CICIMAR-IPN

**INSTITUTO POLITECNICO NACIONAL**

CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

# FEEDING ECOLOGY & THE CHARACTERIZATION OF FEEDING BEHAVIORS IN WHALE SHARKS IN THE COASTAL WATERS OFF THE EL MOGOTE SANDBAR, BAHIA DE LA PAZ, MEXICO

QUE PARA OBTENER EL GRADO DE DOCTOR EN
CIENCIAS MARINAS

PRESENTA
**M. C. DARREN ANDREW WHITEHEAD**

LA PAZ, B.C.S, MEXICO, DECEMBER 2019

SIP-14

# INSTITUTO POLITÉCNICO NACIONAL

## SECRETARIA DE INVESTIGACIÓN Y POSGRADO

## *ACTA DE REVISIÓN DE TESIS*

En la Ciudad de **La Paz, B.C.S.,** siendo las **12:00** horas del día **15** del mes de **Noviembre** del **2019** se reunieron los miembros de la Comisión Revisora de Tesis designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de **CICIMAR** para examinar la tesis titulada:

**"FEEDING ECOLOGY & THE CHARACTERIZATION OF FEEDING BEHAVIORS IN WHALE SHARKS IN THE COASTAL WATERS OFF THE EL MOGOTE SANDBAR, BAHIA DE LA PAZ, MEXICO"**

Presentada por el alumno:

| | | |
|---|---|---|
| **WHITEHEAD** | **-** | **DARREN ANDREW** |
| Apellido paterno | materno | nombre(s) |

Con registro: | **A** | **1** | **6** | **1** | **0** | **2** | **8** |

Aspirante de:

**DOCTORADO EN CIENCIAS MARINAS**

Después de intercambiar opiniones los miembros de la Comisión manifestaron ***APROBAR LA DEFENSA DE LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

LA COMISION REVISORA

Director(a) de Tesis

Rogelio G. A.

**DR. ROGELIO GONZÁLEZ ARMAS**

**DR. FELIPE GALVÁN MAGAÑA**

**DR. AGUSTÍN HERNÁNDEZ HERRERA**

**DRA. ANA HACOHEN DOMENÉ**

**DR. ALISTAIR DUNCAN DOVE**

**PRESIDENTE DEL COLEGIO DE PROFESORES**

**DR. SERGIO HERNÁNDEZ TRUJILLO**

PODER EJECUTIVO FEDERAL
ESTADOS UNIDOS MEXICANOS
MÉXICO, D.F.

I.P.N.
CICIMAR
DIRECCIÓN

# INSTITUTO POLITÉCNICO NACIONAL
## SECRETARÍA DE INVESTIGACIÓN Y POSGRADO

## CARTA CESIÓN DE DERECHOS

En la Ciudad de La Paz, B.C.S., el día 27 del mes de Noviembre del año 2019

El (la) que suscribe M en C. DARREN ANDREW WHITEHEAD Alumno (a) del Programa

DOCTORADO EN CIENCIAS MARINAS

con número de registro A161028 adscrito al CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

manifiesta que es autor(a) intelectual del presente trabajo de tesis, bajo la dirección de:

DR. ROGELIO GONZÁLEZ ARMAS

y cede los derechos del trabajo titulado:

"FEEDING ECOLOGY & THE CHARACTERIZATON OF FEEDING BEHAVIORS IN WHALE SHARKS IN THE COASTAL WATERS OFF THE EL MOGOTE SANDBAR, BAHIA DE LA PAZ, MEXICO"

al Instituto Politécnico Nacional, para su difusión con fines académicos y de investigación.

Los usuarios de la información no deben reproducir el contenido textual, gráficas o datos del trabajo sin el permiso expreso del autor y/o director del trabajo. Éste, puede ser obtenido escribiendo a la siguiente dirección: darren.a.whitehead@gmail.com - rarmas@ipn.mx

Si el permiso se otorga, el usuario deberá dar el agradecimiento correspondiente y citar la fuente del mismo.

M en C. DARREN ANDREW WHITEHEAD

*Nombre y firma del alumno*

# ACKNOWLEDGMENTS

The writing of this PhD dissertation has been one of the most challenging academic responsibilities of my professional career. It has been filled with sacrifice, wonder and achievements on both a personal and professional level. Without the support and patience of the following people and institutes, this dream of mine would have not been possible. So, it is to them that I owe my deepest gratitude in the times of struggle and success we have shared together during this incredible journey.

I would like to initially thank the Instituto Politécnico Nacional (IPN) and the Consejo Nacional de Ciencia y Tecnología (CONACYT) for the fellowship given to me throughout this PhD program. I further take the opportunity to thank the Centro Interdisciplinario de Ciencias Marinas (CICIMAR) for the chance to be part of its academic research team, and for its mobility and personal student grants given to by its different departments and staff. To Georgia Aquarium thank you for the support and care during my internship as it gave me a chance to observe the feeding behaviors of this wonderful species under such premier care. A special thank you to Harry Webb who offered me guidance in the redesign of the fin clamp to suit my needs.

To my supervisory committee. Firstly, Dr. Rogelio González Armas and Dr. Felipe Galván Magaña for their continued support and commitment to my needs at such a challenging locality. This support allowed me to deliver a level of work that has exceeded my expectations and placed me in a position where I am able to move forward with excellence. To Dr. Alistair Dove and Dr. Ana Hacohen-Domené thank you for your rigorous review of this work as it has allowed me to greatly improved the quality of this thesis. Dr. Augustin Hernández Herrera thank you for the academic overview with my thesis and help with the steps of this program as it came to an end.

To all my friends and colleagues, I would like to especially thank, Dr. Abraham Vázquez-Haikin for his support both in the field and through challenging and delightful whale shark conversations. These have taught me that we really do get surprised by these magnificent animals the more we observe them in their natural habitat. To Dr.

James Ketchum I would like to personally thank you for the guidance in all whale shark matters and the continued support to this day with a collaborative effort for all sharks in our magnificent region. To Robert cooper, for his support and guidance with the preparation of my data for the machine learning part of this thesis as without the guidance and technical support I would not have been able complete this part of my thesis. To Jake Levenson thank you for the introduction to open tags and offering me the kind support as I begin this journey. Dr. Raúl Octavio Martínez Rincón and Dr. Damien Olivier thank you for the exceptional help and supervision with my statistical issues and the general tweaks to make my results show the things I needed to show. Dr. Daniela Alejandra Murillo Cisneros and Dr. Ulianov Jakes-Cota for their support and collaborations with scientific articles. To Tania & Francesca thank you for the uncompromised support and help with all the wild field-based tasks I put on you, as without your dedication to my needs the collection of some of the most important data in this thesis wouldn't not have been possible. To all of the rest of my friends at both the Proyecto Tiburones y Rayas and Pelagios Kakunja A.C you know who you are, thank you for the support and help with this work.

At this point I would like to express a special thanks to my family back in my native county of the United Kingdom and here in Mexico, although words cannot express how grateful I am for the support during this wonderful journey. Ultimately, I would give a heartfelt thank you to Xochitl Toledo Lopez for her continuingly encouraging pushes and patience throughout this up and down roller-coaster of a ride with both myself and this great achievement, as without her support I truly don't think it would have been possible to reach the end of this personal triumph.

"All men dream, but not equally. Those who dream by night in the dusty recesses of their minds, wake in the day to find that it was vanity: but the dreamers of the day are dangerous men, for they may act on their dreams with open eyes, to make them possible" **T.E Lawrence**

## THESIS OUTLINE

ABUNDANCE & COMPOSITION OF ZOOPLANKTON IN

THE EL MOGOTE AREA OF BAHIA DE LA PAZ

ISOTOPIC ASSEMENT OFTISSUES IN WHALE SHARKS

IN THE COASTAL WATER OF THE EL MOGOTE, BAHIA DE LA PAZ

# I. LIST OF FIGURES & TABLES

## II. GLOSSARY

**Accelerometers** - Accelerometers are devices that measure acceleration, which is the rate of change of the velocity of an object.

**Aggregation** – An area of habitat were numerous individuals of the same species come together for either a foraging or social behavior.

**Dermal denticles** - Dermal denticles (placoid scales) are tough "scales" that cover the skin of elasmobranchs (sharks and rays).

**Endangered Species** – A species at risk of extinction because of human activity, change in climate or change in predator-prey ratios.

**Epidermal layer** - The epidermis is the outer of the two layers that make up the skin (or cutis), the inner layer being the dermis.

**Filtration** – A term for a liquid that is passed through a filter to extract objects or particles.

**Foraging** – The acquisition of food by predation or gathering.

**Habitat** – The natural environment of an organism; a place that is natural for the life and growth of the species.

**Keel** – In ecology is defined as a longitudinal ridge, as on a leaf or bone; a carina.

**Locomotion** – The movement of such an organism from one place to another, often by the action of appendages such as flagella, limbs or wings. In some animals such as fish, locomotion results from a wavelike series of muscular contractions.

**Machine Learning** – Machine learning is the scientific study of algorithms and statistical models that computer systems use to effectively perform a specific task without using explicit instructions, relying on patterns and inference instead.

**Mass spectrometer** – A device for identifying the kinds of particles present in a given substance.

**Marine megafauna** – Any large aquatic organism, which can be seen with an unaided eye.

**Mesoscale cyclonic eddy** – Mesoscale ocean eddies are characterized by currents that flow in a roughly circular motion around the center of the eddy. The sense of rotation of these currents may either be cyclonic or anticyclonic.

**Non-invasive** – A technique to extract data or a sample from an organism without invasive means such as the mortality of the animal.

**Open-source software** – Open-source software is a type of computer software where source code is released under a license, in which the copyright holder grants users the rights to study, change, and distribute the software to anyone and for any purpose.

**Photo-Identification** - Photo-identification is a technique used into identify and track individuals in their natural habitat to study population over time. It relies on capturing photographs of distinctive characteristics such as skin patterns and scars from the animal.

**Pre-calibrated** – A prior procedure to determine, or rectify the graduation of any instrument giving quantitative measurements.

**Zooplankton** – Zooplankton consists of tiny animals, such as rotifers, copepods, and krill that float in aquatic environments.

# III. RESUMEN

La presencia estacional de tiburones ballena (*R.typus*) en Bahía de La Paz representa una oportunidad espectacular de investigar la ecología alimenticia de este filtrador con protección internacional. El objetivo del presente estudio fue proporcionar una visión actualizada de la dieta y de los comportamientos alimenticios de tiburones ballena juveniles en esta región aplicando varias técnicas científicas durante múltiples épocas de muestreo. Se recolectaron 384 muestras de zooplancton y evidenciaron un cambio estacional en la biomasa total del zooplancton, con diferencias significativas respecto al mes de muestreo, época climática y estación de muestreo. La mayor abundancia de individuos la registró el grupo taxonómico Copépoda seguido de Chaetognatha, con diferencias significativas estacionales y mensuales, y con un pico durante los meses de invierno. Las firmas isotópicas de $\delta^{13}C$ y $\delta^{15}N$ fueron analizadas en 60 biopsias de piel de tiburón ballena y muestras de zooplancton recolectadas durante la alimentación de tiburones. Si bien, no se observaron diferencias significativas entre las firmas isotópicas y la época climática o el sexo de los individuos, los resultados mostraron un aumento significativo en los valores de $\delta^{15}N$ en relación con la talla, revelando un posible cambio en la dieta a medida que la especie madura. Se observó irregularidad en las firmas de zooplancton en comparación con los tejidos de varios tiburones, presentando valores de $\delta^{15}N$ similares o más bajos que el zooplancton muestreado en la bahía. Con precaución, esto significa que estos tiburones se alimentaron en un sistema diferente con una línea isotópica base variable. Finalmente, 11 tiburones ballena fueron marcados para identificar y caracterizar los comportamientos de alimentación. Se aplicó un algoritmo de aprendizaje automático Random Forest para construir un modelo predictivo y fue eficiente en la clasificación de los comportamientos de alimentación, resaltando la importancia de la construcción del modelo y la clasificación precisa de los comportamientos antes del análisis. En conclusión, el presente estudio incrementó nuestro conocimiento actual de los tiburones ballena en Bahía de La Paz, coincidiendo en su mayor parte con estudios previos, sin embargo, los algoritmos aprendizaje automático presentaron una plataforma robusta para estudios futuros sobre cómo y cuándo podríamos ser capaces de observar los comportamientos de alimentación de los tiburones con un impacto mínimo.

# IIII. ABSTRACT

The seasonal presence of whale sharks (*R.typus*) to Bahía de La Paz presents a spectacular opportunity to investigate the feeding ecology of this international protected filter feeder. This present study aimed to provide a new updated look at the diet and foraging behaviors of juvenile whale sharks in this region, applying several different scientific techniques, over multiple sample seasons. A total of 384 samples of zooplankton were collected and reveled a seasonal change in the total biomass of zooplankton, with significant differences observed between total biomass and sample month, climatic season and sample station. The composition of macro-zooplankton gathered was grouped and revealed the greatest abundance of individuals in the taxonomic group Copepoda followed by Chaetognatha, showing significant difference throughout climatic seasons and sample months, with a peak in the total number of individuals recorded during the winter months. Isotopic signatures for $\delta^{13}C$ and $\delta^{15}N$ were analyzed in 60 whale shark skin biopsies and several zooplankton samples collected alongside feeding sharks. While no significant differences were observed between isotopic signatures and sample season or the sex of individuals sampled, results from this study did show a significant increase in $\delta^{15}N$ values when correlated with size, revealing a potential dietary shift as the species matures. Some irregularity appeared in zooplankton signatures when compared to the tissues in sampled sharks, with several individuals presenting similar or lower $\delta^{15}N$ values than zooplankton sampled in the bay. With caution, this signifies that these sharks fed and consequently showed an isotopic value from a different system with a varying isotopic baseline. Ultimately, 11 whale sharks were marked with non-invasive fin clamps equipped with data logging instruments to identify and characterize foraging behaviors. A Random Forest machine learning algorithm was applied to construct a predictive model and was efficient in the classification of foraging behaviors but outlined the importance of both model construction and the accurate classification of behaviors before the analysis. In conclusion, this present study increased our current knowledge of whale sharks in Bahía de La Paz, coinciding for the most part with previous studies, however, forefront machine learning algorithms presented a robust platform for future studies on how, and when, we may be able to observe the feeding behaviors of sharks with minimal impact.

# V. FOREWORD

The need for a foreword to this document is essential, as it offers an understanding of the structure of this thesis.  As this study falls over many areas of scientific research that are distinctively diverse from one another, especially in their methodology and analysis techniques, it is without doubt imperative to provide a clearer thesis outline to the reader to ensure flow and overall interpretation.

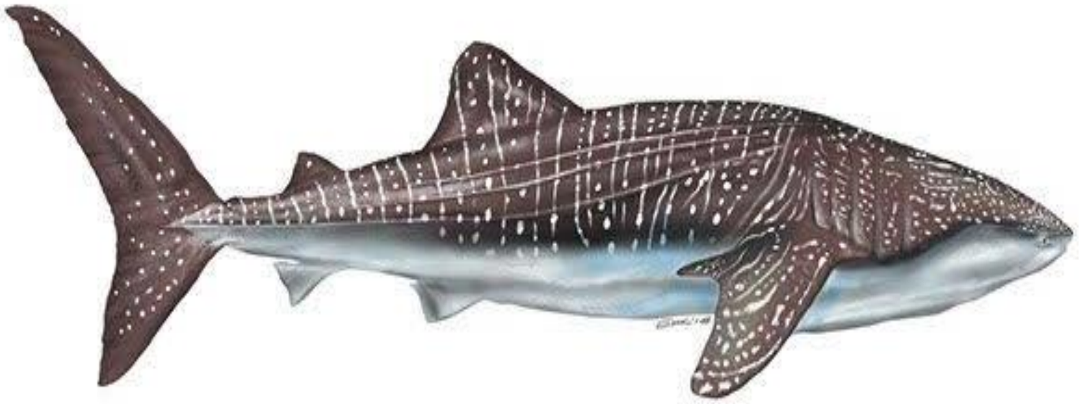
The choice to offer a general introduction of the project and target species followed by a clear research hypotheses and objectives, then robust description of the study area, provides a solid knowledge base to the reader. Succeeding this, the thesis is divided into three appropriate chapters with justifications, methodologies, and results. Then an individual conclusion for each chapter is presented to provide a clear and concise understanding of the results. Ultimately, a final conclusion to the thesis is given to interlock all the separate results and to summarize any final thoughts and future steps, with respect to its feeding ecology and behavior of this charismatic species.

# VI. GENERAL INTRODUCTION

The main goal for this thesis was to investigate the dietary preference of whale sharks in Bahía de La Paz focusing along the El Mogote region and to provide the first characterization of the feeding behaviors, using a recognized machine learning algorithm. Site fidelity in this region and knowledge of the species arrival and departures during specific times of the year makes this easily accessible aggregation site an ideal platform for investigating the foraging behaviors of this species, with minimum logistical effort (Whitehead *et al.,* 2019). Furthermore, the opportunity to interact with whale sharks in coastal habitats such as in Bahía de La Paz may be crucial to understanding and investigating an array of research objectives, which can be applied to harder to reach oceanic sites.

Public views and perceptions of the impact of investigational techniques on animals in their natural habitat are changing and the need for a more non-invasive approach seems to be inevitable. This project demonstrates advantages of using open source technology, and minimal invasive sampling techniques to record or even extract fundamental information or samples from larger marine species that hold national and international protection. While a great deal of scientific attention has been given to this remarkable shark, there still seems to be a lack of published literature on the species foraging ecology and dietary preference at these coastal habitats. Results from this thesis aimed to provide a new updated look at some of those issues in this established, dependable aggregation site of Bahia de La Paz, and present recommendation for future studies that wish to advance our understanding of the world’s largest shark.

## Whale sharks



| | |
|---|---|
| **Kingdom:** | Animalia |
| **Phylum:** | Chordata |
| **Class:** | Chondrichthyes |
| **Order:** | Orectolobiformes |
| **Family:** | Rhindodontidae |
| **Genus:** | Rhincodon |
| **Species:** | *R.typus* |

Figure.1. Illustration of whale shark (*R.typus*) photo credit & copyright: Sam Wilson

### Taxonomy

There are approximately 1,200 known species of cartilaginous fishes, which form the class Chondrichthyes (Campagno, 2005; Rowat & Brooks, 2012; Pierce *et al.,* 2016). This class is divided into two subclasses; Elasmobranchii (sharks, rays, and skates) and Holocephali (chimeras) (Compagno, 2005). The whale shark represents the monotypic family Rhincodontidae and was first described in scientific literature by Dr. Andrew Smith from a 4.6 metre specimen caught off Table Bay, South Africa (Smith, 1828). Originally it was granted the genus name *Rhiniodon typus* (Smith, 1828), but the following year it was amended to *Rhincodon typus* by the same author (Rowat & Brooks, 2012).

### Biology

The whale shark is distinguished by its colossal size, tapering fusiform body type, three prominent longitudinal ridges that run horizontally along its dorsal flanks, an expansive flattened head with a nearly terminal mouth and a pattern of light spots and stripes over a dark body (Compagno, 2005: Rowat & Brooks, 2012). In recent years, the use of these unique external body markings of the species has allowed researchers to identify individuals and build population counts both nationally and on an international scale (Arzoumanian *et al.,* 2005; Holmberg *et al.,* 2008; Marshall & Pierce, 2012). Unlike bony fish, whale sharks and all members of the elasmobranch family do not possess swim bladders and rely on their large livers as an aid for buoyancy control (Gudger, 1915; Gleiss *et al.,* 2011). The whale shark's epidermal layer is comprised of rows of perfectly aligned dermal denticles, which are shaped with three longitudinal ridges, the central one forming a sturdy central keel with deep grooves on either side. These provide exceptional adaptations to the skin that serve as a hydrodynamic aid reducing drag and surface-noise production improving the animal's movement in the water, reducing energy loss during locomotion (Bigelow & Schroeder, 1948; Gleiss *et al.,* 2011). *R.typus* skeletal composition is made up of a durable, lightweight, supple cartilage (Campagno, 2001; Gudger, 1915) and the species also lack a solid rib-cage structure, which reduces the overall weight of the animal (Rowat & Brooks, 2012). A sub-dermal complex mesh corset of collagen fibres serves as a flexible external skeleton, that permits the sub-dermal attachment of the locomotory muscles from the backbone in a light and mechanically efficient system (Martin, 2007; Rowat & Brooks, 2012). The species upholds the title of the world's largest living fish-like vertebrate and has been documented to reach lengths of over 14 meters and weights of more than 20 metric tons (Joung *et al.,* 1996). To date, the largest specimen documented was a Taiwanese fishery record from 1987, which reported the animal to be close to 20 meters in total length with a weight of approximately 34 metric tons (Chang, Leu & Fang, 1997). Current literature and scientific reports have observed sightings of whale sharks around 10-12 meters in total length (Rowat & Brooks, 2012) and the chance of encountering an animal in excess of that is rare, and only chance encounters in the Galapagos islands and sea mounts on the outer area of Bahia de La Paz, has demonstrated larger female sharks on a seasonal basis, but solitary (Hearn *et al.,* 2013; Ramirez-Maricas *et al.,* 2017).

## Distribution

Currently, 16 seasonal aggregation sites exist where whale sharks are observed in high numbers and they are distributed worldwide, confirming that the species circumnavigate both tropical and subtropical waters of the world, with a range of respectively 30° north and 32° south of the equator (Norman & Stevens, 2007; Rowat & Brooks, 2012) (Fig.2). Additionally, *R.typus* is epipelagic with the species exploiting both shallow coastal waters and deeper oceanic regions (Rowat & Brooks, 2012; Ramirez-Marcias *et al.,* 2017). Until recent, the species was thought to be absent in the Mediterranean Sea (Rowat & Brooks, 2012), but sporadic sightings have confirmed their presence (Jaffa & Taher, 2007) Aggregations of whale sharks have been reported at Ningaloo Reef in Western Australia (Taylor, 1996), Philippines (Quiros, 2007), Mozambique (Rohner *et al.,* 2013), Madagascar (Jonason & Harding, 2007), Seychelles (Rowat & Brooks, 2012), Maldives (Riley *et al.,* 2010), Djibouti (Rowat *et al.,* 2011), Belize (Graham & Roberts, 2007), Mexico Caribbean (De la Parra *et al.,* 2011), Gulf of California, Mexico (Ramirez-Marcias *et al.,* 2012; Whitehead *et al.,* 2018) Tanzania; (Rohner *et al.,* 2015), Qatar (Robinson *et al.,* 2013), Gujarat, India (Borrell *et al.,* 2011), Indonesia (Stacey *et al.,* 2012), Red sea, Egypt (Berumen *et al.,* 2014), St. Helena (Clingham *et al.,* 2016) and various others (Rowat & Brooks, 2012; Hearn *et al.,* 2013). These aggregation sites or "hot spots" provide reliable research opportunities for a number of biological studies that otherwise would be logistically difficult due to the sporadic or seasonal appearances of the species, yet historically provided opportunities for targeted fisheries to exploit whale shark populations (Chen & Phipps, 2002; Rowat & Brooks, 2012). Aggregations seem to be related to high concentrations of zooplanktonic food (Hoffmayer *et al.,* 2007; Nelson & Eckert, 2007; Motta *et al.,* 2010; Ketchum *et al.,* 2013) or a number of hydrologic and oceanographic features that may influence the seasonal distribution of whale sharks such as; convergent zones, upwelling, and temperature discontinuities (Burks *et al.,* 2006). The ability of the species to withstand short-term exposure to considerably colder waters does not seem to be a barrier to the species', as they have been recorded making regular dives into water masses with temperatures of below 10°C (Tyminski *et al.,* 2008). With continued knowledge of the species range and extensive comparisons with biophysical factors may provide more information that will help to identify potential areas of occurrence and transitory pathways (Rowat & Brooks, 2012).

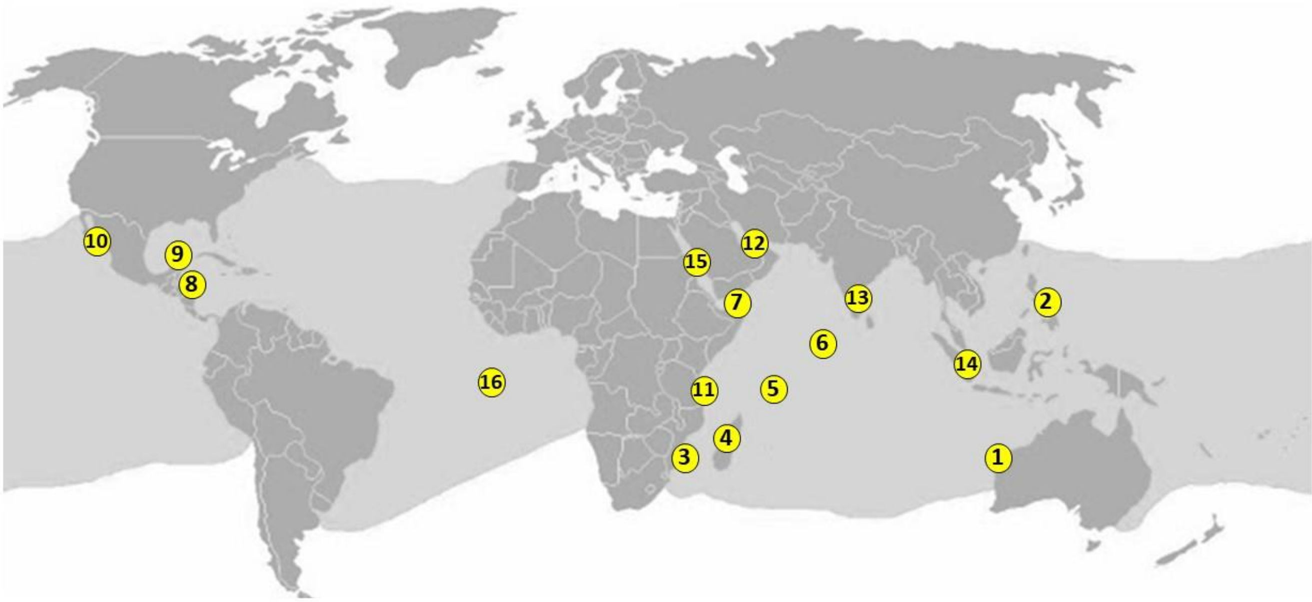


Figure. 2. Global distribution map showing known whale shark aggregation sites (yellow circles): 1. Western Australia; 2. the Philippines; 3. Mozambique; 4. Madagascar; 5. Seychelles; 6. Maldives; 7. Djibouti; 8. Belize; 9. Mexico Caribbean; 10. Gulf of California, Mexico; 11. Tanzania; 12. Qatar; 13. Gujarat, India; 14. Indonesia; 15. Red sea; Egypt 16. St. Helena

## Reproduction

Regarding their reproductive cycle, relatively little is known given the lack of sightings of both larger specimens and neonates, leaving gaps in scientific knowledge regarding these important phases of their existence (Norman & Stevens, 2007; Rowat & Brooks, 2012; Ramirez-Marcia *et al.,* 2017). Very few cases of juvenile sharks less than three metres in total body length have ever been sited globally (Schmidt *et al.,* 2010) and only 19 neonate sharks measuring less than 1.5 metres appear in scientific literature (Schmidt *et al.,* 2010; Rowat & Brooks, 2012) (Fig.3). The species reproductive cycle was unclear until the late 20th century when a pregnant shark of 10.6 meter was landed at Chen-Kung fish market, Taiwan (Joung *et al.,* 1996). This specimen contained a combined total of more than 300 embryos in her twin uteri; many of which were in their egg cases and had external yolk sacs (Joung *et al.,* 1996; Schmidt *et al.,* 2010). This encounter proved that species is aplacental viviparous and ultimately the largest litter ever recorded from any shark species (Rowat & Brooks, 2012). It is uncertain at what size or age whale sharks become sexually mature, although several studies have provided variety of possible indicators such as; clasper morphology (Joung & Chen, 1995; Norman & Stevens, 2007), vertebral growth rings (Wintner, 2000; Hsu et al., 2014) and growth rate analysis on captive specimens (Uchida, Toda, Kamei & Teruva, 2000), although given the species' conservation status it generates problems when trying to obtain samples (Perry *et al.,* 2018; Rowat & brooks, 2012).

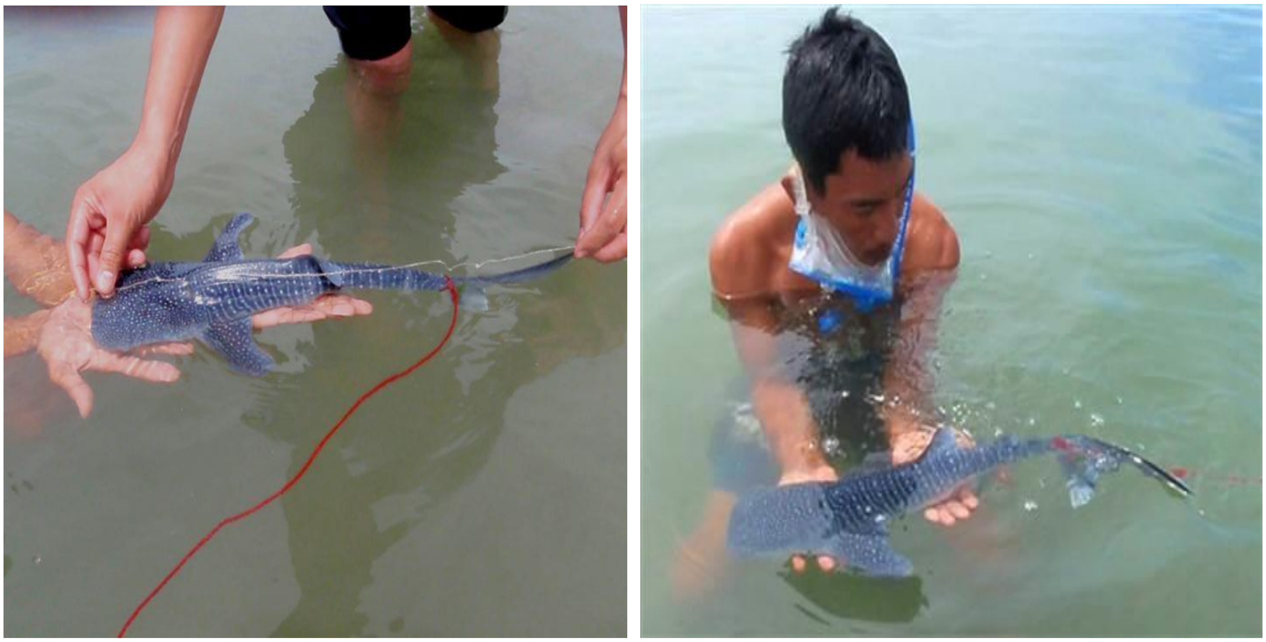


Figure. 3 Neonate whale shark found and reported to WWF- Phillipines, near Donsol, Philippines. Photo credit: Elson Aca, copyright World Wide Fund for Nature-Philippines (WWF-Phillipines).

A number of widespread field-based methods including 'guesstimations' have been used as a means of obtaining animal size by competent in-water swimmers to the nearest half a metre accuracy (Meekan *et al.,* 2006) or a research vessel to estimate size (Hobbs *et* *al.,* 2009), and the use a of rope marked at known intervals and held at either end by swimmers, while it is strung alongside a mobile subject to determine its relative size (Norman & Stevens, 2007). But there seems to be a degree of error between observers when reporting data of the same animal (Rowat & Brooks, 2012; Jeffreys *et al.,* 2013; Perry *et al.,* 2018). Technology now offers new ways in which to obtain measurements of free-swimming sharks, such as the use of paired lasers (Rohner *et al.,* 2011; Jeffreys *et al.,* 2013; Sequeira *et al.,* 2016), and stereo photogrammetry (Shortis, 2015; Delacy *et al.,* 2017) to estimate length and growth of sharks (Perry *et al.,* 2018). But human error still plays an important factor in true growth parameters, although new studies are working to determine true growth estimates based on comparing multiple techniques (Perry *et al.,* 2018).

## Foraging behavior

The species is one of only three filter-feeding shark species, along with the basking shark (Cetorhinus maximus) and the megamouth shark (Megachasma pelagios) (Compagno, 2001; Rowat & Brooks, 2012). Collectively they feed on a wide variety of planktonic (microscopic) zooplankton and nektonic (larger free-swimming) prey (Clarke & Nelson, 1997; *Simms et al.,* 2000; Nelson & Eckert, 2007; Motta *et al.,* 2010; Ketchum *et al.,* 2013). Additional prey includes tiny crustaceans, small schooling fishes such as sardines, anchovies and occasionally small tuna and squid (Campagno, 1984; Motta *et al.,* 2010; De la Parra *et al.,* 2011; Cárdenas-Palomo *et al.,* 2018; Boldrocchi & Bettinetti, 2019). The feeding mechanism of whale sharks is designed to capture their food supply by the filtering of water through special filter pads (Motta *et al.,* 2010; Paig-Tran *et al.,* 2011). An early dissection of a whale shark in South Africa revealed that there is a transverse band of stiff filtration tissue within each gill slit of the species (Beckley *et al.,* 1997). Each pore in this uniquely designed tissue is effectively a tiny canal that is connected to another directly under the surface to form a much larger passage, which eventually opens into the branchial cavity underneath the branchial arches (Beckley *et al.,* 1997; Motta *et al.,* 2010). The overall function of these filtration pads is based upon three methods of filtration (Motta *et al.,* 2010). The first known as this dead-end sieving, where water and floating particles approach approximately perpendicular to the pads with larger prey items being trapped while smaller particles pass through the gill arches and are swept back in the environment (Gudger, 1941; Motta *et al.,* 2010). Hydrosol filtering, uses hydrodynamic processes and allows floating particles to pass into the mouth of the sharks and given the abrasive properties of the filtration pads, prey is retained and shallowed by the shark (Motta *et al.,* 2010). The third filtration technique is known as cross-flow filtration, in this method water and food particles pass relatively parallel to the filtration pads, moving at high velocities as the shark is feeding and drawing the water inwards. As the suspension travels posteriorly, the water is forced back out through the filtration pads and the particles are concentrated in a ball at the rear of the pharynx where they can be swallowed (Brainerd, 2001; Sanderson *et al.,* 2001; Motta *et al.,* 2010). Whale sharks are well-known to display several types of foraging behaviors presumably related to differing concentrations of available food sources (Nelson & Eckert, 2007; Motta *et al.,* 2010; Ketchum *et al.,* 2013) (Fig.4).

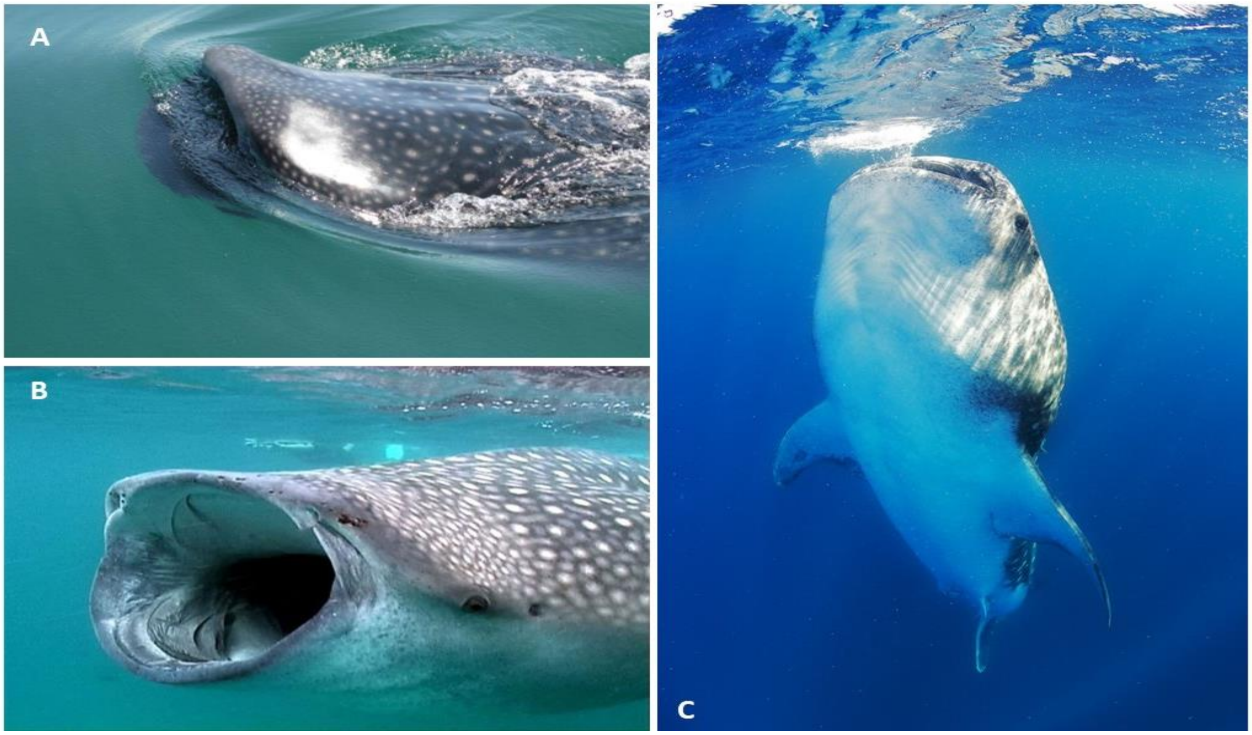


Figure.4. Examples of feeding behaviors in whale sharks; (A) active surface feeding photocredit (Motta *et al.,* 2010), (B) Passive feeding photo credit & copyright: Werner Mischlar, (C) Vertical feeding photo credit & copyright: Shawn Hienricks.

In many feeding aggregations, the most frequently observed feeding behavior is active surface or surface ram feeding (Clark & Nelson, 1997; Nelson & Eckert, 2007; Motta *et al.,* 2010; Ketchum *et al.,* 2013). During this behavior the species may swim almost at the surface with its mouth open and slightly lifted out of the water, repeatedly opening and closing its mouth driving water and food items over its filtering apparatus (Clark & Nelson, 1997; Heyman *et al.,* 2001; Motta *et al.,* 2010). When food concentrations are higher, suction or vertical feeding might occur, where the species can be observed in a diagonal or an almost vertical position while generating a suction in its pharynx drawing in large volumes of water and prey (Nelson & Eckert, 2007; Motta *et al.,* 2010). Lastly, passive feeding can be witnessed within the species and maybe simply be described as the animal swimming slowly through the water with their mouth marginally open, presumably filtering the scarce prey items, closing its mouth every few minutes with the shark appearing to swallow (Heyman *et al.,* 2001; Dove, 2015).

## Threats

The passive nature of these giant sharks, their aggregation behavior at specific times of the year, slow maturation rate and distinctive feeding habits make them very susceptible to targeted and non-targeted fishery impact (Colman, 1997; Norman, 1999; Chen & Phipps, 2002; Rowat & Brooks, 2012; Peirce *et al.,* 2016). Traditionally whale sharks were hunted for food and for their liver oil, used for waterproofing wooden boats (Colman, 1997; Chen & Phipps, 2002; Rowat & Brooks, 2012). The demands for their meat and fins increased, creating a large international trade in whale sharks, that became the main stimulus for new, improved, targeted fisheries (Norman, 2002) (Fig.5). As the cartilage fibres in the sharks' fins are apparently not high quality for making soup, the fins were either discarded or sold for display in shark-fin soup restaurants (Chen & Phipps, 2002). The demand for whale shark meat, increased in value and so did the search effort, ultimately resulting in declines in catch records and the collapse of the fishery (Chen, 2002; Hanfee, 2007; Ziegler, 2010). Currently the largest non-targeted fishery for whale sharks comes from purse-seine fleets (Rowat & Books, 2012). Tuna fisheries have been known to use whale shark sightings as an indicator of tuna presence, and consequently lay their nets around them (Iwasaki, 1970). Fishery records from a number of purse seine fleets, have shown that the individual whale sharks are usually released from the nets alive (Amande et al., 2010; Chassot *et al.,* 2009; Rowat & Brooks, 2012) and now fleets provide whale shark release protocols (Escalle *et al.,* 2016).

Figure.5. Historical photos of whale shark fisheries (A) stacking fins for export photo credit & copyright: Hilton Hofford for WildLifeRisk, (B) whale shark meat for export proto credit & copyright: WildLifeRisk, (C) landed shark being cut into smaller peices in the Philippines 1998 photo credit & copyright: Jürgen Freund, (D) whale shark transported to market in Chine photo credit & copyright: Peter Hess.

The whale shark is listed under the International Union of Conservation of Nature (IUCN) as an endangered species and holds further protection under the Convention of international trade in endangered species (CITES) and the convention of migratory species (CMS) (Rowat & Brooks, 2012; Pierce & Norman, 2016). Conservation efforts and governmental legislations have provided national protection for most countries where known pathways of whale sharks exist, but still, threats are apparent as the species moves into international waters and along busy ocean pathways increasing the chance of boat stripes or bycatch incidents. Currently, in Mexico, the whale shark is protected by Mexican regulations in the NOM- 059-SEMARNAT-2010 and NOM-029-PESC-2006 by a national fishery ban for this species (Ketchum *et al.,* 2013; Whitehead *et al.,* 2019).

In the last two decades there has been an increase in the trend and demand for the species to be utilized as a tourist venture and now as the industry is rapidly growing there are prevailing concerns regarding its management (Quiros, 2007; Pierce & Norman, 2016; Whitehead *et al.,* 2019). Whale shark-related tourism has exploded from only a handful of sites in the 1990s to more than 12 sites internationally distributed throughout the world, allowing it to become a highly lucrative industry based upon this endangered species, without a universal understanding of its carrying capacity (Orams, 2002). In Bahía de La Paz eco-tourism activities have existed for more than a decade (Ramirez-Marcia *et al.,* 2012; Whitehead *et al.,* 2019) and in recent years, pressures from uncontrolled tourism have motivated authorities to implement an area of protection and site-specific regulations for these activities, to regulate this ever-growing industry (SEMARNAT, 2017; Whitehead *et al.,* 2019).

## VII. RESEARCH HYPOTHESES

There is seasonality in the accumulation of biomass in the El Mogote region of Bahia de La Paz with peaks in the winter months and the composition of zooplankton during that time consists primarily of copepods species. Isotopic signatures of whale sharks reveal a correlation between size of sampled animals and the concentration of $\delta^{15}N$, but show no significant difference between sex. Ultimately, the use of machine learning algorithms can provide insights into characterizing foraging behaviors of whale sharks.

## VIII. RESEARCH OBJECTIVES

1. Determine the annual abundance and composition of zooplankton along the El Mogote sandbar over multiple sample years.

2. Investigate the importance of the different zooplanktonic groups to the diet of the species in this region.

3. Compare stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) within the specie and determine if there are significant differences between sex or correlations between size of sampled individuals.

4. Identify & characterize the feeding behaviors of whale sharks and develop a Random forest predictive model for the study area of Bahia de La Paz.

# IX. STUDY AREA

## Bahía de La Paz

Bahía de La Paz (BLP) is an almost entirely enclosed bay with a semi-elliptical shape, lying on the geographical coordinates 24°08'32N -110°18'39W and boasts both shallow and deep-water basins (Cruz-Orozco *et al.,* 1996). The Bay itself is the largest coastal water body in the Gulf of California (GC) with an approximate area of 2,635 km$^2$ and situated on the southeastern coast of the state of Baja California Sur, approximately 180km north of the entrance (Monreal-Gomez *et al.,* 2001) (Fig.6). Connectivity with the GC occurs via two passages, a northeasterly channel named Boca Grande and towards the southern part of the bay connectivity through the San Lorenzo Channel (Hernández-Trujillo *et al.,* 1987; Monreal-Gomez *et al.,* 2001; Sanchez-Velasco *et al.,* 2006).

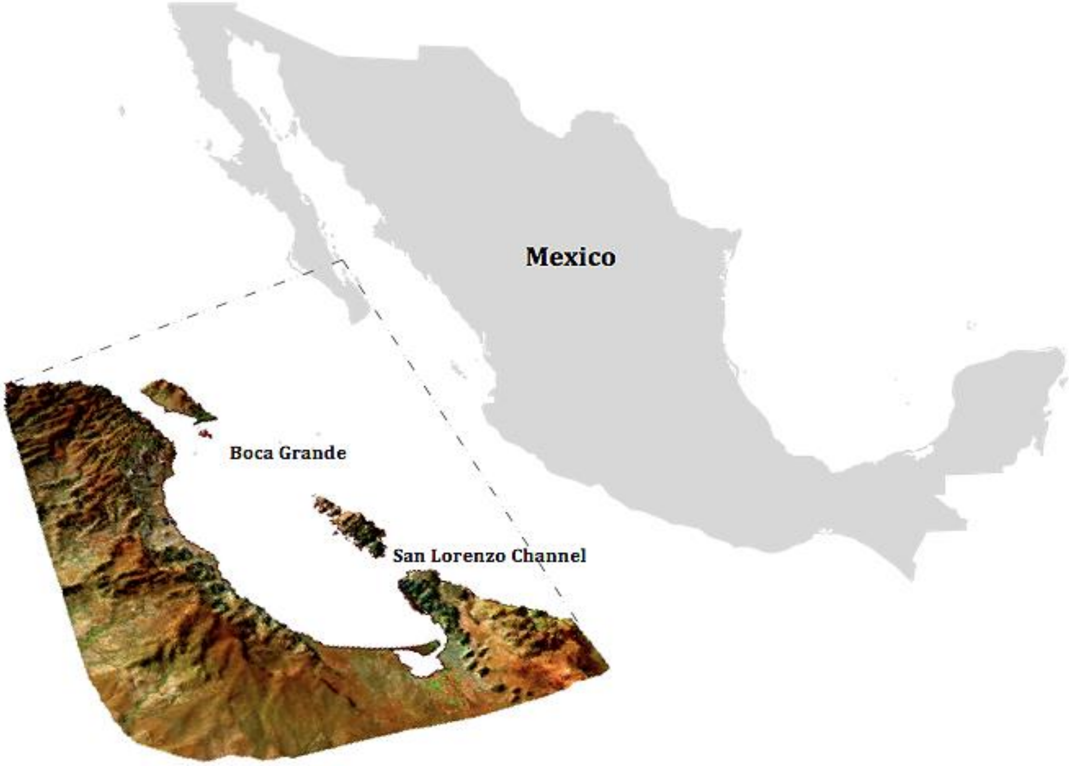


Fig.6. Overview of Mexico and a zoomed illustration of BLP, showing connectively outlets to the GC

Two main seasons occur in BLP: summer (June to September), which provides constant water temperatures ranging from 25–29 °C, a stratified water column and an apparent thermocline and winter (December to March), were average water temperatures range between 19–22 °C, contributing to a mixed layer within the water column and no significant thermocline (Obeso-Nieblas *et al.,* 2004; Sanchez-Velasco *et al.,* 2006). An additional two transitional periods do also occur throughout the year the first in spring (March to June) and the other in the fall (September to December), where the bay features gradual hydrographic changes from summer to winter or winter to summer, and are therefore considered transitional periods of oceanographic change (Robles *et al.,*1987; Ketchum, 2003). BLP is dominated by prevailing winds, which move over the bays surface waters and typically blow in a northwesterly direction throughout most of the year, with moderate intensity (Sanchez-Velasco *et al.,* 2006; Duran-Campos *et al.,* 2015). During the summer months predominant winds shift and commonly move in a southeasterly direction with much lighter intensities (Badan-Dangon *et al.,* 1991). Northerly winds throughout late autumn encourage strong currents that run in a counter-clockwise direction parallel to the coast creating the formation of a semi-permanent cyclonic gyre around the bays central portion (Ketchum, 2003; Coria-Monter *et al.,* 2017). Southerly winds during late spring appear to generate currents in a reverse circular pattern constructing the formation of an anti-cyclonic gyre, which appears to cause a general collapse of the thermocline layer in the bay (Jimenez-Illescas,1996; Coria-Monter *et al.,* 2017). Overall circulation in BLP is driven primarily by water exchange with the GC through its major passage of Boca Grande located in the northeastern corner of the bay and secondly, by the narrow San Lorenzo Channel in the southern portion (Ketchum, 2003; Duran-Campos *et al.,* 2015). The bay presents a bathymetric gradient; with relatively shallow regions across the entire basin with an average maximum depth of around 50m, while areas in the northern portions give home to considerably deeper parts and incorporate the bays deep region known as the Alfonso Basin, which is in excess of 400m (Sanchez-Velasco *et al.,* 2006).

## El Mogote Sandbar

The area to the southern part of the bay encompasses a protruding sandbar attached at one end to the mainland and stretching out approximately 12 km parallel to the mouth of the bay, known as the El Mogote sandbar (Fig.7). The natural formation of this sandbar has created almost two separate bodies of water: the main bay itself and the inlet or lagoon known as the Ensenada de la Paz (León-de la Luz *et al.,* 2006). El Mogote covers approximately 16 km$^2$ of surface vegetation comprised mainly of a sandy coastal environment with periodic patches of mangroves on its inlet side and desert shrub vegetation (León-de la Luz *et al.,* 2006). This unique coastal zone is exposed to seasonal north winds and annual water circulations provided by the eddies in BLP enabling it to provide both primary and secondary productivity of zooplanktonic organisms. Aggregations of whale sharks within the El Mogote area seem to be seasonal and have been documented to occur with a maximum peak during the winter months (Ramírez-Macías *et al.,* 2012; Whitehead *et al.,* 2019). The size of these annual aggregation of juvenile whale sharks in the El Mogote area is suspected to be around 70 individuals (Ramírez-Macías *et al.,* 2012), but this number seems to vary among seasons (Whitehead *et al.,* 2019). Given the knowledge of these aggregating along this shallow coastal bar, the El Mogote was chosen as the focus area for achieving all results in this study.

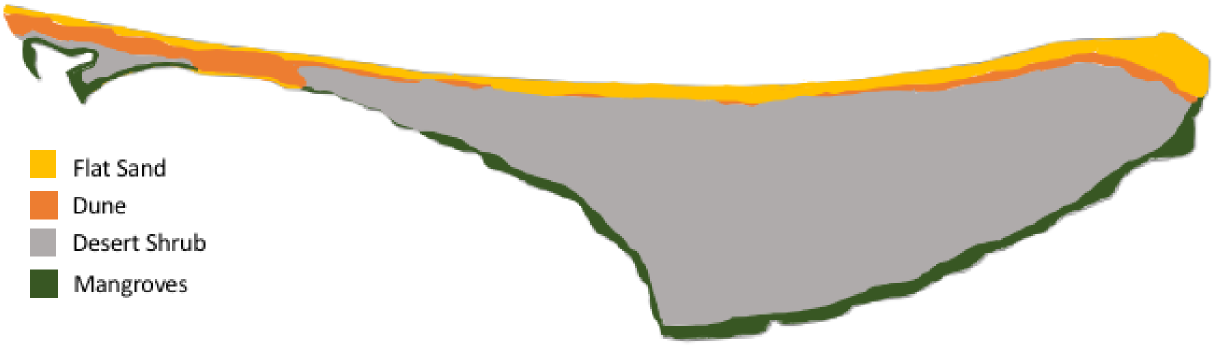


Figure.7 Principle study area the El Mogote Sandbar in BLP

# X. FIELD WORK

All fieldwork throughout the entire project took place over two consecutive years from June 2016 – June 2018, when weather conditions permitting access to the search area, onboard an 8m research vessel equipped with a single outboard engine. All ocean-based observations were conducted during daylight hours approximately between 08:00 – 15:00, with a maximum duration of 7 hours for any trip, which was dependent on the monitoring or fieldwork obligations, and the logistical costs available to support the research objectives.

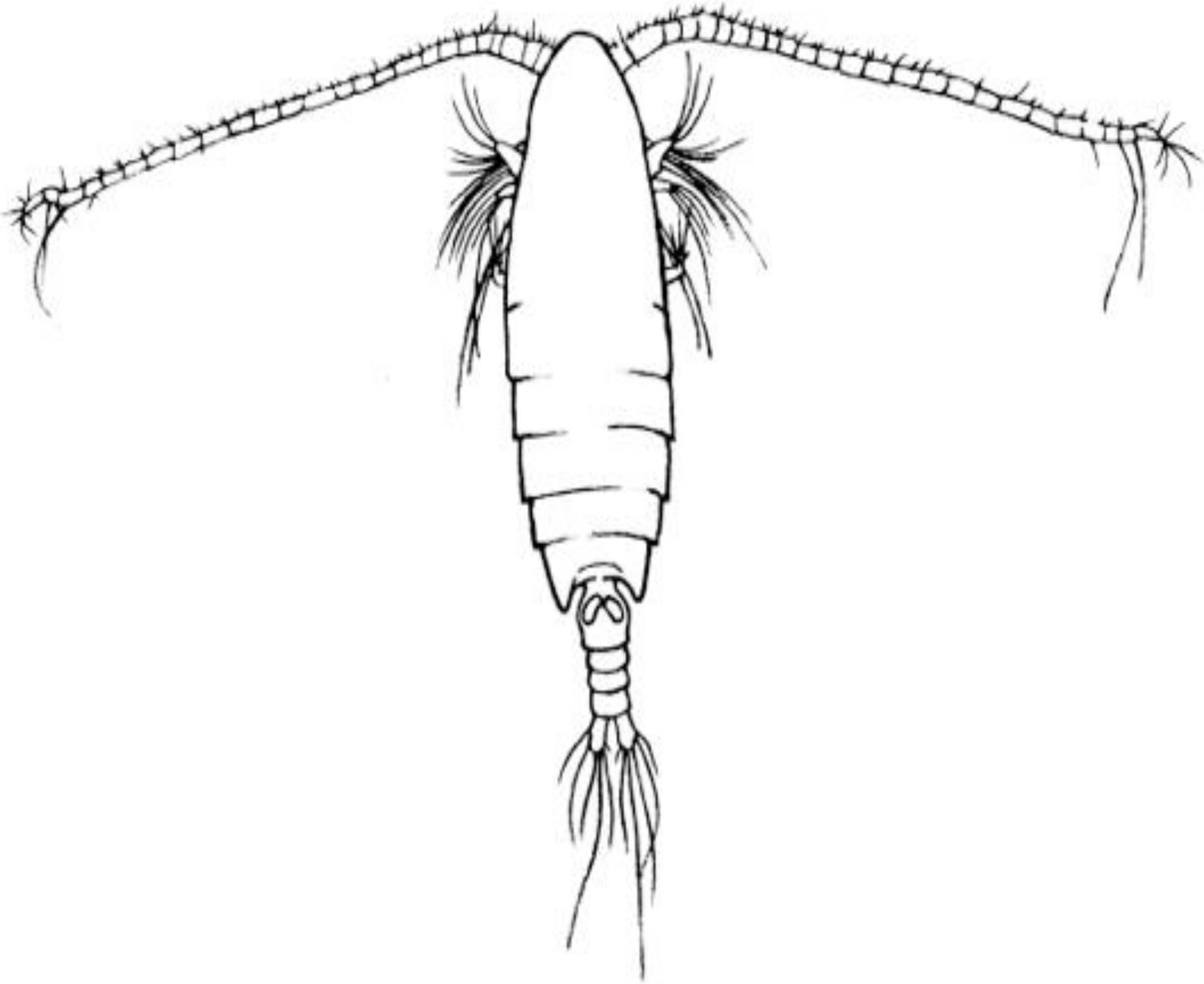
## Laboratory work

All laboratory work was conducted at the Centro Interdisciplinario de Ciencias Marinas (CICIMAR) under the supervision of technicians within their relevant departments. All analysis equipment manual and biogeochemical such as the mass spectrometer of isotopic ratios (IRMS). External direction and statistical support were provided by several technicians from external institutes and a more detailed explanation is provided in the previous acknowledgment section of this thesis.

## Authorized Permits

This study was conducted under the permit SGPA/DGVS 05605/17 and SGPA/DGVS 006924/18 provided by the Dirección General de Vida Silvestre from the Subsecretaría de Gestión para la Protección Ambiental of SEMARNAT.

# CHAPTER 1



# ABUNDANCE & COMPOSITION OF ZOOPLANKTON IN THE EL MOGOTE AREA OF BAHIA DE LA PAZ

## TABLE OF CONTENTS

◆

# 1. THEORETICAL BACKGROUND

◆

A study by Duran-Campos *et al.* (2015) examined the relationship between the cyclonic eddy in the center of BLP and the abundance and distribution of zooplankton and verified that the mesoscale cyclonic eddy can be the dominant physical feature that influences the distribution of all trophic groups of zooplankton in the bay. Furthermore, they described that the trophic group composition in this region exhibits a predominance of more herbivorous zooplankton in the center of the eddy, a predominance of omnivorous zooplankton in its periphery, and a much higher abundance of carnivorous zooplankton in the sample regions that were adjacent to the GC. The area to the southern part of BLP known as the El Mogote sandbar, offers a clear passage to the north and is thought to be influenced dramatically by a number of changing environmental factors which occur throughout the year, such as seasonal changes in wind direction, magnitude and the effect of the circulation of surface waters that moves through this area (Palomares-García, 1996; Durán-Campos *et al.,* 2015; Coria-Monter *et al.,* 2017). This highly productive coastal zone also experiences both primary and secondary productivity of planktonic species and is known as the principle foraging area for seasonally aggregating whale sharks (Ramírez-Macías *et al.,* 2012; Ketchum *et al.,* 2013; Whitehead *et al.,* 2018, 2019).

In the GC, whale shark aggregations are known to occur in both Bahía de Los Ángeles (Nelson & Eckert 2007) and BLP (Ramírez-Macías *et al.,* 2012; Whitehead *et al.,* 2019) and seem to be related to high zooplankton abundance (Hacohen-Domené *et al.,* 2006; Nelson & Eckert, 2007). Initial plankton work on prey preferences for the species have been investigated for Bahia de Los Ángeles (Nelson & Eckert, 2007; Lavaniegos *et al.,* 2012; Hernandez-Nava *et al.,* 2013) and BLP (Clark & Nelson, 1997; Hacohen-Domené *et al.,* 2006; Ketchum *et al.,* 2013) and known prey items on which *R. typus* have been observed to forage on include a number of copepod species, mainly *Acartia clausi*, Acartia spp. and members of the Euphausiidae family, such as *Nictiphanex simplex* (Clark & Nelson 1997; Hacohen-Domené *et al.,* 2006; Hernandez-Nava *et al.,* 2013; Ketchum *et al.,* 2013).

A preliminary study by Clark & Nelson. (1997) observed groups of juvenile whale sharks feeding on zooplankton blooms in the southern parts of BLP. These individuals were witnessed suction feeding on blooms of zooplankton during the winter months coinciding with seasonal sightings reports and shifting environmental conditions. In-water recording of the sharks' showed that when the sharks were feeding in these patches of concentrated surface plankton, they demonstrated swaying movements of their head from side to side, with part of the head being occasionally lifted out of the water and the mouth opening and closing between 7 to 28 times per minute. Analysis of the plankton collected during this study revealed that almost all tows consisted primarily of a single species of copepod (*Acartia clausi*) and the remaining animals within the samples comprising of a range of chaetognaths, hydromedusae, and appendicularians.

Hacohen-Domené *et al.* (2006) examined the abundance of zooplanktonic prey items in BLP and its association with whale sharks. Using several predetermined sample sites both inside and outside the bay, this baseline study revealed that overall zooplankton composition comprised mainly of copepods, representative of 12 genera with the most dominant species being *Acartia*, *Undinula*, and *Corycaeus*. Given the obvious abundance of these copepods during observed whale shark feeding events, the authors speculated that these microscopic animals represent a key and potentially the main prey item for the species during these seasonal aggregations. A larger more comprehensive study by Ketchum *et al.* (2013) examined whale shark segregation, foraging ecology and the abundance of zooplankton in BLP. Following rigorous plankton sampling encompassing the entire bay and its surrounding islands, clear temporal changes in the abundance of zooplankton biomass were observed, with peaks in biomass seemingly coinciding with changes in environmental factors in relation to the shift between climatic seasons. From observational data, the authors concluded that there was also clear segregation of the species by sex and size throughout the bay, which may be associated with a difference in diet between juvenile and adult members. Segregation by sex and size has also been observed by whale sharks in other parts of the gulf such as in Bahia de Los Angeles where larger animals within the population have been witnessed feeding in different areas than the smaller animals (Nelson & Eckert, 2007).

# 2. JUSTIFICATION

Understanding the productivity of a specific habitat especially at the base of the food web may help provide important information on the foraging behaviors of marine organisms that depend on it. Given the size of BLP and the logistical complications that exist to extensively investigate the entire region over a prolonged period, has left this area with limited studies focused on the abundance and composition of zooplankton over multiple sample seasons. To date, previous studies undertaken on zooplankton abundance and community structure have focused on a short term or sporadic sampling over a single sample year or season. This has led to only fundamental data regarding the dynamics of these communities and its influence on the presence or absence of filter feeding marine species who depend on them. Furthermore, concerns are apparent when attempting to provide scientifically robust data for the area along the El Mogote sandbar, where the concentrations of whale sharks exist, as there is no published work focused in this coastal zone. This study aimed to generate the first attempt at sampling two full annual cycles, as a means to investigate changes in the overall abundance and composition of zooplankton in the coastal waters off the El Mogote Sandbar. It focused on investigating whether there is noteworthy changing in the overall biomass and composition of zooplankton within sample seasons, months and seasons, along with a more comprehensive look at the different taxonomic groups of macro-zooplankton and their composition.

# 3. MATERIALS & METHODS

## 3.1 Sampling Equipment

For all plankton sampling, a standardized conical plankton net with a 505 μm mesh size, 60 cm mouth diameter and 1.5 m in length (Fig. 8a). The net was fitted with a General Oceanics mechanical flowmeter (Model 2030R), which assists in the determination of the water volume that passed through the net with each tow (Fig.8b). The device incorporates precision-molded rotors coupled directly to six-digit counters that record each rotor revolution. A single pin and lanyard system located ahead of the lateral center of the mass allows the flowmeter to maintain correct dynamic alignment with the fluid flow in which it is immersed and provide reliable results.

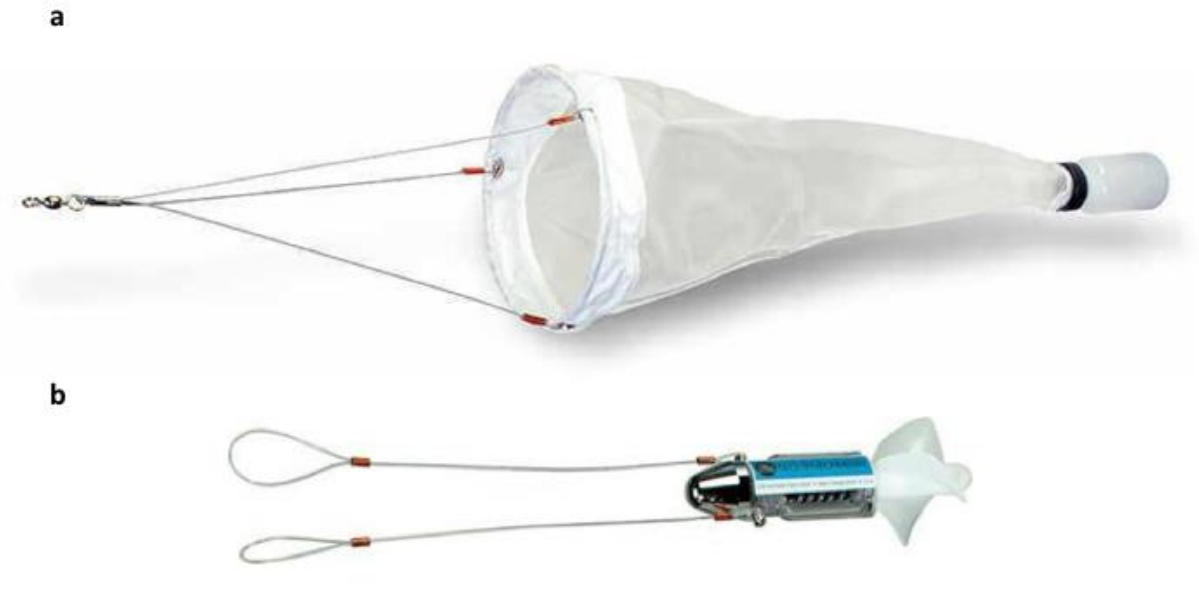


Figure.8 Conical Plankton net (a) and a General Oceanics flowmeter device (b)

## 3.2 Habitat Sampling

A total of 384 zooplankton tows were routinely collected every two consecutive weeks from June 2016 through to May 2018 completing two full 12-month cycle of sampling in the coastal waters of the El Mogote sandbar. Plankton tows were conducted at four predetermined sample stations, registered by GPS coordinates and encompassing the entire length of the sample area (Fig.9). Both surface and vertical tows were completed at each station with the net towed for five minutes behind the boat in a circular motion at approximately 3–4.5 km h−1 for surface tows and sampling of the water column by vertical tows, with the net, lowered at each sample station and manually raised at a constant speed.

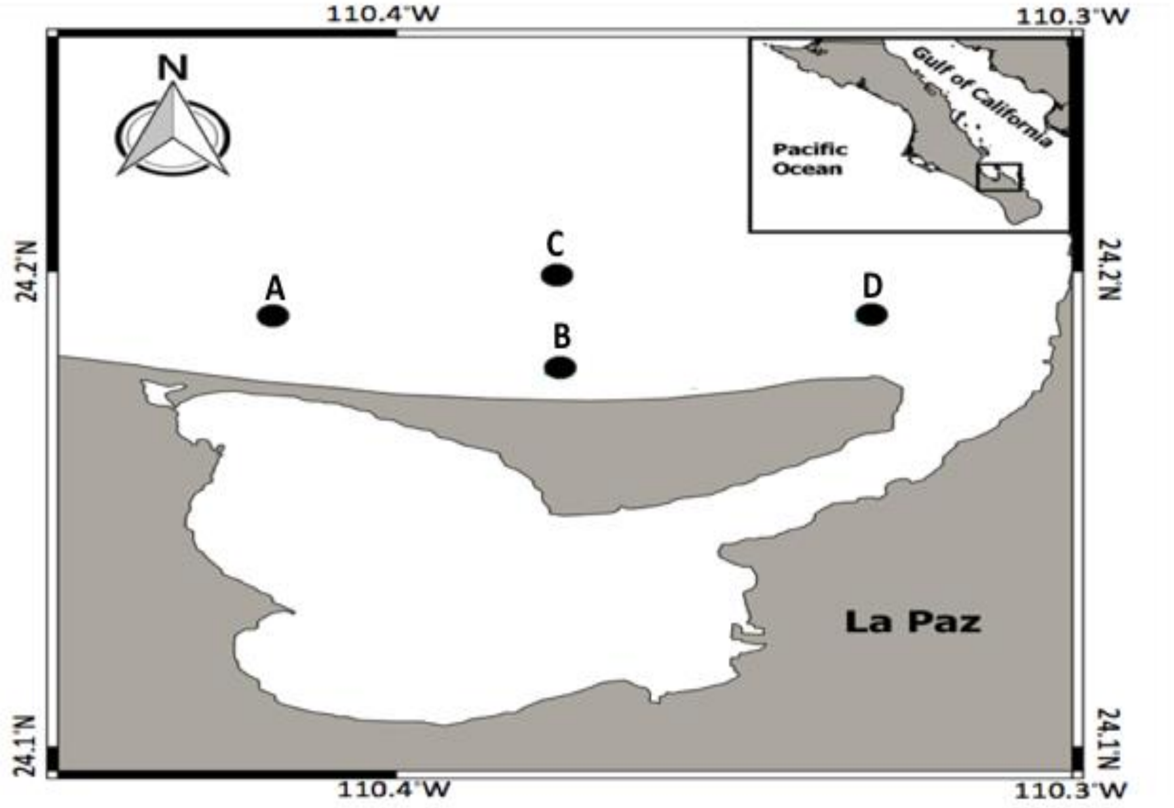


Figure.9 Study area showing sample stations (black circles) along the El Mogote sandbar

Upon the net being removed from the water the collected prey items were washed down into the collecting container at the end, fixed with 10ml of 4% buffed formalin solution, labeled for identification and stored in plastic screw-top containers. Simultaneously, during each tow information was recorded for: date, time, location (station identification), duration of the tow in minutes and a record of the flowmeter digits (initial and final). Also, information on the environmental conditions such as cloud cover over the sample area, sea state using the Beaufort scale and any recent extreme weather conditions were noted.

## 3.3 Volumetric Method

The total volume of wet zooplankton was determined by using the displacement method (Beers, 1976) and the wet biomass was standardized using the standard biomass formula and the records collected from the flowmeter instrument to 100 $m^{-3}$ (Smith & Richards, 1979). In this method, individual zooplankton samples were filtered once more through a piece of clean, dried netting material of the same mesh size as used by the net (505µm). Filtered zooplankton was then transferred with a spatula to a graduated cylinder along with a known volume of 4% buffered formalin solution. The displacement volume was obtained by recording the volume of fixative in the graduated cylinder displaced by the zooplankton (Beers, 1976) and ultimately standardizing the biomass to 100 $m^3$ (Smith & Richards, 1979). Total biomass was calculated for each calendar month for both sampling years and also divided into four climatic seasons: 1) Summer (June, July, August), 1) Autumn (September, October, November), 3) Winter (December, January, February) and 4) Spring (March , April, May) as a means to further investigate any seasonal differences.

## 3.4 Faunal Enumeration & Counting

Enumeration of organisms was performed by using a microscope and a grid-referenced petri dish. Given the larger number of samples collected, identification of the entire samples to species level would have been laborious and time-consuming. All zooplankton samples were stored in plastic containers and diluted to a known volume than using a stampel pipette a subsample was extracted. Zooplankton was grouped into eight taxonomic groups: Cladocera, Copepoda, Chaetognatha, Euphausiidae, Hydrozoa, Decapoda, Fish larvae and “Other”. The counting of individuals within each group was performed by using a tally mark technique on a data sheet then the total numbers of specimens in the sub-samples were later calculated to estimate the total faunal enumeration for the whole sample.

## 3.5 Statistical Analysis

All statistical analysis was performed in the statistical program R-Studio using several packages developed for the platform (R Development Core Team, 2018). Accumulative biomass of zooplankton was determined for each sample and a Kruskal Wallis test was used to investigate differences between sample years and individual sample months as well between sample stations and seasons. Furthermore, Post Hoc Dunn tests using a conservative multiple comparison method based on the Bonferroni inequality were utilized to explain where variance exists and to which degree. Once more non-parametric statistics were used to investigate variance in zooplankton abundance and composition between sample years and climatic seasons as previously mentioned.

# 4. RESULTS

## *4.1 Annual Zooplankton Biomass*

An annual shift in the overall biomass of zooplankton was observed during both sample years and revealed a significant difference between total biomass and sample year (Kruskal-Wallis chi-squared = 26.65, *P* = <0.0001). During the first sample year (June 2016 - May 2017) the lowest zooplankton biomass was recorded in April (94.75 ml/100m$^3$), while the highest values were observed for January (422.27 ml/100m$^3$) followed by December (296.30 ml/100m$^3$). June also showed relatively high variation in total biomass with a cumulative total of (267.29 ml/100 m$^3$) (Fig.10). As for the second sample year (June 2017 - May 2018), the lowest zooplankton biomass was recorded in July (56.41 ml/100m$^3$), while the highest values were observed once more for January (268.56 ml/100m$^3$) followed by February (202.31 ml/100m$^3$) (Fig.10).

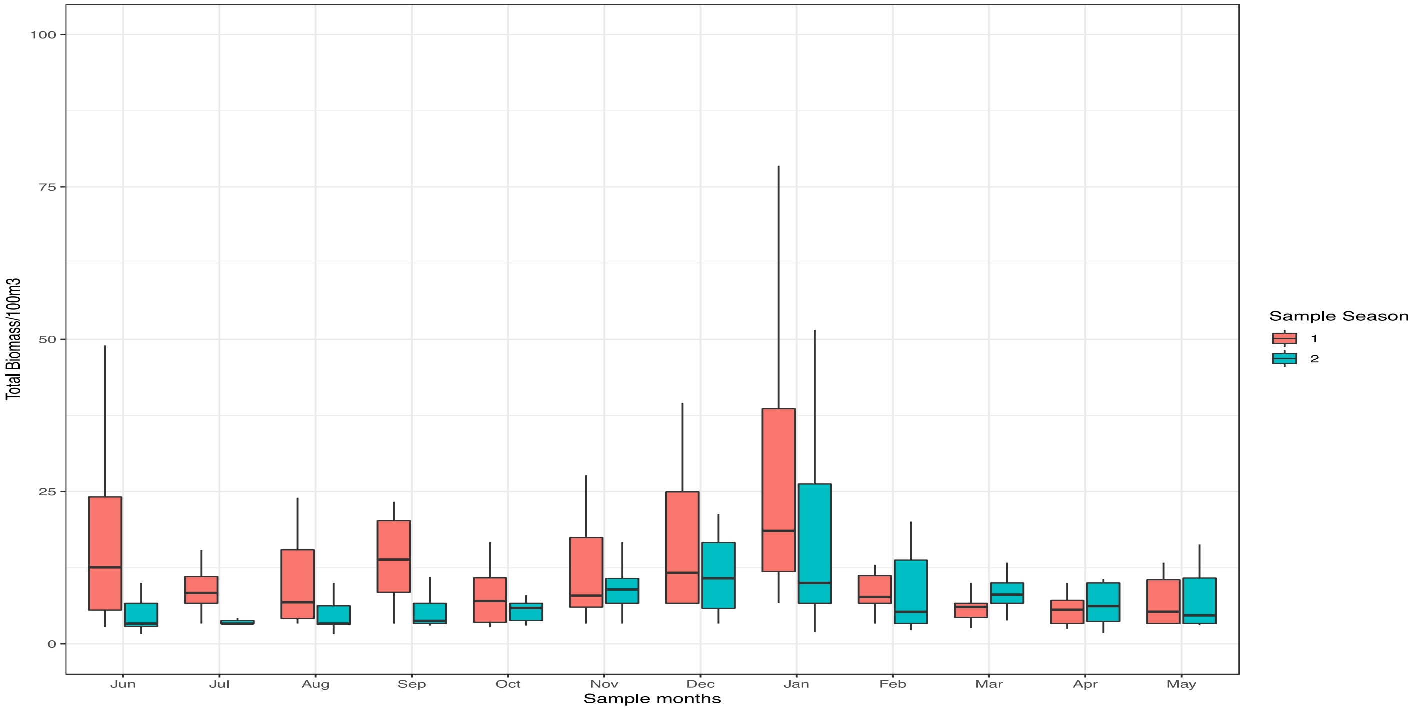
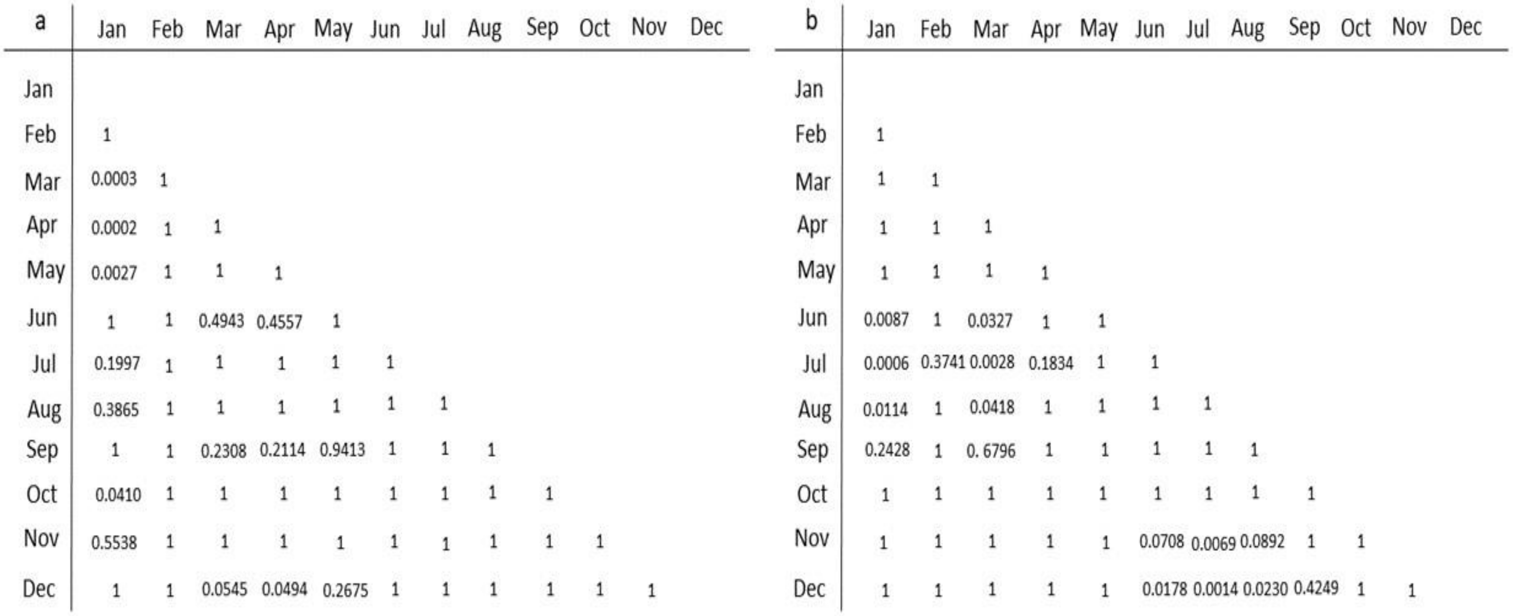


Figure. 10 Mean, max and min values of zooplankton biomass for each sample month during the two sample years

Non-parametric tests revealed a significant difference between total biomass of zooplankton and sample months within both sample year (Year 1: Kruskal-Wallis chi-squared = 42.09, *P* = <0.0001; Year 2: Kruskal-Wallis chi-squared = 51.48, *P* = <0.0001). A Post Hoc Dunn-Test was used using a conservative multiple comparison method based on the Bonferroni inequality explained that the apparent differences between months occurred mainly in January when compared to March, April May and October. December also showed a slight variance when compared to April during the first sample year (Table.1a). As for the second year, January showed significant variance to June, July and August, while March showed variance when compared to June, July and August. December also showed significant variance when compared to June, July, August and September (Table.1b).

Table. 1 Post Hoc Dunn-Test results for total biomass variation between sample months (adjusted p-values presented) (a) represents sample year 1 & (b) represents sample year 2



| a | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jan | | | | | | | | | | | | |
| Feb | 1 | | | | | | | | | | | |
| Mar | 0.0003 | 1 | | | | | | | | | | |
| Apr | 0.0002 | 1 | 1 | | | | | | | | | |
| May | 0.0027 | 1 | 1 | 1 | | | | | | | | |
| Jun | 1 | 1 | 0.4943 | 0.4557 | 1 | | | | | | | |
| Jul | 0.1997 | 1 | 1 | 1 | 1 | 1 | | | | | | |
| Aug | 0.3865 | 1 | 1 | 1 | 1 | 1 | 1 | | | | | |
| Sep | 1 | 1 | 0.2308 | 0.2114 | 0.9413 | 1 | 1 | 1 | | | | |
| Oct | 0.0410 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | |
| Nov | 0.5538 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | |
| Dec | 1 | 1 | 0.0545 | 0.0494 | 0.2675 | 1 | 1 | 1 | 1 | 1 | 1 | |

| b | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Jan | | | | | | | | | | | | |
| Feb | 1 | | | | | | | | | | | |
| Mar | 1 | 1 | | | | | | | | | | |
| Apr | 1 | 1 | 1 | | | | | | | | | |
| May | 1 | 1 | 1 | 1 | | | | | | | | |
| Jun | 0.0087 | 1 | 0.0327 | 1 | 1 | | | | | | | |
| Jul | 0.0006 | 0.3741 | 0.0028 | 0.1834 | 1 | 1 | | | | | | |
| Aug | 0.0114 | 1 | 0.0418 | 1 | 1 | 1 | 1 | | | | | |
| Sep | 0.2428 | 1 | 0. 6796 | 1 | 1 | 1 | 1 | 1 | | | | |
| Oct | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | | | |
| Nov | 1 | 1 | 1 | 1 | 1 | 0.0708 | 0.0069 | 0.0892 | 1 | 1 | | |
| Dec | 1 | 1 | 1 | 1 | 1 | 0.0178 | 0.0014 | 0.0230 | 0.4249 | 1 | 1 | |

Total biomass at each sample station for the first sample year revealed that station D had the highest cumulative annual biomass (815.83 ml/100m$^3$) while station A showed the least abundance during the sample period (486.19 ml/100m$^3$) (Fig.11). As for the second sample year station C showed the highest accumulative annual biomass (410.27 ml/100m$^3$), whereas station B showed the lowest abundance (358.12 ml/100m$^3$) (Fig.11). A slight significant difference was observed between annual biomass at the different sample stations during the first sample year (Kruskal-Wallis chi-squared = 8.15, *P* = 0.04295) but not for the second sample year (Kruskal-Wallis chi-squared = 0.45, *P* = 0.929).

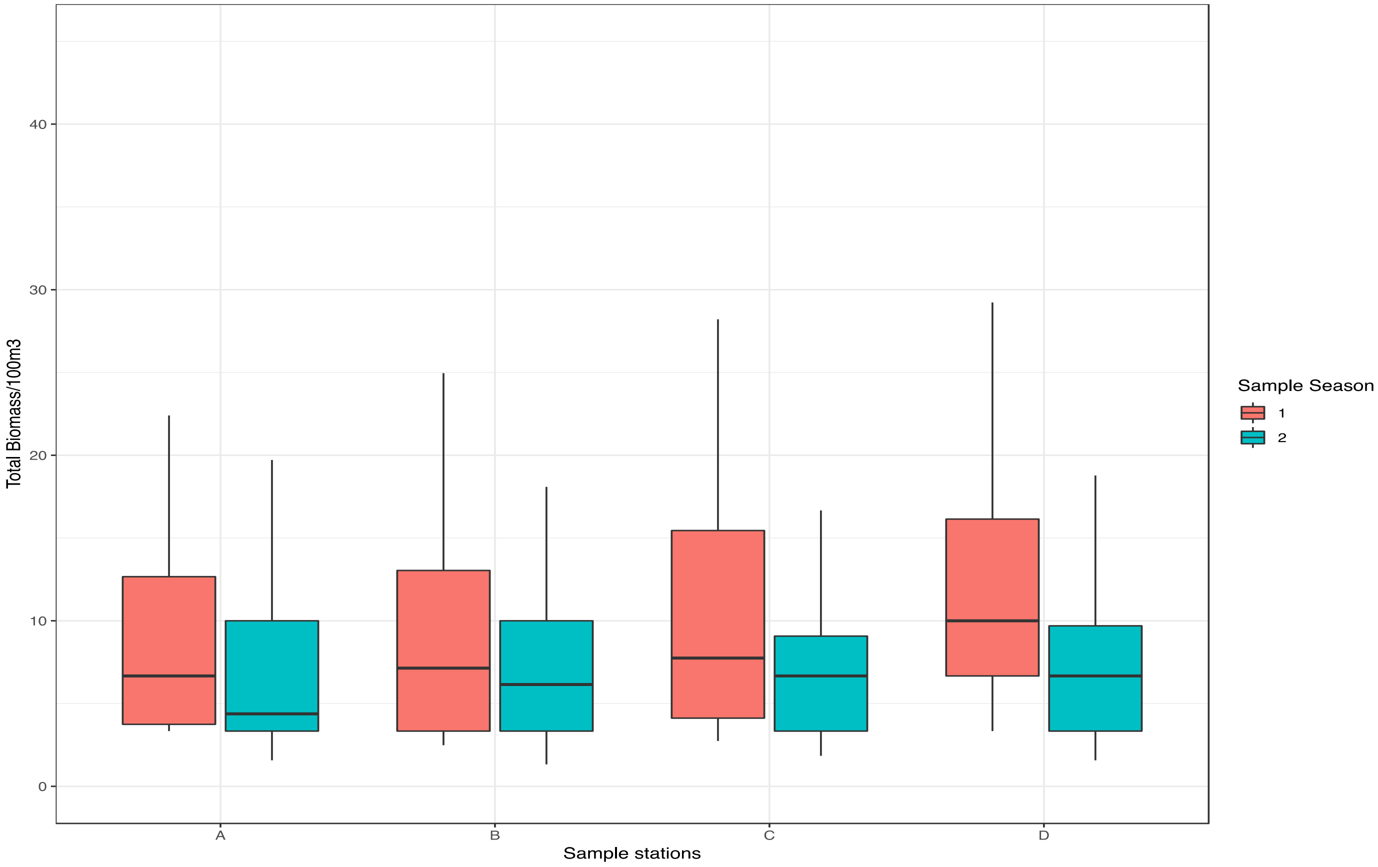
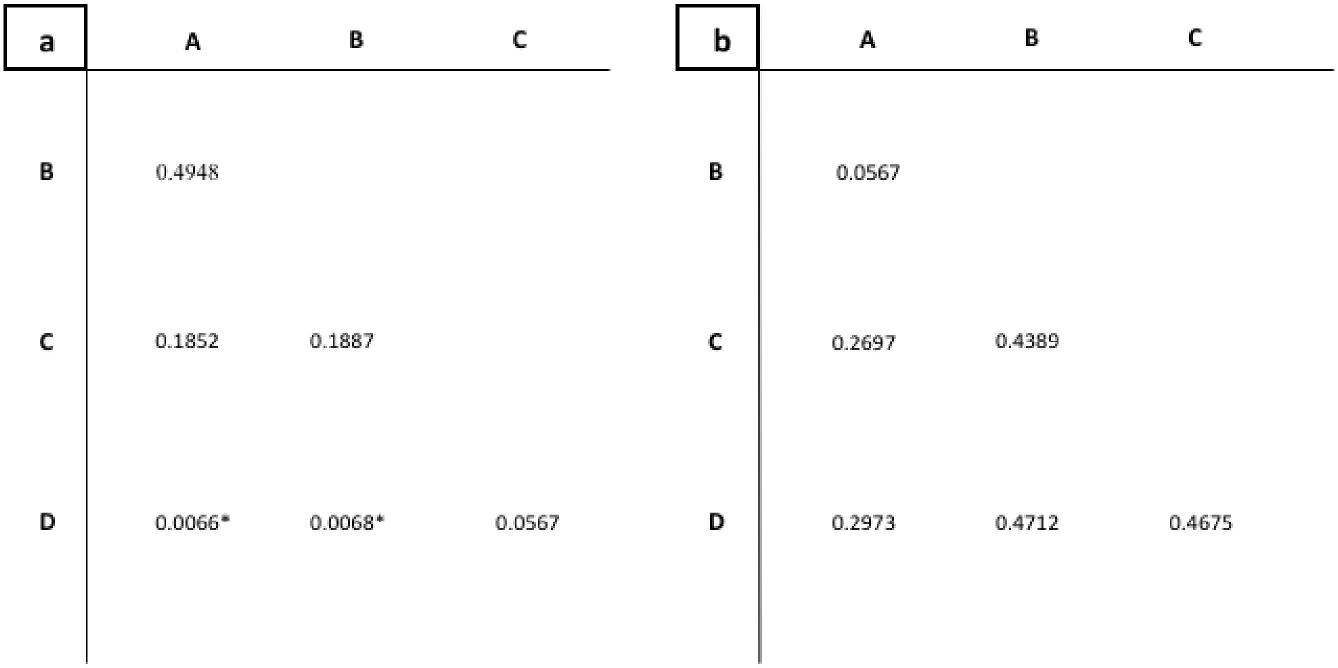


Figure.11. Mean, max and min values of zooplankton biomass for each sample station during the two sample years

Results from the Post hoc Dunn test showed that the difference in biomass was observed between station A and B when compared to station D but not for station C for the first sample year (Table.2a). As for the second year of sampling no significant difference was present when any of the stations were compared to one another (Table.2b).

Table. 2 Post Hoc Dunn-Test results for total biomass variation between sample stations (p-values presented) (a) represents sample year 1 & (b) represents sample year 2



| a | A | B | C |
|---|---|---|---|
| B | 0.4948 | | |
| C | 0.1852 | 0.1887 | |
| D | 0.0066* | 0.0068* | 0.0567 |

| b | A | B | C |
|---|---|---|---|
| B | 0.0567 | | |
| C | 0.2697 | 0.4389 | |
| D | 0.2973 | 0.4712 | 0.4675 |

### *4.2 Zooplankton Groups & Composition*

Both sample years showed different concentrations of zooplanktonic prey item and collectively in all samples (*Year 1* + *Year 2*) the taxonomic group copepoda presented the highest number of individuals (800,320), followed by chaetognatha (311,425), with the lowest number of individuals belonging to the taxonomic group cladocera (62,525). The number of individuals per sample year demonstrated that within the first sample year the highest number of individuals belonged to copepoda (311,710) followed by chaetognatha (177,995), with the lowest number of individuals belonging to the taxonomic group other (30,250) (Fig.12). As for the second year, results revealed that the highest density of individuals belonged to the group copedoda (488,610) followed by chaetognatha (133,470), with the lowest number of individuals belonging to the taxonomic group cladocera (15,420) (Fig.12).

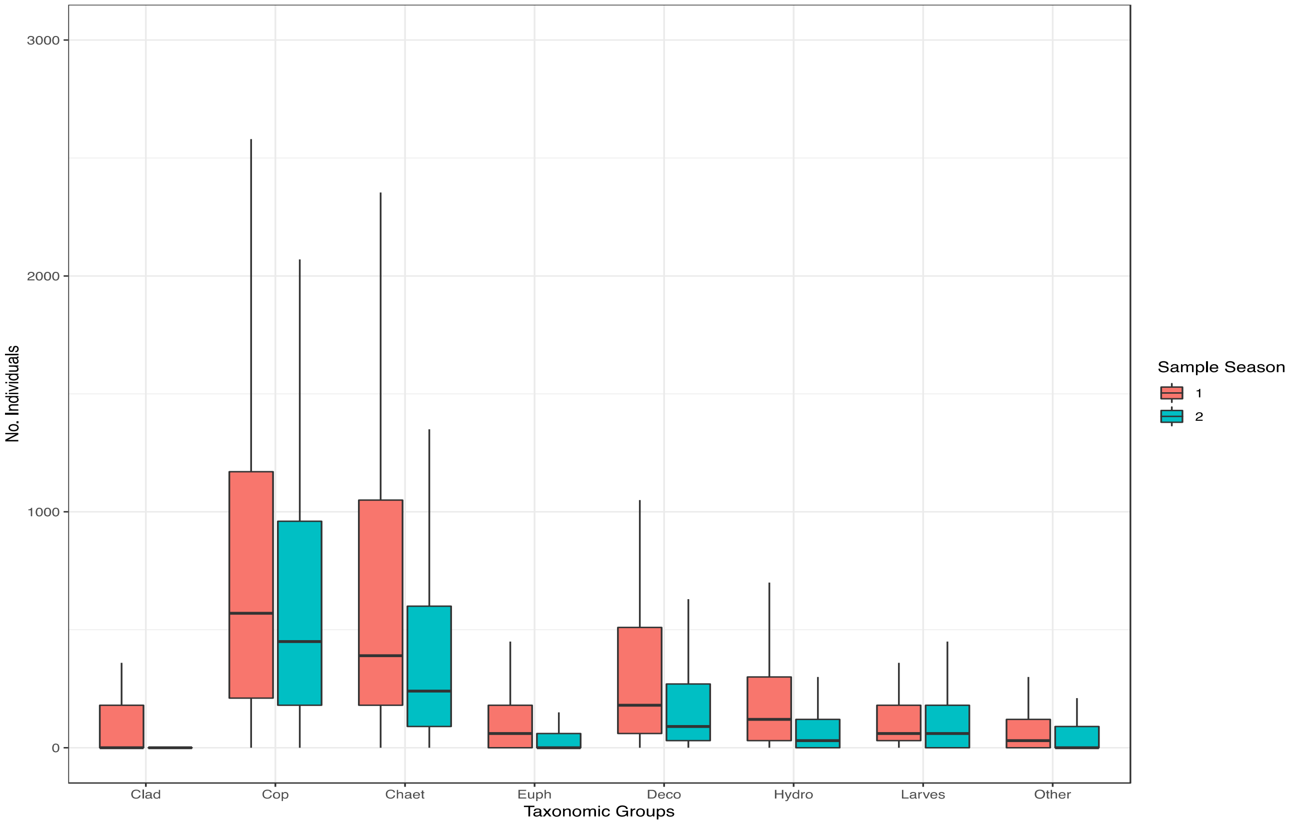


Figure.12. Mean, max and min values of the total number of individuals per taxonomic group during both sample years

Overall composition within the first sample year was largely dominated by three main taxonomic groups: copepoda (40.55%), chaetognatha (23.15%), decapoda (11.23%), with the remaining five groups showing no significant outliners (Table.3). As for the second year the same three groups remained dominant: copepoda (53.19%), chaetognatha (14.53%), decapoda (11.31%) (Table.3). In addition, the relative abundance of zooplankton groups showed variation during monthly samples within both sample years, demonstrating that all taxonomic groups do show variation during the year but was most apparent within certain groups such as copepoda during the winter months compared to the summer and cladocera during the summer compared to the winter. The taxonomic group euphausiidae did show a sudden increase during the month of February during the second sample year compared to the rest of the sample months (Fig.13).

Table.3. Percentage of individuals per taxonomic group during each sample year

| Year | Cladacera | Copepoda | Chaetognatha | Decapoda | Euphausiidae | Hydrozoa | Larvae | Other |
|---|---|---|---|---|---|---|---|---|
| **1** | 6.71% | 40.55% | 23.15% | 11.23% | 4.31% | 6.12% | 3.93% | 3.96% |
| **2** | 2.03% | 53.19% | 14.53% | 11.31% | 4.58% | 1.67% | 5.24% | 7.41% |

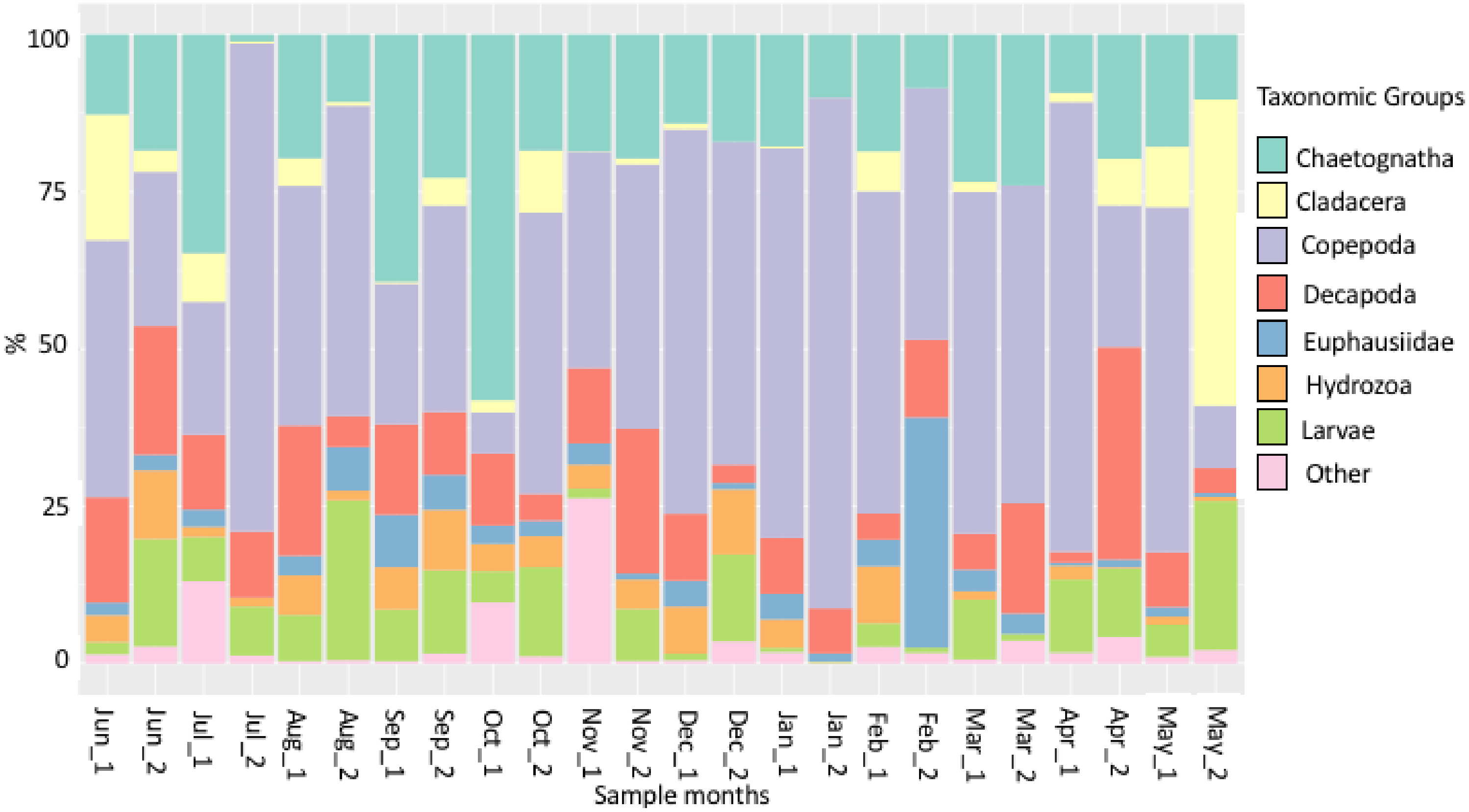


Figure.13. Relative abundance (100%) of individuals within each taxonomic group during sample months during both sample years (1 & 2)

## 4.3 Zooplankton Biomass for Climatic Seasons

A seasonal shift in the overall biomass of zooplankton was observed during the first sample year with the lowest zooplankton biomass recorded in spring (176.28 ml 100m$^{-3}$), 10.13% of the total biomass, while the highest values were observed for winter (703.04 ml 100m$^{-3}$), 40.40% of the total biomass, followed by summer (493.92 ml 100m$^{-3}$), 28.38% of the total biomass and autumn (366.57 ml 100m$^{-3}$), 21.06% of the total biomass (Fig.14a). As for the second sample year, zooplankton biomass was lowest in summer (82.36 ml 100m$^{-3}$),10.43% of the total biomass, while the highest values observed for Winter (411.83 ml 100m$^{-3}$) 52.16% of the total biomass, followed by Spring (196.27 ml 100m$^{-3}$) 24.85% of the total biomass, then Autumn (99.07 ml 100m$^{-3}$) 12.54% of the total biomass (Fig.14a).

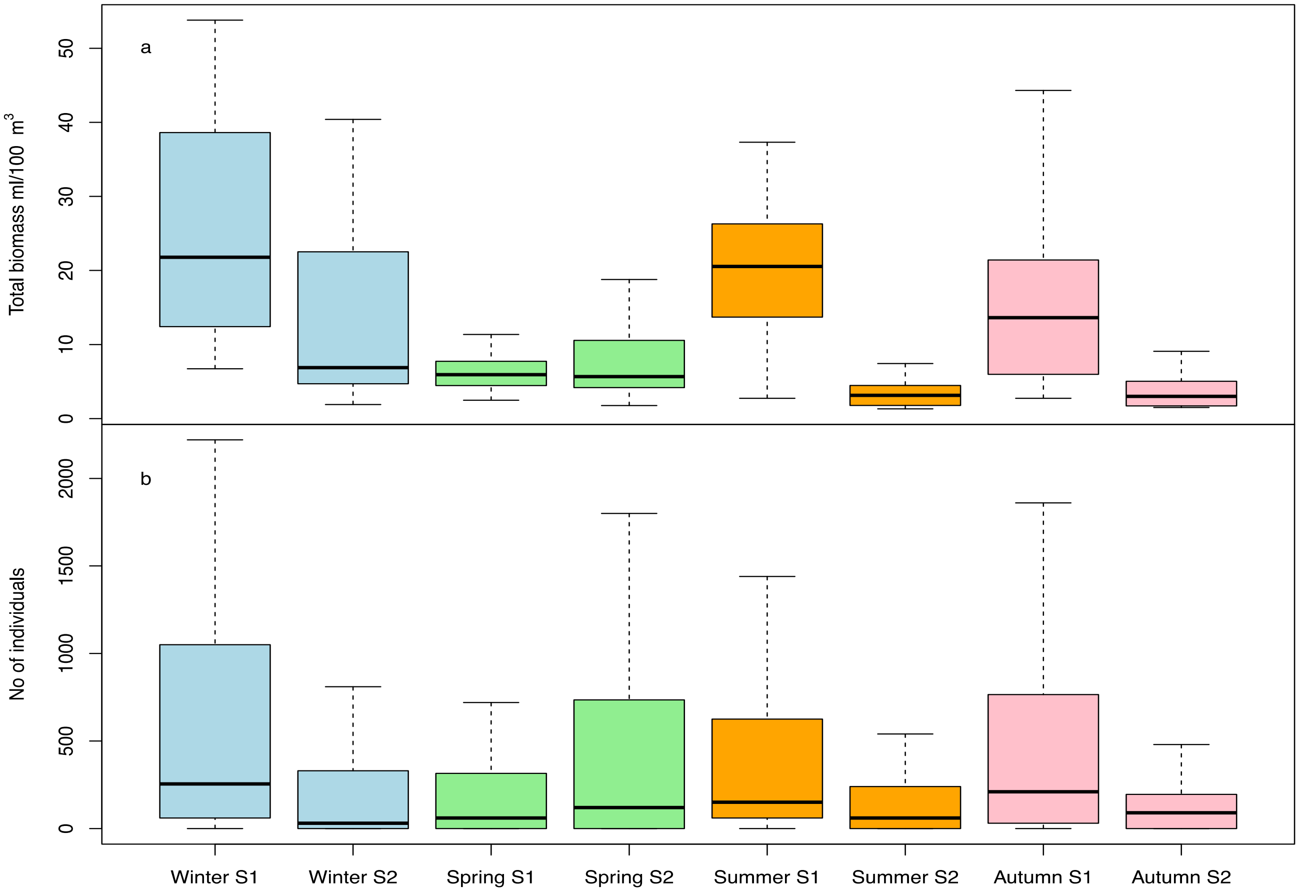
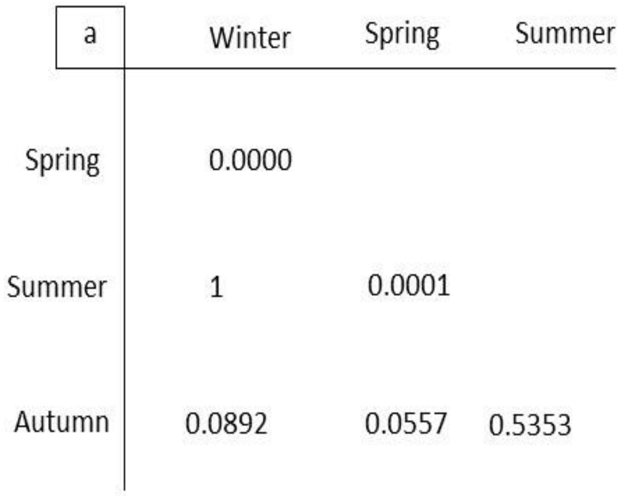


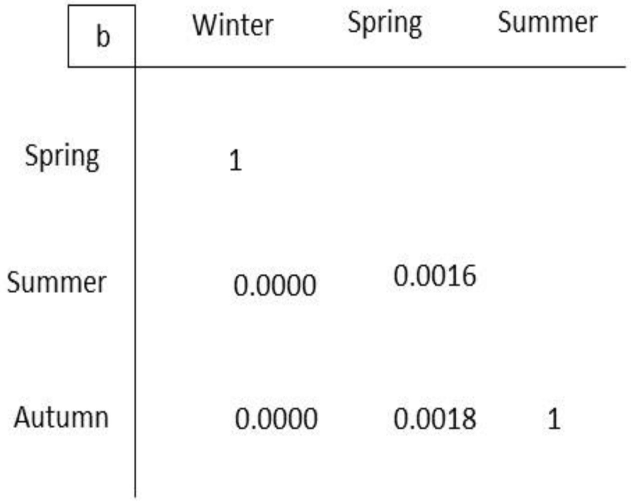
Figure.14. (a) Biomass of zooplankton (ml 100m$^{3}$) for each season during both sample years (b) Total number of individuals (sum of the eight taxonomic groups) between sample years

A Kruskal-Wallis one-way analysis of variance test did show a statistically significant difference in total biomass between the different sample years (Kruskal-Wallis chi-squared = 43.7, *P* = <0.0001) and also between climatic seasons during each sample period (*Year 1*= Kruskal-Wallis chi-squared = 29.993, *P* = <0.0001; *Year 2*= Kruskal-Wallis chi-squared = 32.2, *P* = <0.0001). A Post Hoc Dunn-Test using the Bonferroni correction showed significant variation between winter when compared to spring and summer when compared to spring for the first sample year (Table.4a). As for the second sample year, significant variation between almost all climatic seasons except for summer when compared to autumn and winter when compared to Spring (Table.4b).

Table. 4 Post Hoc Dunn-Test results for total biomass variation between climatic seasons (p-values presented) (a) represents sample season 1 & (b) represents sample season 2



| a | Winter | Spring | Summer |
|---|---|---|---|
| Spring | 0.0000 | | |
| Summer | 1 | 0.0001 | |
| Autumn | 0.0892 | 0.0557 | 0.5353 |



| b | Winter | Spring | Summer |
|---|---|---|---|
| Spring | 1 | | |
| Summer | 0.0000 | 0.0016 | |
| Autumn | 0.0000 | 0.0018 | 1 |

### *4.4 Zooplankton Groups & Composition through Climatic Seasons*

The total number of individuals (sum of the eight taxonomic groups) between sample years showed significant differences (Kruskal-Wallis chi-squared = 48.43, *P* = <0.0001). During the first sample year the highest number of individuals (sum of the eight taxonomic groups) was recorded in winter (237,200), accounting for 41.1% of the total amount of individuals, followed by autumn (128,280), 22.3% of individuals, summer (109,888), 19.1% of individuals, and spring (100,000), 17.4% of individuals (Table.5, Fig. 14b). As for the second sample year the highest concentration of individuals was recorded in winter (404,970), 57.59% of individuals, followed by spring (151,080), 21.48% of individuals, summer (88,620), 12.60% of individuals and autumn (58,410), 8.30% of individuals (Table.5, Fig. 14b). Non parametric tests showed that there was a significance difference in the total number of individuals between climatic seasons during both sample years (*Year 1*= Kruskal-Wallis chi-squared = 29.215, *P* = <0.0001; *Year 2*= Kruskal-Wallis chi-squared = 9.11, *P* = 0.02781).

Table.5. Percentage of the sum of individuals (all taxonomic groups) during each climatic season

| Year | Winter | Spring | Summer | Autumn |
|---|---|---|---|---|
| **1** | 41.10% | 17.44% | 19.16% | 22.30% |
| **2** | 57.59% | 21.48% | 12.60% | 8.30% |

A significant difference was observed between taxonomic groups in first sample year (Kruskal-Wallis chi-squared = 243.56, *P* = <0.0001) and also for the second sample year (Kruskal-Wallis chi-squared = 229.17, *P* = <0.0001). In spring during the first sample year, the total number of individuals between taxonomic groups was significantly different (Kruskal-Wallis chi-squared = 71.045, *P* = 9.083e$^{-13}$), with dominant taxonomic group being copepoda (58,720, 58.72%), followed by chaetognatha (17,640, 17.64%) and Larvae (8,160, 8.16%) (Fig.15). In summer, the total number of individuals between taxonomic groups was also significantly different (Kruskal-Wallis chi-squared = 69.98, *P* = 1.491e$^{-12}$). Copepoda (40,141, 36.52%) remained as the dominant taxonomic group followed by chaetognatha (20,702, 18.83%), decapoda (18,879, 17.18%) and cladocera (14,021, 12.75%) (Fig. 15). In autumn, the total number of individuals between taxonomic groups was also significantly different (Kruskal-Wallis chi-squared = 72.352, *P* = 4.94e$^{-13}$) revealing chaetognatha (50,040, 39%) to be the dominant taxonomic group followed by copepoda (27,030, 21.07%) and decapoda (16,470, 12.83%) (Fig. 15). Finally, in winter, the total number of individuals between taxonomic groups was again significantly different (Kruskal-Wallis chi-squared = 98.083, *P* = 2.2e$^{-16}$) with the dominant taxonomic group being copepoda (141,110, 59.48%) followed by chaetognata (38,910, 16.40%) and decapoda (20,700, 8.72%) (Fig. 15).

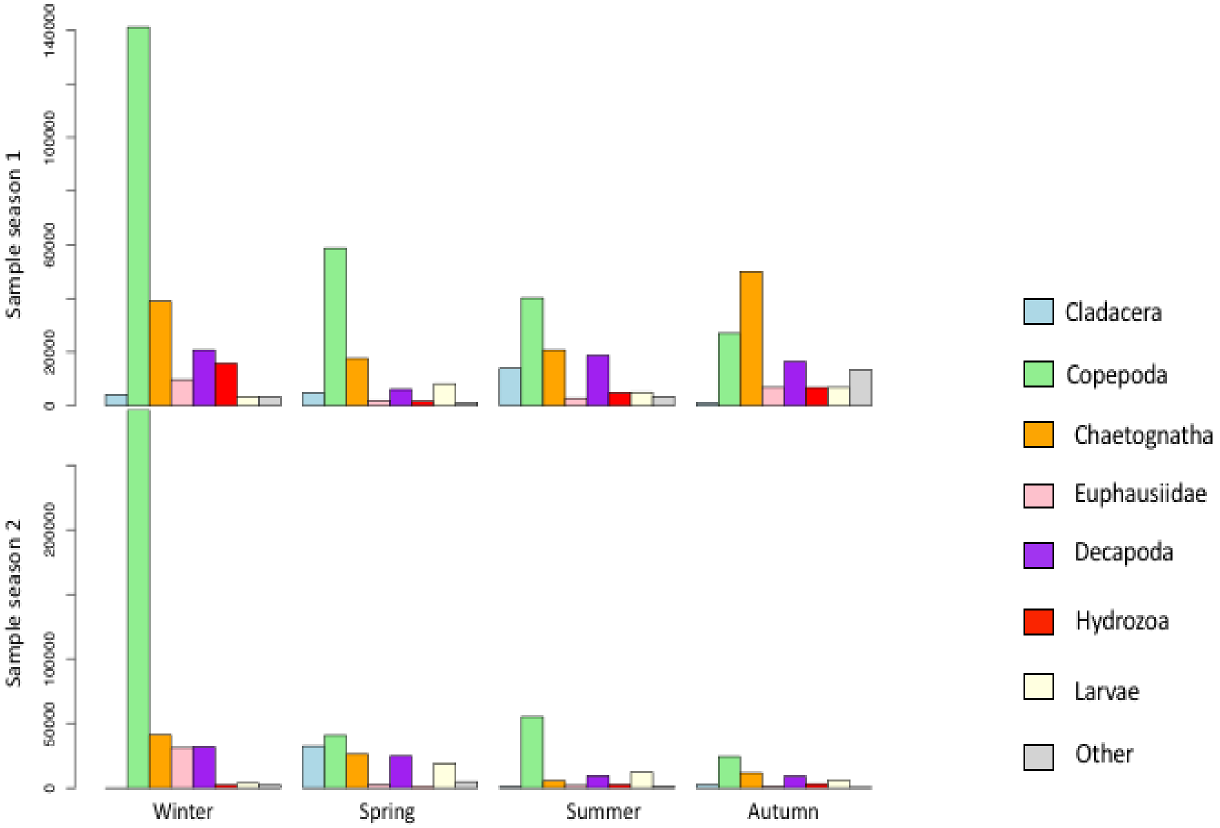


Figure 15. Number of individuals per taxonomic group per climatic season in the waters off the el Mogote sandbar in Bahía de La Paz

As for the second sample year during the spring, the total number of individuals between taxonomic groups was significantly different (Kruskal-Wallis chi-squared = 68.27, $P = 3.296e^{-12}$), with dominant taxonomic group being copepoda (40,650, 26.90%), followed by cladocera (32,370, 21.42%), then chaetognatha (26,310, 17.41%) (Fig. 15). In summer, the total number of individuals between taxonomic groups was also significantly different (Kruskal-Wallis chi-squared = 61.14, $P = 8.917e^{-11}$). Copepoda (55,170, 62.25%) remained as the dominant taxonomic group followed by Larvae (12,210, 13.77%) then Decapoda (9330, 10.52%) (Fig. 15). In autumn, the total number of individuals between taxonomic groups was also significantly different (Kruskal-Wallis chi-squared = 76.28, *P* = $7.885e^{-14}$) revealing copepoda (24,360, 41.70%) to be the dominant taxonomic group followed by chaetognatha (11.520, 19.72%) and decapoda (9030, 15.45%) (Fig. 15). Finally, in winter, the total number of individuals between taxonomic groups was again significantly different (Kruskal-Wallis chi-squared = 76.28, *P* = $7.855e^{-14}$) with the dominant taxonomic group being copepoda (293,100, 72.37%) followed by chaetognata (40,950, 10.11%) and decapoda (31,800, 7.85%) (Fig. 15).

# 5. DISCUSSION

## 5.1 Biomass

Monthly shifts in the total biomass of zooplankton was observed in our data (Fig.10), revealing that the largest accumulation of zooplankton biomass in the coastal waters off the El Mogote occurs in the month of January for both sample seasons and lowest concentrations in April and July respectively. Also, seasonal shifts in the total biomass per climatic season were exposed revealing clear fluctuations of cumulative biomass within the different climatic seasons, with the winter months showing the highest concentrations (Fig.14a). An early study by Jiménez-Pérez and Lara-Lara. (1988) examined zooplankton biomass in the GC and their observations showed that this region seems to present two unique community assemblages that may be related to different environmental factors caused by the shifting of climatic seasons that drive productivity in the entire region. Another study focused on the assemblage of fish larvae in this region expressed that the variations of different species of fish larvae present during different months appears to be related to a strong water exchange with the Gulf (Sanchez-Velasco *et al.,* 2006). Results showed that during the months July – October the geostrophic flow through the entrance of the bay is intensified resulting in the distribution of oceanic prey items across the entire bay, whereas during February–May when the geostrophic conveyance is weak, distribution of the coastal assemblage is dispersed over the whole bay (Sanchez-Velasco *et al.,* 2006). The overall circulation of surface water within BLP is known to be driven by a central mesoscale cyclonic eddy and may be highly influential in the accumulation of biomass during certain times of the year, given the changing intensities in its effect and orientation (Duran-Campos *et al.,* 2015).

Total biomass at our predetermined sample stations did show a slight variation in the first sample year, but given the low significance (p=0.04295) it is difficult to conclude that over a small sample area of less than 12 km, there is differences in the accumulation of biomass. One explanation may be that the there is a high possibility of variation between any two tows at any site, and that this in itself may be masquerading as a pattern of variation between sample stations observed in our first sample year, as it was not observed in the second sample year. The absence of replicate tows during the design of this study adds further doubt that there is clear variation of biomass between sample stations and future studies should consider this approach to confirm the accumulation of biomass at certain parts of the El Mogote sandbar. Although no previous work has been conducted in this specific area using multiple sample stations, work by Hacohen-Domené *et al.* (2006) did observe high densities of biomass at a similar station along the El Mogote sandbar during the same time, compared to other sample stations in other regions of the bay, demonstrating that the El Mogote area has high biomass accumulation.

Aggregations of whale sharks within the El Mogote area seem to be seasonal and have been documented to occur for the most part during the winter months (Ramírez-Macías *et al.,* 2012; Whitehead *et al.,* 2019), seemingly to be related to the availability of planktonic food (Hacohen-Domené *et al.*, 2006; Ketchum *et al.,* 2013). Juvenile whale and their correlation with the abundance of available food sources has also been observed in Bahía de Los Angeles in the autumn months (Nelson & Eckert, 2007) and the Gulf of Mexico in the summer (Hoffmayer *et al.,* 2007; Motta *et al.,* 2010). In numerous other regions, aggregations of whale sharks have also been linked to a sudden increase in zooplankton biomass or fish spawning events at certain times of the year such as in the Seychelles (Rowat *et al.,* 2011), Belize (Heyman *et al.,* 2001), Australia (Meekan *et al.,* 2009) Tanzania (Rohner *et al.,* 2015), Qatar (Robinson *et al.,* 2012) as well as the Mexican Caribbean (De la Parra *et al.,* 2011; Cardenas-Palomo *et al.,* 2014). An early study by Ketchum (2003), proposed that the populations of whale sharks in BLP may be highly influenced by seasonal and inter-annual changes and anomalies of oceanographic patterns in the GC, such as El Niño years and annual hurricanes in the region. Previous work in the GC (Jiménez-Pérez & Lara-Lara, 1988) did show that the effects of shifting between seasons may cause spikes in the productivity in the entire region causing blooms of planktonic food and creating a reliable seasonal food source for visiting sharks.

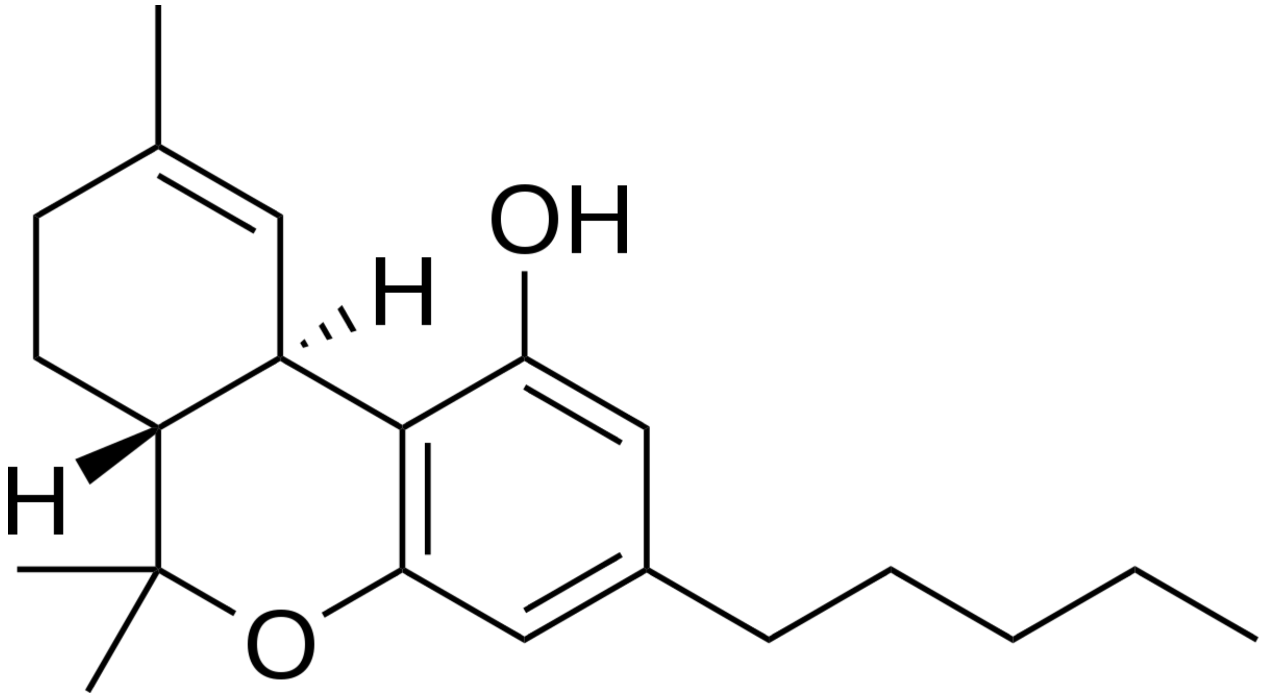
### *5.2 Composition*

Zooplankton composition in our study showed an overall dominance to members of the copepod family (Fig.12). Absolute values of these individuals did show a significant difference throughout climatic seasons with a peak in the number of individuals recorded in the winter months coinciding with the highest biomass of zooplankton as a whole. Similar work on zooplankton and the foraging ecology of whale sharks in BLP have documented high concentrations of copepods during the autumn and winter months (Palomares-García, 1996; Clark & Nelson, 1997; Hacohen-Domené *et al.,* 2006; Ketchum *et al.,* 2013). With observations of juvenile whale sharks foraging on dense patches of zooplankton comprising of more than 90% copepods in the southern regions of the bay near San Juan de la Costa (Clark & Nelson, 1997), and concentrations of up to 80 % copepods in the waters in front of the El Mogote (Hacohen-Domené *et al.,* 2006) similar to our results. In the northern regions of the GC such as Bahía de Los Angeles, the composition of zooplankton, also reported a predominance of copepods, especially in areas where whale sharks were sighted (Lavaniegos *et al.,* 2012; Hernández-Nava & Álvarez-Borrego, 2013). Daily or monthly changes in prey items within BLP and around the coastal waters off the El Mogote seems to be is highly influenced by its connectivity with water from the gulf itself and changes of environmental conditions, which has been shown to be the main driving factor motivating water exchange (Monreal-Gomez *et al.,* 2001).

Historical reports from aggregations in the upper gulf (Nelson & Eckert, 2007; Hernández-Nava & Álvarez-Borrego, 2013) and BLP (Hacohen-Domené *et al.,* 2006; Ramirez-Marcia *et al.,* 2012; Ketchum *et al.,* 2013; Ramirez-Marcia *et al.,* 2017; Whitehead *et al.,* 2019) together with our current study strongly support the perception that the presence of whale sharks is highly related to the density of copepod species available, and that these planktonic organisms play an important role in the dietary preference of whale sharks in the entire GC.

Ultimately, the presence of juvenile whale sharks coupled with the occurrence of an abundant food supply off the coastal waters of the El Mogote sandbar provides a much-needed refuge for the development and fast growth of juvenile sharks (Wintner, 2000; Ketchum *et al.,* 2013). Now as whale shark-related tourism is on the increase in this specific area, it is vital that continued work to monitor and provide good management of this ever-growing industry alongside side scientific research may help to understand the influences seasonal climatic changes have on the availability of food and its visiting sharks. In conclusion, this chapter provides the first multiple annual sampling focused exclusively in the El Mogote area, and exposing that there is a clear fluctuation in zooplankton biomass and communities concerning shifting environmental conditions and ultimately a solid baseline of information for this important aggregation area for future work on whale shark dietary items.

# CHAPTER 2



## ISOTOPIC ASSEMENT OF TISSUES IN WHALE SHARKS

## IN THE COASTAL WATER OF THE EL MOGOTE, BAHIA DE LA PAZ

# TABLE OF CONTENT

# 1. THEORETICAL BACKGROUND

Understanding the dietary preferences of any marine species has historically relied on stomach content analysis as the preferred method of investigation for marine ecologists. This process often requires the death of the specimen and to some degree relatively large sample sizes which is problematic, given that many species that are captured have empty stomachs. This obstacle may be due to either (i) a result of natural feeding behavior or (ii) the regurgitation of the stomach contents due to capture stress responses. Work on stomach content analysis can often lead to additional complications, especially when working with marine fauna that are protected on a national or international level, prohibiting the fishing or capture of the species. Leaving the availability of samples existing only through "by-catch" in fisheries, which in whale sharks is very rare. Observing the feeding behaviors of any marine species in their natural habitat and understanding how intricate food webs relate to one another can similarly be difficult, due to the numerous types of predator-prey relationships that overlap on multiple levels in any food chain, in all marine ecosystems.

Stable-isotope analysis (SIA) is an alternative, cost-effective and relatively non-invasive method, which can be used to investigate the foraging ecology, migratory movements and role of any species in its relative ecosystem (Kim *et al.,* 2011; Hussey *et al.,* 2012). This method of sampling removes the need to slaughter specimens to gain dietary information and also offers a more comprehensive long-term look into an animals' food preferences and therefore avoids the 'snapshot' bias associated with stomach content analysis. SIA can also be more tolerant to reasonably smaller samples sizes allowing for the study of species that are often challenging to interact within their natural habitat or limited in numbers in the wild due to the uncertainties of extinction. This biogeochemical technique exposes an organism's diet and movement over a period of time depending on the incorporation rate of the analyzed tissue and its isotope turnover rate (Vander Zanden *et al.,* 1997; Graham *et al.,* 2010; Hussey *et al.,* 2010; Wyatt *et al.,* 2019). Throughout the metabolic processes, an organism's tissues are enriched relative to their specific diet by ~1‰ for $\delta^{13}C$ and ~3 to 4‰ for $\delta^{15}N$ per trophic level (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Hussey *et al.,* 2010). $\delta^{13}C$ provides clear information on marine vertebrates foraging habitats throughout a shore-offshore gradient (Rau *et al.,* 1990; Rubenstein and Hobson, 2004), while $\delta^{15}N$ is a reliable tool to calculate trophic positions of organisms within food webs (Hussey *et al.,* 2012). In addition, there are $\delta^{13}C$ and $\delta^{15}N$ variations throughout different geographic areas controlled by factors related to primary production at the base of the food web (Graham *et al.,* 2010; Kim *et al*., 2012). Stable isotope analysis provides us with a true representation of how nutritional sources from all levels of the food chain are being transported and can be used to provide a time-integrated description of trophic relationships (Hussey *et al.,* 2010, 2012). The influence of diet with regards to the distribution of nitrogen and carbon isotopes in the tissues of animals, indicates that the isotopic composition of an organism may be determined by the isotopic value of the food consumed (Hussey *et al.,* 2010; Kim *et al.,*2012; Wyatt *et al.,* 2019).

Application of SIA is based on the assumption that the isotopic composition of a consumer's tissue is $^{13}C$ and $^{15}N$ enriched, relative to its prey, due to the differential excretion of the lighter isotope ($^{12}C$ and $^{14}N$) by the consumer (Peterson & Fry, 1987; Vander Zanden *et al*., 1997). Nitrogen values can also provide information on the depth at which the species may forage (epipelagic, benthic or mesopelagic); while the levels of carbon present in the organism's tissue indicates the relative contribution of different potential primary sources in the food web, allowing differentiation between coastal and oceanic zones (Rau *et al.,* 1990; Martínez del Rio *et al.,* 2009). For the presentation of isotopic data, results are generally expressed according to the delta (δ) notation in terms of parts per thousand (‰) deviation from the standard formula by Park and Epstein. (1961):

$$\delta X = \left\lfloor \frac{R\ sample}{R\ standard} - 1 \right\rfloor X\ 1000‰$$

where X is $^{13}C$ or $^{15}N$ and R is the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. R standard is the molecular ratio 45/44 ($^{13}C^{16}O^{16}O$ / $^{12}C^{16}O^{16}O$) of the sample or standard gas ($CO^{2}$)

for the determination of $\delta^{13}C$ in the mass spectrometer. Similarly, for the determination of $\delta^{15}N$, R standard is the molecular ratio 29/28 ($^{15}N^{14}N$ / $^{14}N^{14}N$) of the sample or standard gas ($N_2$).

However, researcher who may wish to venture into stable isotope application must be aware of the limitations that need to be considered with respect to the current knowledge of diet-tissue discrimination factors of different tissues, isotopic turnover rates and sample preparation methods, all of which may bias data providing inaccurate results (Kim et al., 2012; Marcus *et al.,* 2017; Prebble *et al.,* 2018; Wyatt *et al.,* 2019). The general assumption for applying stable-isotopes to ecological studies, particularly when focused on dietary preferences or trophic position is gaining sufficient knowledge of the stable isotope fractionation rates. Fractionation signifies the variation in the stable-isotope composition between a consumer and its diet and it is often referred to as a diet-tissue discrimination factor (DTDF) or a trophic discrimination factor. Understanding DTDF in sharks still presents great challenges due to the lack of available controlled studies (Hussey *et al.,* 2010; Kim *et al.,* 2012; Wyatt *et al.,*2019). Tissue turn-over rate on the other hand is the amount of time needed for a change in diet to be reflected in a consumer's tissues (Hussey et al., 2010, 2012; Wyatt *et al.,* 2019). The urgent need to conduct more experimental validation tests under controlled laboratory conditions is a necessity to help ecologists improve the overall knowledge of DTDF and tissue turn-over rates in a number of shark species (Hussey *et al.,* 2012; Kim *et al.,* 2012; Prebble *et al.,* 2018; Wyatt *et al.,* 2019). Post. (2002) estimated that the carbon discriminator factor for most teleost fishes (bony fishes) to be 0.461.3‰, respectively, while Hussey et al. (2010) found a mean discrimination factor of 0.960.33‰ based on data from a number of elasmobranch species. A study by Kim et al. (2012), developed a controlled feeding study of six Leopard sharks (*T. semifasciata)* that were given a constant diet of a single prey item, squid *Luligo opalescens*, for more than 1000 consecutive days. The results did indicate a higher $^{15}N$ and $^{13}C$ values for elasmobranch muscle tissue and found a discriminating factor of 1.7% for the leopard sharks, which seemed to coincide with previous work by Hussey et al. (2012), revealing that isotope values can vary between species and also different tissue types. However, the authors conclude that the relatively high value observed in the tissues *T. semifasciata* may be biased by the controlled protein-rich diet the sharks were fed on, which may potentially lead to an enriched δ$^{13}$C signature.

The use of Stable isotope analysis on the feeding ecology of sharks and rays has been conducted for a number of species such as: leopard sharks (Kim *et al.,* 2012), megamouth shark (Fulgencio de Moura *et al.,* 2015), bull sharks (Hussey *et al.,* 2010), basking sharks (Estrada *et al.,* 2003), blue-spotted stingray (Burgess *et al.,* 2016), oceanic and reef manta ray (Couturier *et al.,* 2013; Stewart *et al.,* 2016) and a number of smaller devil rays (Sampson *et al.,* 2010). As for whale sharks, published literature was very limited until the last couple of year and now consists of a handful of articles (Borrell *et al.,* 2011; Yu *et al.,*2016; Marcus *et al.,* 2017; Prebble *et al.,* 2018; Wyatt *et al.,* 2019; Marcus *et al.,* 2019). A study by Borrell *et al*. (2011), was the first to evaluated a series of muscle samples obtained from the tissues of deceased whale sharks caught off the coast of India. It presented evidence through the use of a regression analysis that revealed a clear positive enrichment of both δ$^{13}$C and δ$^{15}$N isotopes as the species matures, possibly indicating a greater influence of the species to seek out and consume smaller fish species or much larger zooplanktonic prey as they mature. However, the authors concluded that the sampled tissues did present high δ$^{15}$N values compared to the baseline signatures in the area, which may be a result of the influence of much higher concentrations of organic pollutants that occur in the coastal environment around the Gujarat coastline. In 2016, a study by Yu *et al.* (2016) examined several whale shark tissue samples taken from individuals entangled in set nets in Taiwanese waters. Like the previous study (Borrell *et al.,* 2011), a positive relationship was observed between both δ$^{13}$C and δ$^{15}$N for animal size adding additional weight to the dietary change theory as the species matures. Prebble et al. (2018) investigated the latitudinal ranging of juvenile whale sharks in the Western Indian Ocean at three separate aggregation sites. Using a combination of photo-identification and stable isotope data, the authors tested the assumption that aggregation sites of Mozambique, Tanzania, and Qatar are a mixed stock of animals moving between sites. Results demonstrated that there is clear difference in the stable isotope values at the separate aggregation sites and minimal connectivity. Isotopic profiles also showed high variation in intra-specific profile of individuals within each location and a comparison with latitudinal isotope data, suggesting that sharks at each locality show clear site fidelity over the period of isotopic integration. A recent study by Wyatt et al. (2019) presented the first multi-tissue turnover rates in whale sharks using controlled feeding trails on captive sharks, as a basis for enhanced ecological insights into wild populations. The study showed significant variation in turn-over rates between sampled tissues (plasma and cartilage) with the best estimates for plasma demonstrating a turnover time of approximately 9 months, while fin cartilage a turnover time of 3 years, respectively. Like previous studies on deceased sharks (Borrell *et al.,* 2011, Yu *et al.,* 2016) a significant relationship was evident for $\delta^{15}N$ in both blood plasma and fin cartilage of the aquarium sharks, but showed that growth significantly lowered $\delta^{15}N$ signatures. Captive findings from this robust study allowed for the first multi tissue growth and nutrition corrected analysis of wild populations, suggesting individual foraging specialization on low trophic level mid ocean or coastal prey items.

Unpublished work from the GC has shown that whale sharks in the northern parts of the Gulf seem to show enriched signatures of $\delta^{15}N$ compared to sharks sampled lower in the Gulf (Hachohen, 2007 *unpublished MSc thesis, CICIMAR, IPN*). Although these observed differences in the isotope signatures may suggest that the spatial separation within the whale shark aggregations could be a driving factor and therefore not related to diet, but rather differences in latitudinal lines or unique behavioral strategies of the two separate subpopulations within this region like studies from the Indian Ocean (Prebble *et al.,* 2018). Furthermore, the lack of significant differences in the $\delta^{13}C$ values between foraging areas may suggest that the separate populations of whale sharks in the GC target prey that are foraging in similar areas, drawn together by changing environmental factors, such as in BLP (Monreal-Gomez *et al.,* 2001; Duran-Campos *et al.,* 2015). Additional unpublished work (Hacohen-Domené, 2015 *unpublished Ph.D. thesis, CICIMAR, IPN*), examined the oceanic factors that allow the co-occurrence of both the whale shark and oceanic manta rays in the northern part of the Mexican Caribbean. Results revealed that both species have analogous isotope signatures, clarifying that whale sharks and oceanic manta rays share both habitat and resources in the Mexican Caribbean. This may signify that both species are opportunistic planktivorous feeders and co-exist and share the same ecological niche in a particular habitat.

With all studies on stable isotope analysis, there seems to be lack of standardization within this discipline, especially when referring to the treatment of tissue samples before SIA (Kim *et al.,* 2011; Hussey *et al.,* 2012). Markus *et al.* (2017) from a study in the waters around Ningaloo Reef, Western Australia set out to examine the effects of lipid extraction, acidification for the removal of inorganic carbonate and rinsing with deionized water would have on isotope values of whale sharks and collected prey items. Before the SIA process, the authors went through rigorous dividing of samples into subsamples to be separated by treatments: untreated or a standard control sample, lipid extracted, deionized water rinsed the remaining two a combination of both techniques. The authors compared the different treatments against one another and also presented existing mathematical models for predicting normalization of samples, concerning lipid extraction as a means to save processing costs and time. The study did observe a slight enrichment in $\delta^{13}C$ and $\delta^{15}N$ values following treatment of both lipid extraction and a combination of lipid extraction and a deionized rinse, despite the low lipid content in the tissues analyzed. However, changes in the $\delta^{15}N$ values and the abundant concentrations of urea found in the deionized rinses seemed to be more effective with the removal of nitrogenous waste than the principal lipid extraction (Wyatt *et al.,* 2019). Although contradicting views on extractions and the necessity seems to present further problems, as aforementioned studies on the species at times lack the extraction of lipids, so comparing global work for international standards for the species generates new problems with regards to the affordability and time restraints to standardize protocols. Ultimately, concerns regarding the trophic level of the species compared to higher-level elasmobranch species and the overall level of lipids present in its tissues, it may however not be necessary or easier to use currently available mathematical model for elasmobranch species (Churchill *et al.,* 2015 & Li *et al.,* 2016).

# 2. JUSTIFICATION

Understanding of the stable isotope signatures of any species will allow us to elucidate whether there is a separation in habitat or to determine if any dietary preference exists among sex and maturity within a certain population. The use of conventional stomach content analysis is not feasible given that whale sharks are not fished commercially in Mexico, and are listed as a protected species by the Official Mexican NOM-059 Act (NOM-059-Ecol-2001). Furthermore, the urgent need to constantly develop more non-invasive means to obtain this fundamental information for larger marine species without restraint or mortality is essential regarding the ethics of animal care, particularly when working with protected species to provide accurate management recommendations. Given the lack of published literature in this region, it exposes a necessary need to provide a clear baseline study for whale sharks in this reliable aggregation site and to build a platform for future investigation. This study aimed to generate a comprehensive isotopic assessment of whale sharks in BLP over two sample seasons and to investigate whether there are noteworthy differences for sex of individuals or correlations between the size of sampled individuals within this population.

# 3. MATERIALS & METHODS

## 3.1 Biopsy apparatus

Extraction of connective tissue from individual whale sharks was achieved with the help of a 1.5m rigid aluminum Hawaiian sling, fitted with a removable modified biopsy core (Fig.16a). The biopsy core was custom made from a stainless-steel tube 8cm in total length and fashioned with a sample retaining entry point along its body and also included a stopper spacer to ensure that the instrument did not enter too deep into the body of the shark (Fig.16b). Once extracted, biopsies were placed in sterilized Eppendorf tubes using pincers, labeled for identification and kept on ice until return to the laboratory, where they were preserved and frozen at -80 °C.

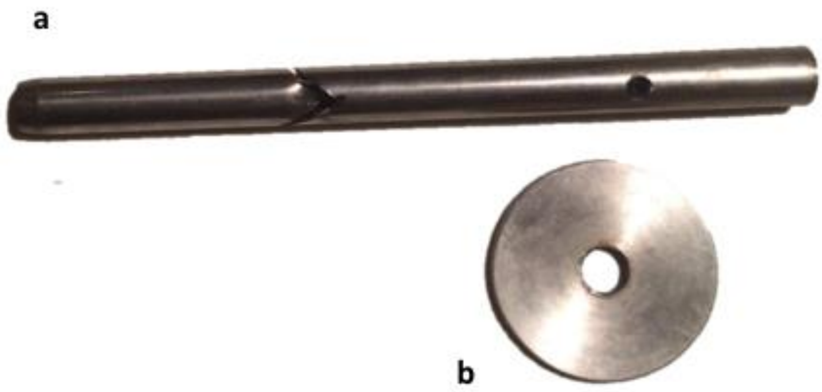


Figure. 16. Showing custom made Biopsy core (a) and stopper (b)

### 3.2 Field sampling

Skin biopsies (*n*=60) were collected from free-ranging whale sharks along the coastal waters of the El Mogote sandbar over two sample periods October 2016 – March 2017 and October 2017 – March 2018. GPS position (using a Garmin global positioning system receiver) were registered for each sampled shark and in-water photographs were taken of each shark identifying unique external body patterns, used for individual identification (Taylor, 1994; Van Tienhoven *et al.,* 2007) as a means to prevent repeatability of animals in the study. The presence or absence of claspers allowed for the determination of the sex of sampled sharks and total length (TL) of animals was estimated to the nearest 0.5m using both in water objects (swimmers) and known length of the research vessel as it was placed along the side of the shark. Ultimately, reaction to the biopsy sampling event was noted falling into either: No reaction, slight reaction or reaction as a means to evaluate the invasiveness of the methodology and its impact on the sharks.

### 3.3 Plankton Sampling

Surface plankton tows (*n*=12) were performed using a 505 μm mesh plankton net towed for five minutes behind the boat in a circular motion at 1-1.5 knots $h^{-1}$ approximately 1m below the water's surface in specific areas where sharks were observed feeding. Once encountering a whale shark, observers onboard the research boat confirmed that the animals they were feeding either actively on the surface or in a vertical position. Following the confirmation of the sharks feeding, the research, boat approached slowly and the animals GPS position was registered. Next, the plankton net was lowered into the water and for incidences of vertical feeding a tow for five minutes was conducted in a circular motion around the animal. As for incidences of active surface feeding, the net was towed alongside the sharks for the same amount of time. Once collected, plankton samples were stored in sample containers and labeled for identification then stored in a cooler with ice on board the research vessel, before frozen at -20◦C back at the laboratory prior to the analysis work.

### 3.4 Analysis of skin & zooplankton

All SIA analysis was conducted at the Centro Interdisciplinario de Ciencias Marinas (CICIMAR), where the analysis of stable isotopes of carbon and nitrogen signatures was carried out using an Elemental Analyzer coupled to an Isotope Ratio Mass Spectrometer (IRMS). All skin samples used during the entire study came from the shark's dermis layer (connective tissue) removing the epidermis layer to avoid the presence of dermal denticles (calcified structures), which may have an effect on isotopic values. For isotopic analysis a subsample of tissue biopsies was lyophilized using a LABCONCO freeze dry system at a constant temperature of -50 ° C for a total of 48 hours to remove all excess moisture in the sample. Upon completion of the drying process all skin samples were ground into a homogeneous powder using agate mortar and pestal. Next using a microbalance (Sartorius) machine a subsample of 1.0 ± 1.2 mg was placed into tin capsules and enclosed ensuring no contamination. All $\delta^{13}C$ and $\delta^{15}N$ values were calculated with a precision estimated to be at 0.2‰ for $\delta^{13}C$ and 0.3‰ for $\delta^{15}N$ respectively. Subsequently, zooplankton samples were subsampled and similarly dried and ground using the same procedures as previously mentioned. As whale sharks do not appear to actively select a specific food source during feeding events within plankton aggregations (Clark & Nelson, 1997; Motta *et al.,* 2010), isotopic signatures for the zooplankton samples were analyzed whole and one $\delta^{15}N$ and $\delta^{15}N$ values presented for each plankton sample.

### 3.5 Stable isotope analysis

Results for this study are presented according to the delta (δ) notation, where all relative variation of stable isotope ratios are expressed in parts-per-thousand relative to atmospheric N for $\delta^{15}N$ and Pee Dee Belemnite for $\delta^{13}C$. Our variation was calculated using the following formula by Park and Epstein. (1961):

$$\delta X = \left[\left(\frac{RS}{RR}\right) - 1\right] * 1000$$

where RS is the ratio of the heavier isotope to the light isotope of each sample, and RR represents the ratio of the heavier isotope to the light isotope in the reference.

### 3.6 Statistical analysis

Statistical tests were performed using several packages in the platform R studio (R Development Core Team, 2018) to gain information on any differences in isotope signatures within sample seasons as well as to observe any significant difference for sex or correlations for size for sampled sharks. Normality and homogeneity of variance were assessed by using Kolmogorov-Smirnov and Bartlett tests. Differences among body size and by sex were analyzed using Student t-test or Mann-Whitney U-test and a linear regression was used to assess the relationship between total body length (TL) and isotopic values ($\delta^{13}C$ and $\delta^{15}N$). Ultimately to detect outliers from each group of data a Grubbs outlier test was performed.

# 4. RESULTS

## 4.1 Descriptive data analysis

Skin biopsies (*n*=60) were collected from free ranging whale sharks (♂42, ♀18) in the study area between October 2016 – March 2018 and their GPS positions were registered (Fig.17). During the first season (October 2016 – March 2017) 32 whale sharks were sampled (23♂, 9♀) with a sex ratio of 72% male and 28% female. As for the second season (October 2017 – March 2018) 28 whale sharks were sampled (19♂, 9♀) with a sex ratio of 68% male and 32% female (Table.6).

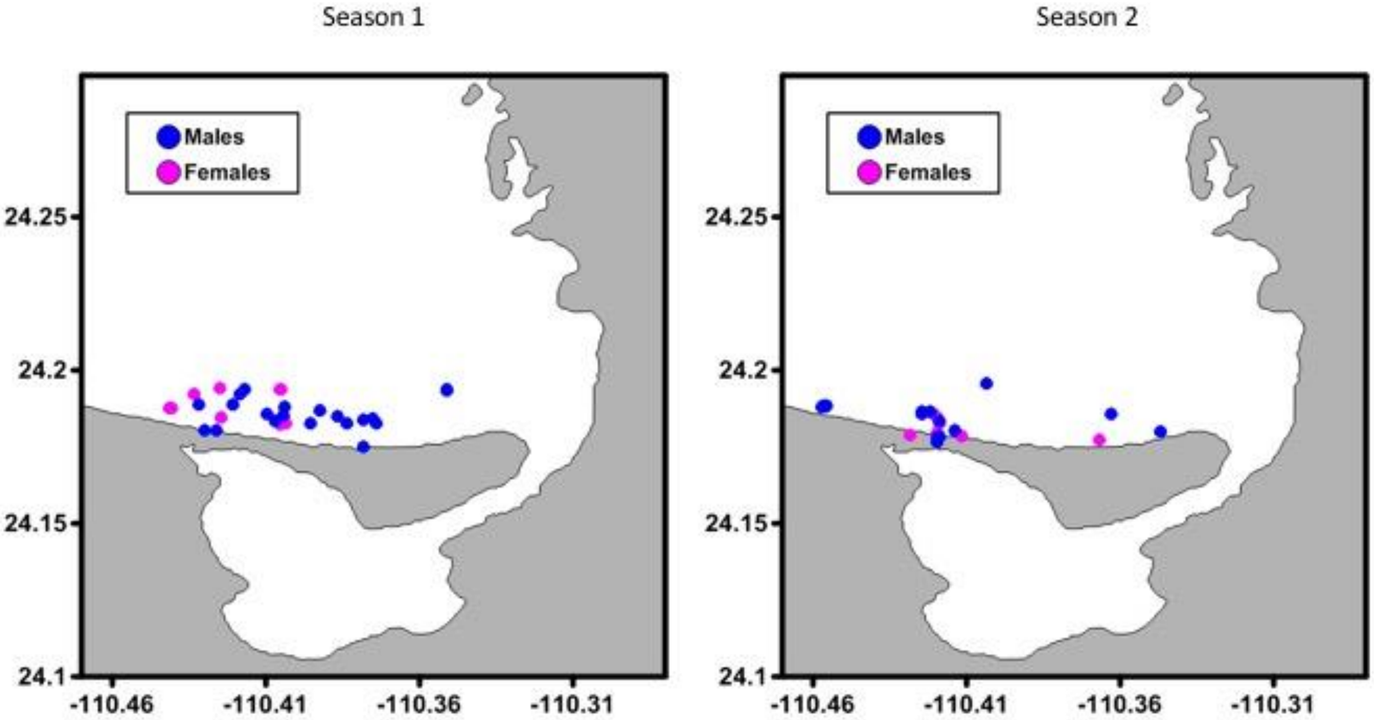


Figure.17. Sampling locations of male and female whale sharks during both sample seasons

Total length (TL) of sampled sharks ranged from 3.5 - 8m with a mean size of 5.41m for the first season and 3 - 6.5m with a mean size of 5.14m for the second respectively (Fig.18). Response of the shark to the biopsy technique showed that 84.5% of sharks showed no reaction, 4% showed a slight reaction and 11.5% of sampled sharks showed a reaction to the biopsy event.

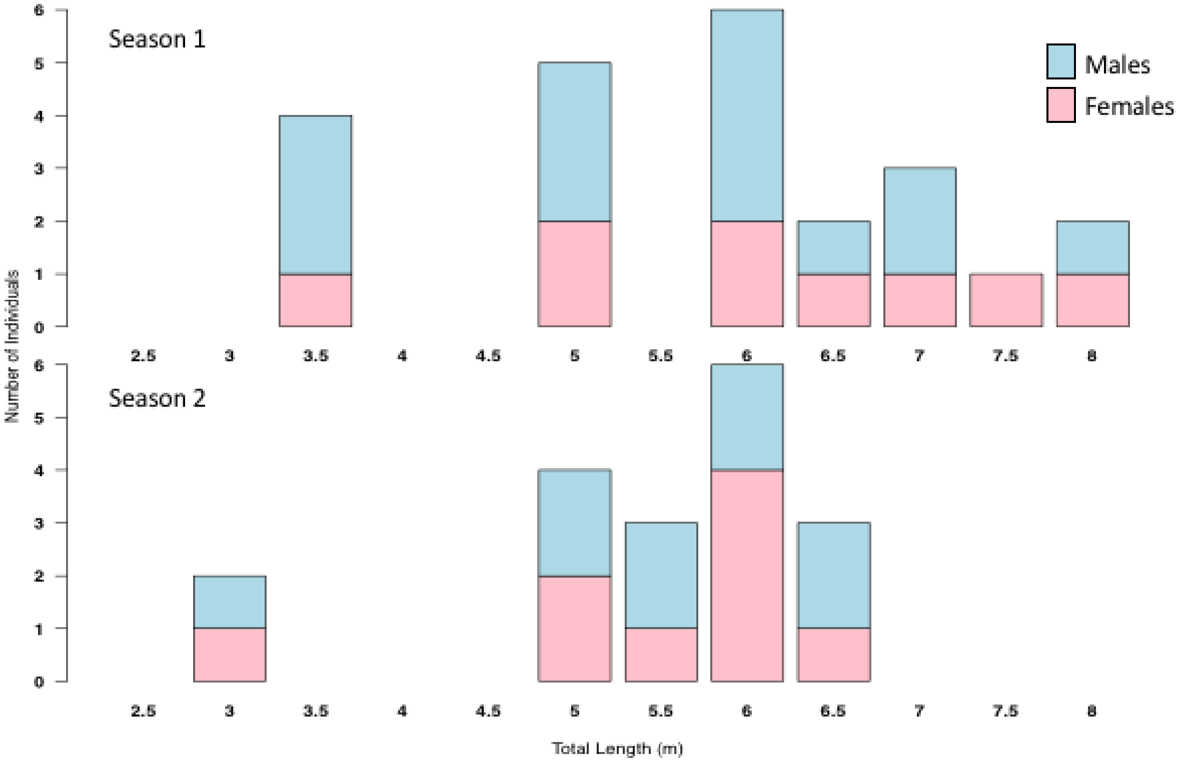


Figure.18. Size range stacked plot for sampled sharks within each sample season

## 4.2 Stable isotope analysis

Isotopic range during the first season ranged from -16.85‰ to -14.30‰ with a mean value of -15.40 ±0.5‰ for $\delta^{13}C$ and 10.55‰ - 15.04‰ with a mean of 13.52±1.3‰ for $\delta^{15}N$. As for the second season, isotope range was 10.31‰ - 15.08‰, with a mean of 12.73±0.1‰ for $\delta^{15}N$ and -17.21‰ to -14.16‰ with a mean value of -15.37±0.1‰ for $\delta^{13}C$ (Figure.19; Table.6). A slight visual variation can be observed in the overall isotopic signatures of sampled sharks a two-sided t-test revealed no significance difference between sample season and isotopic signatures for both $\delta^{15}$N (*p*= 0.05797) or $\delta^{13}$C (*p*= 0.8741).

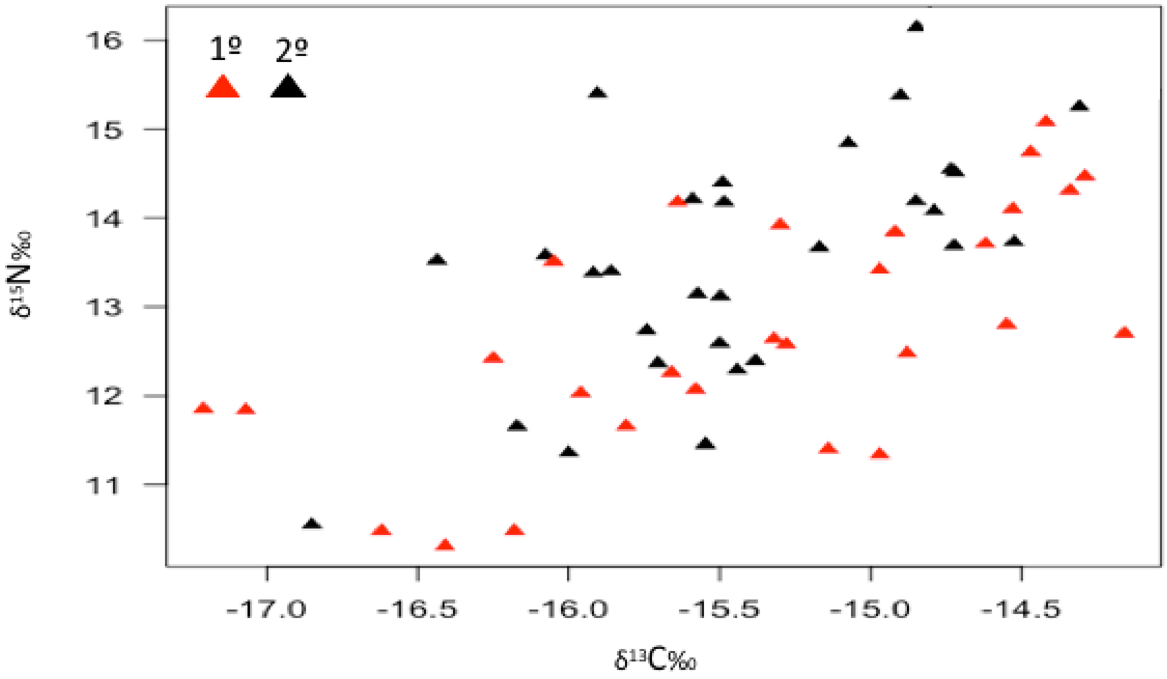


Figure.19 $\delta^{15}$N & $\delta^{13}$C stable isotope values for sampled sharks separated by sample season 1° & 2°

Table.6 Isotopic summary of descriptive data on biopsied sharks over both seasons

| | Male | Female | MeanTL (M) | Mean$\delta^{13}$C (‰) | Mean$\delta^{15}$N (‰) | mean$\delta^{13}$C Zooplankton (‰) | mean$\delta^{15}$N Zooplankton (‰) | n |
|---|---|---|---|---|---|---|---|---|
| **Season 1** | 23 | 9 | 5.41 | -15.04‰ | 13.52‰ | -18.27‰ | 12.77‰ | 32 |
| **Season 2** | 19 | 9 | 5.18 | -15.37‰ | 12.73‰ | -18.08‰ | 13.40‰ | 28 |

Zooplankton isotopic values for the first season *(n*=6) ranged from -18.75‰ to -17.71 with mean values of -18.27‰±0.1‰ for $\delta^{13}C$ and 12.37‰ to 13.50‰ with a mean of 12.77‰±0.1‰ for $\delta^{15}N$. As for the second season (*n*=6) ranged from -18.55‰ to -17.65 with mean values of -18.08‰±0.1‰ for $\delta^{13}C$ and 12.43‰ to 13.96‰ with a mean of 13.40‰±0.1‰ for $\delta^{15}N$ (Figure.20; Table.6). Once more a sight visual variation was observed in the overall isotopic signatures of zooplankton but following a two-sided t-test no significance difference was observed between sample season and isotopic signatures for both $\delta^{15}N$ (*p*= 0.0736) or $\delta^{13}C$ (*p*= 0.4753).

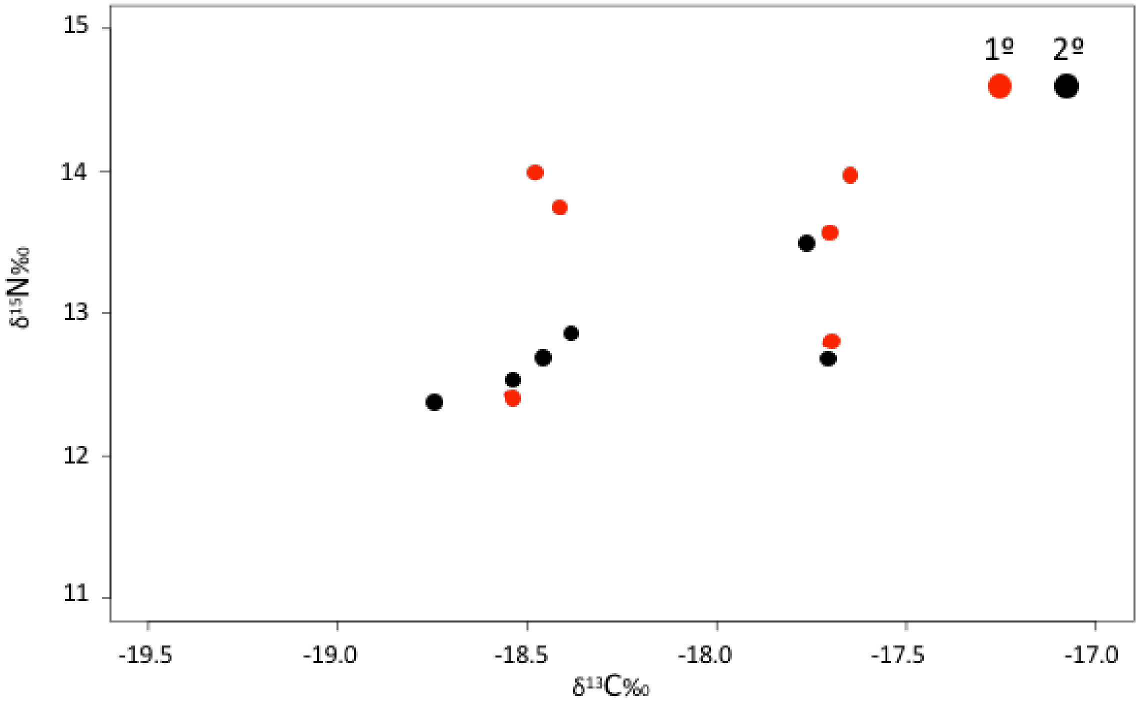


Figure.20. $\delta^{15}N$ & $\delta^{13}C$ stable isotope values for sampled zooplankton (Sample season 1° & 2˚)

When comparing the mean values for sex of sampled individuals, a slight increase in $\delta^{15}N$ for females over males (♀13.47‰ ♂13.36‰) was observed for the first sample season but following a two-sided t-test no significance difference was found in regards to $\delta^{15}N$ values for sex of individual sharks both for the first season (p= 0.2629) and for the second season (p= 0.1463). Furthermore, $\delta^{13}C$ values statistical tests also revealed no significance difference between sex of sampled sharks for the first season (p= 0.2762) and for the second season (p= 0.2606) (Fig.21).

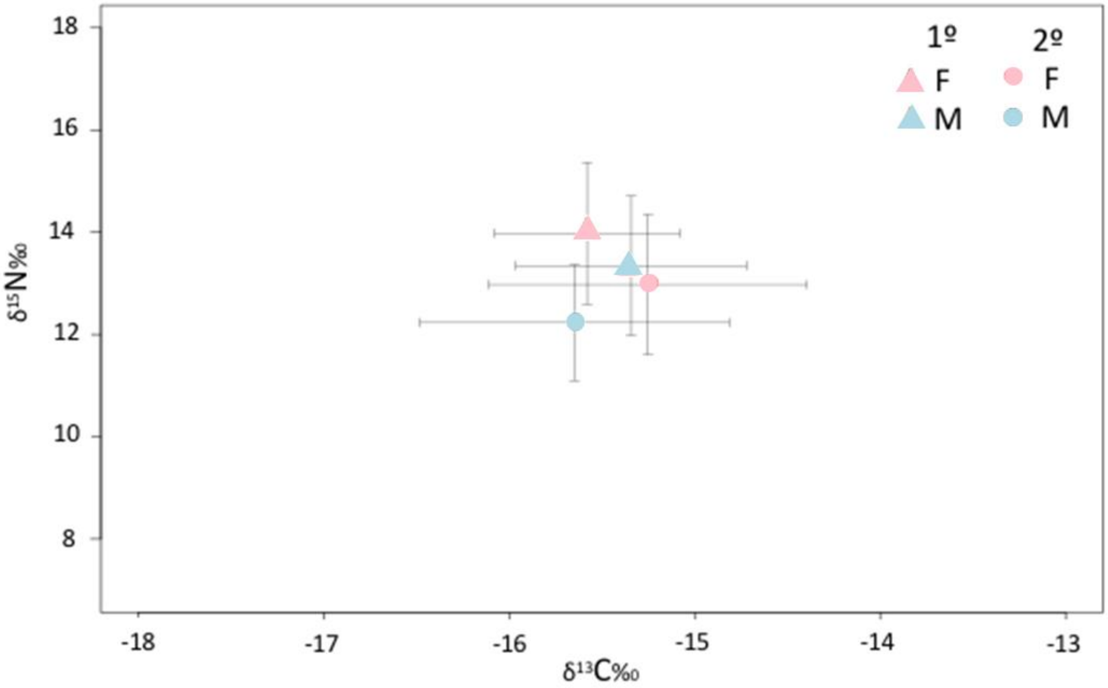


Figure.21. Mean $\delta^{15}N$ & $\delta^{13}C$ for sex of sampled sharks for both sample seasons (M=Male; F=Female)

$\delta^{13}C$ and $\delta^{15}N$ values were analyzed against size of sampled individuals to understand if any correlation exists between the two variables. A Pearson's correlation statistical test revealed no significant relationship for $\delta^{13}C$ with regards to size of the animal for the first season ($p$= 0.384) or the second season ($p$=0.071) (Fig.22). While $\delta^{15}N$ values did show a significant association for the first season ($p$=0.01182) but not for the second season ($p$=0.1352). Given the smaller size range of individuals for the second season (3 - 6.5m) compared to the second season (3.5 - 8m), coupled with the non-significance between signatures between sample seasons, the decision to run a Pearson's correlation on both sample seasons together revealed a highly associated relationship between animal size and $\delta^{15}N$ values ($p$=0.002023) (Fig.23).

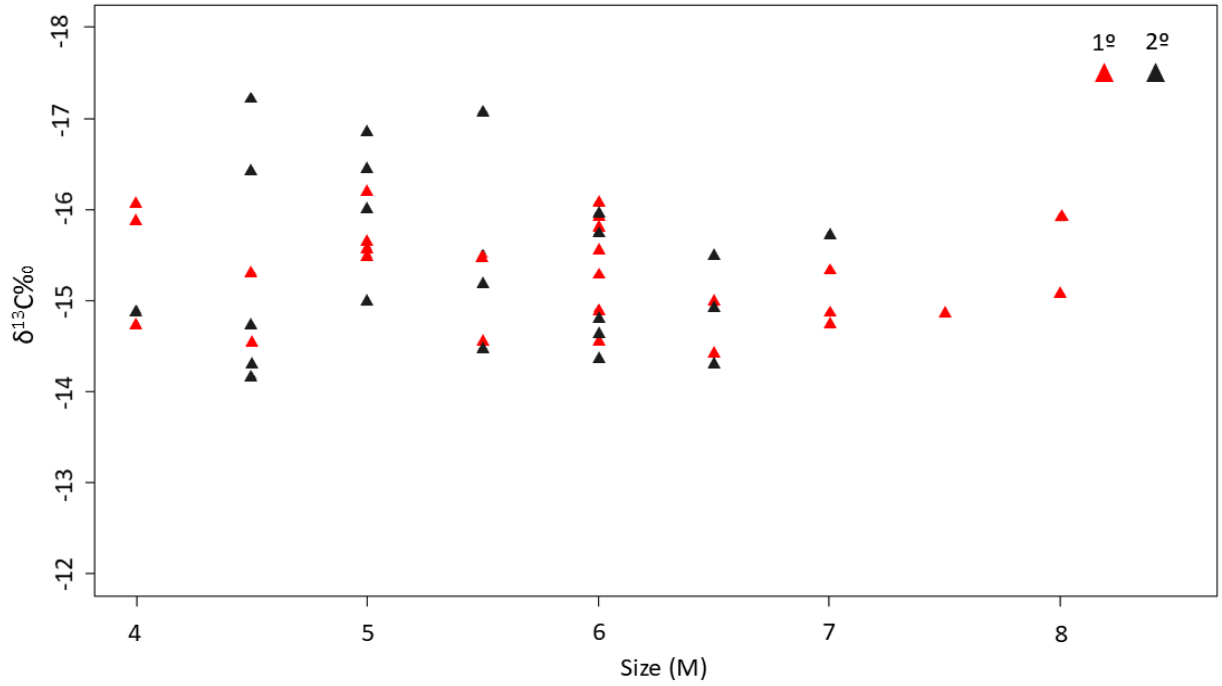


Figure.22 Scatterplot showing $\delta^{13}N$ against the variable size for both seasons

Pearson product-moment correlation coefficient reported a significant linear correlation between the two variables with the coefficient explaining as much as (r=0.4217, 42%) in its model configuration (Table.7). A simple linear regression was calculated to predict $\delta^{15}N$ based on animal size. A significant regression equation was found (F(1, 58) = 10.45, *p*= 0.002023), with an adjusted $R^2$ of .1381. Individual sharks $\delta^{15}N$ value is equal to 10.9497 + 0.4217 (size) parts per thousand (‰) when size is measured in meters (m). Whale sharks $\delta^{15}N$ signatures increased 0.4217 parts per thousand for each meter of total length (Fig.23; Table.7).

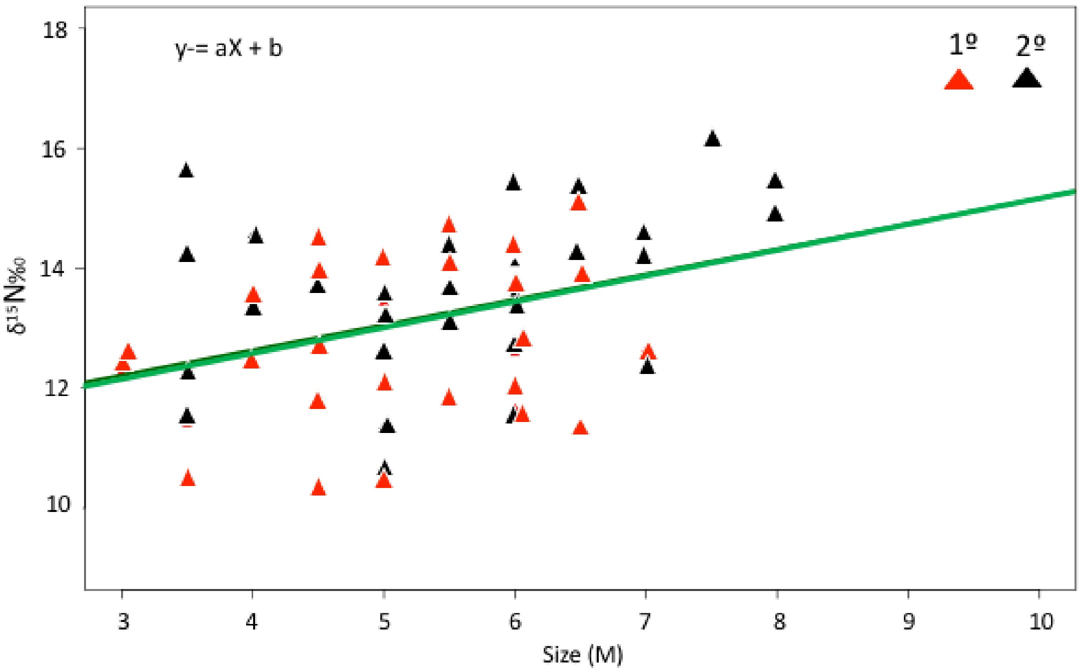


Figure.23. Scatterplot showing δ$^{15}$N values against the variable size within both seasons and the linear regression line

Table.7 Linear regression model output table showing residuals & coefficients

**Linear Regression Model output**

| **Residuals** | | | | | **Coefficients** | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Min | 1Q | Median | 3Q | Max | | Estimate | Std.Error | T value | Pr(>ItI) |
| -2.5783 | -0.9778 | 0.1634 | 0.8484 | 3.1343 | (Intercept) | 10.9497 | 0.7030 | 15.576 | <2e-16*** |
| | | | | | Size | 0.4217 | 0.1304 | 3.233 | 0.00202** |

Residual standard error: 1.299 on 58 degrees of freedom

Multiple R-Squared: 0.1527, Adjusted R-Squared: 0.1381, F-Statistic: 10.45 on 1 and 58 DF, P-Value: 0.002023

# 5. DISCUSSION

Isotopic values in all marine species may be influenced by the species-specific diet, physiology or differing foraging locations (Newsome *et al.,* 2007). This study provides a baseline isotopic assessment of whale sharks from Bahía de La Paz over multiple sample seasons. While no significant differences were observed between isotopic signatures of shark's tissues between sample seasons or the sex of individuals sampled, results from this study did show a significant increase in $\delta^{15}N$ values when correlated with size (Fig.23). The correlation between shark size and $\delta^{15}N$ was present when all animals from both sample seasons were analyzed collectively and not separated by sample season. While a correlation was observed within the first sample season (P=0.01182) the second sample season didn't show significance (P=0.1352). This lack of correlation between $\delta^{15}N$ and size for the second season may be somewhat explained as a result of the small size range of animals (3 – 6.5m) compared to the first sample season (3.5 - 8m), affecting the statistical power rather than an actual homogeneity in signatures (Hoem, 2008). The relationship between animal size and $\delta^{15}N$ in our results provides more evidence to support the theory of a potential dietary shift in the species as they mature as proposed by previous studies (Borrell *et al.,* 2011; Yu *et al.,* 2016). For the whale shark, separation by size has been previously reported in the Gulf of California (Eckert & Stewart, 2001; Ketchum *et al.,* 2013), Belize (Graham & Roberts, 2007), Djibouti and the Seychelles (Rowat *et al.,* 2011). These segregations by size in the species might be driven by differences in dietary preferences as a strategy to reduce intraspecific competition for habitat or by different size-based migratory pathways (Graham & Roberts, 2007). According to Ketchum *et al.* (2013), whale sharks from BLP segregate by size (juveniles <9m and adults >9m), with juveniles observed in shallow coastal waters foraging on a range of copepods, while adults remain in deep offshore waters, foraging on krill. However, the influence of migration to different isoscapes cannot be discarded and our correlations, need to be taken with caution, as work in the Indian Ocean demonstrated that sharks at different sites present different isotopic profiles and may enter a new aggregation area representing isotopic profiles from a different site (Prebble *et al.,* 2018). Seventeen whale sharks (28.34%) within this study showed a $\delta^{15}N$ signature that is suggested to have its origin in a different $^{15}$N-depleted isoscape. These individuals showed $\delta^{15}$N values that were similar or lower than the zooplankton collected in BLP, hence, it is highly likely that these individuals are foraging in areas outside of the bay and outside of the entire GC. Natural variations in stable isotopes of C and N at the base of the food web take place as a result of differences in productivity, upwelling, and other oceanographic factors. These changes provide important information on the habitat use of marine organisms in the ecosystems (Aurioles-Gamboa *et al.,* 2009; Prebble *et al.,* 2018). BLP, and in general the GC, are characterized for having high $\delta^{15}$N values due to a denitrification process and the transport of denitrified waters from the Eastern Tropical North Pacific into the GC (Altabet *et al.,* 1999; Voss *et al.,* 2001; White *et al.,* 2013).

Previous work on the isotope signatures of whale sharks in the coastal waters of India also found a positive correlation in $\delta^{15}$N values with size (Borrell *et al.,* 2011) but suggested that the general coastal ecosystem of the sample area may be influenced by higher levels of organic pollution. Possibly due to the extensive raw urban and industrial sewage released into the area, which has also been mentioned in other studies and known to be an indicator of elevated values (Dolenec *et al.,* 2005). An additional study on tissues collected from whale sharks entangled in set nets in Taiwanese waters (Yu *et al.,* 2016), also showed a similar relationship between animal size and overall isotope $\delta^{15}$N values like the earlier study, but the contamination of samples due to the death of the specimen may have affected the condition of its tissues and overall signatures. In the latest study from captive animals (Wyatt *et al.,* 2019) documented that growth appeared to significantly lower $\delta^{15}$N signatures in contrast to previous studies and our current studies.

An anatomical study by Garrick (1964) presented evidence of the underdeveloped filtration system of neonate whale sharks, showing a lack of development in their filtration apparatus at this early stage of their lives. This may support evidence proving the inability of the species at a young age, to forage on larger prey source, adding weight to the general theory of a dietary change or differences in optimal food choice as the species matures. Juvenile whale sharks observed in Belize are known to forge on a range of fish eggs close to coastal areas, while larger individuals are observed in more oceanic waters foraging of different species of fish larvae (Graham & Roberts, 2007). In BLP a number of studies have observed a segregation by size of the species, with juvenile whale sharks showing tendencies to feed on various species of copepods (Clark & Nelson, 1997; Hacohen-Domené *et al.,* 2006), whereas mature members of the population are seen foraging on blooms of euphausiids species at offshore seamounts at the entrance of the bay (Ketchum *et al.,* 2013). Whale sharks are long-lived and sexual maturity in the species is believed to be reached at around 9 meters (Norman & Stevens, 2007; Rowat & Brooks, 2012). Given that all sampled sharks in this current study were of a juvenile stage, additional caution must be taken into account when predicting the clear feeding strategies of the entire population of whale sharks using isotopic ratios from a subpopulation of juvenile sharks with no adults sampled, as it may present a biased error given that isotopic profiles are dynamic and in constant change (Hussey *et al.,* 2012; Prebble *et al.,* 2018; Wyatt *et al.,* 2019).

Sexual segregation and sex-related biases has also been observed in many shark species and has been reported in whale sharks in places such as the Seychelles (Rowat *et al.,* 2011), Belize (Graham & Roberts, 2007), Australia (Norman & Stevens, 2007) and various other regions (Eckert & Stewart 2001; Wilson *et al.,* 2006) and for the most part more males then females are observed within these coastal aggregations (Norman & Stevens, 2007; Rowat *et al.,* 2011; Ketchum *et al.,* 2013; Whitehead *et al.,* 2019). We found no significant difference with regards to sex (Fig.21), which coincides with all previous stable isotope studies on whale sharks (Borrell *et al.,* 2011; Yu *et al.,* 2016; Prebble *et al.,* 2018; Wyatt *et al.,* 2019). Our current work and with past studies (Borrell *et al.,* 2011, Prebble *et al.,* 2018), there seems to be a clear lack of evenly sampled males and female sharks and our data reinforces that theory (70% male and 30% female). Incidences of mutual foraging events of both juvenile male and female sharks in Djibouti and the Seychelles (Rowat & Brooks, 2012) and here the GC (Ketchum *et al.,* 2013; D. A, Whitehead *pers. obs*.) may be one reason for the lack of variation in the isotope signatures. Both male and female sharks were sampled in the same feeding grounds during the same sampling period and are potentially driven to the same food source during this stage of their lives.

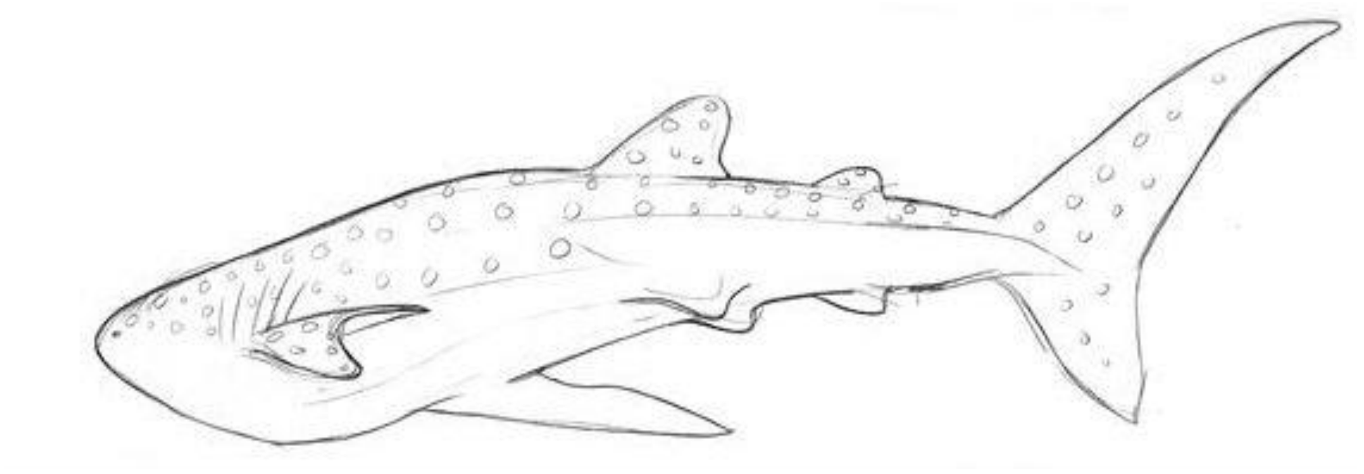
On a more regional level, our results did seemingly compare with previous studies conducted in the GC (*unpublished* Hacohen-Domené, 2007) in which their $\delta^{15}N$ isotope values displayed a similar range and a clear comparable mean signature (~0.45‰) from BLP. Although Hacohen-Domené. (2007) did observe differences in the isotope $\delta^{15}N$ values between sample sites in the GC, but these differences may in fact be due to latitudinal lines, that is, higher values with greater latitude and vice-versa such as in other studies (Prebble *et al.,* 2018). Demonstrating that these separate sub-populations of whale sharks in the GC target the same prey items and forage within similar habitats, where concentrations of zooplanktonic is drawn together. Therefore, differences in isotopic signatures may be associated to geographical variation in the water column like the in aggregations in other parts of the world (Prebble *et al.,* 2018) and its distribution of suspended organic nitrogen particles as previously reported by work focused on the geographical variations of the water column and its distribution of suspended particulates (Saino & Hattori, 1987).

As for isotopic signatures of zooplankton, our results show similar mean values for both $\delta^{15}N$ and $\delta^{13}C$ to previous baseline studies in this area (*unpublished* Hacohen-Domené, 2007) and the Mexican Caribbean (*unpublished* Hacohen-Domené, 2015). Irregularity in the overall zooplankton signatures was observed compared to the values of tissues in sampled sharks. According to the $\delta^{15}N$ values present in this study, there were several individuals from BLP with similar or lower $\delta^{15}N$ values (9.21‰ to 12.39‰) than potential prey (12.40 ± 0.95‰) sampled in the bay. With caution, this signifies that these whale sharks fed and consequently showed an isotopic value from a different system with a varying isotopic baseline probably outside the $^{15}N$-enriched GC (Altabet *et al.,* 1999). The GC is a semi-enclosed sea characterized to be generally enriched in $^{15}N$ compared to neighboring areas outside of the Gulf. This is the result of an intense microbial denitrification process in the suboxic layer (between 300 and 900m) and the influence of the highly denitrified intermediate waters of the eastern tropical Pacific Ocean that is carried northward by the Mexican Occidental Current (Altabet *et al.,* 1999; Aurioles-Gamboa *et al.,* 2009). The variation in isotopic signatures may also shed light on the concept that zooplankton tends to have a more dynamic isotopic signature, since these lower positioned organisms have the tendency to reflect direct changes in the environment over a much shorter period, whereas larger organisms, of organisms placed on a higher trophic level such as filter feeders, that require an integration period for isotopic signals over varying periods of time (Hussey *et al.,* 2010; Kim *et al.,* 2011, 2012; Wyatt et al., 2019). Wyatt et al. (2019) presented the first insight into tissue turnover rates in whale sharks and while there lacks the information on muscle or connective tissue which are generally collected in field-based sampling, a clear variation in the differences in tissue turn-over rates between different tissues is apparent. Blood plasma showing a turn-over rate of approximately 9 months and fin cartilage up to several years. The extensive time need to replace tissues in whale sharks demonstrating that signatures collected at any given time or place may not represent the food at that time or even of that specific aggregation site (Prebble *et al.,* 2018; Wyatt *et al.,* 2019). Prebble et al. (2018) work from the Indian ocean clearly exposed that intra-specific variation is even present in individual from the same aggregation site. Controlled feeding studies on whale sharks are very rare given the limited number of individuals in captively, not to mention the costly and time-consuming efforts to maintain superior care. Until there is more available information on DTDF's for whale sharks especially on its tissues that are most commonly collected in the field, a level of caution must be taken in to account with regards to the tissue samples taken from conventional pole spear biopsies (connective tissue, muscle), and the unlikeliness of those providing clear dietary information over short temporal scales (Wyatt et al., 2019). Seasonal aggregations of whale sharks typically last from weeks to several months and accepting that muscle or connective tissue may fall as an intermediate between plasma and fin cartridge turn over times, it sheds a shadow on assumptions of SIA and its ability to define dietary preference at any given time (Prebble *et al.,* 2018; Wyatt *et al.,* 2019).

The issue in regards to the treatment of samples prior to analysis given the concentration or effects of both urea and lipids in shark tissues is subject for investigation somewhat, due to the lack of standardization within this discipline especially when referring to the treatment of tissue samples prior to SI analysis (Kim *et al.,* 2011, 2012; Hussey *et al.,* 2012; Markus *et al.,* 2017). Several studies have proposed that urea extraction can significantly alter $\delta^{15}$N estimates in shark tissues, including in *R. typus* subdermal tissue (Hussey *et al.,* 2012; Kim *et al.,* 2012; Carlisle *et al.,* 2017, Marcus *et al.,* 2017), although it is unclear to what degree. Previous unpublished work by Hacohen-Domené *et al.* (2007) on the lipid extraction on tissues collected on whale sharks presented evidence that the process of the extraction of lipids on connective tissues of whale sharks was exhaustive and did not significantly change isotope values. But in recent years the effects of different treatments on the tissues of whale sharks have shown conflicting views. Markus *et al.* (2017) set out to examine the effects of lipid extraction, acidification for the removal of inorganic carbonate and rinsing with deionized water would have on isotope values of whale sharks and collected prey items. This publication demonstrated that if future studies choose to treat samples before SIA, a combination of lipid extraction and a deionized water rinse resulted in a slight enrichment in both $\delta^{15}N$ and $\delta^{13}C$ values, despite the low lipid content in the species tissues. Continued work on *R.typus* continue to opt for no chemical treatments for the extraction of lipids (Prebble *et al.,* 2018), so comparing global work for international standards for the species generates continued restraints. Hussey *et al.* (2012) demonstrated in their work on *R.typus* a slight difference in the muscle of whale shark tissue after lipid extraction, although the difference in the $\delta^{13}C$ values observed between connective and muscle tissue may be due to the variable lipid content. The decision to remove or extract lipids from whale shark samples may now be a matter of choice or purpose of investigation based on the type of tissues analyzed, as current conflicting views still present arguments on both sides of this topic. Ultimately, given the trophic level of whale sharks compared to much higher trophic level elasmobranch species and the overall level of lipids present in their tissues, it may however not be necessary to perform a lipid extraction or easier to opt to use a currently available mathematical model for elasmobranch species (Churchill *et al.,* 2015; Li *et al.,* 2016). Although given the previously mentioned contradictory views on the need for this process, there is an urgent need to investigate further to ensure standardized results across aggregation sites.

In conclusion, the use of this biogeochemical technique to investigate the feeding ecology, segregation or dietary preference of sharks is a proven method of investigation and has been used throughout the world on several shark species (Hussey *et al.,* 2010; Kim *et al.,* 2012; Prebble *et al.,* 2018). Like all previously presented studies of whale sharks using stable isotope analysis on the species (Borrell *et al.,* 2001; Yu *et al.,* 2016; Prebble *et al.,* 2018; Wyatt *et al.,* 2019) a level of caution needs to be established in regards to the assumptions of the isotopic analysis, its limitations and potential errors when comparing tissue turn-over rate or DTDF from another species or different tissue types (Hussey *et al.,* 2010; Markus *et al.,* 2017; Wyatt *et al.,* 2019). On a final note the application of stable isotope analysis for investigating elasmobranch feeding ecology shows the capacity to generate noteworthy results, especially within populations of species that are challenging to interact within their natural habitat and may also hold strict international protection. SIA provides an alternative, cost-effective and relatively non-invasive method, which can be applied to investigate the foraging ecology of whale sharks in their relative ecosystem. This method of sampling removes the need to slaughter specimens to gain dietary information promoting survival of an endangered species and also offers a more comprehensive long-term look into whale sharks food preferences and therefore avoids the ‘snapshot' bias associated with stomach content analysis. Continued work both in this region and across the globe focused on SIA, needs to generate more equally balanced datasets of both male and female members to explore the dietary preference in the sex of whale sharks, as well as incorporating the entire maturity stages of the species sampling adult members to establish isotopic profiles for large animals. Although, given the elusive behaviors of this species, its free-ranging properties and the lack of observations of female, neonates and adults members of the global population, this may continue to present challenges to researchers.

# CHAPTER 3



# CHARACTERIZING FEEDING BEHAVIOR OF WHALE SHARKS THROUGH THE APPLICATION OF A RANDOM FOREST MACHINE LEARNING METHOD

## TABLE OF CONTENT

◆

# DISCLOSURE STATEMENT

Results from this chapter would not have been possible without the analytical support by external technicians. At this point I would like to acknowledge in full Robert Cooper for his supervision in writing of some of the analysis code and for his assistance in the integration of the calibration data to its final format. Code used to prepare the data collected from free-swimming sharks is freely available by contacting the author of this thesis.

In addition, the development of our custom-made fin clamps used for attaching tag packages to free-swimming sharks, was modified from an existing fin clamp design that has been pioneered by CATS (http://www.cats.is/). Further support and guidance in the construction of the release section of our fin clamps came from Harry Webb at Georgia Aquarium, U.S.A (https://www.georgiaaquarium.org/).

# 1. THEORETICAL BACKGROUND

According to scientific theory, marine animals should adopt locomotory strategies that maximize the amount of energy they acquire relative to the energy they expend to obtain prey, travel a given distance or to move effectively through the water column (Gudger, 1941b; Gleiss *et al.,* 2011, 2013; Kays *et al.,* 2015;). Quantitative behavioral studies on large, migratory, marine mega-fauna will always be challenging, due to their complex lifestyles and the continually altering environmental factors that affect the means to successfully interact with them (Orams, 2002). Recent technological advances, now allow researchers to collect large data sets on specific areas of behavior such as: animal movement (Tomkiewicz *et al.,* 2010; Kays *et al.,* 2015), swim or dive behaviors (Gleiss *et al.,* 2011, 2013; Meekan *et al.,* 2015) and social interactions (Krause *et al.,* 2013) of free-ranging animals in their natural habitat. Now automatically collecting data sets from animal-borne instruments is becoming a conventional option, but analysis of these enormous data sets is still an intimidating task, mainly due to the lack of accessible analytical tools (Valletta *et al.,* 2017). Machine learning (ML) offers a complementary data platform for modeling techniques that are distinctive from those used in classical statistics (James *et al.,* 2013; Valletta *et al.,* 2017; Brewster *et al.,* 2018). ML is the scientific study of algorithms and statistical models used by computers to perform tasks, without instructions (James *et al.,* 2013). Machine learning incorporates several methodologies that can learn or observe distinct patterns in the acquired data and regression, classification, clustering and dimensionality reduction are some of the most common applications that ML may be used for. A machine (an algorithm) can improve its performance (predictive accuracy) in achieving a task (e.g. classifying a behavior) from experience (data) through a training process (James *et al.,* 2013; Valletta et *al.,* 2017). The ultimate goal is for the predictive model to make accurate projections on previously unseen data, based on its memory, through the success of its training.

For the majority of ML applications, the main focus is typically on prediction; without necessarily assuming a functional distribution for the data, producing an overall optimal performance model (Valletta *et al.,* 2017; Brewster *et al.,* 2018). It is this hypothesis-free approach that makes ML an attractive choice for dealing with complex data sets. While in traditional statistical modeling a hypothesis is put forward and is then accepted or rejected depending on how consistent it is with the measured observations, ML methods learn this hypothesis directly from the training data set. Supervised machine learning is similar to traditional statistical models (e.g. generalized linear models) as it can identify the relationship between an outcome and a set of explanatory variables. By using the data as a starting point, rather than a traditional predefined model structure, the supervised ML algorithm, learns the mapping between a set of features and a continuous outcome (regression) or a categorical variable (classification). Once an appropriate feature set is determined, the observations are then split into training and testing data sets. The training data set is used to build the predictive model, while the testing data set (not used in model construction) is used to compute the expected predictive performance 'in the field' or as a validation test. In statistics, this is similar to making interpretations about the population based on a finite and random sample (Valletta *et al.,* 2017). The application of ML algorithms in behavioral studies has proved successful, especially for an array of species which are at times hard to observe given there elusive behaviors such as nocturnal organisms like badgers (McClune *et al.,* 2014), penguins (Carroll *et al.,* 2014), large marine species such as tiger sharks (Hammerschlag *et al.,* 2011), or those that are hard to follow continuously owing to their speed or covertness like cheetahs and pumas (Grünewalder *et al.,* 2012; Wang *et al.,* 2015). Random forest (RF) is an ensemble machine learning algorithm that uses multiple decision trees to extrapolate a decision through random selection and a voting strategy (James *et al.,* 2017; Olivier *et al.,* 2018). Decision Trees are a non-parametric supervised learning method used for classification and regression models in the form of a tree-like structure. In its basic form, it breaks down a data set into smaller and smaller random subsets while at the same time an associated decision tree is incrementally developed, with the final results presenting a tree with nodes and leaf nodes. A decision node may have two or more branches and leaf nodes, and represents a classification or decision. The topmost decision node in a tree which corresponds to the best predictor for the model is referred to as the root node (James *et al.,* 2013). Ultimately, decision trees can handle both categorical and numerical data, but on their own, they don't hold very strong predictive power so a RF approach offers a solid platform with robust statistical power.

The whale shark is the largest fish in the ocean and has traditionally presented an energy management paradox, by exclusively feeding on small planktonic food items (Meekan *et al.,* 2015). The external characteristics of *R.typus* epidermal layer is comprised of rows of perfectly aligned dermal denticles (Norman & Stevens, 2007). These external denticles have three longitudinal ridges, the central one forming a sturdy central keel with deep grooves on either side providing specially designed adaptations of the skin that serve as a hydrodynamic aid. This diminishes drag and surface-noise production improving the animal's movement in the water, which reduces energy loss during locomotion and improves foraging behaviors (Bigelow & Schroeder, 1948; Gleiss *et al.,* 2009). While there is currently no published work on ML for the species, recent behavioral studies using accelerometry data has shown that whale sharks display a wide variety of behaviors to conserve energy (Gleiss *et al.,* 2011, 2013; Meekan *et al.,* 2015). As elasmobranchs are negatively buoyant, they appear to perform regular deep dives gliding through the water column without beating their tails. This is a convergent behavior that is widely recognized as a strategy used by fish, birds, and mammals to conserve energy while moving vertically (Williams *et al.,* 2000; Davis *et al.,* 2001). Studies by Gleiss *et al.* (2009 & 2011) were the first assessment of *R.typus* dive geometry and cost of transport in the species. The earliest study, which spanned two consecutive seasons, was successful in developed a novel methodology for the attachment and retrieval of motion-sensitive tags to whale sharks using a fin clamp, without any form of restraint. Results from this study were published in Gleiss *et al.* (2011) enlightening the use of animal-borne accelerometers and their ability to construct models of diving geometry, to test how swimming energetics may be related to vertical and horizontal movement in free-ranging whale sharks. They revealed that animal pitch was shown to affect the cost of locomotion in a negatively buoyant animal moving through a three-dimensional environment and is therefore expected to affect the optimality of movement depending on the ecological circumstance. A following study by the same authors (Gleiss *et al.,* 2013) also showed that whale sharks demonstrate a moderate reverse diel vertical migration, but exhibited a clear crepuscular pattern in locomotory activity. Meekan *et al.* (2015) continued to investigate the swimming energetics and foraging efficiency of whale sharks and published results showing that *R. typus* exhibits four strategies to save energy and improve foraging efficiency. These swimming adaptations were found to increase efficiency (>20%) relative to swimming horizontally and resolve the energy-budget paradox of whale sharks. In addition, bio-logging data showed that whale sharks took advantage of their negative buoyancy to incorporate periods of gliding behavior to save energy during the descent part of dives, a pattern reported in previous studies (Gleiss *et al.,* 2011, 2013) and a further energy-conservation tactic, previously documented in marine mammals and birds (Williams *et al.,* 2000; Davis *et al.,* 2001).

*R.typus* are wide-ranging and they spatially aggregate in areas that have higher concentrations of valuable prey items (Nelson & Eckert, 2007; Motta et al., 2010; Ramirez-Marcia *et al.,* 2012; Ketchum *et al.,* 2013). These areas are warm often-shallow basins, with high productivity, were individuals aggregate seasonally, coinciding with peaks in the abundance of available food sources (Hacohen-Domené *et al.,* 2006; Ketchum *et al.,* 2013). While in these highly productive areas, individuals are known to display multiple types of foraging behaviors. To date, research has revealed that whale sharks carry out at least three methods of filter-feeding (Motta *et al.,* 2010) and these behaviors are observed temporally and spatially with the expectation that; individuals choose the foraging intensity depending on the density of food. Ultimately, whale sharks appear to be behaving uniquely at each aggregation site and it is important to understand the variability in energy management strategies, and when individuals display each behavior.

# 2. JUSTIFICATION

Recording information on mobile species via animal attached data-logging tags often requires the animal to be restrained for devices to be solidly attached to the body, which presents great challenges when working with larger marine fauna. Modern technology offers an ideal platform for observing fine-scale behaviors of any species in its natural habitat under both the observation and out of view of researchers. The use of relatively new accelerometer devices has promptly allowed the fine-scale locomotory and energy conservation behaviors of larger marine fauna to be examined and further understood, with relatively non-invasive techniques at an affordable cost. Foraging behaviors are not all equal and whale sharks are only likely to display costlier behaviors when the likelihood of behaviors being beneficial is higher with regards to the energy they acquire, relative to the energy they exert. To date, there is no published data for characterizing whale sharks feeding behavior using accelerometers or the application of machine learning algorithms, as a direct means to define these specific feeding behaviors from one another. Given the limited amount of literature available on the species swimming behaviors and the lack of information on the species when it´s foraging, it justifies the urgent need to investigate and present a baseline of information of such behaviors in foraging grounds. Thus, we aimed to identify and characterize the feeding behaviors of whale sharks through the use of a random forest algorithm on accelerometry data and develop the first predictive model that can serve as a means to identify specific feeding behaviors of whale sharks in a dataset without human observation at the study site.

# 3. MATERIALS & METHODOLOGY

## *4.1 Tag package*

The Open tag is an open-source inertial measurement unit for recording high-speed motion sensor data to a microSD memory card developed by Loggerhead instruments (www.Loggerhead.com). These tags offer a three-dimensional gyroscope, accelerometer, and magnetometer that can be used to understand the movements of the animal, estimate the pitch, yaw, roll and heading, while pressure and temperature sensors can continuously sample the environment the instrument is in (Fig.24A). The open tag is contained in a small waterproof housing fitted with an o-ring to allow access to the memory card and battery, and to reach the power switch (Fig.24B). The rechargeable lithium battery in the device allows for deployments for up to 7 days with the option to recharge, reuse and deploy on numerous occasions, making the instrument cost-effective and easy to use in a challenging environment. The further availability of an internal data script allows the user to program the instrument to record data in numerous ways and of differing intervals. For this project we set the board to record 50 data point per second for the inertial measurement unit (IMU) and 1 data point per second for the pressure and temperature sensors.

Figure.24 Open tag (A) and a custom-made aluminum housing with o-ring (B)

### *4.2 Fin Clamp & Attachment protocol*

For attachment of the Open tags to the whale sharks, a non-invasive fin clamp was constructed based on a pioneered design by CATS (http://www.cats.is/) and placed over the leading edge of the first dorsal fins of sampled sharks, using natural tension in the clamp and the locomotion of the swimming sharks to hold it in place. This fin clamp was constructed from 8mm stainless steel bar, which was fabricated into a custom size torsion spring. The spring measured a total length of 27cm, with a 4cm diameter on the coil edge and spring arms of a total length of 22cm. The Open tag was mounted to one arm of the fin clamp via two stainless steel brackets, which held the instrument securely in place to eliminate vibration and rocking in the tag from the movement of the sharks. A GSM/GPS tracker was placed inside a brightly colored hydrodynamic syntactic foam float and mounted along the second arm of a fin clamp to assist in the recovery of the clamp and provide positive buoyancy of the entire system. Along the internal edges of both the tag mounting brackets and the foam float, a layer of abrasive material was placed to provide extra grip and support utilizing the dermal denticles of the species (Fig.25).

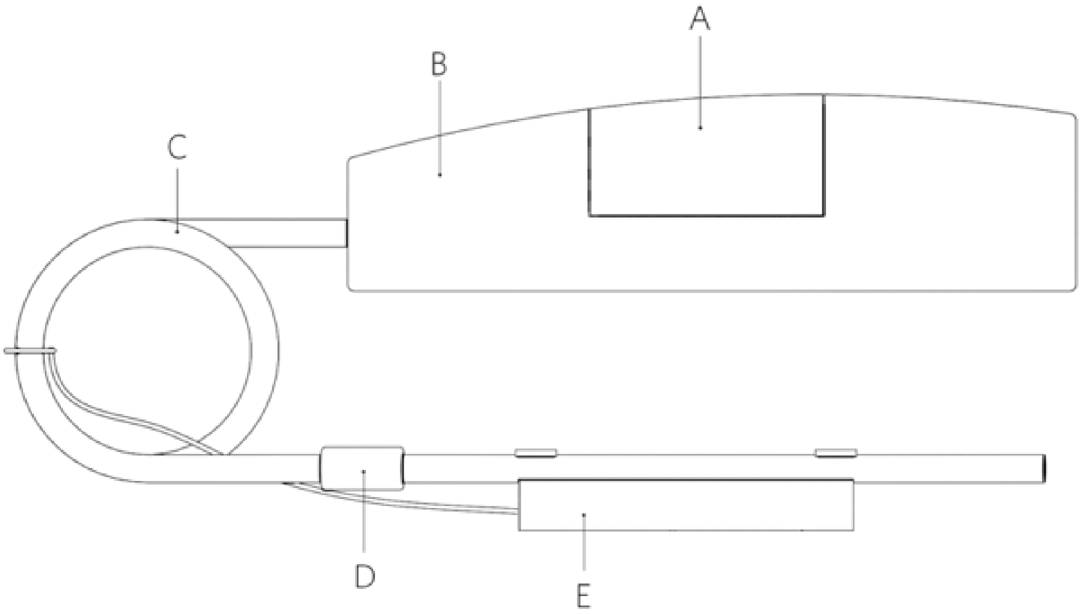


Figure. 25. Sketch of custom-made fin clamp showing (A) GPS/GSM transmitter, (B) buoyant float, (C) torsion spring, (D) galvanic time release and (E) open tag

A galvanic release was incorporated into the fin clamp to allow for a known release time of the complete system and through the use of a small GPS/GSM tracker recovery of the instrument was achieved. Deployment of the clamp was accomplished by an in-water snorkeler who swam down to the shark and manually placed the clamp over the dorsal fin, approaching from behind and above so out of visual capabilities of the animal limiting the disturbance. Upon retrieval of the open tags, microSD cards were removed and data imported for examination and analysis.

### *3.3 Field sampling*

A total of 11 whale sharks were tagged between October 2016 and March 2018 in the coastal waters in front of the El Mogote sandbar. Tagging consisted for the most part as active tracking, where the animals had their GPS position registered every 10 minutes during tag durations, to generate a rudimentary track of their movements. The bathymetry of the area around the sandbar and the fact that the whale sharks spent the majority of their time in the shallow coastal waters allowed us to recreate an almost ¨semi captive environment¨ in free-swimming sharks, ideal for real-time behavioral analysis and constant observation.

### *4.4 Classification of Feeding Behaviors*

The classification of whale shark feeding behaviors was derived from pre-chosen, accepted, feeding behaviors of the species available in current literature (Clark & Nelson, 1997; Norman, 1999; Motta *et al.,* 2010; Ketchum *et al.,* 2013). The principal behaviors classified in this study were the three accepted foraging behaviors (Passive, Active and Vertical) as well as horizontal swimming to provide a natural behavior of the shark when not engaged in a foraging activity. In addition to these, important avoidance behaviors (reaction to swimmers/to boat) were registered as well as banking, a known avoidance behavior in the species (Norman, 1999; Quiros, 2007).

### *4.5 Behavioral observations*

To create a robust and reliable ¨semi-captive¨ environment in a natural habitat, tag package timelines were synchronized to a single watch onboard the research vessel, to ensure that the data from the tags coincided with the real-time behavioral observations. Following this, in-water observers reported behavioral changes in the sharks as they happened using specific pre-arranged hand signals, which were reported in real-time to the observers on board, wearing the digital clock, synchronized to the tags. The exact time to the second was used from the moment the instrument was placed on the shark until the time it was removed or released from the shark. Any data either side of those points were trimmed and removed from the data analysis to avoid confusion with on shark and off shark information. Also, the time between individual behaviors was assigned to the initial behavior until the next registered behavior and grouped for classification (Fig.26).

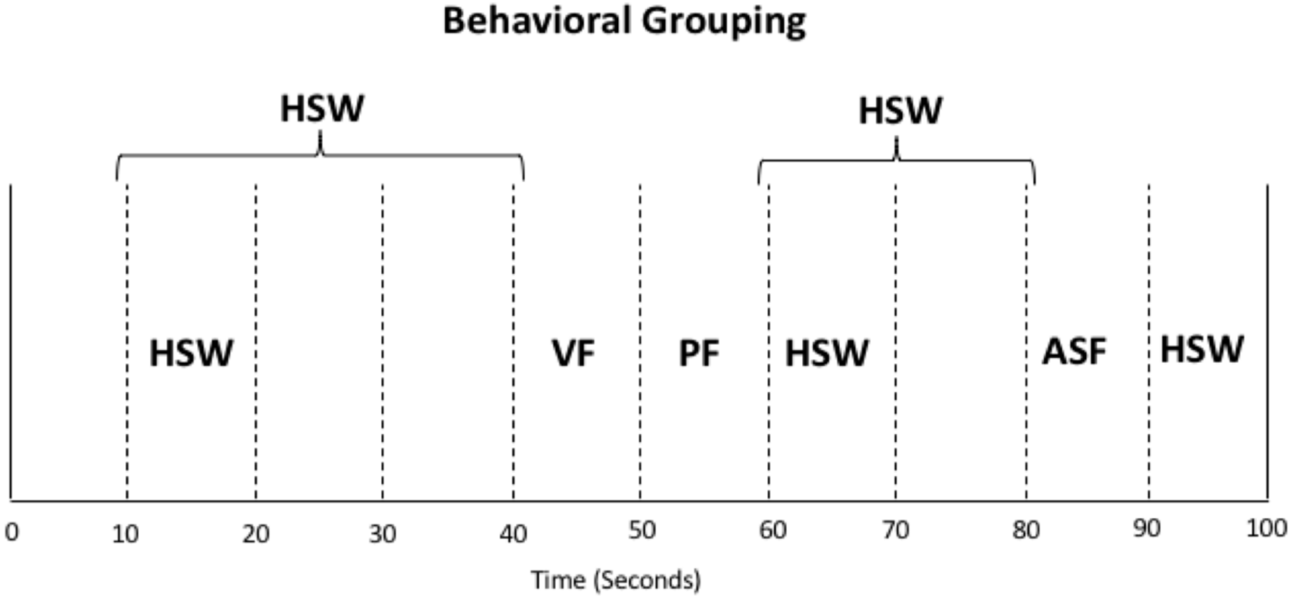
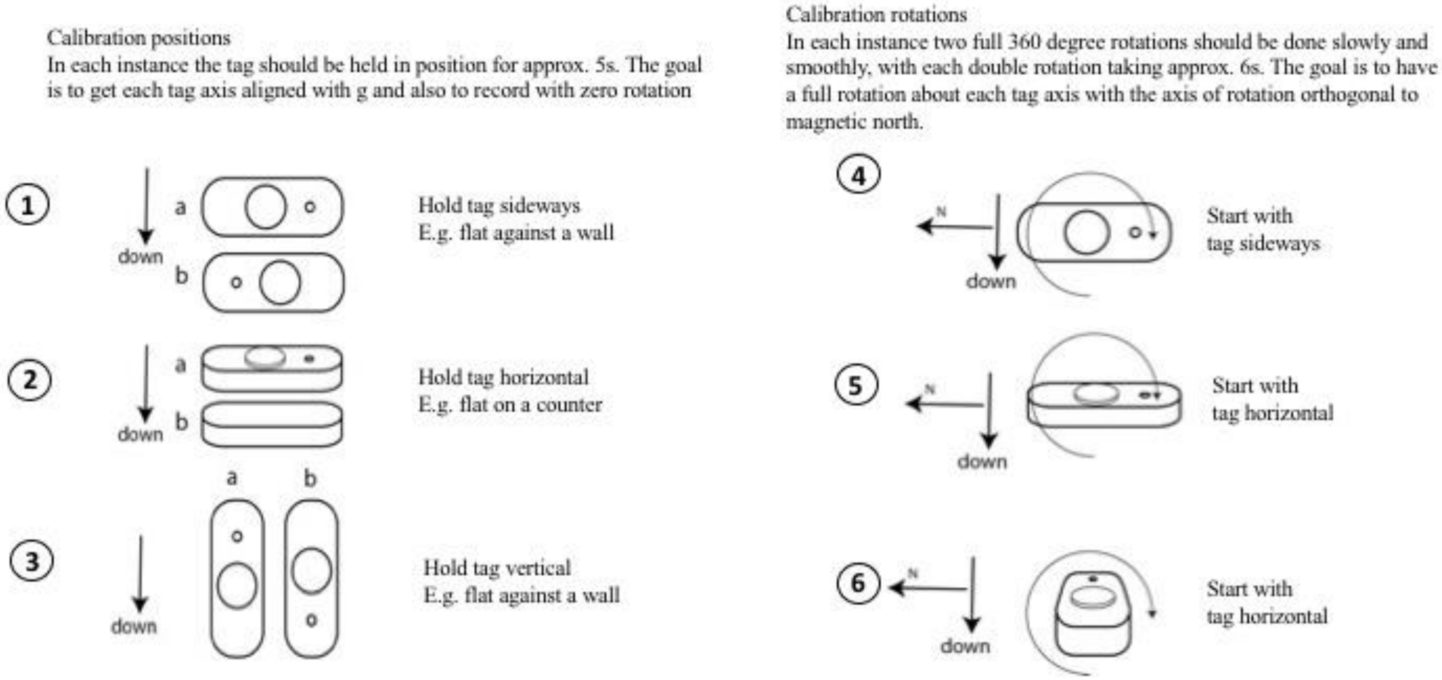


Figure. 26. A visual explanation of the grouping of behaviors from its initial observation to the next behavior registered (HSW= Horizontal swimming, VF=Vertical feeding, PF=Passive feeding)

Following the release or collection of tag packages, observations of the animal the same day and following days revealed no superficial impact to the dorsal fins of all sampled individuals, representing a clean and non-invasive attachment technique.

### *4.6 Software*

All tags were pre-calibrated before deployment and offsets of both the gyroscope and magnetometer were determined using a standard calibration protocol developed by the manufacturer. This calibration protocol consisted of the tag being placed in a series of known orientations for several seconds to gain information on the sensor's offsets for the magnetometer. Following this, the instrument was run through a series of rotations to provide the offsets for the gyroscope to magnetic north (Fig. 27).



Calibration positions
In each instance the tag should be held in position for approx. 5s. The goal is to get each tag axis aligned with g and also to record with zero rotation

Calibration rotations
In each instance two full 360 degree rotations should be done slowly and smoothly, with each double rotation taking approx. 6s. The goal is to have a full rotation about each tag axis with the axis of rotation orthogonal to magnetic north.

① Hold tag sideways
E.g. flat against a wall

② Hold tag horizontal
E.g. flat on a counter

③ Hold tag vertical
E.g. flat against a wall

④ Start with tag sideways

⑤ Start with tag horizontal

⑥ Start with tag horizontal

Figure. 27. Calibration Protocol for the Open Tag developed by Loggerhead instruments

The data from the calibration tests were run through a custom MatLab script provided by the manufacturer using the mathematical program Octave (Eaton *et al.,* 2015), an open-source program capable of running MatLab scripts. Octave generated a new file for each instrument of the offsets for the magnetometer and gyroscope, which is unique for each tag. Each instrument was run separately through the calibration protocol and a file was generated for each tag and used for the entire duration of this study. Automatically collecting data from each tagged shark was decoded from binary, offsets applied, prepared and analyzed using the statistical program R studio (R Development Core Team, 2018). Two separate files were applied to separate the dynamic acceleration from the static acceleration within the IMU and to calculate the overall dynamic body accelerations (ODBA), which is the sum of all axis in the accelerometer minus the static and the vector of the overall dynamic acceleration (VDBA). The second file, allowed for the calculation of the pitch, roll, and yaw of the instrument using the gyroscope coupled with the accelerometer data.

### *4.7 Data analysis*

Several variables were calculated and used from the IMU data. Accelerometer data consists of two portions: the static or the gravity portion and the dynamic portion, which is attributable to the muscle movements of the animal bearing the tag. The static portion of the data allows for the calculation of tag orientation and the dynamic has been correlated to energy output as seen in several species (Wilson *et al.,* 2006). The static values were calculated by moving average calculations, using a six-second window at a rate of 1.5 times the period of a tail beat as suggested by Shepard (Shepard *et al.,* 2008). Furthermore, Pitch, Roll, and Yaw were calculated from the static information. ODBA and VDBA were also calculated from the dynamic portion of the data using the equations by Wilson (Wilson *et al.,* 2006) for ODBA and Qasem (Qasem *et al.,* 2012) for VDBA. The Open tag also provides two additional sensors: ocean temperature stored in degrees Celsius and pressure (Depth) stored as mBar, and our depth calculation was used assuming saltwater (111.377 mBar/m) and a surface pressure of 1017.0 mBar an average of the study period.

### *4.8 Random Forest*

The RF algorithm was selected as the machine learning algorithm to develop the predictive behavioral model after investigating several other ML algorithms such as K-Nearest Neighbor (KNN), Extra trees classifier (ETC) and Decision Tree Classifier (CART). The choice was due to RF´s overall statistical power, the capacity to mitigate the problem of overfitting in decision trees (Breiman, 2001a; Cutler *et al.,* 2007; James *et al.,* 2013) and its ability to distinguish the classification of behaviors over the other algorithms. Construction of the model used the full array of variables from the IMU sensors incorporating additional variables that didn't come directly from the instrument's sensors totaling 24 predictive features for the movement of the sharks (Table. 8). A total of 500 trees was created in its construction, using 6 random variables tried at each split in every individual tree, following methodology from James *et al.* 2013. The statistical approach of bootstrapping was applied to separate the data into a training data set consisting of 2/3 of the data and the remaining 1/3 of the data used as a validation test.

Table.8 List of predictive features used in the Rf model construction

| **Predictive Features** | | |
|---|---|---|
| accelX | accelY | accelZ |
| magX | magY | magZ |
| gyroX | gyroY | gyroZ |
| accelXstatic | accelYstatic | accelZstatic |
| accelXdyn | accelYdyn | accelZdyn |
| accelXsd | accelYsd | accelZsd |
| ODBA | VDBA | Pitch |
| Roll | Yaw | Depth |

# 4. RESULTS

## 4.1 Descriptive data analysis

A total of 11 sharks was tagged (♂10, ♀1) in the study area (El Mogote) between October 2016 – March 2018 and their GPS positions were registered every 10 minutes throughout their tagging duration (Fig.28). Tag time ranged from 1hr 12mins to 5hrs 24mins, with a mean time of tagging of 3hrs 47mins (SD=1hr 35mins). Total length (TL) of sampled sharks ranged from 3.5 to 7m with a mean size of 5.1m (SD=1.00) (Table.9). One shark showed instrument failure and was removed from the analysis. Sharks were observed displaying several different behaviors during the sample period, some displayed all of the classified behaviors (horizontal swimming, passive, active and vertical feeding), whereas others only demonstrated a few (horizontal swimming and passive feeding), while all sharks at some stage demonstrated at least one foraging behavior under direct observation. Evaluation of the tagging event showed that only one shark showed any kind of reaction to the tagging event.

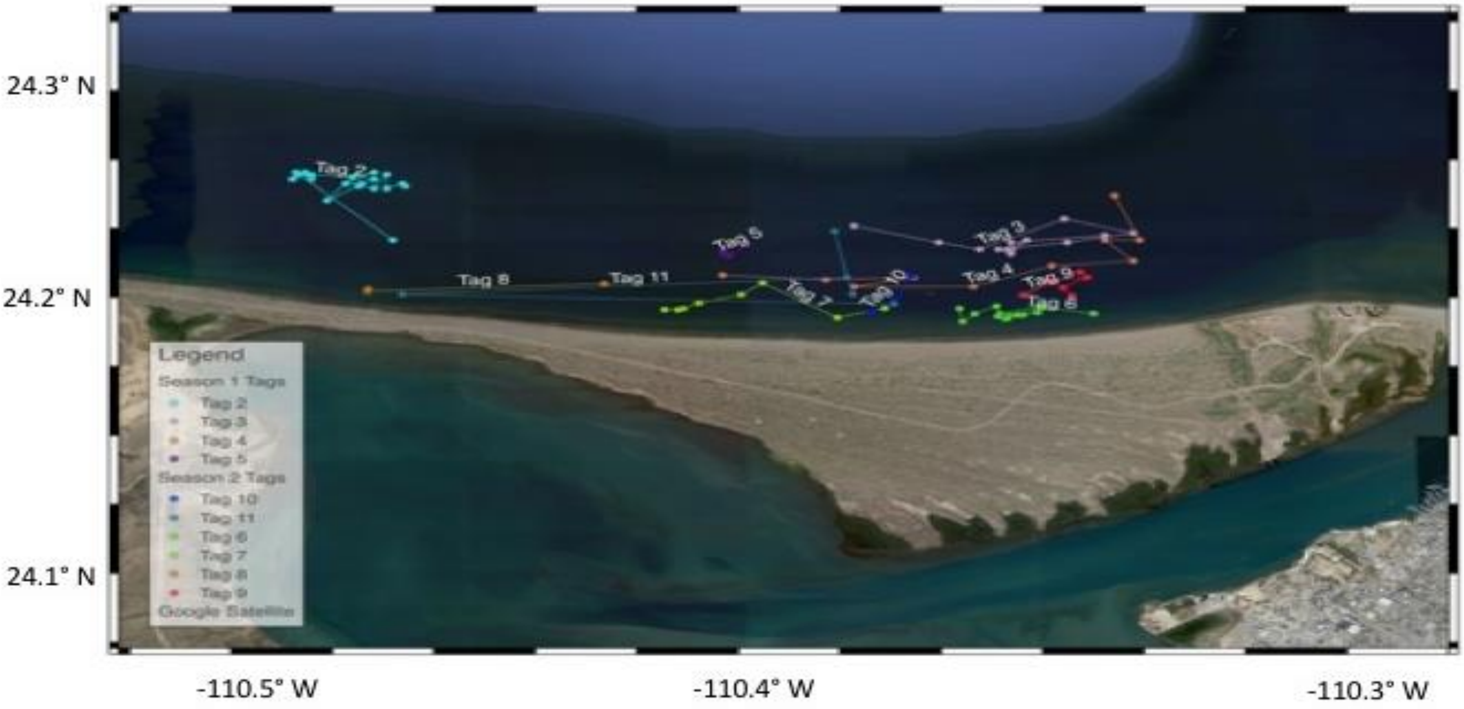


Figure.28. GPS positions of tagged sharks in 10min intervals throughout the tagging durations

Table.9. Biological characteristics of tagged sharks and tagging duration

| Shark ID | Sex | Size (M) | Duration |
|---|---|---|---|
| Shark_1 | Male | 4.5 | Tag Failure |
| Shark_2 | Male | 5.5 | 5hrs 24mins |
| Shark_3 | Male | 7 | 2hr 45mins |
| Shark_4 | Male | 4 | 1hr 12mins |
| Shark_5 | Male | 6 | 2hrs 38mins |
| Shark_6 | Male | 4.5 | 4hrs 11mins |
| Shark_7 | Female | 6 | 1hr 45mins |
| Shark_8 | Male | 5 | 3hrs 35mins |
| Shark_9 | Male | 5.5 | 2hrs 17mins |
| Shark_10 | Male | 3.5 | 5hrs 6mins |
| Shark_11 | Male | 5 | 2hrs 6mins |

### *4.2 Random Forest Model Construction & Classification*

A model comparison test was performed to validate the selection of the RF algorithm incorporating the models KNN, ETC, CART, and RF as a means to validate the model selection. Results showed that RF presented the highest correct classification of behaviors 0.742623 (74%) followed by ETC 0.736574 (73%), KNN 0.729431 (72%) and finally CART 0.666388 (66%) (Fig.29).

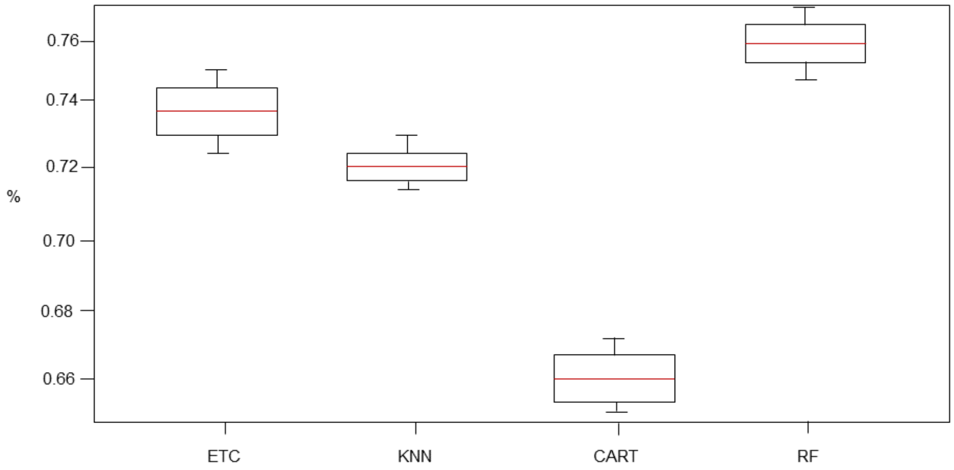


Figure. 29.Comparison test demonstrating predictive accuracy of tested models

A first model used the four classified behaviors: Passive Feeding (PF), Active Surface Feeding (ASF), Vertical Feeding (VF) and Horizontal swimming (HSW). The initial model presented an overall predictive accuracy of 82.5%. However, the accuracy varied between the different behaviors from 91.8 % for HSW to only 40.3% for PF (Table 10).

Table.10. Output and classification report for the first RF model

**Model 1** Overall Accuracy: 82.5%

| Behavior | Accuracy | Error | No. Observations |
|---|---|---|---|
| HSW | 91.8% | 8.2% | 22,258 |
| PF | 40.3% | 59.7% | 1,861 |
| VF | 82.2% | 17.8% | 10,410 |
| ASF | 44.2% | 55.8% | 3,315 |

A second model grouped the behaviors into three classes HSW, VF and OTHER a combination of PF, ASF and the other behaviors recorded (Banking, interaction with boat / swimmer) grouped together, as a means to balance the uneven number of observations of each behavior, some of them having a higher number of observations (HSW) while others very few (PF). The second model had an accuracy of 76.8% (Table.11). Less variation in the correct classification of each behavior was observed even if the HSW was still the best-predicted behavior (Table 11).

Table. 11. Output and classification report for the subsequent RF model

**Model 2** Overall Accuracy: 76.8%

| Behavior | Accuracy | Error | No. Observations |
|---|---|---|---|
| HSW | 83.5% | 16.5% | 22,258 |
| VF | 69% | 31% | 10,410 |
| OTHER | 72.4% | 27.6% | 14,849 |

To provide an evenly weighted model towards each classified behavior, a third model was constructed using an even random number of observations (10,410) for each of the three behaviors (HSW, VF, and OTHER). The third model reported similar overall predictive accuracy of 75.7% as the second model but showed very low variation in the error among behaviors and correct classification of behaviors varying from 80.5% for VF to 73.9% for HSW (Table.12).

Table. 12. Output and classification report for the final RF model

**Model 3** Overall Accuracy: 75.7%

| Behavior | Accuracy | Error | No. Observations |
|---|---|---|---|
| HSW | 73.9% | 26.1% | 10,410 |
| VF | 80.5% | 19.5% | 10,410 |
| OTHER | 73.1% | 26.9% | 10,410 |

An overall importance features plot was generated on this model to provide further evidence on which variables provide more importance to the predictive power of the RF model (Fig.26). The importance features plot demonstrated that depth of the instrument was the strongest predictor of behavior, followed by all three axes of the magnetometer, while the dynamic acceleration of the animal revealed to be the weakest predictor of behavior (Fig.30).

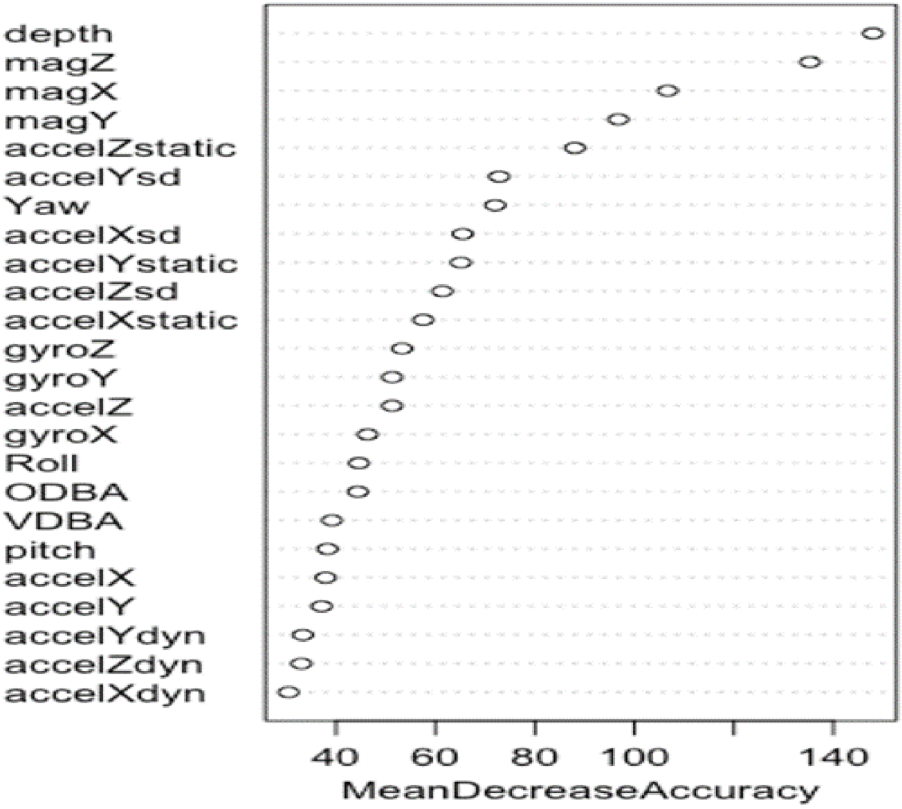


Figure.30 Importance features plot of predictive features in RF model

# 5. DISCUSSION

The main aim of this study was to develop the first ML model to predict foraging behaviors of free-swimming whale sharks using classified behavioral groups. We were successful and able to generate a strong predictive model grouping behavior into three main groups horizontal swimming, vertical feeding and other with an overall precision of 75.7%, showing promise as an effective way to predict classified behaviors, with accuracies falling between 73.1% - 80.5%. While we were successful in the classification of horizontal swimming and vertical feeding, the model was unable to characterize or identify active surface feeding or passive feeding presenting predictability of 40. 3% and 44.2%, respectively. When collecting behavioral information in a semi-captive environment on free-ranging animals like in this present study, a few factors that need to be taken into consideration or addressed. Human error in observing or correctly assigning behaviors needs to be clear and coherent, so accurate data can be gathered. Training before field sampling was an essential part of developing a study of this magnitude, and the choice to provide this to all participants both in-water and on-board vessels allowed for reliable and trustworthy data collections and unbiased data. Also, it is crucial that realistic and favorable environmental conditions are present for field surveys as mentioned in previous studies (Brewster *et al.,* 2018) using observational data for behavioral analysis. In addition, environmental changes in shallow water regions such as; current speed or direction, tidal action, and wind intensity may also potentially affect observational opportunities and the animals' movements, altering the effort or way they perform a specific behavior. The effects of environmental changes have been observed in studies on nurse sharks, were factors such as water temperature affected the swimming speeds of sampled sharks (Whitney *et al.,* 2010; Lear *et al.,* 2017).

Studies of animal movement have been conducted for more than 50 years and are rapidly increasing as tracking technologies make it possible to collect more data of a larger variety of species (Kranstauber *et al.,* 2011). Our study used a relatively small sample size similar to previous studies using accelerometry (Gleiss *et al.,* 2009, 2011, 2013; Meekan *et al.,* 2015), especially when comparing it directly with those typically gathered in both satellite or acoustic telemetry studies. Consideration must be taken into account for the highly detailed and fine-scale behavioral information gathered from data loggers (up to 50 data points a second) and its capacity to provide a much more detailed insight into the movement of an animal, that is not attainable using other conventional tagging equipment. Previous studies that investigated animal movement and habitat use in large marine mega-fauna have, for the most part, been constrained by available technology and its cost (Ropert *et al.,* 2005). The future of tagging studies may be angling towards more open-source approaches with the option of the instruments such as the ones used in this present study, that can be re-calibrated and re-used on multiple occasions or animals. This option may allow more studies in this field which currently lack sufficient funds, an alternative cost-effective approach to traditional tagging studies, that are known to present a high percentage of instrument failure or loss of equipment resulting in no data and costly overheads.

Our model's predictive accuracy improved in its ability to correctly predict behaviors with the balancing of the even number of observations of each behavioral classification, removing the effect of overfitting or overweighting in the model's design. The overfitting of predictive models has also observed in a similar study working with pinnipeds, who reported that care and consideration of the model parameters is an essential part to achieving reliable results (Ladds *et al.,* 2017). The variance between certain behaviors and the instrument or model's ability to classify them may be one of the most complicated parts of effective ML application. In our case the classification of PF, where the shark is swimming slowly through the water with their mouth slightly open, may, in fact, be concealed within other behaviors as such swimming. As the animal's speed does not appear to change when engaged in this particular behavior, nor does its body position and it only appears to slightly open its mouth presenting minimal hydrodynamic drag. These marginal differences between behaviors, coupled with the lack of observations of this particular foraging behavior when comparing to the others, may provide the best explanation of the inability of our model to classify it with any accuracy (40.3%). In contrast, VF, where the animal may position itself in an almost vertical position and almost cease to propel itself in a forward motion, makes it distinctively different from all other behaviors collected. In a similar study that used the application of

ML algorithms for the classification of elasmobranch behavior, foraging events were distinguished by the observation of head-shaking directly after consumption in captive lemon sharks (Brewster *et al.,* 2017). The authors did document the evidence of a single feeding event under observation, that did not result in head-shaking, only a gulping motion on a smaller fish, that was not discernible from swimming behavior or even detected by the mounted tag package. This direct observation of a predation behavior in lemon sharks (*Negaprion brevirostris*) a more dramatically moving species of shark, might illustrate that even observed behaviors can be masked within datasets, and for a passive moving animal such as the whale shark, the marginal change in behavior from swimming to PF, may, in fact, be more difficult to define or even detect. Although further work on the behavioral classification of the species is needed, with more incidences of PF under observation, to clarify this theory. The unfortunate circumstance of not being able to correctly classify ASF the most commonly observed foraging behavior (Motta *et al.,* 2010) was a disappointment to our results. Given the number of literatures documenting this foraging behavior and the personal observations in our study area didn’t concur with tagged sharks. Continued work using machine learning algorithms and accelerometry data should strive to gather more incidences of all foraging behaviors in controlled data collections, allowing for a more robust model construction and ultimately the potential for a more fruitful classification of this important foraging behavior.

The predictive features plot exposed that the depth of the instrument plays an important role in the model's ability to classify behavioral groups. Previous work on whale sharks using several variables such as acceleration and depth, as a means to compare activity patterns and vertical habitat use, did observe contradictory patterns in the activity pulses of depth profiles against those from locomotor activity measured (Gleiss *et al.,* 2011, 2013). These patterns observed in depth data within the water column do not necessarily reflect animal activity and are probably not adequate for defining foraging patterns of pelagic animals, especially those that are planktivorous; this is supported further by work in basking sharks (Whearmouth & Simms, 2009). It is a known phenomenon that zooplankton make daily vertical migrations to and from the surface waters to forage (Folt & Burns, 1999; Valle-Levinson *et al.,* 2004). This behavior is thought to reduce predation pressure as decreased light levels during the night offer a semicryptic environment for these micro-organisms and this has been shown to affect the foraging patterns of a wide range of species (Hays, 2003). Given that whale sharks are filter-feeding predominantly on zooplankton, time of day may be a key factor in the specific activity whale sharks may display, especially with regards to the intensities of foraging behaviors. Peaks in the vertical activity of whale sharks around sunset seen in previous studies (Gleiss *et al.,* 2009, 2013) may be an indication of sharks anticipating the movement of zooplankton, prompting these daily vertical diving behaviors at certain times of the day and may explain the rapid increased pitch angles observed, as opposed to low ascent angles typical of movement in the surface waters or across open oceans (Gleiss *et al.,* 2011).

Swimming energetics and foraging efficiency of whale sharks have shown that *R. typus* exhibits four strategies to save energy and improve foraging efficiency, with these foraging adaptations believed to even increase efficiency (>20%) relative to swimming horizontally and resolve the energy-budget paradox of whale sharks (Meekan *et al.,* 2015). In addition, the negative buoyancy of the species incorporates periods of gliding behavior to further save energy during the decent part of dives a pattern consistent with previous studies (Gleiss *et al.,* 2011, 2013) and a further energy-conservation tactic, previously documented in marine mammals and birds (Williams *et al.,* 2000; Davis *et al.,* 2001). Whale sharks in Western Australia appear to show pronounced phases of ram surface filter-feeding and high activity at dusk and dawn, and it may suggest that temporal dynamics of aggregation of the zooplankton prey represents the critical factor in influencing the behavior of whale sharks at Ningaloo (Gleiss *et al.,* 2013). Although whale sharks are known to feed throughout the day and appear to show a strong correlation with time spent in surface waters feeding during daylight hours such as in the Mexican Caribbean (Motta *et al.,* 2010), Djibouti (Rowat *et al.,* 2007), Belize (Heyman *et al.,* 2001) the GC (Clark & Nelson, 1997; Nelson & Eckert, 2007; Ketchum *et al.,* 2013; Whitehead *et al.,* 2019) and even Western Australia. Given this, a level of caution needs to be considered before suggesting that the species feeds predominately at dusk or dawn in relation to the diel movement of its prey, as the species is believed to be opportunistic in its foraging strategies and may in fact forage whenever it needs to replenishment energy costs, regardless of the time of day.

In our case, the importance of depth as a predictor of behavioral classification may just be due to the position of the animal when engaged in VF compared to HSW, and the placement of the tag on the first dorsal fin of the shark. This constant depth of the instrument given the distance of the fin to the mouth of the shark coupled with the energy requirements of the behavior may generate a pattern in the data used by the model and aid in the classification of this foraging behavior. This theory may also be further supported given the shallow water environment of the study area (Duran-Campos et al., 2015) and the lack of observations of the sharks diving during behavioral observations. Ultimately, the ability of the model to use depth of the instrument as the strongest predictor of VF and HSW by the altering positions of the sensor is an exciting innovative approach to define one distinct foraging behavior against swimming as proved in our results.

In conclusion, this study demonstrates the utility of a voting ensemble ML algorithm such as RF and its effectiveness as a classifier to predict certain behaviors of whale sharks from accelerometry data. While the sample size is relatively low the study does present strong evidence of its application in a shallow habitat with slow-moving megafauna under direct observation, which otherwise would be impractical given the diving ability of the species and its elusive behaviors. By minimizing energetic costs and maximizing food intake animals can increase the amount of energy available to them for growth and reproduction, ultimately increasing their fitness. However, these behaviors are largely antagonistic to each other, with behaviors focused on foraging increasing overall energetic expenditures. Thus, when and where or how often individuals display foraging behavior is very important to their energy budgets. Future work of the species both in this area and across global aggregation sites should address the energy expenditures of the species when engaged in each specific behavior and continue to investigate the utility of new machine learning applications.

# XI. THESIS CONCLUSIONS

Juvenile whale sharks, for the most part, are known to aggregate in somewhat coastal waters to forge, allowing researchers to interact with them and study their feeding behaviors with relative ease. While advancements have been made in the ways we observe and gather information on the species both in their natural habitat and in a captive environment, there still seems to be a limited amount of published information focused on the feeding ecology of this shark. A clear factor that does seem to influence the feeding behaviors of whale sharks, is the availability of zooplanktonic organisms that support their dietary needs. In numerous regions around the world, correlations with the availability of a food source and the occurrence of whale sharks exists, such as off Christmas Island were sharks are witnessed foraging on the spawning on the megalopa larvae of red land-crabs (Meekan *et al*., 2009), in Tanzania where they are observed targeting dense patches of sergestid shrimp (Rohner *et al.,* 2015) and off the Yucatan Peninsula, Mexico where they are observed feeding on fish eggs (De la Parra Venegas *et al*., 2011; Cárdenas-Palomo *et al.,* 2014). This association between high or dense patches of food and the occurrence of whale shark feeding events, is also present here in the GC, with the species witnessed targeting dense blooms of copepods in both Bahia de Los Angeles (Nelson & Eckert, 2007) and BLP (Clark & Nelson, 1997; Hocohen *et al.,* 2006; Ketchum *et al.,* 2013). This present study further highlighted the importance of the productivity of groups of copepods in BLP and especially around the coastal waters along the El Mogote sandbar. We found that when the abundance of available food is at its highest, especially during the winter months, the number of whale sharks sighted in the area seems to reach a peak, concurring with published studies that focused on the sightings of juvenile sharks in the bay and the GC (Ketchum *et al.,* 2013; Ramirez-Marcia *et al.,* 2017; Whitehead *et al.,* 2019). These aggregations of juvenile sharks are without doubt driven by changes in environmental factors that are present in the region, such as wind direction, its magnitude, and the circulation of a central mesoscale cyclonic eddy, which influences the horizontal distribution of all trophic groups of zooplankton in the bay (Obeso-Nieblas *et al.,* 2007; Durán-Campos *et al.,* 2015; Coria-Monter *et al.,* 2017). These factors together may play a significant role in moving smaller scattered food patches into more concentrated patches in certain regions, during particular months of the year resulting in potential hot spots for whale shark sightings as presented in a recent study in this area (Whitehead *et al.,* 2019). The location of BLP and the orientation of its mouth to the GC might even provide a natural plankton trap, with zooplankton being moved by currents and surface winds in the GC, mixing with strong upwellings along landmasses and underwater seamounts at the entrance of the bay, resulting in a healthy water exchange between these two bodies of water. These unique conditions, may also be a behavioral trigger that controls the movement patterns or homing ability of the whale sharks to this region. Studies on whale shark movement usually involve conventional tagging studies either by acoustic or satellite devices to examine large-scale movements or short-term residency and habitat use (Gunn *et al*., 1999; Eckert & Stewart, 2001; Rowat & Gore, 2007), respectively. In recent years, non-invasive approach though photo identification tools (Marshall & Pierce, 2012) has provided an additional platform for monitoring regional whale shark movements and site fidelity. However, overall, there is a lack of information on the different aspects of navigation and movement, and what may control it, especially at this important juvenile stage. Movement in response to environmental conditions may provide physical or physiological benefits, and in some cases maybe a means of avoiding mortality from a lack of a food source, in this case, plankton productivity. This statement in itself may even reinforce how important movement is as a behavioral response in mobile populations, and how finely tuned a species can be to the conditions and changes within their environment.

Three feeding behaviors were observed in BLP during our sampling period and they seem to be in response to zooplankton densities and composition (Ketchum *et al.,* 2013; Whitehead *et al.,* 2019). Vertical feeding, for the most part, appears to occur when concentrations of available food are both at the surface and in high concentrations. Whereas, passive feeding is usually observed when food is scarce or when sharks are theoretically moving from one dense patch to another as a means to take advantage of opportunistic feeding techniques. Throughout the coastal waters off the El Mogote and its uninhabited coastline to the southern regions of the bay, whale sharks have been observed foraging on dense blooms of copepods (Clark & Nelson, 1997; Hacohen-Domené *et al.,* 2006; Ketchum et al., 2013). The importance of this taxonomic group of organisms to the diet and occurrence of whale sharks, needs to be further examined, to understand if the size of available copepods correlates to the size of animals targeting them, or what densities or available food induce the different types of foraging behaviors of whale sharks. Future studies should aim to estimate the daily dietary intake and ingestion per unit of time between the different foraging behaviors in BLP. A study in the Mexican Caribbean (Motta *et al.,* 2010) did successfully estimate the daily intake and ingestion for surface ram filter feeding in whale sharks, but information on the other foraging behaviors was not presented, which may have been due to the lack of observations of the other foraging behaviors in this sample site. The El Mogote region presents the ideal shallow water habitat to develop such a study as all foraging behaviors are present throughout the season as observed in this present study.

The opportunistic feeding behavior of this filter feeder allows it to prey upon a wide variety of planktonic organisms. In the last two decades, several studies have investigated the feeding habits of this species using a variety of methods, such as observations during feeding events (Clark & Nelson, 1997; Heyman *et al.,* 2001; Duffy, 2002; Hacohen-Domené *et al.,* 2006; Hoffmayer *et al.,* 2007; Nelson & Eckert, 2007; Motta *et al.,* 2010; Ketchum *et al.,* 2013), stomach content analysis (Gudger, 1941; Silas & Rajagopalan, 1963; Rohner *et al.,* 2013), DNA of feces to confirm the presence of specific prey items (Meekan *et al.,* 2009), fatty acids (Couturier *et al.,* 2013; Rohner *et al.,* 2013; Cárdenas-Palomo *et al.,* 2018) and stable isotope analysis (Borrell *et al.,* 2011; Marcus *et al.,* 2017; Yu *et al.,* 2016; Prebble *et al.,* 2018; Wyatt *et al.,* 2019). However, studies of this nature in Mexican waters have been limited to direct observations (Hacohen-Domené *et al.,* 2006; Motta *et al.,* 2010; De la Parra Venegas *et al.,* 2011; Ketchum *et al.,* 2013), with only one recent study on fatty acids in whale sharks from the Mexican Caribbean (Cárdenas-Palomo *et al.,* 2018) and currently no published work on stable isotope analysis. Natural variations in stable isotopes of C and N at the base of the food web take place as a result of differences in productivity, upwelling, and other oceanographic factors. These changes provide important information on the habitat use of marine organisms in any given ecosystem (Aurioles-Gamboa *et al.,* 2009). The isotopic work from this study provides an isotopic assessment of tissues in whale sharks from BLP over multiple sample seasons. We observed elevated $\delta^{15}N$ values when compared directly to previous isotopic studies on the species (Borrell *et al.,* 2011; Marcus *et al.,* 2017; Yu *et* *al.,* 2016), which may be a result of differences in the oceanographic conditions that influence this region. BLP, and in general the GC, are characterized for having high $\delta^{15}N$ values due to a denitrification process and the transport of denitrified waters from the Eastern Tropical North Pacific into the GC (Altabet *et al.,* 1999; Voss *et al.,* 2001; White *et al.,* 2013).

While it is a known that sex-segregated aggregations are present in whale sharks with the majority of sites being dominated by juvenile male members (Rowat & Brooks, 2012; Whitehead *et al.,* 2019), we found like many other studies no significant differences between our isotopic signatures and the sex of sampled animals. During our sampling, we did however, observe female sharks foraging amongst male sharks in the same patches of food, illustrating that especially in region, both sexes appear to target the same food sources during feeding events. The lack of observations of female sharks in all aggregation sites around the world, may be due to different foraging behavior rather than a dietary preference. Females may essentially be feeding in a different space in the water column away from the surface waters, out of the visual observation of researchers or during different times of the day or night. But without further evidence, this theory is only speculation and backed only by personal observations to date. Understanding the times of day that females forage using modern technologies equipped with depth and light sensors may shed some light on this theory and offer a platform to solve this unknown phenomenon that currently eludes researchers.

A correlation between shark size and $\delta^{15}N$ was present in our results, but is fragile, when the size range of sampled sharks is small, affecting the statistical power. This relationship between animal size and $\delta^{15}N$ supports the common theory of a probable dietary shift in the species as they mature (Borrell *et al.,* 2011). For the whale shark, separation by size has also once more been previously reported in the GC (Eckert & Stewart, 2001; Ketchum *et al.,* 2013) and in other regions such as Belize (Graham & Roberts, 2007), Djibouti and the Seychelles (Rowat *et al.,* 2011). Juvenile sharks appearing to prefer coastal often shallow water environment to forage, whereas larger adult sharks are observed in open ocean environments or in areas of high productivity, such as around underwater sea mounts and continental shelves (Rowat & Brooks, 2012; Ketchum *et al.,* 2013; Ramirez-Marcia *et al.,* 2017). Like all previously presented studies using stable isotope analysis on the species (Borrell *et al.,* 2001; Markus *et al.,* 2017; *Yu et al.,* 2016; Prebble *et al.,* 2018; Wyatt *et al.,* 2019), a level of caution needs to be established in regards to the assumptions of the isotopic analysis, its limitations and potential errors as exposed by other studies working with sharks (Hussey *et al.,* 2010; Kim *et al.,* 2011, 2012; Wyatt *et al.,* 2019). While we were successful in generating an isotopic assessment of juvenile sharks in BLP over two subsequent sample seasons, future work in this discipline and species should aim to increase the proportion of female to male sharks sampled, investigate size grouping and opt for techniques to increase the accuracy of size estimations, allowing for a more detailed examination of the size ranges of individuals and a likely size of any kind of dietary shift.

The continued use of observational data to record real time behavioral traits of whale sharks can be a costly practice and often requires long days in the field. Also, the issue of human error and biases associated with visual observational techniques raises concerns as the interpretation of behaviors can be different between individuals, although true clarification of this subjection is problematic. Our trail approach of a voting ensemble model generated a baseline of information and a platform for future studies on how, and when, we may be able to observe the feeding behaviors of sharks in BLP, with minimal impact. The learning curve with machine learning is a gradual one, which requires understanding the way in which these mathematical algorithms use and interpret datasets. This present study revealed that certain foraging behaviors of the world's largest shark, may be at times, be masked within other behaviors given the minimal behavioral changes presented by the species. These unique characteristics in the species demonstrate the importance of correctly assigning and classifying behaviors during the field based behavioral observations and the consequence of working with limited observations of behaviors in pre-selected groups. Throughout marine science the adoption of simple approaches to investigate animal behavior, continues to be the most successful, and increased task or equipment complexity has a much greater tendency to incline towards failure (Harvey *et al.,* 2004).

Animal ethics and care given to sampled animals, especially if they hold both national and international protection, is an important and often overlooked area of investigation. Our integrity to this area of investigation, through clean, uncompromised sampling protocols and the development of a non-invasive attachment clamp to mount data logging instruments played an important role in ensuring that animal ethics were of the highest standard. It allowed us to successfully gather important data from free-swimming sharks with minimal impact essential when working in a lively tourism destination. Throughout the last two decades, tourism interactions with whale sharks have become a highly lucrative industry and more localities with habitual whale shark occurrences have themselves formed whale shark activities that generate profitable revenue for local communities (Pierce *et al.,* 2010). In BLP, eco-tourism activities involving whale sharks have existed for more than a decade and now concerns are apparent regarding the effect it may be having on this endangered species (Whitehead *et al.,* 2018, 2019). In recent years, pressures from uncontrolled tourism has motivated authorities to implement an area of protection and site-specific regulations for these activities, as a means to regulate this ever-growing industry (SEMARNAT, 2017; Whitehead *et al.,* 2019). As this industry continues to grow, so does the appearance of the sharks on social media outlets and media channels, in part due to the availability of more economical photographic equipment and smart devices that are constantly connected to the internet. Impactful research techniques from investigators, may in fact, alter the guest satisfaction when encountering a shark during a tourism interaction, if encountering an animal with a damaged body or fin from inadequate monitoring techniques. These photographic opportunities and the power of social media may place researchers in a negative light to the public, potentially affecting future funding and authorized investigation permits. Although it does provide a level of accountability for monitoring techniques on this currently endangered species of shark.

This project pushed forward our knowledge of the feeding ecology of whale sharks in this region, however, there are still some very important questions which remain unanswered regarding the species dietary preference and foraging strategies, which have been raised in this thesis, along with suggestions to further advance our understanding of the world’s largest fish.

Research on whale sharks relies on hard work and the dedication of researchers. Unfortunately, the number of scientists that devote their lives to the protection of these magnificent creatures is still very low compared to other marine megafauna such as cetaceans. While our understanding of the species has greatly increased since 1828, there are still areas we know little or nothing about, such as, where they spent the first and final parts of their lives, the processes involved in their mating rituals and where the majority of the females spend their daily lives. The continued development of new research techniques both in the lab and in the field, as well as the increased awareness of this species among the public, is certainly a step in the right direction towards uncovering the mystery of the world’s largest fish and understanding the best way to protect it.

# XII. PUBLISHED LITERATURE

This thesis and its investigation under the authorized permits were successful in generating a number of published literature and several more currently in revision, all of which are listed below:

Whitehead, D.A., Becerril-García, E.E., Petatán-Ramírez, D., Vázquez-Haikin, A., González-Armas, R & Galván-Magaña, F. 2018. Whale shark *Rhincodon typus* strandings in the Gulf of California, Mexico. *Journal of Fish Biology,* 94(1),165-167. doi: http://dx.doi.org/10.1111/jfb.13845

Whitehead, D., Galván-Magaña, F., Soto-López, K., Juaristi-Videgaray, D., Cervantes-Gutiérrez, F., & Becerril-García, E. (2019). First description of a deceased juvenile whale shark Rhincodon typus in La Paz Bay, Mexico with comments on morphometry and age estimates. *Latin American Journal of Aquatic Research, 47*(3), 575-579. doi:http://dx.doi.org/10.3856/vol47-issue3-fulltext-19

Whitehead, D., Petatán, D., Olivier, D., Gonzalez Armas, R., Pancaldi, F & Galvan-Magana, F. 2019. Seasonal trends in whale shark *Rhincodon typus* sightings in an established tourism site in the Gulf of California, Mexico. *Journal of Fish Biology*, 95(3), 982-984. doi:http://dx.doi.org/10.1111/jfb.14106

Whitehead, D., Murillo-Cisneros, D., Elorriaga-Verplancken, F. R., Hacohen-Domené, A., La Parra, R., Pancaldi, F., Gonzalez-Armas & Galvan, F. 2018. Stable Isotope assessment of whale sharks across two ocean basins: Gulf of California and the Mexican Caribbean. *Journal of Experimental Marine Biology and Ecology* (Currently under third revision).

D. A. Whitehead, D., Jakes-Cota, U., Pancaldi, F., Galván-Magaña, F & González-Armas, R. 2019. The influence of zooplankton communities on the feeding behaviors of whale shark in Bahia de La Paz, Gulf of California. Revista Mexicana de Biodiversidad (Under revision).

Whitehead, D.A., Jakes-Cota, U., Galván-Magaña, F., Palcaldi,F & and González-Armas, R. 2019. Description of the composition and abundance of groups of macrozooplankton in the coastal waters off the El Mogote Sandbar, a known foraging area for juvenile whale sharks. Revista Hidrobiológica (Second revision).

# XIII. FULL REFERENCE LIST

Altabet, M.A., Cynthia Pilskaln, C., Thunell, R., Pride, C., Sigman, D., Chavez, F & Francois, R. 1999. The nitrogen isotope biogeochemistry of sinking particles from the margin of the Eastern North Pacific. - *Deep*-Sea Research. 46: 655-679.

Amande, M. J., Ariz, J., Chassot, E., Molina, A. D., Gaertner, D. & Murua, H. 2010. Bycatch of the European purse seine tuna fishery in the Atlantic Ocean for the 2003–2007 period. *Aquatic Living Resources*, 23, 353–362.

Aurioles-Gamboa, Newsome, S.D., Salazar-Pico, S & koch, P.L. 2009. Stable isotope differences between sea lions (*Zalophus*) from the Gulf of California and Galápagos Islands. - Journal of Mammalogy. *90*(6): 1410- 1420.

Arzoumanian, Z., Holmberg, J & Norman, B. 2005. An astronomical pattern-matching algorithm for computer-aided identification of whale sharks Rhincodon typus. *Journal of Applied Ecology, 42*, 999-1011.

Badan-Dangon A, Dorman C & Merrifield M. 1991. The lower atmosphere over the Gulf of California. J. Geophys. Res. 96: 16887–16896.

Beers, J.R. 1976. Volumetric methods. In: H.F. Steedman (ed.). Zooplankton Fixation and Preservation, *Monographs on Oceanographic Methods* 4, Paris, Unesco: 54-60.

Beckley, L. E., Cliff, G., Smale, M. J & Compagno, L. J. V. 1997. Recent strandings and sightings of whale sharks in South Africa. *Environmental Biology of Fishes* 50, 343–348.

Berumen M.L., Braun, C.D., Cochran, J.E., Skomal, G.B & Thorrold, S.R. 2014. Movement patterns of juvenile whale sharks tagged at an aggregation site in the Red Sea. *PLoS One*. 2014;9(7):e103536.

Bigelow, H. B & Schroeder, W. C. 1948. *Fishes of the Western North Atlantic*. Yale, CT: *Sears Foundation for Marine Research*, Yale University.

Brainerd, E.L. 2001. Caught in the crossflow. *Nature,* 412, 387–388.

Breiman, L. 2001. Random forests. Machine Learning, 45, 5e32. http://dx.doi.org/10.1023/A:1010933404324.

Brewster, L.R., Dale, J.J & Guttridge, T.L. 2018. Development and application of a machine learning algorithm for classification of elasmobranch behavior form accelerometry data, *Marine Biology*, 165(62).

Borrell, A., Cardona, L., Kumarran, R.P & Aguilar, A. 2011. Trophic ecology of elasmobranchs caught off Gujarat, India, as inferred from stable isotopes. *CES Journal of Marine Sciences,* 68, 547–554

Burgess, K.B & Bennett M.B. 2016. Effects of ethanol storage and lipid and urea extraction on δ15 N and δ13C isotope ratios in a benthic elasmobranch, the bluespotted maskray Neotrygon kuhlii. *Journal Fish Biology*, 90, 417–423

Burks, C. M., Driggers, W. B & Mullin, K. D. 2006. Abundance and distribution of whale sharks (*Rhincodon typus*) in the northern Gulf of Mexico. *Fishery Bulletin* 104, 579 – 584.

Cárdenas-Palomo, N., Herrera-Silveira, J., Velázquez-Abunader, I., Reyes, O., & Ordoñez, U. 2014. Distribution and feeding habitat characterization of whale sharksRhincodon typusin a protected area in the north Caribbean Sea. *Journal of Fish Biology, 86(2), 668–686.*

Cárdenas-Palomo, N., Norena-Barroso, E., Herrera-Silviera, J., Galvan-Magana, F & Hocohen-Domene, A. 2018. Feeding habits of the whale shark (Rhincodon typus) inferred by fatty acid profiles in the northern Mexican Caribbean. *Environmental Biology of Fishes*,11,1599-1612.

Carroll, G., Slip, D., Jonsen, I & Harcourt, R. 2014. Supervised accelerometry analysis can identify prey capture by penguins at sea. *Journal of Experimental Biology*, 217,4295–4302.

Chang, W. B., Leu, M. Y & Fang, L. S. 1997. Embryos of the whale shark, *Rhincodon typus*, early growth and size distribution. *Copeia* 1997, 444–446.

Chassot, E., Amande, M. J., Chavance, P., Pianet, R. & Dedo, R. G. 2009. Preliminary results on tuna discards and bycatch purse seine fishery of the eastern Atlantic. *Collective Volume Of Scientific Papers*, 64, 1054-1067.

Chen, V. Y & Phipps, M. J. 2002. Management and Trade of Whale Sharks in Taiwan. Taipei: TRAFFIC East Asia.

Clark, E & Nelson, D.R. 1997. Young whale sharks, Rhincodon typus, feeding on a copepod bloom near La Paz, Mexico. *Environmental Biology of Fishes*, 50, 63–73.

Churchill, D.A., Heithaus, M.R & Grubbs, R.D. 2015. Effects of lipid and urea extraction on δ15 N values of deep-sea sharks and hag- fish: can mathematical correction factors be generated?, *Deep- Sea Research* II, 115,103–108.

Clingham, E., Brown, J., Henry, L., Beard, A & Dove, A.D. 2016. Evidence that St. Helena island is an important multi-use habitat for whale sharks, *Rhincodon typus*, with the first description of putative mating in this species. *PeerJ Preprints 4:e1885v1.*

Colman, J. G. 1997. A review of the biology and ecology of the whale shark. *Journal of Fish Biology* 51, 1219–1234.

Compagno, L.J.V., Dando, M & Fowler, S. 2005. Sharks of the world. Princeton University Press, New Jersey.

Coria-Monter, E., Monreal-Gómez, M., Salas de Leon, D., Durán-Campos, E & Merino-Ibarra, M. 2017. Wind driven nutrient and subsurface chlorophyll-a enhancement in the Bay of La Paz, Gulf of California. Estuarine, *Coastal and Shelf Science*. 196.

Couturier, L.I.E., Rohner, C.A., Richardson, A.J., Pierce, S.J., Marshall, A.D., Jaine, F.R.A., Townsend, K.A., Bennett, M.B., Weeks, S.J & Nichols, P.D. 2013. Unusually high levels of n-6 polyunsaturated fatty acids in whale sharks and reef manta rays. *Lipids*

48,1029–1034.

Cruz-Orozco, R.C, Martinez-Noriega, C & Mendoza-Maravillas, A. 1996. Batimetría y sedimentos de la Bahía de La Paz, B.C.S, *Océanides*, 11(1), 21-27.

Cutler, D. R., Edwards, T. C., Beard, K. H., Cutler, A., Hess, K. T & Gibson, J. 2007. Random forests for classification in ecology. *Ecology*, 88(11), 2783e2792.

Davis, R. W., Fuiman, L. A., Williams, T. M & Le Beouf, B. J. 2001. Three-dimensional movements and swimming activity of a northern elephant seal. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 129, 759–770.

De la Parra Venegas R., Hueter R., Gonzalez Cano J., Tyminski J & Gregorio Remolina J.J. 2011. An unprecedented aggregation of whale sharks, Rhincodon typus, in Mexican coastal waters of the Caribbean Sea. *PloS one,* 6: e18994.

Delacy, C. R., Olsen, A., Howey, L. A., Chapman, D. D., Brooks, E. J. & Bond, M. E. 2017. Affordable and accurate stereo-video system for measuring dimensions underwater: a case study using oceanic whitetip sharks Carcharhinus longimanus. *Marine Ecology Progress Series,* 574, 75–84.

DeNiro, M. J and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. - Geochimica et Cosmochimica Acta. 42: 495-506

Dolenec T, Vokal B & Dolenec, M. 2005. Nitrogen—15 signals of anthropogenic nutrient loading in Anemonia sulcata as a possible indicator of human sewage impacts on marine coastal ecosystems: a case study of Pirovac Bay and the Murter Sea (Central Adriatic). *Croat Chemica Acta,* 78(4),593–600.

Dove, A. D. M. (2015). *Foraging and Ingestive Behaviors of Whale Sharks, Rhincodon typus, in Response to Chemical Stimulus Cues. The Biological Bulletin, 228(1), 65–74.*

Duffy, C. A. J. 2002. Distribution, seasonality, lengths, and feeding behaviour of whale sharks (*Rhincodon typus*) observed in New Zealand waters. *New Zealand Journal of Marine and Freshwater Research,* 36, 565–570.

Duran-Campos, L, Salas-de-Leon, D. A, Monreal-Gomez, M. A, Aldeco-Ramírez, J & Coria-Monter, E. 2015. Differential zooplankton aggregation due to relative vorticity in a semi-enclosed bay, Estuarine, *Coastal and Shelf Science*, 164, 10-18.

Eaton, J. W., Bateman, D., Hauberg, S & Wehbring, R. 2015. GNU Octave version 4.0.0 manual: a high-level interactive language for numerical computations. Retrieved From http://www.gnu.org/software/octave/doc/interpreter

Eckert, S.A & Stewart, B.C. 2001. Telemetry and satellite tracking of whale sharks, *Rhincodon typus*, in the sea of Cortez, Mexico, and the North Pacific Ocean. - *Environmental Biology of Fishes*, 60, 299-308.

Escalle, L., Murua, H., Amande, J. M., Arregui, I., Chavance, P., Delgado de Molina, A., … & Merigot, B. 2016. Post-capture survival of whale sharks encircled in tuna purse-seine nets: tagging and safe release methods. *Aquatic Conservation: Marine and Freshwater Ecosystems, 26(4), 782–789.*

Estrada, J.A., Rice, A.N., Lutcavage, M.E & Skomal, G.B. 2003. Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. *Journal Marine Biology Association,* UK, 83, 1347-1350.

Folt, C.L & Burns, C.W. 1999. Biological drivers of zooplankton patchiness. Trends in Ecology & Evolution,14, 300–305.

Fulgencio de Moura, J., Merico, A., Montone. R.C., Silva, J., Seixas, T.G., de Oliveira Godoy, J.M., Saint-Pierre, T.D., Hauser-Davis,R. A., Madeira Di Beneditto, A.P., Reis, E.C., Tavares, D.C., Lemos, L.S & Siciliano, S. 2015. Assessment of trace elements, POPs, $^{210}$Po and stable isotopes ($^{15}$N and $^{13}$C) in a rare filter-feeding shark: The megamouth, *Marine Pollution Bulletin.*

Garrick, J.A.F. 1964. Additional information on the morphology of an embryo whale shark. *Proceeding of the united states national museum*, 115,10

Gleiss, A. C., Norman, B., Liebsch, N., Francis, C & Wilson, R. P. 2009. A new prospect for tagging large free-swimming sharks with motion-sensitive data-loggers. *Fisheries Research* 97, 11–16.

Gleiss, A., Norman, B & Wilson, R. 2011. Moved by that sinking feeling: variable diving geometry underlies movement strategies in whale sharks. *Functional Ecology,* 25, 595–607.

Gleiss, A. C., Wright, S., Liebsch, N., Wilson, R. P & Norman, B. 2013. Contrasting diel patterns in vertical movement and locomotor activity of whale sharks at Ningaloo Reef. *Marine Biology*, 160(11), 2981e2992.

Graham, R.T & Roberts, C.M. 2007. Assessing the size, growth rate and structure of a seasonal population of whale sharks (*Rhincodon typus* Smith 1828) using conventional tagging and photo identification*. Fisheries Research*, 84, 71-80.

Grünewälder, S., Broekhuis, F., Macdonald, D.W., Wilson, A.M., McNutt, J.W., Shawe-Taylor, J & Hailes, S. 2012. Movement activity-based classification of animal behaviour with an application to data from cheetah (*Acinonyx jubatus*). *PLoS One,* 7:e49120.

Gudger, E. W. 1915. Natural history of the whale shark *Rhincodon typus* Smith. *Zoological: New York Zoological Society, 1,* 349-389.

Gudger, E.W. (1941). The food and feeding habits of the whale shark, *Rhineodon typus*. J. Elisha Mitchell Sci. Soc. 57, 57–72.

Gunn, J. S., Stevens, J. D., Davis, T. L. O & Norman, B. D. 1999. Observations on the short term movements and behaviour of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia. *Marine Biology* 135, 553–559.

Hacohen-Domené, A, Galván-Magaña, F & Ketchum, J. 2006. Abundance of whale shark (*Rhincodon typus*) preferred prey species in the southern Gulf of California, Mexico, *Cybium, 30(4), 99-102.*

Hacohen, A.D. (2007). Ecología trófica alimentaria del tiburón ballena (Rhincodon typus, Smith, 1828) en la costa occidental del Golfo de California, México. *Tesis de maestría. CICIMAR-IPN, 57 pp*

*Hacohen*-Domené*, A.D. 2015. Uso de hábitat trófico del tiburón ballena (Rhincodon typus) yla manta gigante (Manta cf. birostris) en el norte del caribe mexicano, Tesis de Doctorado, CICIMAR-IPN 114pp.*

Hammerschlag, N., Gallagher, A. J & Lazarre, D. M. 2011. A review of shark satellite tagging studies. *Journal of Experimental Marine Biology and Ecology,* 398, 1–8.

Hanfee, F. 2007. Indian whale shark fishery. In The First International Whale Shark Conference: Promoting International Collaboration in Whale Shark Conservation, Science and Management (Irvine, T. R. & Keesing, J. K., eds), p. 74 Perth: *CSIRO Marine and Atmospheric Research*, Australia.

Harvey, E. S., Shortis, M. R., Seager, J. W & Hall, N. 2004. Refining non-intrusive stereo video techniques & protocols for SBT transfers. *Report on AFMA Research Contract Number R03/1428. Australian Fisheries Management Authority Research,* 1-*37.*

Hays, G.C. 2003. A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations, *Hydrobiologia,* 503,163–170.

Hearn, A.R., Green, J.R., Espinoza, E., Peña-Herrera, C., Acuña, D & Klimley, A.P. 2013. Simple criteria to determine detachment point of towed satellite tags provide first evidence of return migrations of whale sharks (Rhincodon typus) at the Galapagos Islands, Ecuador, *Animal Biotelemetry,* 1:11.

Hernández-Trujillo, S., Esquivel-Herrera, A & Saldierna-Martínez, R. 1987. Biomasa zooplanctónica en la costa oeste de Baja California Sur (1982-1985), en: Ramírez-Rodríguez E. M. (Ed.), *Mem. Simp. Invest. Biol. Ocean. Pesq. Méx*., CICIMAR-IPN, La Paz, B.C.S, México, 161-170.

Hernández-Nava, M. F & S. Álvarez-Borrego. 2013. Zooplankton in a whale shark (Rhincodon typus) feeding area of Bahía de los Ángeles (Gulf of California), *Hidrobiológica,* 23 (2), 198-208.

Heyman W., Graham R., Kjerfve B & Johannes R. 2001. Whale sharks Rhincodon typus aggregate to feed on fish spawn in Belize. *Marine Ecology Progress Series,* 215, 275–282.

Hobbs, J.-P. A., Frisch, A. J., Hamanaka, T., McDonald, C. A., Gilligan, J. J. & Neilson, J. 2009. Seasonal aggregation of juvenile whale sharks (Rhincodon typus) at Christmas Island, Indian Ocean. Coral Reefs, 28, 577 Hoem, J .2008. The reporting of statistical significance in scientific journals. *Demographic Research*, 18,437–442.

Hoffmayer, E.R., Franks, J.S., Driggers, W.B., Oswald, K.J & Quattro, J. M. 2007. Observations of a feeding aggregation of whale sharks, Rhincodon typus, in the north central Gulf of Mexico. Gulf Caribbean Research, 19,69–74.

Holmberg, J., Norman, B & Arzoumanian, Z. 2008. Estimating population size, structure, and residency time for whale sharks Rhincodon typus through collaborative photo-identification. *Endangered Species Research*, 7, 39–53.

Hussey, N.E., Brush, J., McCarthy, I & Fisk, A.T. 2010. $\delta^{13}$C and $\delta^{15}$N diet-tissue discrimination factors for large sharks under semi- controlled conditions. *Comparative Biochemistry and Physiology Part A Molecular and Integrative Physiology,* 155, 445–453.

Hussey, N.E., MacNeil, M.A., Olin, J.A., McMeans, B.C., Kinney, M.J., Chapman, D.D & Fisk, A.T. 2012. Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions, *Journal of Fish Biology*, 80,1449–1484.

Iwasaki, Y. 1970. On the distribution and environment of the whale shark, Rhincodon typus, in skipjack fishing grounds in the western Pacific Ocean. *Journal of the College of Marine Science Technology, Tokai University*, 4, 37-51 (in Japanese with English abstract).

Jaffa, K.S, & Taher, N. A. B. A. 2007. Whale sharks in Palestinian waters: a whale shark (Rhincodon typus, Smith 1828) rescued near the Tantura Beach, Carmel Coast, North Palestine. The first record from the Palestinian Mediterranean Coast. Gazelle: *The Palestinian Biological Bulletin,* 71, 22–23.

James, G., Witten, D., Hastie, T & Tibshirani, R. 2013. An Introduction to Statistical Learning with Applications in R. Springer Texts in Statistics. Springer, New-York (2013).

Jeffreys, G. L., Rowat, D., Marshall, H. & Brooks, K. 2013. The development of robust morphometric indices from accurate and precise measurements of free-swimming whale sharks using laser photogrammetry. Journal of the Marine Biological Association of the United Kingdom, Journal of the Marine Biological Association of the United Kingdom, 1-12.

Jiménez-Illescas, A.R. 1996. Análisis de procesos barotrópicos y baroclínicos en la Bahía de La Paz, BCS. *Tesis doctoral, Universidad Nacional Autónoma de México, DF*, 212 pp.

Jiménez-Pérez, L.C & J.R. Lara-Lara. 1988. Zooplankton biomass and copepod community structure in the Gulf of California during the 1982–1983 El Niño event. CALCofi Fish. Inv. Rep,12,122-128.

Jonahson, M & Harding, S. 2007. Occurrence of whale shark (Rhincodon typus) in Madagascar. Fisheries Research, 84(1), 132-135.

Joung, S.J., Chen, C.T., Clark, E., Uchida, S & Huang, W.Y. 1996. The whale shark, *Rhincodon typus*, is a livebearer: 300 embryos found in ‘megamamma’ supreme. *Environmental Biology of Fishes*, 46(3), 219-223.

Kays, R., Crofoot, M. C., Jetz, W & Wikelski, M. 2015. Terrestrial animal tracking as an eye on life and planet. *Science*, 348(6240).

Ketchum, J.T. 2003. Distribución espacio-temporal y ecología alimentaria del Tiburón ballena (*Rhincodon typus*) en la Bahía de la Paz y zonas adyacentes en el Suroeste del Golfo de California. *Master's thesis, Centro Interdisciplinario de Ciencias Marinas-IPN, La Paz*

Ketchum, J. T, Galván-Magaña, F &Klimley, P. 2013. Segregation and foraging ecology of whale sharks, Rhincodon typus, in the southwestern Gulf of California, *Environmental Fish Biology*, 96(3), 779-755.

Kim, S. L., Casper, D. R., Galvan-Magana, F., Ochoa-Dıaz, R., Hernandez-Aguilar, S. B & Koch, P. L. 2011. Carbon and nitrogen discrimination factors for elasmobranch soft tissues based on a long-term controlled feeding study. *Environmental Biology of Fishes*, 95, 37-52.

Kim S. L., del Rio, C. M., Casper, D & Koch, P. L. 2012. Isotopic incorporation rates for shark tissues from a long-term captive feeding study. *The Journal of experimental biology,* 215, 2495-2500.

Kranstauber, B., Cameron, A., Weinzerl, R., Fountain, T., Tilak, S., Wikelski, M & Kays. R. 2011. The Movebank data model for animal tracking, *Environmental Modelling & Software*, 26(6),834-835.

Krause, J., Krause, S., Arlinghaus, R., Psorakis, I., Roberts, S & Rutz, C. 2013. Reality mining of animal social systems. *Trends in Ecology & Evolution*, 28(9), 541e551.

Ladds, M.A., Thompson, A.P., Kadar, J.P., Slip, D., Hocking, D & Harcourt, R. 2017. Super machine learning: improving accuracy and reducing variance of behaviour classification from accelerometry. Animal Biotelemetry, 5,8.

Lavaniegos, B.E., G. Heckel, G & Ladrón de Guevara, P. 2012. Seasonal variability of copepods and cladocerans in Bahía de Los Ángeles (Gulf of California) and importance of *Acartia clausi* as food for whale sharks. Ciencias Marinas, 38(1A), 11-3.

Lear, K.O., Whitney, N.M., Brewster, L.R., Morris. J.J., Hueter, R.E & Gleiss, A.C. 2017. Correlations of metabolic rate and body acceleration in three species of coastal sharks under contrasting temperature regimes. Jounral of Experimental Biology, 220(3), 397-407.

León-de la Luz, J., López, B.E & De la Cruz, G. 2006. Estructura y composición de la barra arenosa El Mogote, Baja california Sur, México. *Boletín de la Sociedad Botánica de México.* 79, 21-32.

Li, Y., Zhang, Y., Hussey, N.E & Dai, X. 2016. Urea and lipid extraction treatment effects on $\delta^{15}$N and $\delta^{13}$C values in pelagic sharks. *Rapid Communications in Mass Spectrometry,* 30,1-8.

Marcus, L., Virtue, P., Pethybridge, H.R., Meekan, M.G., Thums M & Nichols, P.D. 2017. Intraspecific variability in diet and implied foraging ranges of whale sharks at Ningaloo Reef, Western Australia, from signature fatty acids. *Marine Ecology Progress Series*, 554, 115-128.

Martin, R. A. 2007. A review of behavioural ecology of whale sharks (Rhincodon typus). *Fisheries Research,* 84, 10–16.

Martínez del Rio, C., Wolf, N., Carleton, S. A & Gannes, L. Z. 2009. Isotopic ecology ten years after a call for more laboratory experiments. *Biological Reviews* 84, 91-111.

Marshall, A. D & Pierce, S. J. 2012. The use and abuse of photographic identification in sharks and rays. *Journal of Fish Biology,* 80, 1361-1379.

McClune, D.W., Marks, N.J., Wilson, R.P., Houghton, D., Montgomery, W., McGowan, N.E., Gormley, E & Scantlebury, M. 2014. Tri-axial accelerometers quantify behaviour in the Eurasian badger (*Meles meles*): towards an automated interpretation of eld data. Animal Biotelemetry, 2:5.

Meekan, M. G., Bradshaw, C. J. A., Press, M., McLean, C., Richards, A. & Quasnichka, S. 2006. Population size and structure of whale sharks (Rhincodon typus) at Ningaloo Reef, Western Australia*. Marine Ecology Progress Series*, 319, 275-285.

Meekan M., Jarman S., McLean C & Schultz M. 2009. DNA evidence of whale sharks (Rhincodon typus) feeding on red crab (Gecarcoidea natalis) larvae at Christmas Island, Australia, *Marine and Freshwater Research* 60, 607–609.

Meekan M., Fuiman, L., Davis, R., Berger, Y & Thums, M. 2015. Swimming strategy and body plan of the world's largest fish: implications for foraging efficiency and thermoregulation. *Frontiers in Marine Science*, 2, 64.

Minagawa, M. et al. 1984. Comparison of Kjeldahl and combustion methods for measurement of nitrogen isotopes ratios in organic matter. - Analytical Chemistry. 56: 1859-1861.

Monreal-Gomez, M.A., Molina-Cruz, A & Salas-de-Leon, D. 2001. Water masses and cyclonic circulation in Bay of La Paz, Gulf of California, during June 1998, *Journal of Marine Systems,* 30, 305–315.

Motta, P. J., Maslanka, M., Hueter, R.E., Davis, R.L., De la Parra, R., Mulvany, S.L., Habegger, M.L., Strother, J.A., Mara, K.R., Gardiner, J.M., Tyminski, J.P & Zeigler, L.D. 2010. Feeding anatomy, filter-feeding rate, and diet of whale sharks *Rhincodon typus* during surface ram filter feeding off the Yucatán Península, Mexico. *Zoology*, 113, 199-212.

Nelson, J.D & Eckert, S.A. 2007. Foraging ecology of whale sharks (*Rhincodon typus*) within Bahía de los Angeles, Baja California Norte, *Mexico. Fisheries Research*, 84, 47-64.

Newsome, S.D., Martinez del Rio, C., Bearhop, S 7 phillips, D.L. 2007. A Niche for isotopic ecology. *Frontiers in the Ecology and the Environment*, 5(8), 429-436.

Norman, B. 1999. Aspects of the biology and ecotourism industry of the whale shark *Rhincodon typus* in north-western Australia. *Master's Thesis, Murdoch University, Western Australia.* Available at http://researchrepository.murdoch.edu.au/231/1/01Front.pdf/

Norman, B & Stevens, J. D. 2007. Size and maturity status of the whale shark (*Rhincodon typus*) at Ningaloo Reef in Western Australia. *Fisheries Research,* 84, 81–86.

Obeso-Nieblas, M., Shirasago, B., Sanchez-Velasco, L & Rodríguez, J.H. 2004. Hydrographic variability in Bahia de la Paz, BCS, Mexico, during the 1997-1998 el Nino. Deep-Sea Reseach, Part II, 51, 689e710.

Olivier, D., Loiseau, N., Petatán-Ramírez, D., Trujillo Millán, O., Suárez Castillo, A.N., Torre, J., Munguia-Vega, A & Reyes-Bonilla, H. 2018. Functional biogeography of the reef fishes of the islands of the Gulf of California: Integrating functional divergence into marine conservation, *Global Ecology and Conservation,* 16.

Orams, M. B. 2002. Feeding wildlife as a tourism attraction: A review of issues & impacts. *Tourism management*, 23, 281-293.

Paig-Tran, E, M., Bizzarro, J. J., Strother, J. A., & Summers, A. P. 2011. Bottles as models: predicting the effects of varying swimming speed and morphology on size selectivity and filtering efficiency in fishes. *Journal of Experimental Biology*, 214(10), 1643-1654.

Palomares-García, J.R. 1996. Estructura espacial y variación estacional de los copépodos en la Ensenada de La Paz. *Oceánides,* 11,29-43.

Park, R & S. Epstein. 1961. Metabolic fractionation of $^{13}C$ and $^{12}C$ in plants. *Plant Physiology*, 36, 133-138.

Perry, C. T., Figueiredo, J., Vaudo, J. J., Hancock, J., Rees, R., & Shivji, M. 2018. *Comparing length-measurement methods and estimating growth parameters of free-swimming whale sharks (Rhincodon typus) near the South Ari Atoll, Maldives. Marine and Freshwater Research,* 69(10) 1487-1495.

Peterson, B. J & Fry, B. 1987. Stable isotopes in ecosystems. *Annual Review of Ecology Systematics,* 18, 293-320.

Pierce, S.J., Mendez-Jimenez., A., Collins, K., Rosero-Caicedo, M & Monadjem, A. 2010. Developing a Code of Conduct for whale shark interactions in Mozambique. *Aquatic Conservation, 20*, 782-788.

Pierce, S.J & Norman, B. 2016. *Rhincodon typus*. *The IUCN Red List of Threatened Species 2016*, [http://dx.doi.org/10.2305/IUCN.UK.2016-1.RLTS.T 19488A2365291.en]. Reviewed: 5 April 2018.

Pinnegar, J. K., Jennings, S., O'Brien, C. M & Polunin, N. V. C. 2002. Long-term changes in trophic level of the Celtic Sea fish community and fish market price distribution. *Journal of Applied Ecology,* 39, 377-390.

Qasem, L., Cardew, A., Wilson, A., Griffiths, I., Halsey, L. G & Shepard, E. L. C. 2012. Tri-Axial Dynamic Acceleration as a Proxy for Animal Energy Expenditure; Should We Be Summing Values or Calculating the Vector?. *PloS One*, *7*(2), e31187.

Quiros, A. L. 2007. Tourist compliance to a code of conduct and the resulting effects on whale shark (*Rhincodon typus*) behavior in Donsol, Philippines. *Fisheries Research,* 84, 102-108.

R Development Core Team. 2018. R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Ramírez-Macías, D., Vázquez-Haikin, A & Vázquez-Juárez, R. 2012. Whale shark Rhincodon typus populations along the west coast of the Gulf of California and implications for management. Endangered Species Research, 18,115-128.

Ramírez-Macías, D., Queiroz, N., Pierce, S.J., Humphries, N.E., Sims, D.W., Brunnschweiler, J.M. 2017.Oceanic adults, coastal juveniles: tracking the habitat use of whale sharks off the Pacific coast of Mexico, *PeerJ,* 5:e3271

Rau, G.H., Tetssie, J.L., Rassoulzadegan, F & Fowler, S.W. 1990. $^{13}C/^{12}C$ and $^{15}N/^{14}N$ variations among size fractionated marine particles: implications for their origin and trophic relationship, *Marine Ecology Progress Series,* 59, 33-38.

Riley, M. J., Hale, M.S., Harman, A & Rees, R.G. 2010. Analysis of whale shark *Rhincodon typus* aggregations near South Ari Atoll, Maldives Archipelago. *Aquatic Biology,* 8, 145-150.

Robinson, D.P., Jaidah, M.Y., Jabado, R.W., Lee-Brooks, K., Nour El-Din, N.M & Malki A.A.A. 2013. Whale Sharks, *Rhincodon typus*, aggregate around offshore platforms in Qatari Waters of the Arabian Gulf to Feed on Fish Spawn. *PLoS ONE* 8(3): e58255.

Robles-Pacheco, J.M & Marinone, S.G. 1987. Seasonal and inter- annual thermohaline variability in the Guaymas Basin in the Gulf of California. *Continent Shelf Research,* 7, 715-733.

Rohner, C.A., Richardson, A.J., Marshall, A.D., Weeks, S.J. & Pierce, S.J. 2011. How large is the world’s largest fish? Measuring whale sharks Rhincodon typus with laser photogrammetry. *Journal of Fish Biology,* 78(1), 378-385.

Rohner, C.A., Couturier, L.I., Richardson, A.J., Pierce, S.J., Prebble, C.E., Gibbons, M.J & Nichols, P.D. 2013. Diet of whale sharks *Rhincodon typus* inferred from stomach content and signature fatty acid analyses. *Marine Ecology Progress Series,* 493, 219-235.

Rohner, C.A., Armstrong, A.J & Pierce S.J. 2015. Whale sharks target dense prey patches of sergestid shrimp off Tanzania. *Journal of Plankton Research*, 37(2):352-362.

Ropert-Coudert, Y & Wilson, R.P. 2005. Trends and perspectives in animal-attached remote sensing. *Frontiers in Ecology and the Environment*, 3, 437-444.

Rowat, D & Gore, M. 2007. Regional scale horizontal and local scale vertical movements of whale sharks in the Indian Ocean off Seychelles. *Fisheries Research* 84, 32–40.

Rowat, D., Brooks, K., Marc,h A., McCarten, C., Jouannet, D., Riley, L., Jeffrey, G., Perri, M., Vely, M & Pardigon B. 2011. Long-term membership of whale sharks (*Rhincodon typus*) in coastal aggregations in Seychelles and Djibouti, *Marine and Freshwater Research*, 62, 621-627.

Rowat, D & Brooks, K. 2012.. A review of the biology, fisheries and conservation of the whale shark *Rhincodon typus*. *Journal of fish biology*, 80, 1019-1056.

Saino, R & Hattori, A. 1987. Geographical Variations of the water column distribution of suspended particulate organic nitrogen and its 15N natural abundance in the Pacific and its marginal seas. *Deep Sea Research*, 34, 807-827.

Sampson, L., Galvan-Magana, F., De Silva-Davilla, R., Aguíniga-García, S & O'Sullivan, J.B. 2010. Diet and trophic position of the devil rays *Mobula thurstoni* and *Mobula japanica* as inferred from stable isotope analysis. *Journal of the Marine Biological Association of the United Kingdom,* 90, 969-976.

Sanchez-Velasco, L., Beier, E., Avalos-Garcia, C & Lavin, M.F. 2006. Larval fish assemblages and geostrophic circulation in Bahia de La Paz and the surrounding southwestern region of the Gulf of California, *Journal of Plankton Research,* 28(11), 1-18.

Sanderson, S.L., Cheer, A.Y., Goodrich, J.S., Graziano, J.D., & Callan, W.T. 2001. Crossflow filtration in suspension-feeding fishes. *Nature,* 412, 439–441.

Schmidt, J., Chen, C., Sheikh, S.I., Meekan, M., Norman, B. & Shoou-Jeng, J. 2010. Paternity analysis in a litter of whale shark embryos. Endangered Species research, 12, 117124

Secretaría de Medio Ambiente y Recursos Naturales (SEMARNAT). 2017. Plan de Manejo de Rhincodontypus (tiburón ballena) para real- izar la actividad de aprovechamiento no extractivo a través de la observación y nado en Bahía de La Paz, B.C.S.,temporada 2017. Retrieved from https://www.gob.mx/semarnat/documentos/plan-de- manejo-de-tiburonballena-rhincodontypus-en-bahia-de-la-paz-bcs

Sequeira, A. M., Thums, M., Brooks, K. & Meekan, M. G. 2016. Error and bias in size estimates of whale sharks: implications for understanding demography. *Royal Society Open Science,* 3, 150668.

Shepard, E., Wilson, R. P., Halsey, L. G., Quintana, F., Gómez Laich, A & Gleiss, A.C. 2008. Derivation of body motion via appropriate smoothing of acceleration data. *Aquatic Biology*, *4*, 235–241.

Shortis, M. 2015. Calibration techniques for accurate measurements by underwater camera systems. *Sensors*, 15(12), 30810–30826.

Silas, E.G & Rajagopalan, M.S. 1963. On a recent capture of a whale shark (Rhincodon typus Smith) at Tuticorin, with a note on information to be obtained on whale sharks from Indian waters. Journal of Marine Biology Association of India, 5, 153-157.

Sims, D., Southall, E., Quayle, V. & Fox, M. 2000. Annual social behaviour of basking sharks associated with coastal front areas. *Proceedings of the Royal Society of Biology,* 267, 1897–1904.

Smith, P.E & Richards, S.L. 1979. Standard techniques for pelagic fish eggs and larval surveys. FAO Fisheries Technical Paper, 175, 100.

Stacey, N.E., Karam, J., Meekan, M.G., Pickering, S. & Ninef, J. 2012. Prospects of whale sharks conservation in the Eastern Indonesia through bajo traditional ecological knowledge and community-based monitoring. *Conservation & Society*, 10, 63-75.

Stewart, J. D., Beale, C. S., Fernando, D., Sianipar, A.B., Burton, R.S., Semmens. B.X & Aburto-Oropeza, O. 2016. Spatial ecology and conservation of *Manta birostris* in the Indo-Pacific, *Biological Conservation,* 200, 6-7

Taylor, G. 1994. Whale Sharks: Marine wildlife conservation and community-based ecotourism. *Angus & Roberston Publishers, Sydney.*

Taylor, J.G. 1996. Seasonal occurrence, distribution and movements of the whale shark, Rhincodon typus, at Ningaloo reef, Western Australia. *Marine and Freshwater Research*, 47, 637-642.

Taylor, G. 2007. Ram filter-feeding and nocturnal feeding of whale sharks (*Rhincodon typus*) at Ningaloo Reef, Western Australia. *Fisheries Research* 84, 65–70.

Tomkiewicz, S. M., Fuller, M. R., Kie, J. G & Bates, K. K. 2010. Global positioning system and associated technologies in animal behaviour and ecological research. Philosophical Transactions of the Royal Society B: *Biological Sciences*, 365(1550), 2163e2176.

Tyminski, J. P., Hueter, R. E. & de la Parra, R. 2008. The vertical movements of whale sharks tagged with pop-up archival satellite tags off Quintana Roo, Mexico. In Second International Whale Shark Conference. Holbox, Mexico. Cancun: *Comision Nacional de Areas Naturales Protegidas.* Available at http://www.domino.conanp.gob.mx/doc_conf/John_T_Holbox_Vertical_Movements_Talk_Final.pdf

Uchida, S., Toda, M., Kamei, Y. & Teruva, H. 2000. The husbandry of 16 whale sharks Rhincodon typus, from 1980 to 1998 at the Okinawa expo aquarium, In abstract of the *American Elamsobrach Society 16th annual meeting*, La Paz, B.C.S, Mexico.

Valle-Levinson, A., Trasviña Castro, A., Gutiérrez de Velasco, G & González Armas, R. 2004. Diurnal vertical motions over a seamount of the southern Gulf of California, *Journal of Marine Systems*, 50(1–2), 61-77.

Valletta, J.J., Torney, C., Kings, M., Thornton, A & Madden, J. 2017. Applications of machine learning in animal behaviour studies. Animal Behaivor,124, 203–220.

Vander-Zanden, M.J., Cabana, G & Rasmussen, J.B. 1997. Comparing the trophic position of litoral fish estimated using stable nitrogen isotopes ($\delta^{15}N$) and dietary data. *Canadian Journal of Fisheries and Aquatic Sciences*, 54, 1142-1158.

Van Tienhoven, A.M., Den Hartog, J.E., Reijns, R.A & Peddemors, V.M. 2007. A computer-aided program forpattern-matchingof naturalmarksonthe spottedraggedtooth shark *Carcharias taurus*. *Journal of Applied Ecology, 44*(2), 273-280.

Voss, M., Dippner, J.W & Montoyer, J.W. 2001. Nitrogen isotope patterns in the oxygen-deficient waters of the Eastern Tropical North Pacific Ocean. - Deep-Sea Research I. *48*(8): 1905-1921.

Wang, Y., Nickel, B., Rutishauser, M., Bryce, C.M., Williams, T.M., Elkaim, G & Wilmers, C.C. 2015. Movement, resting, and attack behaviors of wild pumas are revealed by tri-axial accelerometer measurements. Movement Ecology, 3:1.

Wearmouth, V.J & Sims, D.W. 2009. Movement and behaviour patterns of the critically endangered common skate *Dipturus batis* revealed by electronic tagging. Journal of Marine Biology and Ecology, 380,77-87.

White, A. E., Foster, R.A., Benitez-Nelson, C.R., Pasque, P., Verdeny, E., Arthur, K.E., Popp, B.N & Prahl, F.G. 2013. Nitrogen fixation in the Gulf of California and the Eastern Tropical North Pacific. - Progress in Oceanography. 109: 1-17.

Whitehead, D.A., Becerril-García, E.E., Petatán-Ramírez, D., Vázquez-Haikin, A., González-Armas, R & Galván-Magaña, F. 2018. Whale shark *Rhincodon typus* strandings in the Gulf of California, Mexico. *Journal of Fish Biology,* 94(1), 165-167.

Whitehead, D., Petatán, D., Olivier, D., Gonzalez Armas, R., Pancaldi, F & Galvan-Magana, F. 2019. Seasonal trends in whale shark *Rhincodon typus* sightings in an established tourism site in the Gulf of California, Mexico. *Journal of Fish Biology*, 95,

Whitney, N.M., Pratt, H., Pratt, T & Carrier, J. 2010. Identifying shark mating behaviour using three-dimensional acceleration loggers. Endangered Species Research, 10, 71-82.

Wilson, R. P., White, C. R., Quintana, F., Halsey, L. G., Liebsch, N., Martin, G. R & Butler, P.J. 2006. Moving towards acceleration for estimates of activity specific metabolic rate in free-living animals: the case of the cormorant. *Journal of Animal Ecology, 75*(5), 1081-1090.

Williams, T. M., Davis, R. W., Fuiman, L. A., Francis, J., Le, B. J & Horning, M. 2000. Sink or swim: strategies for cost-efficient diving by marine mammals. *Science,* 288, 133–136.

Wintner, S.P. 2000. Preliminary study of vertebral growth rings in the whale shark, *Rhincodon typus*, from the east coast of South Africa. *Environmental Biology of Fishes,* 59, 441– 451.

Yu, C.J., Joung, S.J., Liu, K.M., Hsu, H.H & Lin, C.Y. 2016. Stable isotope analysis for the whale shark in the waters of Taiwan. QScience Proceedings, *The 4th International Whale Shark Conference*, 2016: iwsc4.68 http://dx.doi.org/10.5339/qproc.2016.iwsc4.68

Ziegler, J. 2010. Assessing the sustainability of whale shark tourism: a case study of Isla Holbox, Mexico. *Unpublished masters of Science dissertation, University of Victoria*.