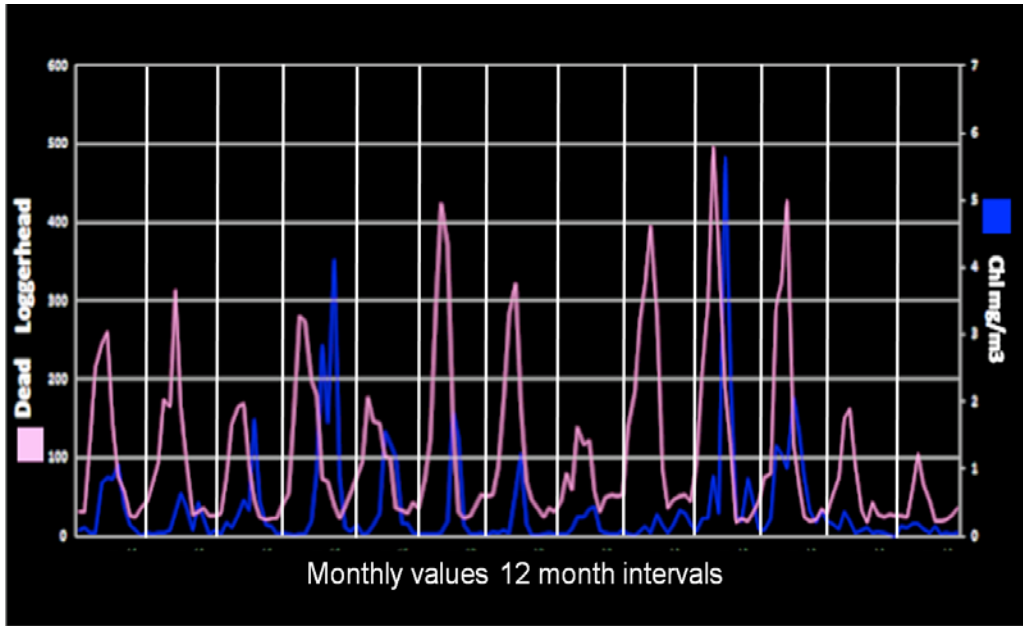


Fig. 3. Time series regarding strandings *Caretta caretta* and chlorophyll concentrations from 2003 to 2015.

Finally cross correlation measure how the temporal behavior or mortality of the number of stranding to changes in productivity as a function of offset relative to each other is simulated performed. It is noted that three months before productivity reaches its highest values in the area, stranding numbers *Caretta caretta* reach their high rate, but surprisingly collapse as productivity decreases in the area (Fig. 4).

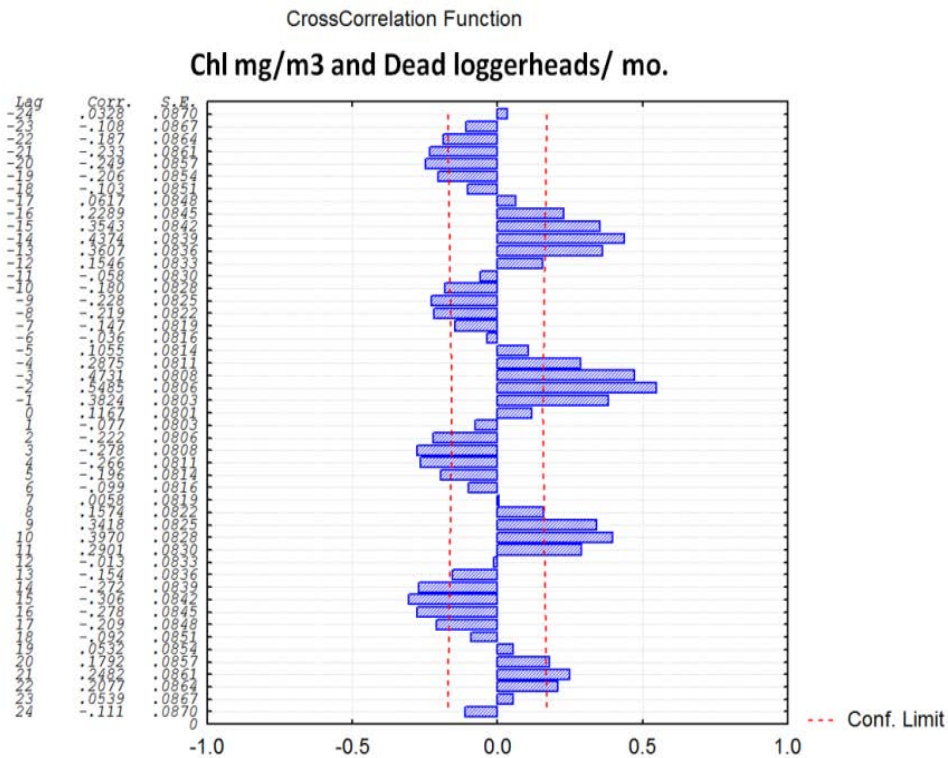


Fig. 4. Cross correlation, simulates the temporal behavior or the number of strandings mortality to changes in productivity over 13 years.

Discussion and conclusion

Temperature and productivity (chlorophyll proxy).

Upwelling are recognized as oceanographic processes characterized by the rise of subsurface waters with high nutrient content (Barnes and Mann 1998). This condition generates significant impacts in areas where its intensity is greater, as in the eastern edges of the oceans, consolidating systems in which the kinetic energy of the sea is particularly effective at encouraging primary production, to the extent that conditions are established that supercharged define ecosystems (Margalef 1985). Thus, arise as a consequence the presence of short food webs and relatively low efficiency and high levels of phytoplankton biomass and high primary production (Longhurst 1981; Margalef 1985; Lalli and Parsons 1997). Therefore, the results show proxy chlorophyll different from the temperature variability scenario. The SST shows a pattern consistent with the presence of upwelling events that cool the coastal strip from December to March. In comparison, only contain chlorophyll concentrations values consistent with the

occurrence of upwelling during the months of March through July. According autocorrelations SST and Chlorophyll though the winds in the region are able to cool the coastal area from February to March is when all necessary for the development of phytoplankton conditions occur. These conditions remain until the summer months when the SST begins to reach its maximum temperature. On the other hand in the month of July it is when the proxy Chlorophyll drops dramatically. That is, when it begins to decrease chlorophyll begins the season of high temperatures and during cold weather start high levels of chlorophyll. This coincides with the invasion of the entire region of tropical waters of very high temperature. An interesting fact is that the highest values of Chlorophyll proxy are observed in the central part of GU, mainly in May and July, not in the northern coastal area where upwellings are more intense (Gonzales 2006).

Differences in biological oceanography give rise to differences in movement patterns and diet observed in turtles. The consistently high primary productivity in the neritic of Baja California probably translates into a greater abundance of prey. Recent studies telemetry and aerial surveys allowed to determine that juvenile loggerheads are concentrated in an area of 15.194 km² with its center just 32 km off the coast of Baja California Sur (Peckham *et al.* 2007).

Feeding

Draws attention to the change of diet in recent years, from langostilla only eat a more varied diet in which fish discarded by the fishery and cephalopods (Peckham *et al.* 2011) are included. It is important to understand the reason for that change, and to help understand why loggerheads feed on discards or the same gear, with the risk of becoming trapped; and if such a strategy is by necessity (food decreased by changes in the environment) or opportunism (easy access to food). It is not known whether the search for food in fishing gear, it is a habit that has acquired the entire population of juveniles of Baja California, or only a portion of these. Stomach contents in neritic habitats BCP (Pacific Baja California) differed from those of ocean habitats CNP (Parker *et al.* 2005). The species that occur most frequently in the stomachs of turtles were fish BCP: searobins *Prionotus* spp. (30% of stomachs), *Diplectrum* spp. (23%), and *Synodus* spp (11%), and crustaceans, pelagic red crabs *Pleuroncodes planipes* (14%), *Platymera gaudichaudii* (6%) and *Hemisquilla ensigera* (5%). From the results obtained in this research the relationship of

high concentrations of chlorophyll (principalemente fitoplancton) from March to July, and the inicio fishing flake May to August is attributed by higher productivity which in turn generates food for fish . All fish are commonly caught and discarded as bycatch in gill local networks (Peckham unpubl.). Therefore, the data content of the stomach and informal observations indicate that fishing discards and bycatch are an important part of their diet in the BCP. It is likely that this behavior is exposed to extremely high levels of incidental mortality of different non-target species, in local small-scale fisheries (Peckham unpubl.).

Bycatch

Since 2003, some authors have observed a strong correlation between the scale fishing season in the area (May-August) and an increase in strandings of dead turtles (Koch *et al.* 2007 and Peckham *et al.* 2007). However, several studies mentioned that the bodies found on the beaches mostly died of unknown causes (Koch *et al.* 2006), and only 1.8% of 594 sampled corpses showed clear signs of injuries from fishing gear (hooks / network marks or signs of entanglement).

Along the 2,700 kilometers of the Pacific coast they are distributed around 11,000 fishermen who exploit various fisheries. In the five municipalities of Baja California Sur, there are coastal fishing cooperatives focused on catching fish (flake) and shrimp (in the case of Magdalena Bay in the municipality of Comondu) employing a lot of people. According to INEGI (2010) together, fisheries contribute approximately 3% of the state's GDP and employ 10% of the economically active population of the entity (Cortes *et al.* 2006). From the above the problem of bycatch of sea turtles and their high mortality rates that according to the results of course there is a relationship with the largest peak stranding *Caretta caretta* (May-Oct) and season shooting scale fishing (May-Aug).

Upwelling is a process characterized by rising ground water with high nutrient content, which favors high primary production, ecosystems as the Gulf of Ulloa. The temperature data (SST) showed a pattern consistent with the presence of upwelling in the Gulf of Ulloa from December to March. However chlorophyll concentrations are higher from March to July. Therefore, it appears that the conditions for the development of phytoplankton accumulate from January to

March. Contrary to the perception that the mortality rates of *Caretta caretta* higher occur during conditions of conditions of low productivity, the results suggest otherwise, the number of strandings begin to rise three months before the peak of higher productivity in the area. These results require a detailed analysis including fishing effort exerted in the area.

Literatura citada

Arévalo-Martínez D. y Franco-Herrera A. 2008. Características oceanográficas de la surgencia frente a la ensenada de Gaira, departamento de magdalena, época de Magdalena, época seca menor de 2006. Universidad de Bogotá Jorge Tadeo Lozano, Facultad de Ciencias Naturales, Programa de Biología Marina.

Barnes, R.S.K. y K. Mann (Eds.). 1998. Fundamentals of aquatic ecology. 2nd edición. Blackwell Science, Oxford. 217 p.

Caldwell D.K. 1963. The Sea Turtle Fishery of Baja California, Mexico. California Fish and Game 49(3):140-151.

Cliffon K., Cornejo D.O., Felger R.S. 1982. Sea turtles of the Pacific coast of Mexico. pp. 199-209 in K. Bjorndal (ed). Biology and conservation of sea turtles. Smithsonian Institution Press, Washington, D.C.

Conant TA, Dutton PH, Eguchi T, Epperly SP, Fahy CC, Godfrey MH, MacPherson SL, Possardt EE, Schroeder BA, Seminoff JA. 2009. Loggerhead sea turtle (*Caretta caretta*) 2009 status review under the US Endangered Species Act. Report of the Biological Review Team to the National Marine Fisheries Service.

Diario Oficial de la Federación. 2011. Ley General de Vida Silvestre. Congreso General de los Estados Unidos Mexicanos.

Fleming E.H. 2001. Swimming against the tide: Recent surveys of exploitation, trade, and management of marine turtles in the Northern Caribbean. TRAFFIC North America, April 2001.

- Koch, V.; W.J. Nichols; H. Peckham & V. de la Toba. 2006. Estimates of sea turtle mortality from poaching & bycatch in Bahía Magdalena, Baja California Sur, México. *Biol. Conserv.* 128:327-334.
- Lalli, C. y T. Parsons. 1997. *Biological Oceanography: An introduction*. 2nd edition. Butterworth-Heinemann, Oxford. 314 p.
- Longhurst, A. (Ed.). 1981. *Analysis of marine ecosystems*. Academic, London. 741.
- Margalef, R. 1985. Primary production in upwelling areas: Energy, global ecology and resources. Simposio internacional sobre áreas de afloramiento en el oeste africano. Barcelona. Instituto de Investigaciones Pesqueras, 1: 225-232.
- Nietschmann, B. 1995. The cultural context of sea turtle subsistence hunting in the Caribbean and problems caused by commercial exploitation. pp. 439-445. In: K.A. Bjorndal (Ed.). *Biology and conservation of sea turtles*. Smithsonian Institution Press, Washington, D.C.
- Norma Oficial Mexicana NOM-059-SEMARNAT-2010. Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo.
- O'Donnell J. 1974. Green turtle fishery in Baja California waters: history and prospect. Master's thesis, California State University, CA. 119 p.
- Peckham S. H. y W. J. Nichols. 2002. Pelagic red crabs and loggerhead turtles along the Baja California coast. En: Seminoff, J. (Comp.) *Proceedings of the Twenty-Second Annual Symposium on Sea Turtle Biology*.
- Peckham SH, Maldonado D, Walli A, Ruiz G, Nichols WJ, Crowder L. 2007. Small-scale fisheries bycatch jeopardizes endangered Pacific loggerhead turtles. *PLoS One* 2(10) doi 10.1371/journal.pone.0001041.
- Peckham SH, Maldonado-Diaz D, Koch V, Mancini A, Gaos A, Tinker MT, Nichols WJ. 2008. High mortality of loggerhead turtles due to bycatch, human consumption and strandings at Baja California Sur, Mexico, 2003-7. *Endangered Species Research* 5: 171-183 doi 10.3354/esr00123.

Peckham SH, Maldonado-Diaz D, Tremblay Y, Ochoa R, Polovina J, Balazs G, Dutton PH, Nichols WJ. 2011. Demographic implications of alternative foraging strategies in juvenile loggerhead turtles *Caretta caretta* of the North Pacific Ocean. *Mar Ecol Prog Ser* 425: 269-280 doi 10.3354/meps08995

Secretaria de Agricultura, Ganadería, Desarrollo rural, Pesca y Alimentación (SAGARPA), 2014. Programa integral de ordenamiento pesquero en el Golfo de Ulloa. Baja California Sur.

Seminoff, J. A. 2004. *Chelonia mydas*. En: IUCN 2011. IUCN Red List of Threatened Species. Versión 2011.2. [En línea] Disponible en Internet desde: <http://www.iucnredlist.org/apps/redlist/details/4615/0> [Consulta: diciembre de 2011].

Selection on the Major Histocompatibility Complex of killer whales (*Orcinus orca*) in the Gulf of California

Acy Wood¹

¹1021 24th Apartment 14th, Bellingham, Washington, 98225, acywood@gmail.com,

Keywords: Mexico, Local Adaptations, Immune System, Cetaceans, Genetic Analysis

Word Count: 3107

Possible Reviewers: Robin Kodner, Alejandro Acevedo-Gutierrez, Benjamin Miner, Sergio Flores-Ramirez, Dan Pollard, and Merrill Peterson

Selection on the Major Histocompatibility Complex of killer whales (*Orcinus orca*) in the Gulf of California

Abstract, English

The Major Histocompatibility Complex (MHC) is an important component of the immune system in vertebrates. Although regarded as highly diverse amongst terrestrial mammals, the diversity of MHC sequences of marine mammals are low in comparison. Previous studies have postulated that positive selection may have led to local populations of globally distributed marine taxa to be less diverse at their MHC sequences. The killer whales of the Gulf of California are an example of a local population of a cosmopolitan marine mammal. Little is known about the Gulf of California killer whales or their adaptations. It was hypothesized that Gulf of California killer whales and their ancestors have experienced positive selection in the past and that they have continued to have experience positive selection since their divergence from related taxa. To test this, a reconstructed ancestral sequence created from MHC sequences of Gulf of California killer whales and MHC sequences of related taxa was compared to the Gulf of California killer whales MHC sequences for similarities and dissimilarities. Analyses suggest that both Gulf of California killer whales and their ancestor have experienced positive selection in the past, but have not experienced positive selection since they have diverged from their related taxa. Instead, the results suggest that neutral selection has been the acting force on the MHC of the Gulf of California killer whales since they diverged from related taxa.

Abstract, Spanish

El Complejo de Histocompatibilidad Mayor (CHM) es un componente importante del Sistema inmune de los vertebrados. Pese a ser considerado como diverso entre los mamíferos terrestres, el CMH resulta ser poco diverso en los mamíferos marinos. Estudios anteriores han postulado que la selección positiva podría haber llevado a las poblaciones locales de taxas marinos globalmente distribuidos a ser más diversos a nivel de las secuencias del MHC. Las orcas del Golfo de California son un ejemplo de una población local de un mamífero marino globalmente distribuido. Poco es sabido acerca de las adaptaciones de las orcas del Golfo de California. En un principio se pensaba que estas y sus ancestros habían experimentado una selección positiva desde su divergencia a partir de taxas relacionados a ellas. Para comprobar esto, un estado ancestral fue reconstruido a partir de las secuencias del CHM de orcas del Golfo de California así como de taxa relacionados, los cuales fueron comparados entre sí con el objetivo de encontrar similitudes y disimilitudes. El análisis sugiere que tanto las orcas del golfo de California como su ancestro han experimentado selección positiva en el pasado, no así desde el momento en el que divergieron del taxon relacionado. Se sugiere que la selección neutral ha sido la fuerza principal que ha actuado en el CHM de las orcas del Golfo de California desde el momento en el que divergieron de los otros taxa relacionados.

Introduction

Interspecific genetic analysis is a powerful tool for determining spatial and temporal genetic diversity between related taxa. Identification of evolutionary forces on local populations with inherently low levels of diversity is important for diagnosing potential conservation concerns, such as locally adapted populations of a global species. Loss of genetic variation for

sequences that encode for immune response in local populations may increase the risk of extinction due to increased disease susceptibility. Biological and anthropomorphic pressures can impact genetic variation of immune system genes. For example, the Chinese White Dolphin has been proposed to have experienced a decrease in genetic diversity due to pollutants in its primary habitat, the Pearl River Estuary (Zhang et al., 2016). It is important to understand what selective forces act on diverse loci and how diversity can be conserved in the future.

The Major Histocompatibility Complex (MHC) is a complex of genes that transcribe for a series of glycoproteins that bind to and present antigens to T cells (Zhang et al., 2016). Two classes of MHC genes are transcribed to interact with antigens of different origins: Class I MHC proteins bind to endogenous antigens and present to cytotoxic T cells while Class II MHC proteins bind to exogenous antigens and present to helper T cells to initiate a an immune response (Gillet et al., 2014). MHC sequences of mammals are regarded as extremely diverse. More than 500 different polymorphisms exist for human MHC, which is more than any other known protein (*Infection*, 2007). Most polymorphisms occur at sites that determine the Peptide Bind Region (PBR) (Vassilakos et al., 2009). The PBR of MHC proteins determine what antigens the MHC proteins can bind to, which in turns determines what T cells respond to (Yang et al., 2013). Balancing selection and small genetic drift are believed to be the primary drivers of variation of MHC sequences (Hayashi et al., 2006). The MHC is comprised of alleles inherited from both parents. An individual with two different sets of alleles can respond to a wider range of antigens, which leads to a larger number of observed heterozygotes in populations than expected in Hardy-Weinberg equilibrium (Vassilakos et al., 2009).

In contrast to their terrestrial counterparts, marine mammals possess a low level of diversity for MHC sequences. This could be due to a less of a selective pressure from marine-borne pathogens compared to terrestrial environments (Nigenda-Morales et al., 2008). As evidence for this pattern, oceanic marine mammals tend to have less MHC diversity than closely related coastal marine mammals, possibly due to coastal marine mammals experiencing both marine and terrestrial based pathogens (Zhang et al., 2016). For example, the oceanic Common Bottlenose Dolphin, (*Tursiops truncatus*), is closely related to the coastal Indo-Pacific Bottlenose Dolphin, (*Tursiops aduncus*). The Indo-Pacific Bottlenose Dolphin has greater MHC diversity than the oceanic Common Bottlenose Dolphin. It has been proposed that positive selection is responsible for the fixation of novel alleles into local populations of marine mammals (Xu et al, 2010). Positive selection is when an allele mutates into a polymorphism and becomes fixed into a population rather than be eliminated. Positively selected MHC sequences can be retained due to MHC heterozygotes being more fit than homozygotes. This favoring of heterozygotes is referred to as ‘balancing selection’. Balancing selection can lead to a rapid increase of local alleles that have been selected for by the environment. Because of this, the MHC is a useful tool for identifying local adaptations of global species, such as killer whales, (*Orcinus orca*).

The killer whales found in the Gulf of California are an example of a locally adapted population of a global species. Little is known about the populations of killer whales found in the Gulf of California. It is assumed that 3 distinct ecotypes of killer whale exist in the Gulf: A fish-eating ecotype related to the Northeastern Pacific Transients, an ecotype related to the Eastern Tropical Pacific and Oceanic ecotypes, and an independent lineage whose origins remain unknown (Sergio Flores-Ramírez, personal communication, July 17, 2016). Little to no gene

flow is experienced by the Gulf of California killer whales. If a novel allele mutated in one of the ecotypes, that allele would be constrained to just the oceanic basin of the Gulf of California.

Previous research suggesting low marine mammal MHC class II *DQB-1* diversity opens the question of whether the Gulf of California killer whales experienced positive selection at their MHC class II *DQB-1* sequences before in their genetic history. It is hypothesized that the Gulf of California killer whales have experienced positive selection due to other Cetaceans reportedly experiencing positive selection in their genetic histories. The second question investigated was whether the PBR of the Gulf of California killer whales have experienced positive selection since diverging away from related taxa. It was hypothesized that the Gulf of California killer whales have experienced positive selection at their PBR as they have adapted to their local environment. To test these questions, MHC sequences of killer whales from the Gulf of California and related taxa were gathered to recreate ancestral states of MHC sequences. These ancestral states were used to determine if positive selection had been experienced previously or since the taxa diverged.

Methods

MHC Sequence Acquisition

The *DQB-1* exon of the MHC Class II sequence was chosen for comparison based off previously published Cetacean MHC studies (Nigenda-Morales et al., 2008). MHC Class II *DQB-1* sequences for the tribe Delphininae and the tribe Globicephalinae were located using the Nucleotide search function on NCBI. MHC Class II *DQB-1* sequences for the Gulf of California killer whales were obtained from Sergio Francisco Flores Ramírez of Universidad Autónoma de

Baja California Sur. The sequences were trimmed to 172 bp after comparison of homologous sequences of other Cetaceans from previously published work (Zhang et al., 2016). After all sequences were trimmed, they were aligned using ClustalX 2.1 (Thompson et al., 1997). Three alignments were constructed: Delphininae, Globicephalinae, and Gulf of California killer whales.

Phylogenetic reconstruction

Each of the three alignments had a best model of evolution selected by a test of Best Model using MEGA 6 (Tamura et al., 2013). Maximum Likelihood phylogenetic trees were constructed on the resulting best models of evolution for each alignment using MEGA 6 (See Supplementary Figures 1-3). Alignments and Maximum Likelihood phylogenetic trees were used to recreate possible ancestral state sequences for each data set using MEGA 6. The most probable of the possible ancestral state sequence created for each data set was determined. Ancestral state sequences were added to the initial alignments they were derived from and realigned using ClustalX 2.1.

Population Genetic Analysis

The three sequence alignments were translated into amino acids based off the second nucleotide in sequence using MEGA 6. Amino acid sequences were aligned with previously published Cetacean MHC Class II *DQB-1* sequences to verify that the sequences of interest were MHC Class II *DQB-1* and to identify which codons of the MHC Class II *DQB-1* affected the PBR (Nigenda-Morales et al., 2008). dN and dS were determined for each PBR-affecting codon for each data set by using MEGA 6. dN/dS ratios were determined for each codon and selective force was determined by testing for significant Z statistics using MEGA 6.

Common Ancestor Analysis

A fourth alignment was created using MHC Class II *DQB-1* sequences from the Delphininae and the Gulf of California killer whales alignments using ClustalX 2.1. Previously determined ancestors were retained within the new alignment. The Globicephalinae tribe sequences were left out of the fourth alignment due to a lack of robustness in the data set. A best model of evolution was determined and a fourth Maximum Likelihood phylogenetic tree constructed using MEGA 6 (See Supplementary Figures 4). The new alignment and Maximum Likelihood phylogenetic tree were used to recreate possible ancestral state sequences for the common ancestor of Delphininae and the Gulf of California killer whales. The most probable ancestral state sequence was determined from the possible ancestral states and was added to the alignment. The sequence were realigned using CLustalX 2.1. dN and dS for PBR-affecting codons were determined using MEGA 6. dN/dS ratios were calculated for each codon and tested for selective force by using Z statistics.

Divergence Analysis

Genetic distance comparing the common ancestor to each individual of Delphininae and Gulf of California killer whales was calculated using MEGA 6. Average genetic distance per taxa was determined for each taxa within Delphininae and the Gulf of California killer whales. Taxa that had less than 10 individuals were ignored. An ANOVA comparing the genetic distance of each taxa was conducted to test for selective force between populations since divergence from the common ancestor using RStudio (Team, 2015).

Results

The three initial data sets revealed that Delphininae, Globicephalinae, and the Gulf of California killer whales have not experienced positive selection at PBR-affecting codons when compared to their derived ancestors (Table 1, Table 2, and Table 3). Any change in codon sequence was attributed to neutral selection. Comparisons of the Delphininae and the Gulf of California killer whales to the common ancestor revealed that positive selection has been experienced at 4 of the 12 amino acids that determine the PBR since the groups have diverged (Table 4).

The common ancestor for the Delphininae tribe and killer whales was calculated to have experienced a dN/dS ratio greater than 1. This implies that at some point in their genetic history, the common ancestor experienced positive selection.

No significant difference in selective force was found between killer whales or taxa of the Delphininae tribe, $F(2,82) = 2.795$, $p = 0.067$. A comparison of the Gulf of California killer whales to the Indo-Pacific Bottlenose Dolphin resulted in a determination of marginally significant effect, indicating positive selection since the two taxa diverged away from each other ($p = 0.06$).

Discussion

Each of the three alignments, Delphininae, Globicephalinae, and Killer whale, have only experienced neutral selection since the time of their derived ancestor (Table 1, Table 2, and Table 3). This could be due to a lack of a pathogenic pressure selecting for an allele or it could be due to a previous allele already capable of binding to novel pathogens that the taxa

experience. However, there was an inherit bias against diversity due to a lack of MHC Class II *DQB-1* sequences available for killer whales and Globicephalinae. NCBI possessed far more Delphininae MHC Class II *DQB-1* sequences than either Globicephalinae or Gulf of California killer whales along with a more global distribution of sequences. Essentially, a comparison was made between a global distribution and a local distribution. This could lead to a more probable common ancestor for the Delphininae being compared to a less defined common ancestor for killer whales. As more killer whale MHC Class II *DQB-1* sequences become available, the common ancestor for killer whales should become more robust leading to a less biased comparison of selective forces.

It was determined that at some point in their genetic past, killer whales of the Gulf of California and their ancestor experienced positive selection. The determined dN/dS ratios for each of the Gulf of California killer whales along with their ancestor were greater than 1, which indicates positive selection. Further reconstruction with more MHC Class II *DQB-1* sequences from broader marine mammal taxa would be needed to determine at what point positive selection was experienced on the MHC Class II *DQB-1*. Once a molecular clock is determined, a possible reason could be postulated. For example, a change in atmospheric conditions could have changed oceanic conditions or a change in range could have led to an expansion in MHC Class II *DQB-1* diversity.

Neutral selection has been the selective force acting upon Delphininae and the Gulf of California killer whales since they have diverged. The Indo-Pacific Bottlenose Dolphin and the Gulf of California killer whales were determined to have a marginally significant effect, which suggests that positive selection may have been experienced. Killer whales are regarded as an

oceanic marine mammal while the Indo-Pacific Bottlenose Dolphin is regarded as a coastal marine mammal. Coastal marine mammals are perceived to be under more pathogenic selective pressure due to being exposed to both terrestrial and marine derived pathogens (Nigenda-Morales et al., 2008). This could account for why positive selection may have been experienced since the two taxa diverged.

The killer whales of the Gulf of California have experienced positive selection before in their genetic history, but not since they diverged from their related taxa in Delphininae. This suggests that novel alleles have not mutated within the Gulf of California populations. This lack of novel alleles could make replacement of killer whales in the Gulf of California more successful if the populations currently residing in the gulf suddenly went extinct. For example, the Eastern Tropical Pacific ecotype of killer whales could colonize the Gulf of California and possess the needed MHC adaptations to survive pathogenic pressure within the gulf.

Reconstruction of ancestral MHC sequences and comparison with modern taxa advances the understanding of how selective forces shape local adaptation. As more MHC sequences for Cetaceans become available, more robust ancestral states can be derived. These sequences can be used to estimate the time at which selective pressures were applied and for how long. Patterns based off previous events can be determined and used to project future development of local populations as they are effected anthropogenic activity. As future research accumulates and progresses our knowledge of human impact of MHC diversity, greater contribution efforts can be made to conserve local adaptations and populations of cetaceans.

1 Reference

2 4-3 The MHC and Polymorphism of MHC Molecules. (2007). In *Infection* (pp. 8–9). New
3 Science Press Ltd.

4 Gillett, R. M., Murray, B. W., & White, B. N. (2014). Characterization of class I – and class II –
5 like major histocompatibility complex loci in pedigrees of north Atlantic right whales. *Journal of*
6 *Heredity*, 105(2), 188–202.

7 Hayashi, K., Yoshida, H., Nishida, S., Goto, M., Pastene, L., Kanda, N., ... Koike, H. (2006).
8 Genetic variation of the MHC DQB locus in the finless porpoise (*Neophocaena phocaenoides*).
9 *Zool Sci*, 23, 147–153.

10 Nei, M., & Gojoborit, T. (1986). Simple Methods for Estimating the Numbers of Synonymous
11 and Nonsynonymous Nucleotide Substitutions ', 3(5), 418–426.

12 Nigenda-Morales, S., Flores-Ramírez, S., Urbán-R., J., & Vázquez-Juárez, R. (2008). MHC
13 DQB-1 polymorphism in the Gulf of California fin whale (*Balaenoptera physalus*) population.
14 *Journal of Heredity*, 99(1), 14–21.

15 Piertney, S. B., & Oliver, M. K. (2005). The evolutionary ecology of the major
16 histocompatibility complex. *Heredity*, 96(1), 7–21.

17 Tamura, K., Stecher, G., Peterson, D., Filipski, A., & Kumar, S. (2013). MEGA6: Molecular
18 Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution*, 30, 2725–2729.

19 Team, Rs. (2015). RStudio. Boston: Inegrated Development for RStudio, Inc. Retrieved from
20 MA URL

- 21 Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., & Higgins, D. G. (1997). The
22 ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality
23 analysis tools. *Nucleic Acids Research*, 25, 4876–4882.
- 24 Vassilakos, D., Natoli, A., Dahlheim, M., & Hoelzel, A. R. (2009). Balancing and directional
25 selection at exon-2 of the MHC DQB1 locus among populations of odontocete cetaceans.
26 *Molecular Biology and Evolution*, 26(3), 681–689.
- 27 Xu, S., Ren, W., Zhou, X., Zhou, K., & Yang, G. (2010). Sequence polymorphism and
28 geographical variation at a positively selected MHC-DRB gene in the finless porpoise
29 (*Neophocaena phocaenoides*): Implication for recent differentiation of the Yangtze finless
30 porpoise? *Journal of Molecular Evolution*, 71(1), 6–22.
- 31 Yang, W.-C., Hu, J.-M., & Chou, L.-S. (2013). Sequence analyses of MHC Class II DQB gene in
32 bottlenose dolphins (*Tursiops* spp.) and the other delphinid species from the Western Pacific.
33 *Taiwan Vet J*, 39(2), 100–109.
- 34 Zhang, X., Lin, W., Zhou, R., Gui, D., Yu, X., & Wu, Y. (2016). Low Major Histocompatibility
35 Complex Class II Variation in the Endangered Indo-Pacific Humpback Dolphin (*Sousa*
36 *chinensis*): Inferences About the Role of Balancing Selection. *Journal of Heredity*, 1–10.

37 Table 1. Maximum Likelihood analysis of natural selection codon-by-codon for Delphininae and
 38 Delphininae ancestor MHC sequences.

Codon Number	Codon Start	dS	dN	P-value
6	16	0	1.949428254	0.218839604
8	22	12.69533419	5.785581196	0.971330528
10	28	0	1.182167367	0.318023745
17	49	1.984797886	0	1
18	52	0.778085991	0	1
27	79	0	0	N/A
37	109	1	1.090354659	0.714386615
41	121	0.518058551	0.77501986	0.667258564
47	139	1.865379225	2.561332397	0.532210873
50	148	0.998632209	7.004797111	0.019247501
51	151	0	0	N/A
54	160	0	1.949428254	0.218839604

39

40

41 Table 2. Maximum Likelihood analysis of natural selection codon-by-codon for Globicephalinae
 42 and Globicephalinae ancestor MHC sequences.

Codon Number	Codon Start	dS	dN	P-value
6	16	0	2.412438572	0.157384671
8	22	0	2.336990354	0.392860581
10	28	0	1.416833757	0.638562762
17	49	0	1.239998383	0.616264187
18	52	0	1.006217264	0.444898087
27	79	0	0.751363444	0.78964259
37	109	0	2.388135866	0.345005316
41	121	0	0	N/A
47	139	0	0.810267617	0.720644022
50	148	0	2.622658398	0.154573107
51	151	0	2.263011079	0.299782814
54	160	0	1.892844457	0.418234192

43

44

45 Table 3. Maximum Likelihood analysis of natural selection codon-by-codon for the Gulf of
 46 California killer whales and the Gulf of California killer whale ancestor MHC sequences.

Codon Number	Codon Start	dS	dN	P-value
6	16	0	0.9772	0.465467
8	22	0	0.7588	0.7719229
10	28	0	0.4445	0.9521253
17	49	0	0.8188	0.7992924
18	52	0	0.4979	0.6699686
27	79	0	0	N/A
37	109	0	0.7638	0.7617936
41	121	0	0	N/A
47	139	0	0.7673	0.8730343
50	148	0	0.9708	0.4901892
51	151	0	0.915	0.609091
54	160	0	1.3703	0.7289649

47

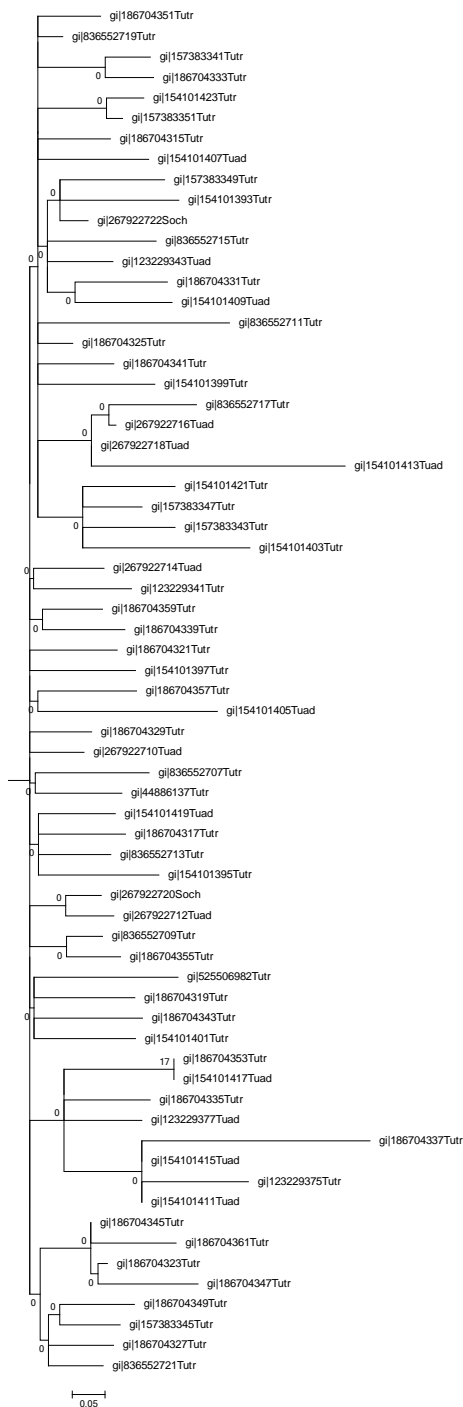
48

49 Table 4. Maximum Likelihood analysis of natural selection codon-by-codon for Delphininae, the
 50 Gulf of California killer whales, and their common ancestor MHC sequences.

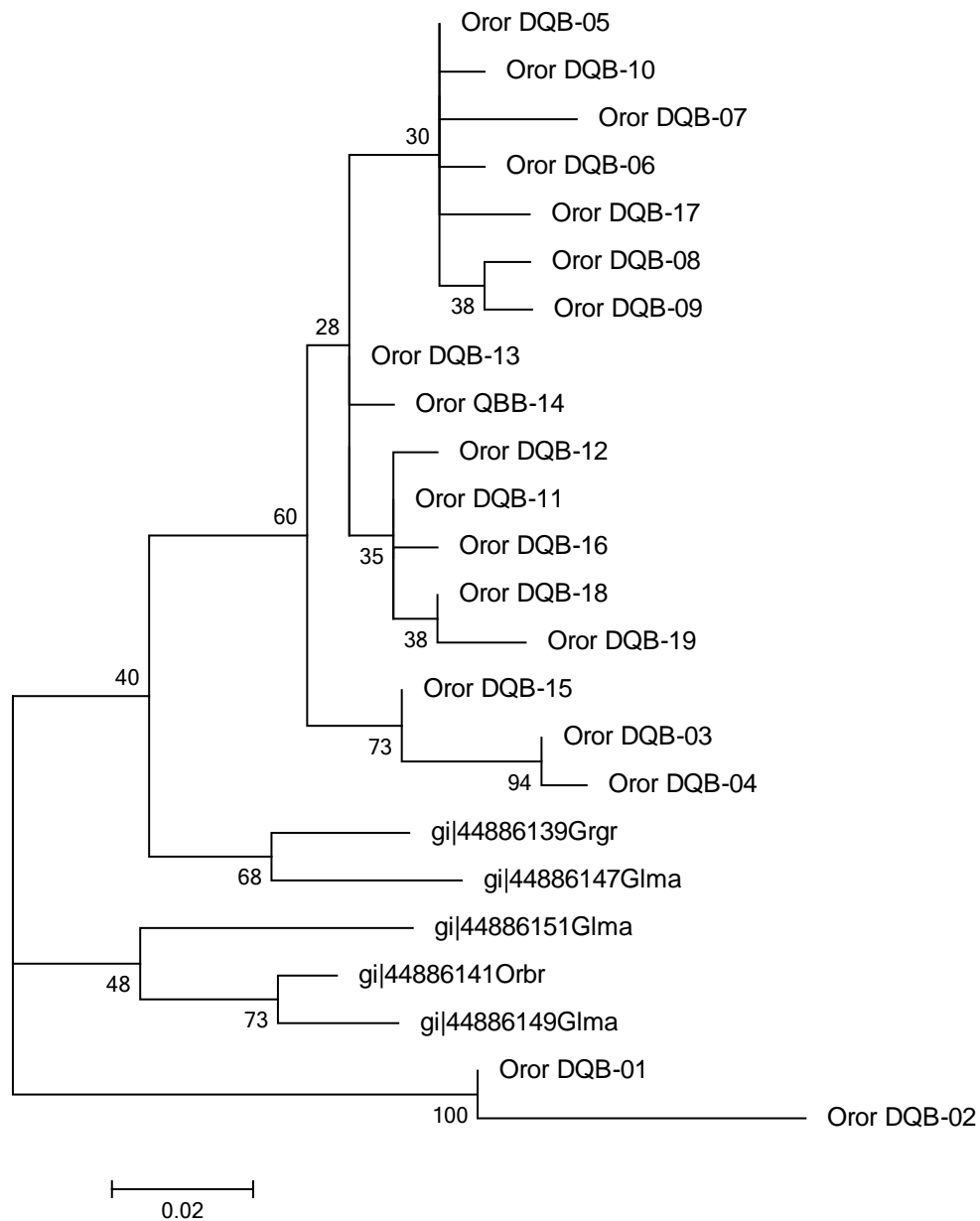
Codon Number	Codon Start	dS	dN	P-value
6	16	0	12.09725927	0.000403393
8	22	2.196643196	9.73326842	0.064842328
10	28	0	8.789124117	0.04005487
17	49	2.344307383	6.209916668	0.285262803
18	52	0	1.499071289	0.297012533
27	79	0	2.344438608	0.467979749
37	109	0	4.78684679	0.115911244
41	121	0	1.160109956	0.956473864
47	139	0	4.669276236	0.076848337
50	148	0	8.531084215	0.001226267
51	151	0	7.544898735	0.023418992
54	160	0	12.09725927	0.000403393

51

52 Supplementary Index:

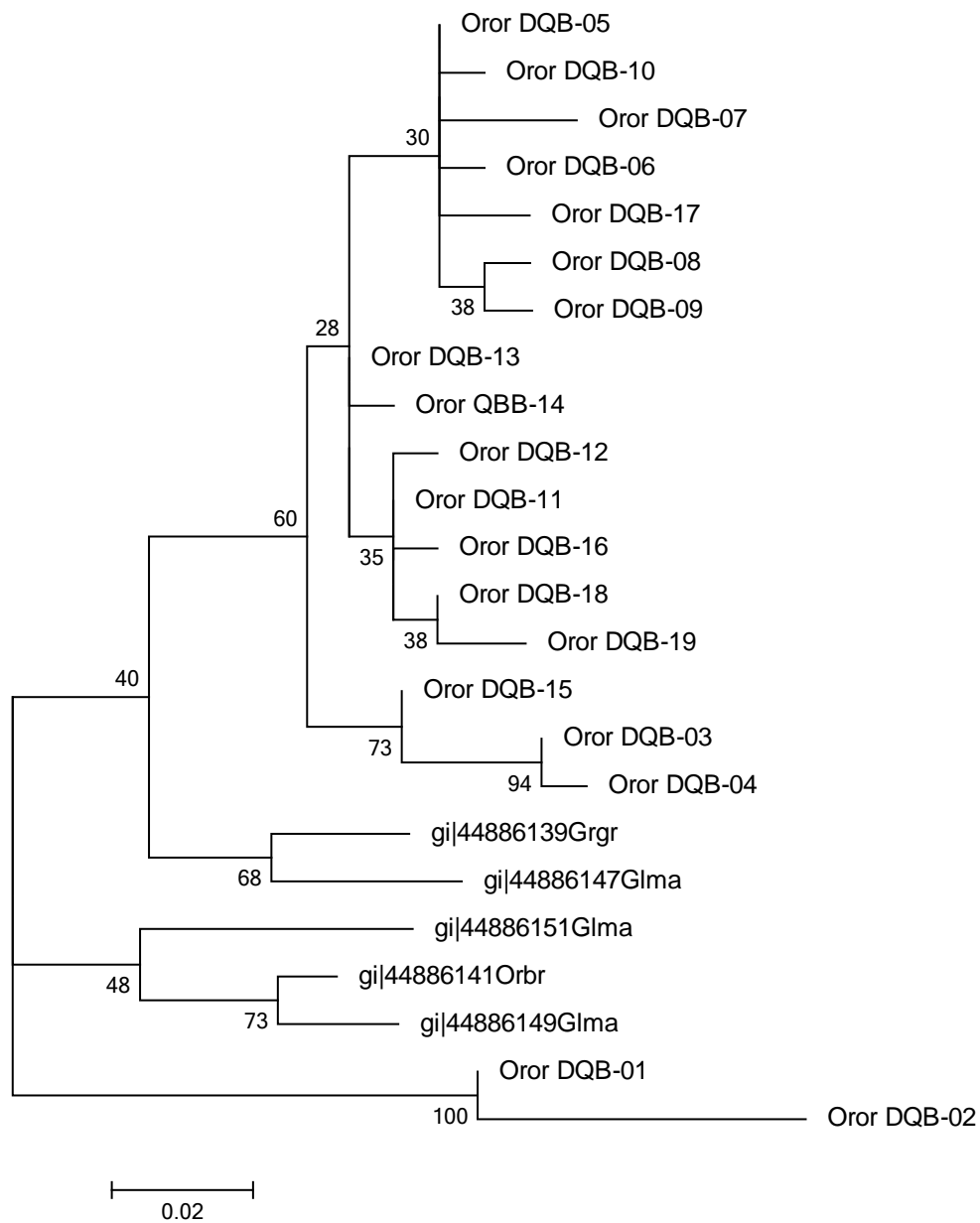


54 Supplementary Figure 1. Maximum Likelihood phylogenetic tree for Delphininae MHC Class II
 55 *DQB-1* sequences constructed with MEGA 6.



56

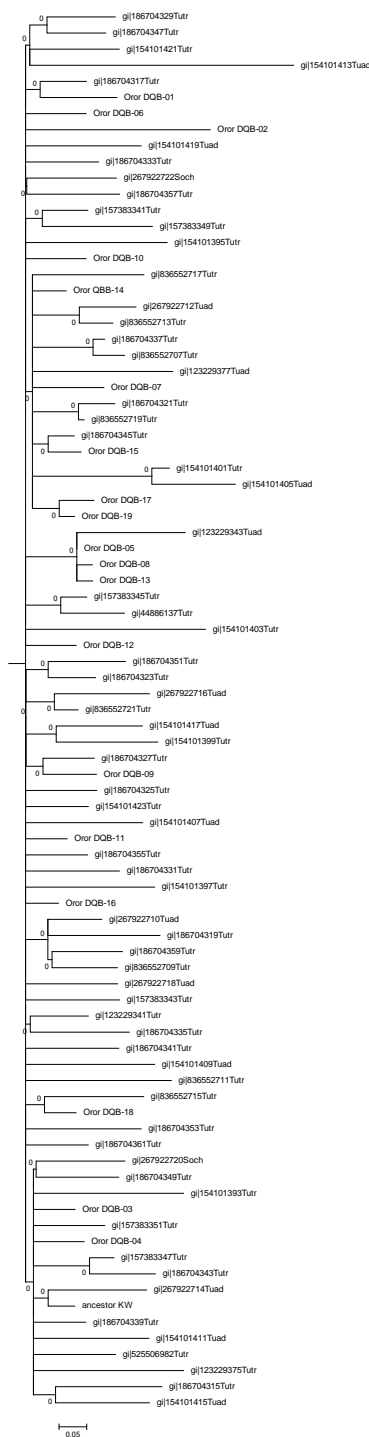
57 Supplementary Figure 2. Maximum Likelihood phylogenetic tree for Globicephalinae MHC
 58 Class II *DQB-1* sequences constructed with MEGA 6.



59

60 Supplementary Figure 3. Maximum Likelihood phylogenetic tree for the Gulf of California killer

61 whale MHC Class II *DQB-1* sequences constructed with MEGA 6.



62

63 Supplementary Figure 4. Maximum Likelihood phylogenetic tree for Delphininae and the Gulf
 64 of California killer whale MHC Class II *DQB-1* sequences constructed with MEGA 6.

Phylogenetic diversity of Sharks at three points of Mexico

Hugo Sánchez Gómez¹

Universidad Autónoma de Baja California Sur¹

Department of Marine and Coastal Science. Carretera al Sur Km 5.5, 23080 La Paz, B.C.S, México

65
66
67
68
69
70

Summary: *Diversity can be measured at three levels: Ecosystem, species and genetic. In México some research at species level diversity of sharks has been done. However, most of this studies only use convencional indeces such as shannon and simpson but not differ between taxonomic diversity. This work belongs to taxonomic diversity and distintiveness indeces which are useful in ecological purposes. In order to recognize the phylogenetic diversity of sharks in México, a taxonomic analysis was done. Data publisehd from three different locations in México was analyzed (Baja California Sur, Oaxaca and Campeche). A taxonomic matrix of all species was created and after that, analyzed in software R (Vegan package) among their abundances. Dendograms of species for each location were built. In order to compare the taxonomic diversity, an analysis of DELTA and DELTA was done. Results shown that BCS presented the highest values of taxonomic indices, due to their highest specie composition because of their overlaped location between two marine regions (Panamic and Californian). While Oaxaca needs more information to get a better analysis. The lowest values presented in Campeche were due to the abundance of a single secie that was also part of the dominant taxa. In order to know the shark species composition among time and locations this kind of information is useful, so we can improve management plans ideal to each region*

KeyWords: Taxonomic diversit, Taxonomic disintivness, Elasmobranchs, Condrichtyes, Shark Fisheries

89

Resumen: *La diversidad puede ser medida en tes niveles: Ecosistemica, de especies y geneticamente. En México, se han hehco investigaciones al nivel de diversidad de especies, incluyendo tiburones. Sin embargo, la mayor parte de estos estudios solo utilizan indices convencionales como Shanon y Simpson que do diferencian entre niveles taxonómicos. Esta tarearecae en los indices de diversidad y Distintividad taxonómica, los cuales son utiles para propósitos ecológicos. Con el objetivo de reconocer la diversidad filogenética de tiburones en México, se realizó un análisis de diversidad taxonómica. Se tomaron datos de las pesquerias de tiburones de Baja California Sur, Oaxaca y Mpexico, creando una mtriz taxonómica de todas las especies. Esta información fue procesada conjunto a las abundancias de cada especie en el programa R, utilizando la paqueteria VEGAN. Se obtuvieron los dendogramas para cada una de las regiones, así como los valores de los indices de diversidad y distintividad taxonómicas. Se encontró que Baja California Sur posee los valores mayors valores, debido a que se encuentra en una zona de transción entre la región panámica y californiana. Oaxaca por su lado, carece de mucha informacion en cuanto a pesuqerias de tiburón para realizar un análisis mas profundo. Campeche, mostró los valores menores debido a la dominancia de una sola especie que*

105 *además se encpntró en el taxón dominante. Este tipo de estudios son de utilidad para conocer el*
106 *ensamblaje de especies, y como esta puede ir variando a lo largo del tiempo y localidades,*
107 *información útil a la hora de realizar planes de manejo*

108 **Palabras clave:** Diversidad Taxonómica, Distintividad taxonómica, Elasmobranquios,
109 Condrictios, Pesquerías de tiburón

110

111 **Introduction**

112 Biological diversity can be characterized in three different levels: diversity among ecosystems,
113 phylogenetic diversity (diversity of species within an ecosystem) and genetic diversity (Agardi,
114 2000).

115 In Mexico there are many studies about shark diversity in both Pacific and Gulf of Mexico
116 (Ramirez-Amaro, 2011. Rochin Alamillo, 2011, Pérez-Jiménez & Mendez-Loeza, 2015),
117 however if certainly shark fisheries research has increased in the last years, little information
118 about changes in shark species composition since the fishery began its known because
119 government management plans do not distinct between species.

120 In order to improve management plans, identification of organisms is necessary at any level
121 (Vecchione & Collette, 1996).

122 However if its true that many shark fisheries papers make evident the species diversity among
123 different regions and sites by using conventional indices such as Shannon and Simpson, its
124 necessary to mention that these indices do not necessary take in consideration the taxonomic
125 distinctivness between species (Warwick & Clarcke, 1998), in other words, they cannot identify if
126 a particular specie is the only representative of a complete evolutionary line or share this
127 characteristic with similar close species (Vecchione *et al.* 2000)

128 Fortunately, taxonomic distinctiveness can be measured by taxonomic distinction indices: Δ^+ and
129 Δ^* . The first one Δ^+ (Taxonomic diversity index) is defined as the mean taxonomic distance
130 between two different organisms chosen randomly (Lineann distance that bonds both of them);
131 by the other hand, Δ^* is defined as the mean length of the distance of two organisms chosen
132 randomly, which corresponds to divide taxonomic diversity by value given if there is no
133 taxonomic hierarchy (all species in the same genus) (Clarcke & Warwick, 1999)

134 **Methods**

135 In order to compare the phylogenetic structure of sharks in three points of Mexico, fisheries
136 published data from Ramirez-Amaro *et al.* (2011) in the occidental coast of Baja California Sur,
137 Alejo Plata *et al.* (2006) in Oaxaca, and Pérez-Jiménez & Méndez-Loeza (2015) in Campeche
138 was analyzed.

139 Information about species composition and abundances was taken. With species information, a
140 matrix which includes taxonomic description of every single specie was done. Five hierarchy
141 levels were used in this analyses: Species, Genus, Family, Order and Class

142 Taxonomic characteristics and abundances of species per site were analyzed in program R, using
143 the package “Vegan” . Dendograms showing taxonomic relationship of species were maken for
144 each region by using “hclust” command.

145 After that, diversity and distinctiveness indices were also calculated.

146 **Taxonomic diversity (Δ):** Average taxonomic distance between any two organisms (Clarcke &
147 Warwick, 1998)

148 **Taxonomic distinctness (Δ^*):** Average path length between any two randomly chosen
 149 individuals from different species (Clarcke & Warwick, 1998)

$$\Delta^* = \frac{\sum_{i=1}^n \sum_{i<j} \omega_{ij} x_i x_j}{\sum_{i=1}^n \sum_{i<j} x_i x_j}$$

150

151 Where:

152 X= abundance distribution

153 ω = taxonomic distance between species *i* and *j*

154

155 **Mean taxonomic distinctivness (Δ^+):** Average taxonomic path between any 2 randomly chosen
 156 species traced throug a linnean or phylogenetic classification of the full set of species involved
 157 (Clarcke & Warwick, 1999)

$$\Delta^+ = \frac{\sum_{j=1}^n \sum_{i<j} \omega_{ij}}{S(S-1)/2}$$

158

159 Where:

160 S= number of species in the studio

161 ω = taxonomic distance between species *i* and *j*

162 Finally, indices value for all three regions were plotted together in order to visualize their results

163 Results

164 A total number of 45 shark species were registered as total sampling. Of these, 30 species
 165 can be found in Baja California Sur (BCS), 11 in Oaxaca and 21 in Campeche (Table I). 20
 166 species belonged only to BCS region, 2 to Oaxaca and 11 to Campeche. By the other hand, 4
 167 species (*Carcharhinus leucas*, *Carcharhinus limbatus*, *Galeocerdo cuvier* and *Sphyrna lewini*)
 168 could be found in all regions

169 Total shak phylogenia is shown in figure 1. Total species composition were culstered in
 170 22 genera, 13 families and 7 orders. Order Carcharhiniforme was the most abundant with 30
 171 species, while order Squatiniformes was the lowest with just one specie. Genus *Carcharhinus*
 172 was also the most abundant with 13 species.

173 Abundancy of sharks per specie and region are shown in table II. Total shark sampling
 174 was 40430 individuals; 10,748 corresponding to BCS; 1,208 to Oaxaca and 28,474 to
 175 Campeche. *Prionace glauca* was the most abundat specie in BCS (N= 3,455), *Carcharhinus*
 176 *falciformis* in Oaxaca (N=424), and *Rhizoprionodon longurio* in Campeche (19,106)

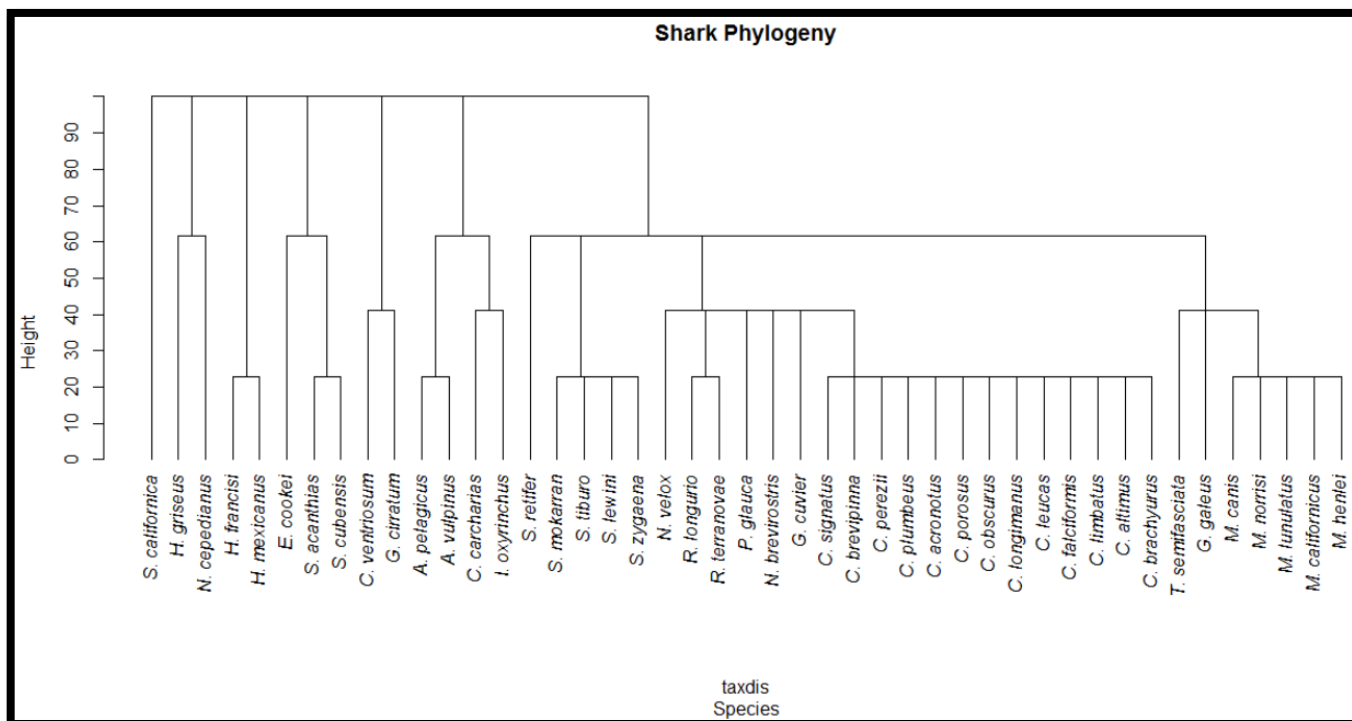
177 **Table I.** List of shark species presence/ausence per location (Presence of a specie in a
 178 determinated location is marked)

Species name	BCS	Oaxaca	Campeche
<i>Alopias pelagicus</i>	*	*	
<i>Alopias vulpinus</i>	*		
<i>Carcharhinus acronotus</i>			*
<i>Carcharhinus altimus</i>	*		
<i>Carcharhinus brachyurus</i>	*		

Carcharhinus brevipinna			*
Carcharhinus falciformis	*	*	
Carcharhinus leucas	*	*	*
Carcharhinus limbatus	*	*	*
Carcharhinus longimanus	*		
Carcharhinus obscurus	*		
Carcharhinus perezii			*
Carcharhinus plumbeus			*
Carcharhinus porosus		*	*
Carcharhinus signatus			*
Carcharodon carcharias	*		
Cephaloscyllium ventriosum	*		
Echinorhinus cookei	*		
Galeocerdo cuvier	*	*	*
Galeorhinus galeus	*		
Ginglymostoma cirratum		*	*
Heterodontus francisi	*		
Heterodontus mexicanus	*		
Hexanchus griseus	*		
Isurus oxyrinchus	*		*
Mustelus californicus	*		
Mustelus canis			*
Mustelus henlei	*		
Mustelus lunulatus	*	*	
Mustelus norrisi			*
Nasolamia velox		*	
Negaprion brevirostris	*		*
Notorhynchus cepedianus	*		
Prionace glauca	*		
Rhizoprionodon longurio	*	*	
Rhizoprionodon terranova			*
Scyliorhinus retifer			*
Sphyrna lewini	*	*	*
Sphyrna mokarran			*
Sphyrna tiburo			*
Sphyrna zygaena	*		
Squalus acanthias	*		
Squalus cubensis			*
Squatina californica	*		
Triakis semifasciata	*		

180

181



182

183

Figure 1. General Shark Phylogeny dendrogram

184 Baja California Sur presented de highest number of shark species (N=30), clustered in 19 genus,

185 12 families and 7 orders (Figure 2). Carcharhiniformes order was the most abundant in number

186 of species (N=26), while Carcharhinidae and Triakidae were the most rich families in number of

187 species of this order (19 and 7 species respectively). Lamniformes order was constriuided by two

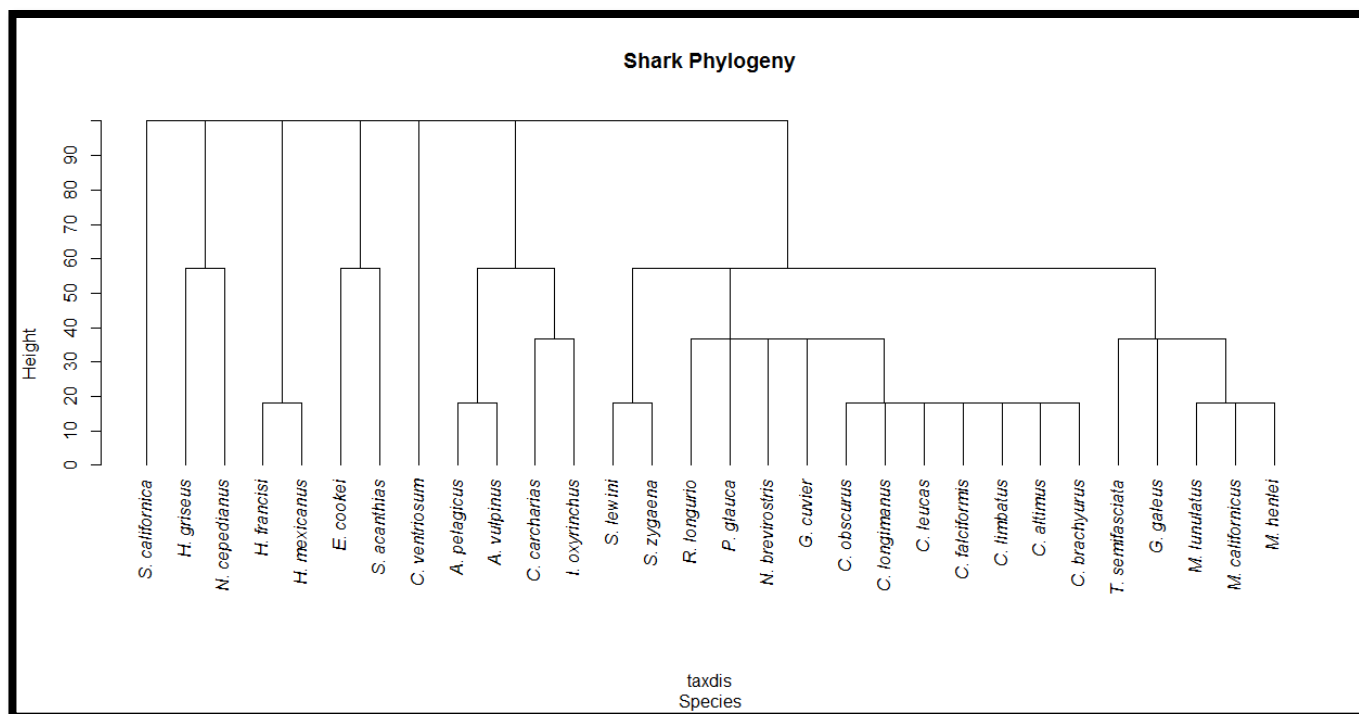
188 families (Alopiidae and Lamnidae), with two species each one. Squaliformes, Heterodotiformes

189 and Hexanquiformes presented two especies each one. By the other hand, angel shark (*Squatina*

190 *californica*) was the only representant of an entire taxa (Squatinifomes), similar to Cat shark

191 (*Cephaloscyllium ventriosum*), the only representant of Orectolobiformes order.

192



193

194

Figure 2. Shark phylogeny dendrogram of Baja California Sur

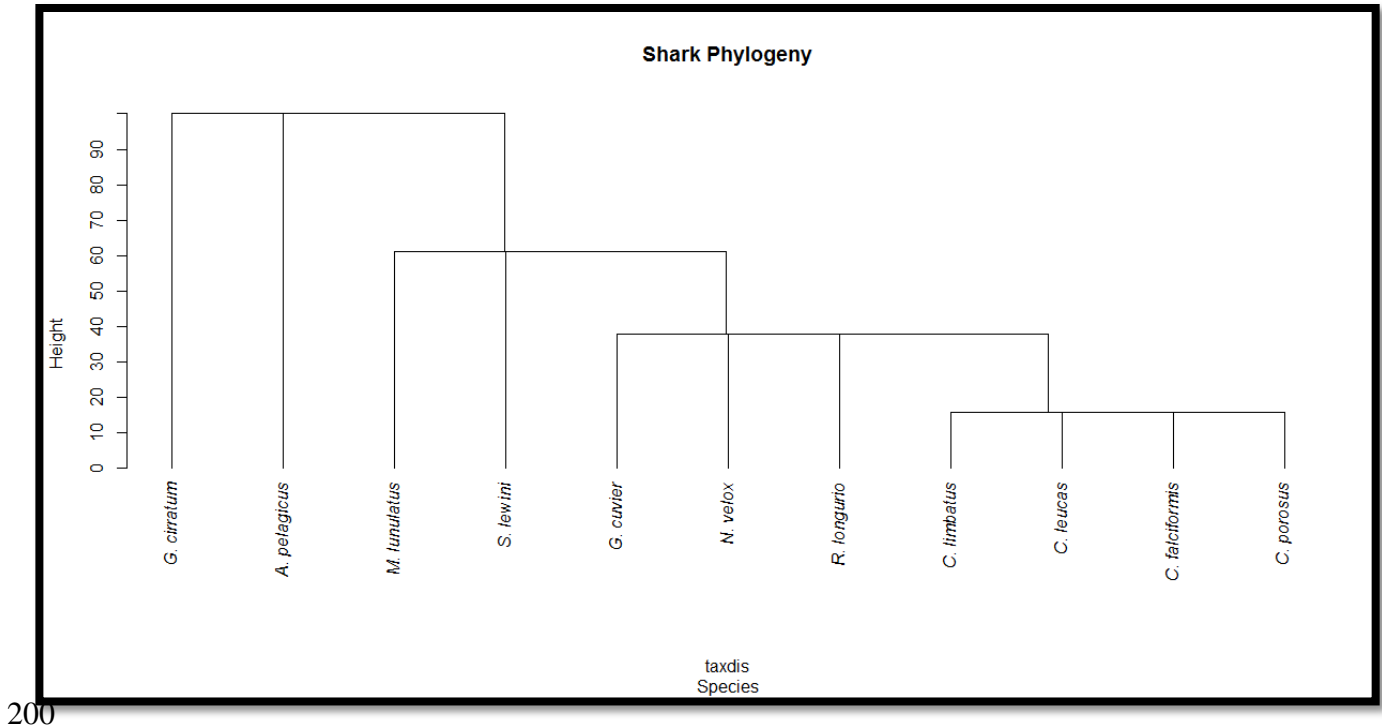
195 Oaxaca presented the lowest number of species (N=11) clustered in 8 genus, 5 families and 3

196 orders. Carcharhiniformes order was the most rich with 9 species, 7 species belonged to

197 Carcharhiidae, 1 species to Triakidae family, 1 specie to Sphyrinidae family. *Ginglymostoma*

198 *cirratum* and *Alopias pelagicus* were the only representants of Orectolobiformes and

199 Lamniformes orders



200

201

Figure 3. Shark phylogeny dendrogram of Oaxaca

202

203 Campeche's shark phylogenies are presented in Figure 4. Species richness was 21, clustered in 10

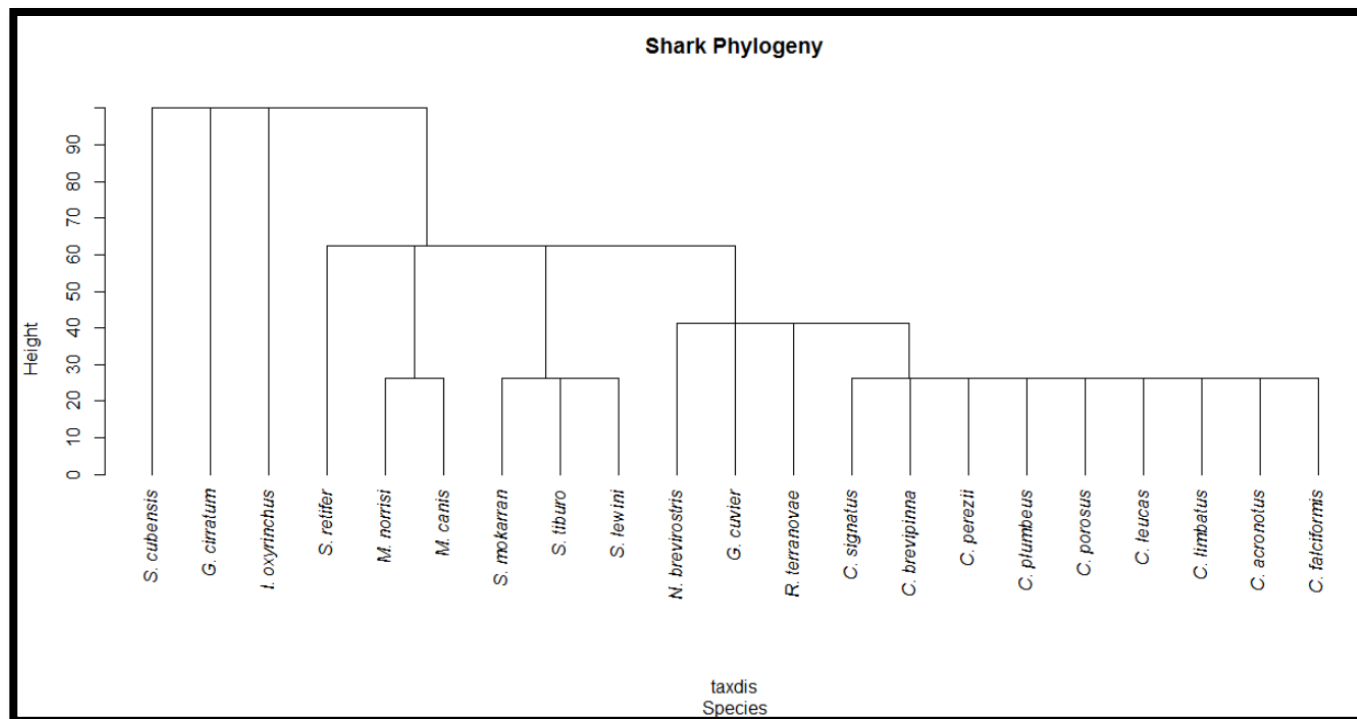
204 genus, 7 families and 4 orders. Carcharhiniformes order was the most rich (18 species), 12

205 species were clustered in Carcharhinidae family, 3 in Sphyrinidae family, two in Triakidae

206 family, and 1 in Scyliorhinidae family. By the other hand, *Isurus oxyrinchus*, *Ginglymostoma*

207 *cirratum* and *Squalus cubensis* were the only representants of Lamniformes, Orectolobiformes,

208 and Squaliformes orders.



209

210

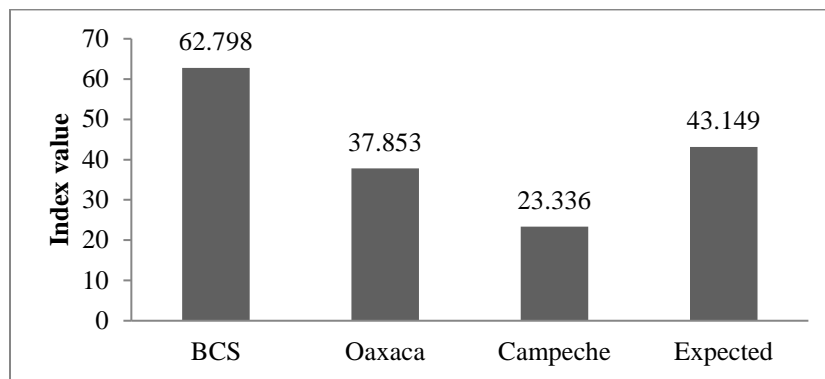
Figure 4. Shark phylogeny dendrogram of Campeche

211

212 Results of taxonomic diversity indices (Figure 4) shown that BCS phylogenetic diversity presents
 213 the highest values ($\Delta = 62.798$), being superior than the expected results (43.149). By the other
 214 hand, both Oaxaca and Campeche had lower values than the expected (37.853 and 23.336
 215 respectively).

216 Results of taxonomic distinctiveness are shown in in figure 5. Baja California Sur presented the
 217 highest value (80.303), while Campeche presented the lowest (46.869). By the other hand,
 218 Oaxaca presented a $\Delta^* = 49.121$. All results shown to be over the expected results of the
 219 analyses.

220 Results of mean taxonomic distinctivness are shown in figure 6. Once again Baja California Sur
 221 presented the highest values of the index ($\Delta^+ = 81.26$), followed by Oaxaca (65.005) and
 222 Campeche (59.624). Baja California Sur was the only location over the expected value.

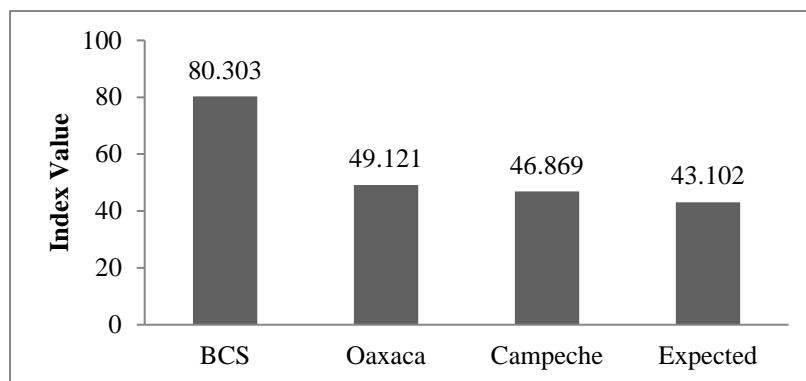


223

224

Figure 5. Taxonomic diversity (Δ) comparisson of diferent locations

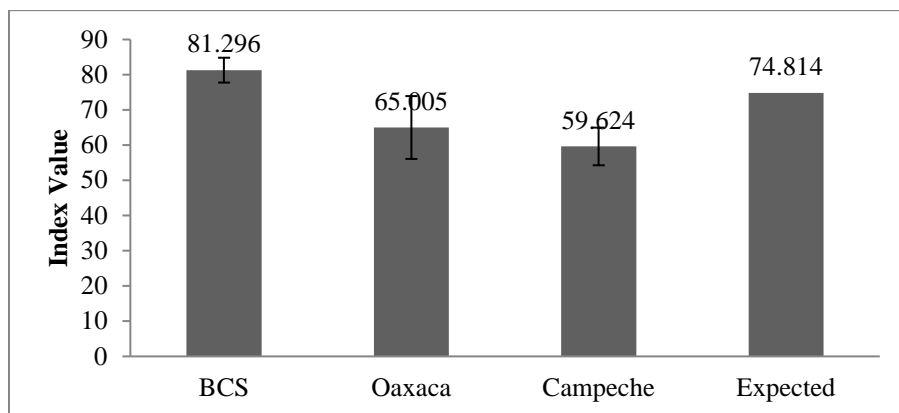
225



226

227

Figure 6. Taxonomic distinctivness (Δ^*) comparisson of different locations



228

229 **Figure 7.** Mean taxonomic distinctivness ($\Delta+$) comparison of different locations

230

231 **Discussion**

232 Taxonomic diversity and distinctivness indices shown to be useful while incorporating taxonomic
233 information of species. Even in this analyses, where data from diferents sources was compared,
234 indeces shown to be useful because they do not take care about effort, normality or a determinate
235 sample size (Warwick & Crick 1998), so information about shark fisheries that use different
236 scales of time and effort can be compared.

237 Results shown that Baja California Sur location presented the highest values of taxonomic
238 indeces, wich means there are more species distributed in a more quantity of branches. Also,
239 BCS presented the more quantity of endemic species, including the angel shark, *Squatina*
240 *californica*, whic is the only representant of Squatinidae family (Smith, 2009) in the area.

241 The highest taxonomic diversity found in BCS is probably due to the overlap of 2 biogeographic
242 marine regions: the Californian province and the Panamic province (Wilkinson *et al.* 2009)
243 These regions, among the influence of the Californian current and North-Equatorial current make
244 this zone to be concurrent to both tropical species, such as white tip shark (*Carcharhinus*
245 *limbatus*), and temperate species such as blue shark (*Prionace glauca*) (Holts *et al.* 1998).
246 Considerig this, it is not surpringsing to found endemic species restricted to this area, such as
247 angel shark (*Squatina Californica*) and small nurseBshark (*Cephaloscyllium ventriosum*) (Smith
248 *et al.* 2009).

249 Oaxaca presented the lowest richness and abundance. Unfortunately, considering the small
250 relative time of sampling of data obtained from Alejo-Plata *et al.* 2006 while comparing it to the

251 other two studies, and the lack of an acumulative curve of species it is possible that the potential
252 quantity of species that can be found could increase. However, to the location of Oaxaca, little
253 research about shark fisheries have been done.

254 Even if Campeche presented the highest abundances of shark it was not the site that had the
255 highest values, but the lowest. In the phylogenetic analysis it was shown that there were some
256 species that could only be found in this region, for example the great hammerhead shark
257 (*Sphyrna mokarran*) (Bonfil, 1997). Results given by the indeces could be explained by two
258 reasons together, the first is that many of the sharks were clustered together not just at
259 Carcharhiniformes order, but at Carcharhinus genus (9 species) which implies a reduction in
260 taxonomic diversity. Also, the most dominant specie caught in this area, *Rhizoprionodon*
261 *terranovae* (Pérez-Jiménez & Mendez-Loeza, 2015) which presented about 67 percent of total
262 catches at this region, was a carcharhinid shark. Both, the dominance of a single taxa and the
263 highest values of one of the specie that comose the same taxa make the value of the indeces to
264 decrease.

265 To sum up, Baja California Sur presented the highest values of taxonomic diversity, presenting
266 also a high quantity of species that are endemic to this region, Oaxaca nees more information in
267 their shark fisheries in order to make a better analysis, while taxonomic diversity of sharks in
268 Campeche is ruled by a single specie. All this information is useful if we want to compare
269 changes on species composition on time and also to know what species, if lost, could cause a
270 bigger reduction on taxonomic information of sharks.

271 **Acknowledgements**

272 I am really glad to Sergio, Alejandro, Benjamin and Debora to all their time and effort given to
273 us: Nothing words to say but Thank You!

274 **References**

275 Alejo-Plata, M., S. Ramo-Carrillo and J.L. Cruz-Ruiz . 2006. La pesquería artesanal del tiburón
276 en Salina Cruz, Oaxaca, México. *Ciencia y Mar*. 10(30): 37-51

277 Bonfil, R. 1997. Status of Shark resources in the Southern Gulf of Mexico and Caribbean:
278 Implications for management. *Fisheries research*. 29:101-117

279 Clarcke, K.R., and R.M. Warwick . 1998. A taxonomic distinctness index and its statistical
280 properties. *Journal of Applied Ecology*. Vol 35. 523-531

281 Clarcke, K.R., and R.M. Warwick. 1999. The taxonomic distinctness measure of biodiversity:
282 weighing of steps lengths between hierarchical levels. *Marine Ecology Progress Series*.
283 Vol. 184. 21-29

284 Holts, B., A. Julian., O. Sosa-Nishizaki and M.W. Bartoo. 1998. Pelagic Shark Fisheries along
285 the west coast of the United States and Baja California, México. *Fisheries research*.
286 39:115-125

287 Pérez-Jiménez, J.C. and I. Mendez-Loeza . 2015. The small scale shark fisheries in the southern
288 Gulf of Mexico: Understanding their heterogeneity to improve their management.
289 *Fisheries research*. 172: 96-104

290 Ramírez-Amaro, S.G. 2011. Caracterización de la pesquería artesanal de elasmobranquios en la
291 costa occidental de Baja California Sur, México. Tesis de Maestría. Centro
292 Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, La Paz. 82pp.

293

294 Smith W.D., J.J. Bizarro, and G.M. Cailliet. 2009. The artisanal elamobranch fishery on the east
295 coast of Baja California, Mexico: Characteristics and managements considerations.
296 *Ciencias Marinas*. 35(2): 209-236

297 Stevens, J.D., R. Bonfil, N.K. Dulvy and P.A. Walker. 2000. The effects of fishing on sharks,
298 rays, and chimaeras (Chondrichthyans), and the implications for marine ecosystems.
299 *Journal of Marine Science*. 57: 476-494

300 Vecchione, M., M.F. Mickevich, K. Fauchauld, B.B Collette, A.B. Williams, T.A. Munroe and
301 R.E. Young. 2000. Importance of assessing taxonomic adequacy in determining fishing
302 effects on marine biodiversity. *Journal of Marine Science*. 57:677-681

303 Warwick, R.M., and K.R. Clarcke. 1998. Taxonomic distictness and environmental assesment.
304 *Journal of Applied Ecology*. 35. 532-543

305 Wilkinson, T., J. Wiken, B. Creel, T. Hourigan, T. Agardy, H. Hermann, L. Janishevski, C.
306 Madden, L. Morgan and M.Padilla. 2009. Ecorregiones marinas de América del Norte,
307 Comisión para la Cooperación Ambiental Montreal. 200pp

308

309

310 **Anexos**

311

Table II. Abundances of shark per specie and location

Specie Name	BCS	Oaxaca	Gulf of Mexico
<i>Alopias pelagicus</i>	15	12	0
<i>Alopias vulpinus</i>	36	0	0
<i>Carcharhinus altimus</i>	12	0	0
<i>Carcharhinus brachyurus</i>	1	0	0
<i>Carcharhinus limbatus</i>	3	11	75
<i>Carcharhinus falciformis</i>	258	424	201
<i>Carcharhinus leucas</i>	2	24	67
<i>Carcharhinus longimanus</i>	7	0	0
<i>Carcharhinus obscurus</i>	53	0	0
<i>Carcharodon carcharias</i>	4	0	0
<i>Cephaloscyllium ventriosum</i>	151	0	0
<i>Galeocerdo cuvier</i>	3	15	7
<i>Echinorhinus cookei</i>	1	0	0
<i>Galeorhinus galeus</i>	152	0	0
<i>Heterodontus francisi</i>	378	0	0
<i>Heterodontus mexicanus</i>	57	0	0
<i>Hexanchus griseus</i>	3	0	0
<i>Isurus oxyrinchus</i>	1277	0	17
<i>Mustelus californicus</i>	192	0	0
<i>Mustelus henlei</i>	3235	0	0
<i>Mustelus lunulatus</i>	76	15	0
<i>Negaprion brevirostris</i>	1	0	1
<i>Notorhynchus cepedianus</i>	1	0	0
<i>Prionace glauca</i>	3455	0	0
<i>Rhizoprionodon longurio</i>	3	145	0
<i>Sphyrna lewini</i>	26	270	2024
<i>Sphyrna zygaena</i>	527	0	0
<i>Squalus acanthias</i>	1	0	0
<i>Squatina californica</i>	755	0	0
<i>Triakis semifasciata</i>	63	0	0
<i>Carcharhinus porosus</i>	0	243	52
<i>Nasolamia velox</i>	0	39	0
<i>Ginglymostoma cirratum</i>	0	10	16

Sphyrna tiburo	0	0	6130
Rhizoprionodon			
terranovae	0	0	19106
Carcharhinus acronotus	0	0	564
Sphyrna mokarran	0	0	76
Carcharhinus plumbeus	0	0	46
Carcharhinus perezii	0	0	45
Carcharhinus brevipinna	0	0	37
Mustelus norrisi	0	0	3
Scyliorhinus retifer	0	0	3
Squalus cubensis	0	0	2
Mustelus canis	0	0	1
Carcharhinus signatus	0	0	1

312

313

314
315
316
317
318
319
320
321
322
323
324
325
326
327
328
329
330
331
332
333
334
335
336
337
338
339
340
341

The Affects of Human Activity on the Diversity of Species Inhabiting the Prop Roots of Mangrove Fringes in La Paz, Mexico

Emily Schultz, Juhi LaFuente, Carlee Bock

Biology Department, Western Washington University

516 High Street, Bellingham, WA 98225, U.S.A., email: schultze4@wwu.edu

Acknowledgments: We would like to acknowledge, A. Acevedo-Gutierrez, B. Miner, S. Florez-Ramírez, and D. Donovan for their expertise and guidance throughout this study.

Keywords: Sessile organisms, bio-indicators, Rhizophoraceae, Macrobenthos, Baja California Sur, La Paz, Mexico, coastal ecosystem

Palabras Clave: organismos sésiles, bioindicadores, Rhizophoraceae, macrobentos, Baja California Sur, La Paz, Mexico, ecosistemas costeros.

Word Count: 4,182

342 **The Affects of Human Activity on the Diversity of Species Inhabiting the Prop Roots of**
343 **Mangrove Fringes in La Paz, México**

344

345 **Abstract**

346 As the global population size increases, higher demand is put on the environment to meet
347 resource needs. Coastal regions, such as those in Baja California Sur (BCS) have increased
348 environmental pressure, because of their appeal to local communities and tourists alike.
349 Mangrove ecosystems are amongst the most degraded in BCS, due to human activities.
350 Mangroves provide many resources and economic benefits to their surrounding communities.
351 They are unique ecosystems that attract tourism, commercial and recreational fishing, and
352 protection coastal regions from erosion and natural disasters. Mangroves also support many
353 terrestrial and marine organisms, such as macrobenthos. Macrobenthic organisms are an
354 important component in the cycling of nutrients between the trophic levels within mangroves.
355 For this reason, macrobenthos have been used in many studies as bioindicators for the condition
356 of the mangrove. The purpose of our study was to evaluate the biodiversity of macrobenthos
357 found on the mangrove fringe prop roots in La Paz, México. To do this we quantified the
358 macrobenthic biodiversity and human activity at 6 sites. We hypothesized that high human
359 activity would have a negative affect on the diversity of macrobenthos found on the mangrove
360 roots. Our results and analysis supported this hypothesis, as indicated by the negative
361 relationship we found between human activity and the macrobenthic biodiversity at all sites. As
362 human activity increases, macrobenthic communities suffer from sedimentation and pollution.
363 Mangroves are essential to many coastal economies. However, activities such as tourism and

364 harvesting can be unsustainable and might result in long-term detriments to the entire mangrove
365 structure. Through the implementation of sustainable practices the affects of these activities
366 might be lessened. Conservation and restoration projects might also help to restore degraded
367 mangrove ecosystems.

368

369 **Resumen**

370 Conforme aumenta el tamaño de la población humana a nivel global, los ecosistemas se
371 ven afectados por la demanda de recursos. En regiones costeras como las de Baja California Sur,
372 México, la presión al ambiente ha aumentado considerablemente debido a la cercanía de las
373 comunidades y a la actividad turística. Los manglares proveen recursos económicos y protegen
374 las costas de la erosión y desastres naturales, sin embargo, son uno de los ecosistemas más
375 afectados por actividades humanas como el turismo y la pesca comercial o recreacional. Los
376 organismos macrobentónicos son un componente importante en el ciclo de los nutrientes entre
377 los diferentes niveles tróficos en los manglares, por lo que han sido estudiados y usados como
378 bioindicadores de la condición de estos ecosistemas. El objetivo de este estudio fue evaluar la
379 biodiversidad macrobentónica encontrada en las raíces de los manglares en la Bahía de La Paz,
380 México realizando conteos y cuantificando la diversidad en 6 sitios distintos. La hipótesis de este
381 estudio es que la actividad humana tendrá un efecto negativo en la diversidad macrobentónica en
382 todos los sitios muestreados. Los resultados obtenidos demuestran que conforme aumenta la
383 actividad humana, la comunidad macrobentónica sufre de sedimentación y contaminación
384 reduciendo la diversidad. Debido a que los manglares son ecosistemas esenciales para la
385 economía de la zona costera, las actividades como el turismo y la recolecta de organismos no son

386 sustentables y a largo plazo pueden causar daños en la estructura de la comunidad de los
387 manglares. Es importante implementar planes de manejo sustentable para disminuir el efecto de
388 estas actividades en estos ecosistemas y proponer proyectos de conservación y restauración de
389 los manglares afectados.

Introduction

The global population continues to grow, increasing the pressure on the environment due to higher demands for natural resources (Ehrlich 1988; Vitousek et al. 1997; McKinney 2002). Rising food shortages have driven the expansion of crops, to cover nearly 15% of land worldwide (Vitousek et al. 1997). The oceans are not exempt from degradation; overfishing is quickly depleting the world's stock of fishes and excessive harvest techniques have led to large-scale destruction of marine habitats (Ortiz-Lozano et al. 2005; UNEP 2006; Halpern et al. 2015). Development of infrastructure is rapidly increasing with population demands and is considered to be the most long-term and widespread form of habitat loss (McKinney 2002). The anthropogenic transformation of our planet's surface is the primary culprit leading to widespread loss of species, i.e. biodiversity (Ehrlich 1988; Vitousek et al. 1997). No ecosystem has escaped human affects on biodiversity, yet as a regional population grows the stress on that environment becomes more pronounced (Vitousek et al. 1997).

Human affects in Baja California Sur (BCS) are elevated due to high population densities (Vitousek et al. 1997). BCS was secluded from the mainland of México prior to the construction of the Baja California Transpeninsular Highway in 1973 (Whitmore et al. 2005). The creation of the highway resulted in an increase in immigration and travel into BCS (Whitmore et al. 2005). Tourism quickly became the second largest source of foreign income for the state (Ortiz-Lozano et al. 2005). In 1982 the federal government of México funded the construction of many coastal resorts, in hopes of expanding the tourism industry (Ortiz-Lozano et al. 2005). Coastal development continued to expand as job opportunities in tourism and marine industry flourished (Whitmore et al. 2005). Vitousek et al. (1997) found that 60% of México's population lives

within 100 km of the coast, causing large-scale degradation to the habitats within (Ortiz-Lozano et al. 2005).

Mangroves provide many economic benefits to humans (Whitmore et al. 2005; Kabir et al. 2014). Mangroves are a unique ecosystem that attracts tourism, providing income for the local economy (Macintosh and Ashton 2002). Mangroves are common sites for commercial and recreational fishing, as well as nurseries that sustain local fish populations (Whitmore et al. 2005; Obade et al. 2009). In addition to fishing, the annual harvest of crabs, prawns, and bivalves generates over \$4 billion USD in revenue (Ellison 2008). In addition to economic benefits, mangroves provide sediment stabilization along shorelines, preventing erosion (Thampanya et al. 2006). They also serve as a buffer to natural disasters, mitigating injury to the inhabitants and coastal infrastructure (Das and Vincent 2009). Despite the many benefits humans gain from mangroves, they continue to threaten them (McKinney 2002).

Mangroves are rapidly disappearing in BCS (Macintosh and Ashton 2002; Whitmore et al. 2005; Aburto-Oropeza et al. 2008). Coastal regions of México have lost nearly 65% of their mangrove stands. Areas surrounding La Paz alone have suffered a loss of 23% between 1973-1981 (Whitmore et al. 2005; Aburto-Oropeza et al. 2008). Today an estimated 2% of BCS mangroves are lost each year due to deforestation and other anthropogenic disturbances (Aburto-Oropeza et al. 2008). While there are some traditional uses for mangrove trees, commercial development has played a much stronger role in the loss of acreage in the region (Whitmore et al. 2005). The Pichilingue port expansion in the 1970's resulted in the destruction of many thriving mangroves (Whitmore et al. 2005). Shrimp aquaculture threatens mangrove ecosystems regionally in México, which accounts for 50% of their loss nationally (Kabir et al. 2014; Ortiz-

Lozano et al. 2005). The shrimp industry has become increasingly prevalent in BCS, bringing in over 300 million USD in 2001 (Búrquez and Martínez-Yrizar 1997; Ortiz-Lozano 2005). As panga tours grow in popularity, channels are cut into the mangroves causing habitat fragmentation (Whitmore et al. 2005). Tourist and locals alike commonly visit the mangroves for activities such as beach camping and barbequing (Whitmore et al. 2005). In the short-term, mangroves can serve in boosting the local economy. However, many of these activities are unsustainable and can result in long-term damage (Macintosh and Ashton 2002).

Mangroves are very productive ecosystems that support many terrestrial and marine organisms (Kumar 2000; Macintosh et al. 2002; Whitmore et al. 2005; Nagelkerken et al. 2008). Mangrove root systems provide shelter for fish, shrimp, green sea turtles, and other marine organisms. This protection makes them highly functional nurseries (Kumar 2000; Whitmore et al. 2005; Nagelkerken et al. 2008; Aburto-Oropeza et al. 2008; Kabir et al. 2014). Marine animals mainly feed on macrobenthic organisms that encrust mangrove prop roots and the surrounding sediment (Whitmore et al. 2005). Macrobenthic assemblages of molluscs and crustaceans are integral to the cycling of nutrients through the mangrove ecosystems (Kabir et al. 2014; Ellison 2008). Macrobenthic communities occupy intermediate trophic levels and play an important ecological role for secondary consumers such as fish and birds (Kabir et al. 2014; Ellison 2008; Ashton et al. 2003; Macintosh et al. 2002). Macrobenthic organisms consume detritus, making organic matter available to higher trophic levels (Kabir et al. 2014; Ellison 2008; Ashton et al. 2003). Loss of the macrobenthic communities, can initiate a trophic cascade through the entire ecosystem (Kabir et al. 2014).

Human activities can negatively impact the diversity and abundance of the macrobenthos in mangroves (Macintosh et al. 2002; Whitmore et al. 2005; Kabir et al. 2014). Macrobenthic communities are affected by increases in sedimentation and chemical pollutants (Lee 2008). Molluscs, primarily oysters, are commonly harvested leading to a decrease in their abundance, as well as causing damage to the prop roots (Kabir et al. 2014). Researchers have previously used macrobenthic communities as indicators of a changing ecosystem (Skilleter and Warren 2000; Macintosh et al. 2002; Paez-Osuna et al. 2003; Kabir et al. 2014).

The goal of our study was to compare the diversity within the macrobenthos associated with mangrove fringes to the amount of human interaction in mangrove systems north of La Paz, México. We evaluated the diversity of macrobenthic communities found on the mangrove prop roots at six different locations. We also measured levels of human visitation to our research sites in order to approximate levels of interaction. We predicted that high human activity would have a negative affect on the diversity of macrobenthos.

Methods

Study Sites

We conducted surveys of six mangrove sites near La Paz, BCS, México. All sites were similar in size, in order to control for differences in biodiversity due to a larger ecosystem, and visited within a week period in July. Site 1 was directly across the road from a shrimp farm. Site 2 and 3 were inlets surrounded by a heavily traveled road, and were easily accessed. Site 5 bordered the Universidad Autónoma de Baja California Sur (UABCS) Pichilingue lab, which contained an

educational shrimp farm among other experimental marine projects. This site was also in close proximity with the BC ferries terminal. Sites 5 and 6 were both secluded inlets we reached by kayak, inaccessible by land (**Figure 1**).

Experimental Protocol

At each site we ran a 60m transect along the fringe of the mangrove. The transect began at the edge of the grove by the most obvious entrance into the water. Seven locations were randomly chosen along the transect. At each location, a quadrat was placed at the base of a prop root; this was done to avoid making observations of free hanging roots, too young to support growth. Quadrants of 1 x .15 m were sectioned off with twine. Macrobenthos found on the roots were identified and counted by species, from the root tips to the high tide mark, indicated by a lack of marine organisms. To determine human impact, on Sunday, July 24, 2016 three people counted the number of visitors at each mangrove site between hours 14:00-16:00.

Data Analysis

Species counts for the seven quadrats were averaged for each site, as were visitor counts. We calculated species diversity from these averages using the Shannon-Wiener index. A linear regression analysis was run using the Shannon diversity indices and visitor averages, to determine the relationship between biodiversity of macrobenthos found on the roots of mangroves and human impact. A one tailed t-test was used to determine if the regression line slope was significantly less than zero. Percent differences between species and sites were also calculated.

Results

We found a negative relationship between human activity and the macrobenthic diversity at sites. Our analysis of the linear regression indicated that the slope was significantly less than zero ($p=0.038$, $t\text{-value}_{df=4}=-2.38$). This suggests that as the amount of human activity at a site increased the macrobenthic diversity score decreased.

The greatest macrobenthic diversity occurred between site 5 and site 1. There was a lower diversity of macrobenthic organisms at site 1, which had the highest amount of human activity (Figure 1). Conversely our results indicate that there was the highest diversity score at site 5, which had the lowest amount of human activity (Figure 1). Our results regarding human activity among sites suggest that as the site location was closer to La Paz city the amount of human activity increased (Figure 1; Figure 2). Similarly, the two sites farthest from La Paz city had the relatively lowest diversity scores as compared to the closer four sites, which had the highest diversity scores (Figure 1). Acorn barnacles were the most abundant taxa at every site, with the exception of site 3 (Table 1). Conversely, crabs had the lowest abundances across all sites, with an exception to site 3 and 5, which had slightly lower abundances of gastropods (Table 1).

Discussion

There was a negative correlation between the diversity of macrobenthic organisms found on the prop roots of mangrove fringes and the human activity at our study sites. This supported our hypothesis that human activity at each mangrove site would have an effect on the diversity of

macrobenthic organisms. Our results are supported by the findings of Macintosh et al. (2002), who found that mangroves with higher amounts of human interaction demonstrated variation in the communities of molluscs and crustaceans present. In a related study, Skilleter and Warren (2000) found that alterations and destruction of mangrove pneumatophores affected the diversity and abundance of macrobenthic species present. We examined a smaller range of species within the macrobenthic community in order to better understand the relationship between diversity and human activity.

Several studies use the abundance and diversity of molluscs as a biological indicator for the status of a mangrove forest (Kabir et al. 2014; Macintosh et al. 2002; Paez-Osuna et al. 2003). Molluscs can represent the mangrove productivity due to their role as a principal component in the nutrient cycle within the mangrove trophic levels (Macintosh et al. 2002; Kabir et al. 2014). Our study did not indicate a trend regarding molluscan diversity and human activity throughout all sites. The results found in sites 5 and 1 might be due to the affects that humans have on mollusc assemblages. Coastal communities commonly harvest oysters from mangroves, which can lead to a decrease in the abundance of molluscs, as well as inflict damage to the mangrove root systems (Kabir et al. 2014). If the harvest of molluscs is extensive and widespread, the resulting damage to the mangrove ecosystem will remain indefinitely (Obade 2009). Anthropogenic pollution can also affect the assemblages of molluscs (Kabir et al. 2014). All of these human interactions cause extensive disturbances that cascade through mangrove ecosystems affecting species dependent on molluscs for nutrient cycling.

In addition to molluscs, studies suggest that crustaceans can also be indicative of a changing mangrove ecosystem (Macintosh et al. 2002, Macintosh and Ashton 2002). The most

abundant crustaceans we observed at our research sites were acorn barnacles (*Chthamalus fissus*). Macintosh et al. (2002) suggests that an overdominance of a particular species might be due to stressful environmental conditions. At Site 1 with the lowest diversity and highest human activity levels we observed only barnacles. Large abundances of barnacles can inhibit mangrove seedlings by interfering with respiration and photosynthetic capabilities, resulting in smaller mangrove forests (Macintosh and Ashton 2002). The least abundant crustacean we observed were crabs because they are challenging to compare across studies due to the wide variability in data collection methodology (Ashton et al. 2003).

At Site 1 our lowest biodiversity scores could be attributed to the adjacent shrimp farm. Our results are comparable to previous findings, that mangroves in close proximity to shrimp aquaculture ponds have low biodiversity (Macintosh et al. 2002). Runoff from shrimp farms can cause many detrimental affects to nearby mangroves and their inhabiting organisms (Páez-Osuna et al. 2003, Ortiz-Lozano et al. 2005). The sheer amount water caused by levees, controlling input and discharge from shrimp ponds, can alter natural tidal and seasonal water levels within mangroves (Páez-Osuna et al. 2003). In addition, due to the high rate of evaporation in shrimp ponds, runoff into nearby ecosystems typically has high concentration of saline (Páez-Osuna et al. 2003). Hypersaline runoff into mangroves has been shown to cause mortality in the organisms of mangroves (Páez-Osuna et al. 2003). Eutrophication is also a driving factor in the damage caused by shrimp aquaculture on mangroves (Páez-Osuna et al. 2003). Shrimp aquaculture is widely accepted to be one of the largest threats to mangroves in the Gulf of California (Búrquez and Martínez-Yrizar 1997; Macintosh et al. 2002; Páez-Osuna et al. 2003; Ortiz-Lozano et al. 2005; Whitmore et al. 2005; Ellison 2007; Aburto-Oropeza 2008).

At Site 5 our highest biodiversity might be due to how difficult it was to access, as it could only be reached by boat. Throughout all three of our visitations made to this site, no human activity was observed. A study conducted by Ashton et al. (2003) in Malaysia also found that there was high biodiversity in mangroves with no human activity. As for the Pichilingue UABCS lab mangrove (Site 4), the many anthropogenic factors affecting this site could be the cause for the low diversity scores seen. An educational shrimp pond borders the mangrove, potentially resulting in eutrophic and hypersaline conditions (Páez-Osuna et al. 2003). However the affect could be reduced in comparison with the first site due to more conservative techniques employed by the university. Fragmentation was caused by a pier extending through the entirety of the grove, which has been suggested to cause degradation of diversity in mangroves (Whitemore et al. 2005). When the university is in session, there is high human traffic through the mangrove utilizing the pier as a boat launch, resulting in damage to the ecosystem. This site was also in close proximity with the BC Ferry terminal. Ports have been shown to negatively affect ecosystems through pollution and eutrophication (Páez-Osuna et al. 2003).

The mangrove forests we sampled in BCS were seemingly less diverse in comparison with studies done on prop root communities around the world. A study done on the Ranong mangrove forest in the Andaman Sea had considerably higher flora and fauna diversities (Macintosh et al. 2002). There were a total of 42 mollusc and 55 crustacean species identified, whereas only 6 molluscan and 4 crustacean species were found in BCS mangroves (Macintosh et al. 2002). This could be accounted for in part by the higher diversity of mangrove species (13) found in the Ranong mangroves, contrasted with only 3 species common to BCS (Macintosh et al. 2002, Whitmore et al. 2005). Another study of prop root diversity was conducted in Moreton Bay, Australia, which had more similar biodiversity levels to BCS (Skilleter and Warren 2000).

There were 5 mollusc, 2 crab and 4 mangrove species found among all sites sampled, which maintains the trend of diminished macrobenthic diversity in regions with low mangrove diversity (Skilleter and Warren 2000). The diversity of mangrove species appears to be an important factor in the diversity of fauna found in the prop roots.

Mangrove rehabilitation has proven to be an effective strategy in global mangrove recovery (Whitmore et al. 2005). Restoring mangroves has many apparent benefits to the biodiversity of species inhabitants, as well as economic benefits to humans (Macintosh et al. 2002; Aburto-Oropeza et al. 2008). Raising awareness to the crucial ecological role of mangroves has inspired enthusiasm, from local communities to global politics (Macintosh et al. 2002). At Laguna de Balandra, a mangrove lagoon in Baja California Sur, a restoration project targeting diminished black mangroves was 74% successful in restoring a clear-cut area (Whitmore et al. 2005). One concern with rehabilitation of existing stands as well as the creation of new mangroves is the lack of biodiversity in the plantings. These human efforts may result in the formation of mangroves with reduced function and community dynamics (Macintosh et al. 2002).

Our study on mangrove system biodiversity and human activity can further the field of conservation by drawing attention to the importance of minimizing human interactions within stands. Sampling across a larger spatial scale, for example the entirety of BCS coastal regions, can further our research. This would allow for a broader understanding of the affects human activities have on mangrove ecosystems. Unrestricted human interactions can be source of myriad negative impacts on fragile ecosystems such as mangroves, and further understanding of these alterations are crucial to the design and implementation of effective biological

conservation.

References:

- Aburto-Oropeza, O., E. Ezcurra, G. Danemann, V. Valdez, J. Murray, E. Sala. 2008. Mangroves in the Gulf of California increase fishery yields. *National Academy of Sciences*. **105**: 10456-10459.
- Alongi, D.M., Sasekumar, 2000. Benthic communities. *Coastal and Estuarine Studies*. **41**: 1-35.
- Ashton, E.C., D.J. Macintosh, and P.J. Hogarth. 2003. A baseline study of the diversity and community ecology of crab and molluscan macrofauna in the Sematan mangrove forest, Sarawak, Malaysia. *Journal of Tropical Ecology*. **19**: 127-142.
- Búrquez, A., A. Martínez-Yrizar. 1997. The ecological importance of mangroves in Baja California Sur. Pages 323-324 in J. E. Cartron, G. Ceballos, R.S. Felger, editors. *Biodiversity, Ecosystems, and Conservation in Northern México*.
- Das, S., J.R. Vincent. 2009. Mangroves protected villages and reduced death toll during Indian super cyclone. *Proceedings of the National Academy of Sciences of the United States of America*. **106**: 7357-7360.
- Ehrlich, P.R. 1988. Loss of Diversity. Pages 21-23 in E. O. Wilson, F.M. Peter, editors. *Biodiversity*.
- Ellison, A.M. 2008. Managing mangroves with benthic biodiversity in mind: moving beyond roving banditry. *Journal of Sea Research*. **59**: 2-15.

- Halpern, B.D, et al. 2015. Spatial and temporal changes in cumulative human impacts on the world's ocean. *Nature communications*. **6**: 1-7.
- Kabir, M., M. Abolfathi, A. Hajimoradloo, S. Zahedi, K. Kathiresan, S. Goli. 2014. Effect of mangroves on distribution, diversity and abundances of molluscs in mangrove ecosystem: a review. *Aquaculture, Aquarium, Conservation & Legislation International Journal of Bioflux Society*. **7**: 286-299.
- Kumar, R.S. 2000. A review of biodiversity studies of soil dwelling organisms in Indian mangroves. *Zoo's Print Journal*. **15**: 221-227.
- Lee, S.Y. 2008. Mangrove macrobenthos: assemblages, services, and linkages. *Journal of Sea Research*. **59**: 16-29.
- Macintosh, D.J., E.C. Ashton. 2002. A review of mangrove biodiversity conservation and management. Centre for Tropical Ecosystems Research, University of Aarhus, Denmark.
- Macintosh, D.J., E.C. Ashton, and S. Havanon. 2002. Mangrove rehabilitation and intertidal biodiversity: a study in the Ranong mangrove ecosystem, Thailand. *Estuarine, Coastal, and Shelf Science*. **55**: 331-345.
- McKinney, M.L. 2002. Urbanization, Biodiversity, and Conservation. *BioScience*. **52**: 883-890.
- Nagelkerken, I., et al. 2008. The habitat function of mangroves for terrestrial and marine fauna: A review. *Aquatic Botany*. **89**: 155-185.
- Obade, P.T., N. Koedam, K. Soetaert, G. Neukermans, J. Bogaert, E. Nyssen, F. Vannedervele, U. Berger, and F. Dahdouh-Guebas. 2009. Impact of anthropogenic disturbance on a

mangrove forest assessed by A 1D cellular automaton model using lotka-volterra-type competition. *International Journal of Design and Nature and Ecodynamics*. **3**: 296-320.

Ortiz-Lozano, L., Granados-Barba, A., Solís-Weiss, V., García-Salgado, M.A. 2005.

Environmental evaluation and development problems of the Mexican coastal zone. *Ocean & Coastal Management*. **48**: 161-176.

Páez-Osuna F., A. Garcia, F. Flores-Verdugo, L.P. Lyle-Fritch, R. Alonso-Rodríguez, A. Roque, A.C. Ruiz-Fernández. 2003. Shrimp aquaculture development and the environment in the Gulf of California ecoregion. *Marine Pollution Bulletin*. **46**: 806-815.

Skilleter, G.A., S. Warren. 2000. Effects of habitat modification in mangroves on the structure of mollusc and crab assemblages. *Journal of Experimental Marine Biology and Ecology*. **244**: 107-129.

Thampanya, U., J.E. Vermaat, S. Sinsakul, N. Panapitukkul. 2006. Coastal erosion and mangrove progradation of Southern Thailand. *Estuarine Coastal and Shelf Science*. **68**: 75-85.

UNEP (United Nations Environment Programme). 2006 *Challenges to International Waters - Regional Assessments in a Global Perspective*. UNEP, Nairobi, Kenya.

Whitmore, R.C., et al. 2005. The ecological importance of mangroves in Baja California Sur: conservation implication for an endangered ecosystem. Pages 298-333 in J.E. Cartron, G. Ceballos, and R.S. Felger, editors. *Biodiversity, Ecosystems, and Conservation in Northern México*.

- Vitousek, P.M., H.A. Mooney, J. Lubchenco, and J.M. Melillo. 1997. Human domination of earth's ecosystems. *Science*. **227**: 494-499.
- Ortiz-Lozano, L., Granados-Barba, A., Solís-Weiss, V., García-Salgado, M.A. 2005. Environmental evaluation and development problems of the Mexican coastal zone. *Ocean & Coastal Management*. **48**: 161-176.
- Páez-Osuna F., A. Garcia, F. Flores-Verdugo, L.P. Lyle-Fritch, R. Alonso-Rodríguez, A. Roque, A.C. Ruiz-Fernández. 2003. Shrimp aquaculture development and the environment in the Gulf of California ecoregion. *Marine Pollution Bulletin*. **46**: 806-815.
- Skilleter, G.A., S. Warren. 2000. Effects of habitat modification in mangroves on the structure of mollusc and crab assemblages. *Journal of Experimental Marine Biology and Ecology*. **244**: 107-129.
- Thampanya, U., J.E. Vermaat, S. Sinsakul, N. Panapitukkul. 2006. Coastal erosion and mangrove progradation of Southern Thailand. *Estuarine Coastal and Shelf Science*. **68**: 75-85.
- UNEP (United Nations Environment Programme). 2006 Challenges to International Waters - Regional Assessments in a Global Perspective. UNEP, Nairobi, Kenya.
- Whitmore, R.C., et al. 2005. The ecological importance of mangroves in Baja California Sur: conservation implication for an endangered ecosystem. Pages 298-333 in J.E. Cartron, G. Ceballos, and R.S. Felger, editors. *Biodiversity, Ecosystems, and Conservation in Northern México*.

Vitousek, P.M., H.A. Mooney, J. Lubchenco, and J.M. Melillo. 1997. Human domination of earth's ecosystems. *Science*. **227**: 494-499.

Table 1. The average number of molluscs and crustaceans found at each mangrove site.

Site	Molluscs		Crustaceans	
	Bivalves	Gastropods	Crabs	Barnacles
1	0	0	0	235
2	22	10	9	550
3	176	4	5	0
4	13	1	0	239
5	30	4	6	218
6	82	502	1	536

Figure 1. The average amount of human activity (n=3) plotted against the average macrobenthic Shannon-Weiner diversity index score (n=7) for each site. Standard error is the gray area on the graph.

Figure 2. A map showing the 6 sample sites that were located in Baja California Sur.

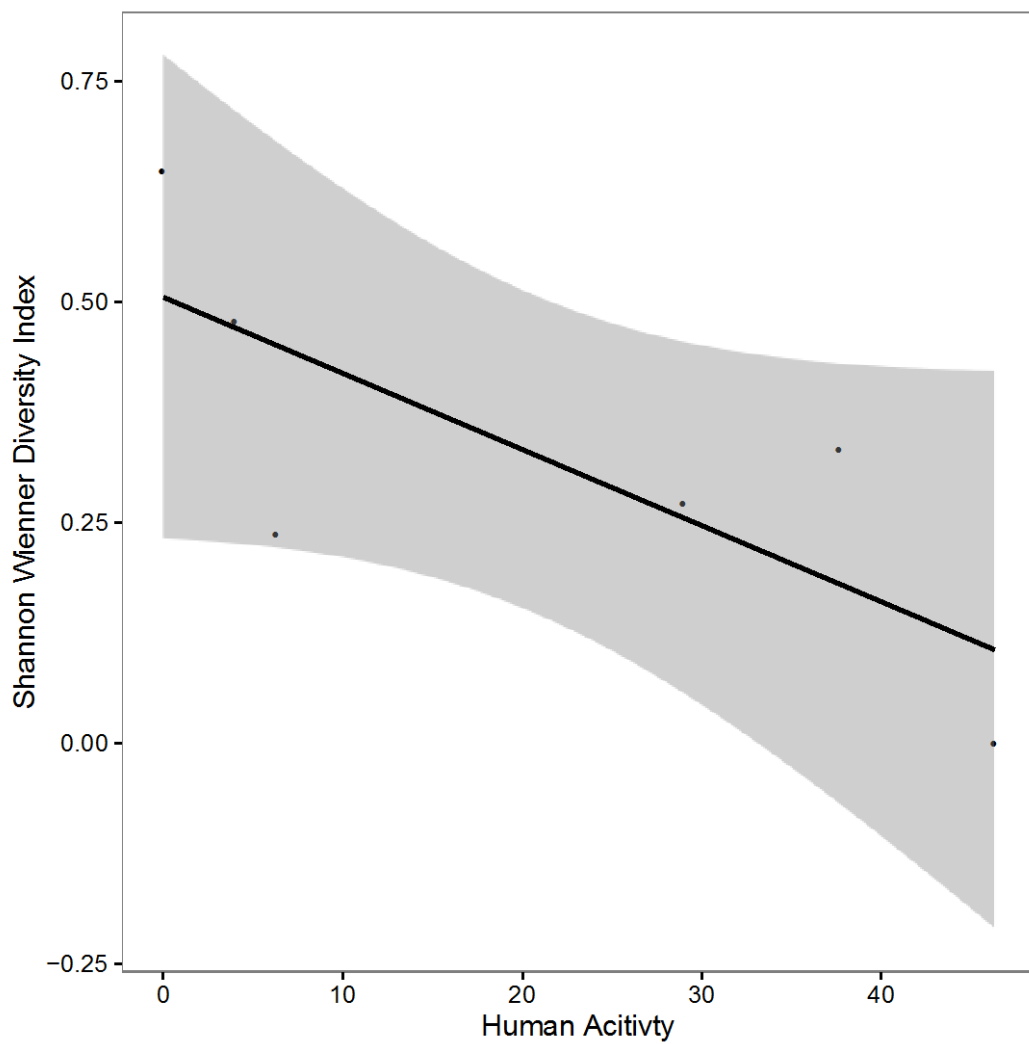


Figure 1.



Figure 2.