INSTITUTO POLITECNICO NACIONAL

CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

Habitat use of juvenile white sharks (*Carcharodon carcharias*) and shortfin mako sharks (*Isurus oxyrinchus*) in a nursery area in the west coast of Baja California, México.

TESIS

QUE PARA OBTENER EL GRADO DE DOCTOR EN CIENCIAS MARINAS

PRESENTA

Elena Tamburin

LA PAZ, B.C.S., JUNIO DEL 2018

SIP-14 BIS

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 05 del mes de Marzo 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:

"HABITAT USE OF JUVENILE WHITE SHARKS (*Carcharodon carcharias*) AND SHORTFIN MAKO SHARKS (*Isurus oxyrinchus*) IN A NURSERY AREA IN THE WEST COAST OF BAJA CALIFORNIA, MEXICO"

Presentada por el alumno:

TAMBURIN	-----	ELENA
Apellido paterno	materno	nombre(s)

Con registro:

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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.

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En la Ciudad de La Paz, B.C.S., el día 14 del mes de Marzo del año 2019

El (la) que suscribe MC. ELENA TAMBURÍN Alumno (a) del Programa

DOCTORADO EN CIENCIAS MARINAS

con número de registro A150176 adscrito al CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

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

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"HABITAT USE OF JUVENILE WHITE SHARKS (*Carcharodon carcharias*) AND SHORTFIN MAKO (*Isurus oxyrinchus*) IN A NURSERY AREA IN THE WEST COAST OF BAJA CALIFORNIA, MEXICO"

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MC. ELENA TAMBURIN

Nombre y firma del alumno

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TABLE OF CONTENTS

Figures

Tables

Glossary

Habitat: union of the resources and conditions present in a specific area that an organism needs to ensure its presence (e.g. food resources, water quality), and to guarantee the survival and reproduction success of the species (Krausman, 1999).

Habitat use: the way of animals to use the physical and biological resources inside the habitat, including foraging, cover, nesting, mating, or protection from threats (Krausman, 1999; Heithaus, 2007).

MS: mako shark

NEP: Northeast Pacific Ocean

RBCs: red blood cells

SCB: Southern California Bight

SIA: stable isotope analysis a technique based on natural tracers of carbon and nitrogen isotope values (δ13C and δ15N) to track resource flow within and across ecosystems.

SVB: Sebastian Vizcaino Bay

TP: Trophic position

WS: white shark

TB: tiburón blanco

TM: tiburón mako

YOY: Young of the years. The animals which are younger or equal to one years old.

SIBER: Stable Isotope Bayesian Ellipses in R

MIXSIAR: Bayesian isotopic mixing models in R

Abstract

White sharks (*Carcharodon carcharias*; WS) and shortfin mako sharks (*Isurus oxyrinchus*; MS) are globally distributed apex predators and keystone species. However, regional information regarding juvenile biology, such as habitat preferences and trophic ecology, is lacking. This study investigates habitat use and feeding ecology of juvenile shortfin mako and white sharks in a nursery area; the Sebastian Vizcaino Bay (SVB; Baja California, Mexico) using stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) using a multiple tissues approach. During 2015 and 2016 we collected muscle (n=165), blood samples (n=147), red blood cells (RBC; n=62) and plasma (n=70) young MS and muscle (n=6), RBC (=2) and plasma (n=2) of young WS from Sebastian Vizcaino Bay (SVB). We also collected local preys, to develop a conceptual foraging framework based on stable isotope analysis. In addition, during 2015 to 2017 we obtained catches and direct observations data of 12 neonates and juveniles around Isla Cedros, in the western coast of Baja California, Mexico suggesting this island as new important nursery area for white sharks. The size range for MS was 64.5-196 cm of total length (TL) and 130-292 cm TL for WS, indicating that the individuals are newborn, young of the year (YOY) and juveniles. Isotopic values for MS muscles ranged from -19 ‰ to -16.4 ‰ for $\delta^{13}C$ and from 13.6 ‰ to 20.1 ‰ for $\delta^{15}N$. For whole blood samples of MS ranged from -23.2 ‰ to -16.2 ‰ for $\delta^{13}C$ and 11.7 ‰ to 19.6 ‰ for $\delta^{15}N$, for RBCs the $\delta^{13}C$ values ranged from -19.5 ‰ to -16.5 ‰ and for $\delta^{15}N$ from 13.5 ‰ to 18.7 ‰ (mean 16.5 ± 1.2 ‰). The plasma of MS showed isotopic values ranged from -19.9 ‰ to -16 ‰ for $\delta^{13}C$ and from 14 ‰ to 20.7 ‰ for $\delta^{15}N$. Whereas in WS muscles, values ranged from -18 ‰ to -15.4 ‰ for $\delta^{13}C$ and from 16.2 ‰ to 18.9 ‰ for $\delta^{15}N$. For the WS whole blood, we obtained -18.3 ‰ to -14.2 ‰ for $\delta^{13}C$ and 16.4 ‰ to 17.1 for $\delta^{15}N$. For RBCs of WS the $\delta^{13}C$ values ranged from -16.3 ‰ to -15.4 ‰ and for $\delta^{15}N$ from 16 ‰ to 17.1 ‰. The plasma of WS showed isotopic values ranged from -16 ‰ to -15.9 ‰ for $\delta^{13}C$ and from 17.4 ‰ to 17.5 ‰ $\delta^{15}N$. MS and WS with similar total length (MS> 102 cm TL and WS < 186 cm TL) reflected in their tissues (muscle, blood, RBC and plasma) the regional baseline 15-N enriched isotopic signature of SVB. These results suggest that SVB have a unique isotopic signature (isoscape), which is trackable in the animals that feed for long times in its trophic chain and that both species showed similar habitat in SVB. The similarity in isotopic niche and isotopic composition between MS and WS in multiple tissues reflecting large and short time periods suggest shared resource and common habitat use inside SVB throughout extended times confirming their residency in this area during their earlier life stages. In fact, the increase in $\delta^{15}N$ values with shark sizes suggest an ontogenetic shift from maternal source to a long-term use of prey resources within SVB, particularly for MS. In addition, this the data of neonate and juvenile WS around Isla Cedros combined with the unique record of the adult female pregnant of MS in the same area, pointed out that the region of Isla Cedros is a critical habitat and possible pupping ground for both sharks species.

Resumen

Los tiburones blancos (*Carcharodon carcharias*; TB) y los tiburones mako de aleta corta (*Isurus oxyrinchus*; TM) son depredadores topes distribuidos a nivel mundial y representan especies claves. Sin embargo, la información regional sobre la biología de los juveniles, como las preferencias de hábitat y la ecología trófica es aun escasa. En este estudio se investiga el uso del hábitat y la ecología trófica de los tiburones mako de aleta corta juveniles y de los tiburones blancos juveniles en un área de crianza: la Bahía Sebastián Vizcaíno (SVB; Baja California, México) mediante el análisis de isótopos estables (SIA) del carbono ($\delta^{13}C$) y nitrógeno ($\delta^{15}N$) utilizando un enfoque con múltiples tejidos. Durante 2015 y 2016 se recolectaron muestras de músculo (n = 165), de sangre entera (n = 147), de glóbulos rojos (RBCs; n= 62) y de plasma (n = 70) de TM juveniles y músculo (n = 6), glóbulos rojos (= 2) y plasma (n = 2) de juveniles de TB en Sebastián Vizcaíno Bay (SVB), en Baja California, México. También se recolectaron presas locales para desarrollar un marco conceptual de alimentación basado en el análisis de isótopos estables. Además, durante el 2015 a 2017, se obtuvieron datos de captura y observación directa de 12 neonatos y juveniles alrededor de Isla Cedros, en la costa occidental de Baja California, México, lo que sugiere que esta isla es un área importante de crianza de tiburones blancos. El rango de tamaño para MS fue de 64.5-196 cm de longitud total (TL) y de 130-292 cm TL para WS, lo que indica que los individuos son neonatos, jóvenes del año (YOY) y juveniles. Los valores isotópicos para los músculos de la TM variaron de -19 ‰ a -16.4 ‰ para $\delta^{13}C$ y de 13.6 ‰ a 20.1 ‰ para $\delta^{15}N$. Para muestras de sangre completa en TM variaron de -23.2 a -16.2 ‰ para $\delta^{13}C$ y de 11.7 ‰ a 19.6 ‰ para $\delta^{15}N$, para RBCs los valores de $\delta^{13}C$ variaron de -19.5 ‰ a -16.5 ‰ y para $\delta^{15}N$ de 13.5 ‰ a 18.7 ‰ (media 16.5 ± 1.2). El plasma de TM mostró valores isotópicos entre -19.9 ‰ a -16 ‰ para $\delta^{13}C$ y de 14 ‰ a 20.7 ‰ para $\delta^{15}N$. Mientras que en los músculos TB, los valores variaron de -18 ‰ a -15.4 ‰ para $\delta^{13}C$ y de 16.2 ‰ a 18.9 ‰ para $\delta^{15}N$. La sangre completa de TB mostró valores de -18.3 ‰ a -14.2 ‰ para $\delta^{13}C$ y 16.4 ‰ a 17.1 ‰ para $\delta^{15}N$ y los valores de globúlos rojos de WS oscilaron entre -16.3 ‰ y -15.4 ‰ para $\delta^{13}C$ y entre 16 ‰ a 17.1 ‰ para $\delta^{15}N$. El plasma de TB mostró valores isotópicos entre -16 ‰ y -15.9 ‰ para $\delta^{13}C$ (media -16 ± 0 16) y de 17.4 ‰ a 17.5 ‰ $\delta^{15}N$ (media de 17.4 ± 0 ‰). Los tiburones mako de aleta corta y los tiburones blancos con longitud total similar (tiburones makos de aleta corta> 102 cm TL y tiburones blancos <186 cm TL) reflejaron en sus tejidos (músculo, sangre, glóbulos rojos y plasma) la línea isotópica base enriquecida en ^{15}N de SVB. Estos resultados sugieren que en SVB se tiene una firma isotópica única (isoscape), que se puede rastrear en los animales que se alimentan durante largos períodos de tiempo en su cadena trófica y que ambas especies de tiburones mostraron un hábitat similar en SVB. La similitud en el nicho isotópico y la composición isotópica entre los tiburones de aleta corta y los tiburones blancos en múltiples tejidos, indican un uso compartido de hábitat y de recursos dentro de SVB a lo largo del tiempo, lo que confirma su residencia en esta área durante sus etapas tempranas. De hecho, el aumento en los valores de ^{15}N con el tamaño de los tiburones sugiere un cambio ontogenético de origen materno a un uso a largo plazo de recursos de presa dentro de SVB, particularmente para los tiburones mako de aleta corta. Además, los datos de neonatos y juveniles de WS alrededor de Isla Cedros,

combinados con el único registro de las hembras adultas de mako de aleta corta en la misma área, indica que la región de Isla Cedros es un hábitat crítico y un posible lugar de nacimiento para ambas especies de tiburones.

CHAPTER 1 – GENERAL INTRODUCTION

In the past few years the researches on elasmobranch habitat use had significantly increased, because of the critical role played by these predators and their importance in marine communities (Heithaus, 2004; Baum *et al.,* 2009; Dulvy *et al.,* 2014). Differences in habitat uses generate different intraspecific and interspecific interactions, which affect the community structure and the sharks' distribution. For this reason, understanding their habitat uses and preference is critical to better understand their ecology (Heithaus *et al.,* 2002). Habitat use is generally is difficult to study in marine environment and for large vertebrate like sharks, because they are highly migratory species with large home ranges (Heithaus *et al.,* 2002; Heithaus, 2007).

The habitat is defined as the union of the resources and conditions present in a specific area that an organism requires to ensure its presence (e.g. food resources, water quality), and to guarantee the survival and reproduction success of the species (Krausman, 1999). The habitat use is defined as the way animals use the physical and biological resources inside a particular space, including foraging, cover, nesting, mating, or protection from threats. So, species can use the same habitat during their life history, or the habitat used vary among different species and life stages (e.g. juveniles and adults), with partial overlap throughout time and space and biological (Krausman, 1999; Heithaus, 2007).

The habitat selection is a hierarchical process, involving innate and learned behavioral that can be influenced by a high number of factors, such as food availability, environmental factors, predation risks, refuge, competition, reproductive and social behavior (Hutto,1985; Johnson, 1980). The difficulty in studying habitat use increase for some life stages, like the juveniles of large and migratory shark species, for which the knowledge about their habitat use is generally sparse (Dahlgren *et al.,* 2006; Heithaus, 2007; Huepel *et al.,* 2007). However, recent studies have focused on the characterization of the habitat used by juvenile sharks, because juvenile survival rates influence strongly the population growth rates, especially for long lifespans and low

fecundity rates of species, like shortfin mako (*Isurus oxyrinchus*) and white sharks (*Carcharodon carcharias*) (Castro, 1993; Simpfendorfer & Heupel, 2004). Most of juvenile sharks have a different trophic ecology from adults, because their metabolic requests and their predatory ability are changing in time (Griffiths, 1975; Dill, 1983; Gerritsen, 1984; Bres, 1993; Guttridge *et al.,* 2009), previous studies suggest that habitat selection for young sharks is driven by high food availability and predator avoidance (Krausman, 1999, Heithaus, 2007). For juvenile white sharks and shortfin mako sharks coastal nursery grounds and juvenile aggregation areas have been observed, but further details are not well characterized (Vélez-Marín *et al.*, 2009; Oñate-González *et al.,* 2017). Then, understanding ecological niche, habitat preference, and resource use of both shark species have direct implications to better understand their habitat use and their distribution, to successively develop optimal management and conservation strategies (Bethea *et al.,* 2009; Kinney & Simpfendorfer, 2009).

1.1 General Background

Shortfin mako and white sharks are two of the more charismatic and studied species in the world in their adult stages, but very few researches are focus on their young age stages (Huveneers *et al.,* 2018). Researchers hypothesize that YOY (Young of the year) and juvenile white and shortfin mako sharks share common prey resource and metabolic needs (Ezcurra *et al.,* 2012, Semmens *et al.,* 2013) that could contribute to co-occurring nursery areas. However, the hypothesis of spatial and resource overlap among YOY and juvenile white and shortfin mako sharks lacks of quantitative evidence. For these reasons, it is important to generate more knowledge about this age classes for both species, starting from their feeding habits and resource use, which allow to infer habitat use inside nursery areas and to better define them.

1.2 Nursery areas

These areas are critical and essential habitats, where the physical and biological characteristics are essential for species development (U.S. Fish and Wildlife Service,

2015). These habitats are often related with high productivity areas, where the resources are abundant, meaning that they can play an important role by increasing the species population size (Hall *et al.,* 1997; Beck *et al.,* 2001; Dahlgren *et al.,* 2006). In fact, nursery areas allow to increase the survival rates of young sharks, improving the species fitness (Heupel & Simpfendorfer, 2002; Kinney & Simpfendorfer*,* 2009; Knip *et al.,* 2010; Heupel *et al.,* 2018). Define a nursery area is quite difficult, so Heupel *et al.,* (2007) proposed strict criteria to identify and define them, however for large migratory species it is difficult to have always all these criteria accomplished.

- Newborn and young of the year sharks are more commonly recorded in these areas relative to other areas (high density of organisms);
- These age classes show a tendency to remain in the considered area for extended time periods (weeks or months);
- The considered area is used the area is used repeatedly across years, showing site fidelity.

Nursery areas are normally found in coastal regions, characterized by shallow protected waters with a high primary productivity sustaining a rich trophic chain (Castro, 1993; Dahlgren *et al.,* 2006; Heithaus, 2007; Knip *et al.,* 2010). These regions normally show intense anthropogenic activities, such as fisheries, so the individuals that inhabit these regions are more affected than others (Lyons *et al.,* 2013).

Then, understanding the habitat use of young animals in these areas is fundamental to ensure the species conservation and to identify the key areas for sharks' development. In this way will be possible to implement management or conservation measures and to avoid declines in shark populations (Heupel *et al.,* 2007; Knip *et al.,* 2010). A proposed nursery area for white and shortfin mako sharks, is Sebastian Vizcaino Bay (SVB) in northern Baja California Sur, México (Cartamil *et al.,* 2011; Santana-Morales *et al.,* 2012; Medina-Trujillo 2013; Oñate-González *et al.,* 2017; Conventional Tagging program of INAPESCA, unpubl. data), where both species can derive fitness benefits from lower predation offered by the use of communal nurseries (Heupel *et al.,* 2018).

1.3 Techniques to study habitat use

Habitat use is generally determined through the quantification of animal presence and frequency across space and over time (Heupel *et al.,* 2007; Heupel *et al.,* 2018) and it is possible to use numerous techniques, depending from on the study area and the species under analysis (e.g. telemetry, BRUVS, direct captures and observation, etc.).

1.4 Direct observations and fishery data

The direct observation is the simplest method to register the species found in a certain area throughout time. This method allows to directly record the presence of sharks during sampling or record the observations from videos or photographs (Marshall *et al.,* 2012. Furthermore, it also can be applied to fishery catches, particularly for the species which are incidentally caught in the different fishery. The use of these direct observations recorded with the support of fishermen communities allows researchers to use data from remote areas logistically difficult to sample and to improve the possibility to detect the presence of protected species, which are not a direct target in fishery. In this way researchers can take advantaged from the artisanal fishermen presence in the sea, providing the sea, and can give important information about shark aggregations, as previously documented by De la Parra-Venegas *et al.,* (2011). These methods are not highly precise and cannot define the fine scale in habitat use, but they are useful to get first approximations about the species presence in a determined area, or to identify possible new nursery areas and to plan further researches and sampling efforts.

1.5 Stable Isotopes Analysis

Traditionally, shark diet characterization is based on stomach content analysis (SCA), which provides a short snapshot of a shark's diet over the timescale of one or several days (Shiffman *et al.,* 2012), then ontogenetic shifts are difficult to assess over large spatial and temporal scales.

More recent shark diet studies use stable isotope analysis (SIA), a technique based on natural tracers of carbon and nitrogen isotope values ($\delta^{13}C$ and $\delta^{15}N$) to track resource flow within and across ecosystems. The analysis of these compounds provides information on the structure of trophic networks, feeding habits and on the diet integrated by animals throughout time and space, allowing to determine ontogenetic changes and animals' habitat use during long-term periods. In fact, stable isotopes provide information about the relationship between the isotopic values of consumers and sources (Peterson & Fry, 1987; Post, 2002; Wolf *et al.,* 2009; Logan & Lutcavage, 2010). Individuals acquire the isotopic composition of consumed prey, which varies depending on environmental conditions (i.e., productivity regimes) and trophic level (Post 2002, Graham *et al.,* 2010), allowing to infer the foraging grounds and to track the animal movements (Hobson, 1999; Shiffman *et al.,* 2012), especially for juvenile sharks which movement and habitat preferences are driven by foraging behavior animals.

The $\delta^{13}C$ values in marine ecosystems are related to primary production and in the eastern Pacific. Higher $\delta^{13}C$ values are typically related to coastal regions (upwelling zones) while lower $\delta^{13}C$ values are more frequent in less productive, offshore regions (Niño-Torres *et al.,* 2006, Graham *et al.,* 2010, Layman *et al.,* 2012). Baseline $\delta^{15}N$ values are dictated by different nitrogen sources in aquatic systems (e.g., nitrate, ammonium, N_2, etc.), higher $\delta^{15}N$ values are typical of regions where intense denitrification processes recycle nitrates (high upwelling regions) while lower $\delta^{15}N$ values are generated from N_2-fixation processes from cyanobacteria (oligotrophic regions) (Vanderklift & Ponsard*,* 2003; Graham *et al.,* 2010). In addition to these baseline differences, biochemical reactions during metabolism cause fractionation in isotope composition, causing systematic increases in $\delta^{15}N$ and $\delta^{13}C$ from prey to predator, defined as trophic enrichment factors (TEFs). Tipically, trophic level estimates use $\delta^{15}N$ values because TEFs are generally 3-4‰ compared to $\delta^{13}C$ TEFs of 0-1‰ (Post, 2002; Martínez del Rio *et al.,* 2009).

These relations between stable isotopes of carbon and nitrogen between consumers and they dietary sources allow to infer the foraging areas and habitat use; hence, and SIA has become a powerful non-lethal technique tool in shark species

(Peterson & Fry, 1987; Hobson, 1999; Post, 2002; Graham *et al.,* 2010; Bird *et al.,* 2018), particularly using different tissues with different turn-overt rates. In fact, the comparison of isotopic signatures between metabolically slow tissues and metabolically faster tissues, with large differences in turnover rates allows for the detection of changes in predators' diet over time and the resources used (Shiffman *et al.,* 2012). In this way, it is possible to detect eventual ontogenetic shifts in diet, to quantifying diet specialization and to infer habitat use through large time frames (Shiffman *et al.,* 2012; Bond *et al.,* 2016). Overall the use of multiple tissues in SIA has become a popular method, because it provides more precise dietary information and a greater trophic resolution than the one inferred by only one tissue, because of exposed biochemical variations among them (MacNeil *et al.,* 2005). In addition, quantitative analyses using Bayesian statistics on stable isotope data are available to describe niche width (e.g. Newsome, 2007, Parnell *et al.,* 2008, Jackson *et al.,* 2011) and estimate relative contribution of prey source inputs (e.g. Moore & Semmens 2008, Parnell *et al.,* 2013). All these characteristics make of SIA a very useful method to characterize and to compare habitat and resource use in juvenile large sharks, like shortfin mako and white sharks, which are difficult to sample and to track in long term periods.

1.6 White Shark-*Carcharodon carcharias*

White shark is one of the more charismatic species with high media profile (Huveneers *et al.,* 2018), so we cannot longer define the white sharks as a little-known species, however numerous aspects about its ecology are open to research. Indeed, the scientific information about the white shark is principally concentrated on the adult stages of this specie and particularly on its movement patterns and migrations, because this species has separate populations showing regional differentiation in long-distance migrations (Huveneers *et al.,* 2018). Despite this geographically separation, white sharks share similar biological and ecological traits across their global distribution, so increase the scientific information in one population will be useful to better understand others, due to the similar biological and ecological traits across their global distribution.

While the main effort of published studies is focused on sub-adult and adult stages a scarce knowledge exists for juvenile and young-of-the-year stages (Dewar *et al.,* 2004; Weng *et al.,* 2007b; Bruce & Bradford, 2012; Lyons *et al.,* 2013; White, 2016; Oñate-González *et al.,* 2017; Curtis *et al.,* 2018), but it is imperative that new studies are implemented to better understand the preferred habitats frequented by juvenile sharks, considering the importance of this age classes in population conservation and recruitment the species (Huveneers *et al.,* 2018). Research priorities included the identification of nursery areas for this species, understanding the early life movements and habitat inside of these areas, and which areas are important for the ontogenetic shifts in diet between juveniles and sub-adults (Domeier, 2012a; Skomal *et al.,* 2017). Juveniles are difficult to sample, because their nursery and aggregation sites are not easy to identify, in fact they prefer surface and coastal water but they are not associated to pinnipeds colonies, which make adults easier to observe (Pyle at al., 2002, Brown *et al.,* 2010). However, some nursery areas are identified, where young white sharks are affected by fisheries bycatch (Lowe *et al.,* 2012; Santana-Morales *et al.,* 2012; Lyons *et al.,* 2013; Ramirez-Amaro *et al.,* 2013; Oñate-González *et al.,* 2017), despite the fact that this species is protected by Mexican regulations (NOM-029-PESC-2006) and they are classified as Vulnerable based on the International Union for Conservation of Nature (IUCN) (Fergusson *et al.,* 2009). Due to the strong relationship between habitat use and prey availability (Dewar *et al.,* 2004), the study of feeding habits to detect eventual ontogenetic changes and related habitat use in a known nursery area in the Northeast Pacific Ocean (NEP) will be a good research priority.

1.6.1 Biology and Ecology

The white shark, *Carcharodon carcharias,* is a cosmopolitan and epipelagic species, which inhabits temperate oceans and normally aggregated near pinniped colonies during its adult stage (Klimley *et al.,* 2001; Le Boeuf, 2004; Martin *et al.,* 2005; Hoyos-Padilla, 201; Weng *et al.,* 2007;), remaining capable of seasonally making long-distance, offshore migrations (Boustany *et al.,* 2002; Bonfil *et al.,* 2005).

The white shark is present around the world with four different populations: South Africa, Australia, North of Atlantic and Northeast Pacific, and some seasonally aggregation points used during off-shore migrations (e.g. Guadalupe Island, SOFA; Domeier & Nasby-Lucas, 2013; Hoyos-Padilla, 2009). The populations are genetically separated and for many of them the abundance estimates remain data-limited, particularly for life-history stages, so estimating white shark population size remains challenging and uncertain for many cases, suggesting in all cases low abundances of animals in all cases (Huveneers *et al.,* 2018).

The maximum size reached by white sharks is still debated, because it is estimated at around 6 m., but Cailliet *et al.,* (1985) reported a maximum length of about 7 m. In general, the most common length for free-swimming specimens is around 5-6 m. (Compagno, 2002).

Maturity sizes for both sexes remain uncertain, due to limited age-growth data, and it possible that biological characteristics like maximum lengths, maturity length and birth size change across the different populations, due to local adaptation and different environment pressures. So far, the more reliable data for white sharks are that birth size is TL 120-150 cm, YOY is reported at TL <175 cm (Bruce & Bradford, 2012), and minimum size-at-maturity are reported TL 350-410 cm for males (8-10 year; Pratt 1996) and 450-500 cm for females (12-18 years; Francis, 1996; Uchida *et al.,* 1996; Bruce & Bradford, 2012).

White shark is a protected species in many regions of the worlds, meaning that getting samples by sacrificing the animals is not possible, which increase the difficult to collect some tissues like vertebrae and develop age and growth studies. Cailliet *et al.,* (1985) developed one of the most complete study of age and growth for white sharks and they reported a generalized age of maturity of 10-12 years and a reported an oldest age of about 14-16 years. A recent study based on radiocarbon ($\Delta^{14}C$) values in vertebrae estimated the maximum age of 40 years for females and 73 years for males, which dramatically extend the maximum age and longevity range of white sharks, with important implications for conservations and management plans (Hamady *et al.,* 2014).

Due to the same problematic to obtained samples of white sharks, particularly from different life stages the data available regarding the white shark reproduction are limited. White sharks present aplacental viviparity with intrauterine cannibalism in the form of oophagy (ingestion of unfertilized eggs), with a gestation period of 12-18 months, strictly related with the migration pattern and parturition apparently occurs between late spring and late summer in warm-temperate neritic waters (Gilmore, 1993; Francis 1996; Uchida *et al.,* 1996; Compagno 2002; Domeier & Nasby-Lucas*,* 2013). The general reproductive age is about at 17 years, but first breed could occur at 9-10 years (Cailliet *et al.,* 1985). White sharks have a three-year reproductive cycle with a litter size of 2 -18 embryos, comparable only with the litter size for shortfin mako sharks (Gilmore, 1993; Francis, 1996; Uchida *et al.,* 1996).

The white sharks is a highly migratory species with long transoceanic migration (Anderson *et al.,* 2011; Weng *et al.,* 2007; Domeier and Nasby-Lucas, 2008; Domeier & Nasby-Lucas *,* 2013), which in some cases can connect different populations (Bonfil *et al.,* 2005) and aggregation points within the same population, as in the Northeast Pacific (California, Guadalupe, SOFA) (Chapple *et al.,* 2011; Weng *et al.,* 2007; Domeier and Nasby-Lucas, 2008; Jorgesen *et al.,* 2009; Chapple *et al.,* 2011).

These connections through different habitats are very important because they show how habitat use changes across life-stages, according to their different ecological and biological requests (Griffiths, 1975; Dill, 1983; Bres, 1993; Guttridge *et al.,* 2009). In many cases this habitat use in the different areas is still unclear, but for in some it has been possible to clarify how white sharks use some of these regions and during which part of life. Different researches demonstrated a connection between California and Mexico; particularly Baja California Sur seems to have a relevant importance for newborns and juveniles of this specie (Dewar *et al.,* 2004; Weng *et al.,* 2007; Weng *et al.,* 2012; Lowe *et al.,* 2012). In fact, previous tagging studies and data from fisheries captures in the eastern Pacific Ocean suggest that YOY and juvenile white sharks utilize surface waters off the California and Baja California coast, showing a tendency to remain close to shore (Dewar *et al.,* 2004; Weng *et al.,* 2007; Weng *et al.,* 2012). Diving abilities and endothermic adaptations seem to improve with size and growth, so young sharks probably prefer warmer waters than adults, in fact telemetry

studies indicated the migration of some juvenile specimens from California costs to Baja California, precisely into Sebastian Vizcaino Bay, which is a confirmed nursery area for young white sharks (Weng *et al.,* 2007; Santana-Morales *et al.,* 2012; Domeier & Nasby-Lucas, 2013; Lyons *et al.,* 2013; Malpica-Cruz *et al.,* 2013; Oñate González, 2017). These studies and also the fishery registers documented an increase of newborn and juvenile white sharks during summer months, coinciding with the parturition season (Weng *et al.,* 2007; Santana-Morales *et al.,* 2012; Domeier & Nasby-Lucas, 2013; Oñate González, 2017) indicating that this bay has an essential role in the life-history of the white sharks, however so far we were not still able to define their habitat use and residency time inside SVB.

The majority of the studies were focused on the ontogenetic shift between sub-adult and adults around the 3 m of total length, with the dietary switch from teleost fishes to marine mammals as principal prey (Tricas and McCosker, 1984; Carlisle *et al.,* 2012; Jaime-Rivera *et al.,* 2013). The other ontogenetic dietary shift was proposed by Estrada *et al.,* (2006) and referred to the shift from the yolk of unfertilized eggs, produced by mothers to feed the embryos during gestation, to the incorporation of exogenous food when the sharks born and start to forage in the outside environment.

The few information existing for earlier age classes indicated that juvenile white sharks NEP, particularly in California and Baja California Sur, feed mainly on teleost fishes and small benthic elasmobranchs (Weng *et al.,*2007; Santana-Morales *et al.,* 2012). However, these studies were developed using stomach contents analysis, because the researches using SIA were focused on the ontogenetic dietary shifts, demonstrating that feeding preferences of the adult white sharks are related to their habitat use throughout their complex migratory patterns that they are influenced by the individual preferences (Carlisle *et al.,* 2012; Kim *et al.,* 2012). Until now one study has been documented, on juvenile white sharks from the NEP, from samples collected in the nursery area of SVB in Baja California Sur (Malpica-Cruz *et al.,* 2013), which represent an important background for early stages. This research inferred that young white sharks showed benthic foraging inside SVB as a consequence of a different feeding strategy in order to decrease the competition for food resource with pelagic feeding strategy of shortfin mako sharks that co-occur in the area.

1.7 Shortfin Mako Shark-*Isurus oxyrinchus*

Worldwide the scientific researches focusing on shortfin mako are generally sparse and particularly concentrated on the adult stage. This is due because this species does not have known aggregation areas, like the white sharks. The knowledge generate until now is principally due to the sample effort in the NEP, particularly close to Japan, and north Atlantic, with the longline fishery data and along the NEP thanks to the intense tagging program developed throughout the years (Casey & Kohler, 1992; Sippel *et al.,* 2004; Semba *et al.,* 2018).

Despite the difficult to identify the aggregation points of this pelagic species to develop intense sampling program, it is regularly caught in artisanal and commercial fisheries. In fact, shortfin mako represents one of the first target species in the artisanal fishery in Mexico, and as by-catch by the sport fishery in in Mexico and U.S (Sippel *et al.,* 2004; Cartamil *et al.,* 2011; Ramirez-Amaro, *et al.,* 2013). However, it has been recently changes from Vulnerable to Endagered in the Red List of International Union for Conservation of Nature (IUCN) ranking system (Rigby *et al.,* 2019).

We know, so far, that in the NEP there is a unique interconnected population (Casey & Kohler, 1992), which is apparently a stable stock without evidences of overfishing (ISC SWG, 2018). However, the globally decline of shortfin mako populations across the world, as the result of combined exploitations by directed fisheries and bycatch captures, established the of knowledge about this species as a priority. In particular, we have to increase the scientific information for vulnerable stages which are essential for the stocks conservation (ISC SWG, 2018; Rigby *et al.,* 2019), such as juveniles.

1.7.1 Biology and Ecology

The shortfin mako, *Isurus oxyrinchus*, is a regionally endothermic lamnid shark with a circumglobal distribution in temperate and tropical waters (Duffy & Francis, 2001; Velez-Marin and Maquez-Farias, 2009). This species occurs from the surface to at

least 500 m depth (Compagno, 2002). They are a highly migratory species of shark that show movements related to differences in water temperature (Semba *et al.,* 2011; Vaudo *et al.,* 2016) and seasonal prey abundance (Wood *et al.,* 2009). Adult shortfin mako sharks are primarily oceanic and epipelagic in the Pacific Ocean (Holts & Bedford*,* 1993; Abascal *et al.,* 2011; Sippel *et al.,* 2004).

The two biggest populations reported for this species, are in the Atlantic and in the Northeast Pacific Ocean. The Atlantic population seems to be separated in two different sub populations genetically distinct (Casey & Kohler, 1992). In fact, mitochondrial DNA data indicate segregation of female makos between the western and eastern north Atlantic, but a lack of differentiation in nuclear DNA suggests male mixing across the north Atlantic (Heist *et al.,* 1996; Schrey & Heist, 2003). Meanwhile, in the NEP is present a unique population, with sub populations not genetically separated and strongly interconnected (Casey & Kohler, 1992).

The shortfin mako shark is probably the fastest shark and is among the most active and powerful of fishes. Like other lamnid sharks, the shortfin mako is endothermic and it uses a heat-exchanging circulatory systems of small veins and arteries (retia mirabilia) to maintain the temperatures of certain areas higher than the ambient water temperature (Bernal *et al.,* 2001a; Sepulveda *et al.,* 2007). These retia mirabilia are distributed and produced heat around the body part that is essential for predation like red locomotor muscles to other muscles, the eyes, brains, and viscera (Bernal *et al.,* 2011; Lowe & Goldman, 2001). The increase of the internal temperatures cause a corresponding increment in heart rates and a consequently raise volume and speed of the blood being pumped through the body, increasing swimming speed and strength (Bernal *et al.,* 2011). In order to maintain this active, regionally endothermic metabolism, shortfin makos have developed highly specialized gills that increase oxygen consumption and show larger hearts than other shark species, increasing the volume of oxygenated blood pumped to their aerobic muscles and their body speed. These evolutionary adaptations make this species in a strong and effective predator, but generated great metabolic demands (Bernal *et al.,* 2011; Lowe & Goldman, 2001) At parturition, shortfin mako pups are around 61-80 cm TL (Semba *et al.,* 2011; Bustamante & Bennett, 2013), and they grow rapidly during their first year to reach the

maximum size reached from the shortfin mako, which is about 4 m (Compagno 2002), with a rapid decrease in growth rate and a consequently slow steady growth until reaching maturity. Age at maturity is different from females and males in the NEP: male mature at 270-300 TL, which means at 5-9 years, and females at 200-256 around 16-21 years (Semba *et al.,* 2011; Bustamante & Bennett, 2013), with a mean size for both sexes of 180 cm TL (Mollet *et al.,* 2000; Conde-Moreno & Galván-Magaña*,* 2006). The birth size is around 70 cm of total length and the gestation period is reported about 9-13 months (Semba *et al.,* 2011) or 15-18 months (Bustamante & Bennett, 2013), depending from the population, with a three-year reproductive cycle (Mollet *et al.,* 2000; Conde-Moreno *et al.,* 2006; Vélez-Marín *et al.,* 2009).

The shortfin mako is aplacental viviparous and oophagous with a gestation period of 9-13 months (Semba *et al.,* 2011) or 15-18 months (Bustamante & Bennett, 2013), depending from the population. Shortfin mako sharks have three-year reproductive cycle and the litter size is around 12-18 pups (Mollet *et al.,* 2000; Conde-Moreno & Galván-Magaña, 2006; Vélez-Marín *et al.,* 2009). There are comparatively few records of pregnant females in all the word, due to offshore and oceanic areas that they frequent and because their migrations are not known as well as for white sharks (Mollet *et al.,* 2000; Cailliet *et al.,* 2009; Francis *et al.,* 2019).

Initial age and growth studies in the western north Atlantic suggested that two pairs of growth bands are laid down each year in their vertebral center, at least in young shortfin makos (Pratt and Casey, 1983). However, counting one vertebral band per year, the maximum age observed for females was 29 years and for males was 28 years (Bishop *et al.,* 2016). More researches on age estimation are needed and the accurately estimating ages is imperative for proper stock assessment and management because it provides information about population demographics, growth rates, age at maturity, and longevity (Bishop *et al.,* 2016).

The shortfin mako is a pelagic species that are categorized as oceanic nomads, randomly moving wander in open Pacific Ocean, and occurring from shallow coastal waters to the open oceans (Holts & Bedford*,* 1993; Abascal *et al.,* 2011; Sippel *et al.,* 2004). In fact, the thermal circulatory adaptations of shortfin mako allow this species to occupy a broad thermal niche, sustaining high swimming speeds, and to be very

highly migratory (Dickson & Graham, 2004; Corrigan *et al.,* 2018). However shortfin makos inhabit different areas of the water column at different life stages: juveniles seem preferred to move in surface waters, which also make them more susceptible to direct fishing pressure (Holts & Bedford*,* 1993; Bustamante & Bennett, 2013).

Past tagging researches indicates that shortfin mako sharks move widely around the southwest Pacific Ocean, make long-distance oceanic movements, but a tendency was also evident to remain and frequent specific regions of ocean basins (Sippel *et al.,* 2011; Byrne *et al.,* 2017; Vaudo *et al.,* 2017). However, the information on their habitat use or mobility in the Pacific Ocean is still lacking. In fact recent studies have been focused to test the hypothesis that shortfin mako sharks have predominantly oceanic nomad behaviors or if there is a component of residency behavior (Francis *et al.,* 2019).The results indicated that small-to-medium shortfin mako sharks (newborns and juveniles) tend to remain within a restricted coastal area for several months, indicating a relatively high degree of residency and suggesting a the foraging as main activity in these areas (Corrigan *et al.,* 2018; Francis *et al.,* 2019). These findings and the presence of juveniles for surface waters should be considered in fishery managements, considering the impact of fishery on coastal zones and consequently on young sharks, in order to generate conservation strategies targeted at an appropriate spatial scale (Bustamante & Bennett, 2013; Francis *et al.,* 2019). So far, Mexican regulations for elasmobranch fishery consist in the interruption of fisheries activity for three months (May, June and July; NOM-029-PESC-2006), because they are the months when most of species give birth. However, there are no management plans for the capture of shortfin mako sharks, despite they are target in longline fishery and the young size classes are target of artisanal fisheries, which is mainly developed in coastal areas (Cartamil *et al.,* 2011; Ramírez-Amaro *et al.,* 2013; Castillo *et al.,* 2014; Sosa-Nishizaki *et al.,* 2014; Castillo-Géniz *et al.,* 2016).

Juvenile individuals dominate shortfin mako shark catches in temperate waters throughout the world (Bustamante & Bennett 2013; Runcie *et al.,* 2016) and the habitat and behavior of mature females are unknown (Corrigan *et al.,* 2018; Francis *et al.,* 2019). Few areas showing significant proportions of adult mako, as well as the record

of pregnant females are almost inexistent throughout the world (Mollet *et al.,* 2000; Compagno, 2002; Cailliet *et al.,* 2009; Francis *et al.,* 2019).

Conventional/electronic tagging studies and data from fisheries captures in the eastern Pacific Ocean suggest that YOY and juveniles of shortfin mako sharks are distributed close to shore, using surface waters off the California and Baja California coast, which are reported as important nursery areas (Holts & Bedford*,* 1993; Dewar *et al.,* 2004; Weng *et al.,* 2007; Weng *et al.,* 2012; Medina-Trujillo 2013). These researches also suggest high numbers of newborn and juvenile shortfin mako during summer months, probably because the efficient of retia mirabilia and diving abilities improved with size and growth, so they have minor tolerance to low temperature and occupying narrow thermal niche, with a preference for superficial water and particularly the mixture of cold and tropical water of Baja California (Holts & Breadfort, 1993; Sepulveda *et al.,* 2004; Abascal *et al.,* 2011).

The entire coast of Baja California is reported as an important nursery area for this species, however the region of SVB is a critical habitat for the shortfin mako sharks, based on high artisanal fisheries catches for this species (Cartamil *et al.,* 2011; Medina-Trujillo *et al.,* 2013; Castillo-Géniz *et al.,* 2014; Conventional Tagging program of INAPESCA, unpubl. data).

In many areas, the diet of shortfin mako sharks consists of 98% teleost fishes, but they also prey on cephalopods, crustaceans, marine mammals, sea turtles, and other sharks (Campana *et al.,* 2005; Wood *et al.,* 2009) and an ontogenetic chance is described in diet, despite being less marked relative to white sharks (Velasco-Tarelo, 2005, Malpica-Cruz *et al.,* 2013). In fact, this species expands its prey spectrum during growth, including larger size items (e.g. tuna fishes and swordfishes) (Mucientes-Sandoval & Saborido-Rey*,* 2008; Preti *et al.,* 2012). The information about their dietary and feeding habits indicate that adults and large shortfin mako sharks feed on larger pelagic fishes and cephalopods, but occasionally can forage on marine mammals (Preti *et al.,* 2012; Lyons *et al.,* 2015). The few information existing for earlier age classes in the northeast Atlantic the YOYs sharks seemed to show a preference for other elasmobranchs (Maia *et al.,* 2006). In the California current shortfin makos have opportunistic diet preferences, without great competition with other shark species

(Rubinas *et al.,* 2017), using as principal preys like cephalopods and teleosts. I the northeast Pacific shortfin makos feed primarily on large squids (Rubinas *et al.,* 2017; Preti *et al.,* 2012; Lyons *et al.,* 2015). The differences in prey choices are affected not only from seasons, but also by class sizes. Juveniles of shortfin mako sharks seem to prefer small teleosts, however the information about their dietary habits is limited. In Baja California Sur, studies of SIA focused on juvenile shortfin mako sharks indicated that juvenile stages preferred to forage on benthic preys (e.g. benthic fishes and invertebrates) and that they showed an ontogeny shift ontogenetic diet shifts to larger fishes (Velasco-Tarelo, 2005; Malpica-Cruz *et al.,* 2013).

1.8 Hypothesis and justification

Due to the strong relationship between habitat use and prey availability, which drive the habitat selection in juvenile sharks (Krausman, 1999; Dewar *et al.,* 2004; Heithaus, 2007), the definition of the feeding habits is a research priority. The juveniles and YOYs of shortfin mako and white sharks use protected and coastal areas as nursery areas to growth, such as SBV in the North of Baja California (Mexico) (Medina-Trujillo 2013, Oñate González, 2017). Conventional/acoustic/satellite tagging studies, fish records and direct observations suggest that YOYs and juveniles of both species frequent SVB for several months after birth (Medina-Trujillo 2013; Lowe *et al.,* 2012, Oñate González, 2017; Conventional Tagging program of INAPESCA, unpubl. data, Castillo-Géniz, pers. comm).

The habitat use and residency time inside SVB remain unknown for both species and their estimation will represent priority information for juvenile stages useful also for other population worldwide. The researches so far (Malpica-Cruz *et al.,* 2013) explored the hypothesis of resources parturition as a consequence of different feeding strategies (benthic vs pelagic). Nevertheless, the hypothesis of a high habitat use overlap and of sharing feeding resource between both shortfin mako and white sharks remain unexplored, despite this strategy was previously reported and generated higher benefit, particularly in a communal nursery like SVB (Heupel *et al.,* 2018). In fact, if food sources do not represent a limiting factor like in rich biological hot-spot like SVB, the competition for feeding resources decrease and different species took more

advantaged sharing resources in a communal nursery area compared to a single-species nursery (Heupel *et al.,* 2018).

The number of studies using SIA in multiple tissues has increased in the last years, because this technique demonstrated to be powerful to compare movements and resource use among shark species and across size classes, as isotopic differences reflect habitats and/or trophic differences integrated over a period of time (Peterson & Fry, 1987; Hobson, 1999; Post, 2002; Graham *et al.,* 2010; Bird *et al.,* 2018). In addition, researchers were not able to evaluate if SVB is also a pupping ground for both species, because tagging studies indicated the presence of adult pregnant female of white sharks in the off-shore waters of the bay, close to Isla Cedros, but any adult pregnant female of shortfin mako or white sharks was observed or caught close or inside SVB.

1.9 General Objective

The main goal of this study is to investigate the habitat use and compare habitat and resource use between shortfin mako and white sharks in the nursery and juvenile aggregation area of SVB, Baja California (Mexico), using stable isotopes analysis of carbon and nitrogen, in multiple tissues with different turn-over rates.

1.9.1 Specific objectives

- To compare $\delta^{13}C$ and $\delta^{15}N$ values between the shortfin mako and white sharks using different tissues, in order to study the diet and habitat use in different time scales.
- To compare the isotopic composition of both species across life-stages (newborns vs juveniles), to detect eventual ontogenetic shifts in diet.
- To quantify isotopic niche for white and shortfin mako, in order to assess the overlap of habitat use between both predators
- To determine the food resource and evaluate the isotopic contribution of their potential prey to assess ontogenetic changes in habitat use.

- Estimate the trophic position (TP) of juvenile of both species in the study area.

2. Study area

The study area is SVB (28°14'52" N, 114°04' 09.7" W to 27°41'30" N, 114°53'00'' W; Fig. 1), located in the Western coast of Baja California Sur. SVB is the largest wildlife refuge in all Latin America with a landmass of over 26,310 km^2 (2,546,790-25-00 hectares) and it is included in the Natural Protected Reserve of Vizcaino Biosphere (REBIVI) in the municipality of Mulegé, which is located in Northern Baja California Sur (260° 36' North and 112° 20' East), and the marine part of the reserve includes a coastal strip of 5 km along its 450 km of coast (Arriaga *et al.,* 2000). SVB is part of REBIVI and it is a semicircular bay with a hooked form, where Punta Eugenia is the southern extreme of this hook, determining the southern border of the bay. The bay is approximately 100 km wide and 200 km long, with a large shallow and gradual continental shelf (20 km of wide), extending from 5 to 20 km offshore, and a soft bathymetry with a mean depth is around mean depth of 25-30 m and maximum depth is 200 m, close Isla Cedros, which is located 20 km to the north of Punta Eugenia, where the depth is around 140 m (Amador-Buenrostro *et al.,* 1995; Hernández-Rivas *et al.,* 2000) (Fig. 1 A). SVB maintains a connectivity with the NEP in the northern part, meanwhile in the south the two channels of Dewey (between Punta Eugenia and Isla Natividad) and Keller (between Natividad and Cedros) created a physical barrier. In fact, both channels are shallow and narrow: the Dewey channel has an approximate width of 10 km and a depth of 40-45 m, the Keller of 20 km and a maximum depth of 25-30 m; however the strong currents colliding in these regions make difficult to reach Isla Natividad and Isla Cedros (Peraza *et al.,* 1995; Hernandez-Rivas *et al.,* 2000). In addition, this region and Punta Eugenia are reported as the maximum south boundary for the distribution of norther species and the maximum north boundary for southern species, because the collision of the different water bodies and currents create a physical and biological barrier for species distribution (Hernández-Rivas *et al.,* 2000).

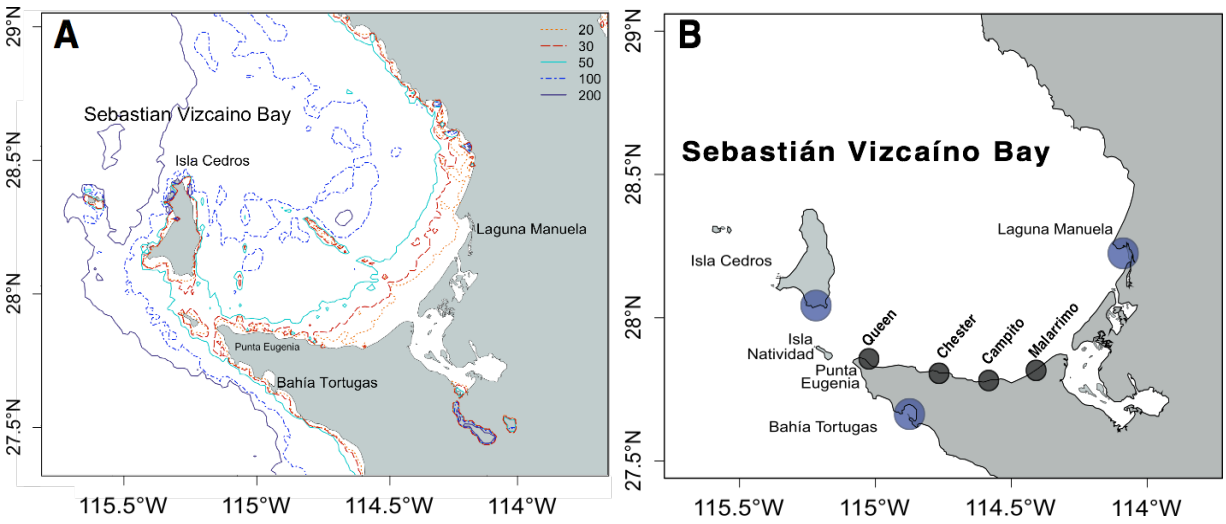

Figure 1. Map of the study area in Sebastian Vizcaino Bay (SVB), Baja California Sur, Mexico. (A) map of SVB with the bathymetry lines, (B) map of SVB with the main landing points for elasmobranch captures and the permanent fishery camps used to set out in the central part of the bay

SVB presents a complex of internal lagoons: Laguna Manuela (6 miles), Laguna Ojo de Liebre (156 miles) and Laguna Guerrero Negro (41 miles). Laguna Manuela is the northern and the smaller one, Laguna Guerrero Negro is the middle one and Ojo de Liebre lagoon is the largest and most famous, because it represents the main recruitment and refuge region for gray whales (Eberhardt, 1966), and because of this SVB and the Ojo de Liebre Lagoon are declared World Heritage Sites (Arriaga *et al.,* 2000).

SVB is a biological hotspot with high productivity (BAC: Centros de Actividad Biológica) due to coastal topography, winds, the presence of oceanic gyres and the collision of different currents, with a consequently strong upwelling (Hernandez-Rivas *et al.,* 2000). The waters of this region have very different characteristics: inside the lagoon the waters are characterized to have high temperatures and salinities, while close the region of Punta Eugenia waters are cold and with strong upwelling. SVB is located and influenced by the region of California Current (CC) and influenced by it (Hernandez-Rivas *et al.,* 2000), however its particularly shape, coastal topography and oceanographic conditions create a restricted and unique system where the dominant northwest winds caused a strong coastal upwelling, one of the most intense of the all Baja (Bakun & Nelson, 1977). In fact, SVB showed which high primary productivity, high chlorophyll-a concentrations and a large phytoplankton community Amador-

Buenrostro *et al.,* 1995; Palacio-Hernández *et al.,* 1996; Hernández-Rivas *et al.,* 2000).

These winds combined to the bay topography its bathymetric configuration generate one of the main characteristic of SVB: an anti-cyclonic gyre present in its central area (Amador-Buenrostro *et al.,* 1995; Hernández-Rivas *et al.,* 2000; Fig.2), which was reported for the first time in 1916 and estimated with a diameter of 50 and 70 km and between 40 and 70 m of extension (Amador-Buenrostro *et al.,* 1995; Palacio-Hernandez *et al.,* 1996; Hernandez-Rivas *et al.,* 2000). This anti-cyclonic gyre causes anoxic water and a decrease of primary production in the central part of the bay as showed by the lower level of chlorophyll-a concentrations during time (Fig. 2; https://coastwatch.pfeg.noaa.gov/erddap/griddap/erdVHNchla1day.graph), compared with the rest of SVB, and the consequently recycling of nutrient inside the system.

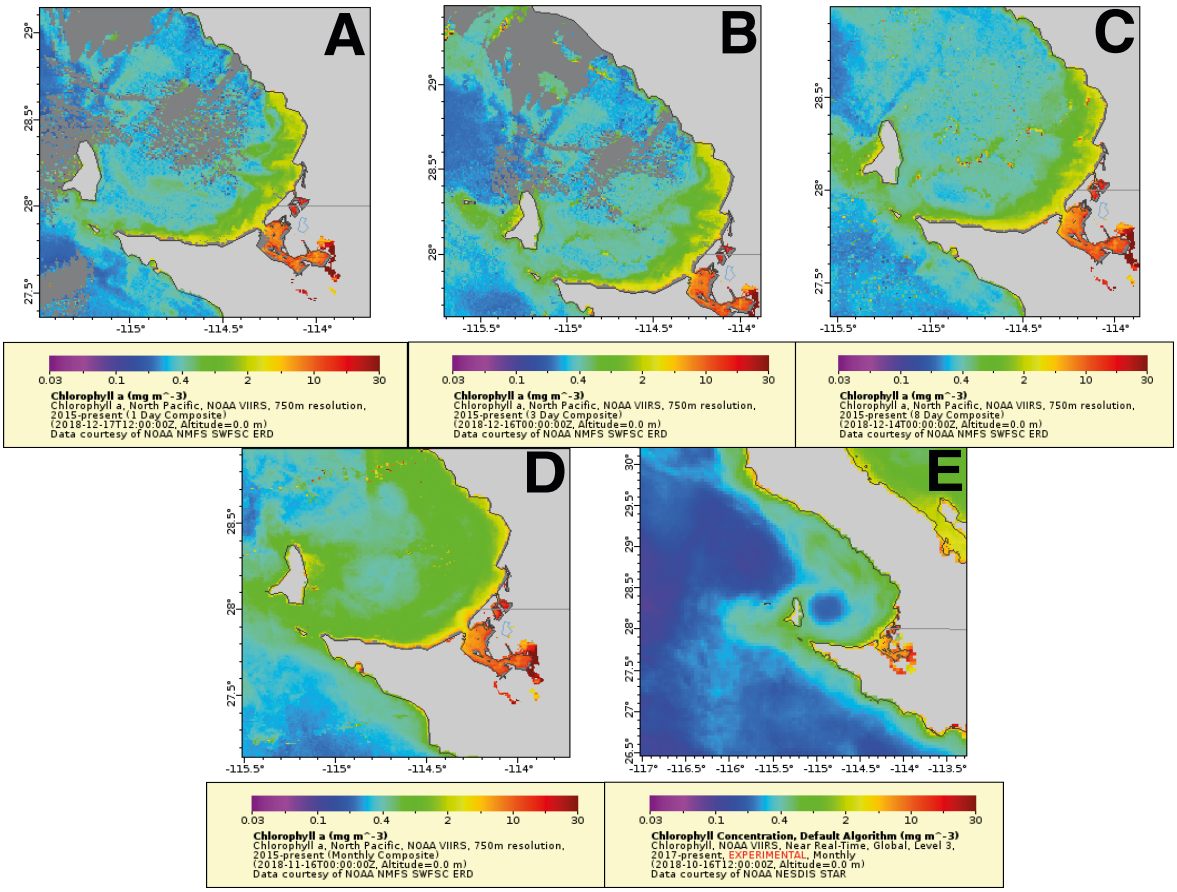

Figure 2. Map of the study area in Sebastian Vizcaino Bay (SVB), with simulation of chlorophyll-a concentrations (mg/m3) with NOAA VIIRS, 750m resolution. (A) 1-day simulation, (B) 3-days simulation, (C) 8-days simulation, (D) Monthly simulation, (E) Monthly simulation with NOAA VIIRS, Near Real-Time, Global.

The physical and biological processes that occur in the zone support a very rich trophic chain, allowing the development of economic activities and ecological process. In fact, highly valued commercial species are found in its waters (for example, lobster and abalone) and for some of these, such as sardines, SVB represent an important area for spawning throughout the entire year, meaning that this is area is critical for their recruitment (Groves & Reid, 1958). Some of the most important economic activities are the extraction and exportation of salt, mainly developed in Guerrero Negro, the ecotourism with the gray whales and the artisanal fishery, which is principally focused on lobsters, abalone, clams and other mollusks, teleost fishes and elasmobranchs (sharks and rays) (Hernandez-Rivas *et al.,* 2000). The elasmobranch species that are target include 18 species of sharks and 14 species of manta rays and

the principals are *Mustelus henlei*, *Mustelus californicus*, *Mustelus lunulatus*, *Myliobatis californica*, *Pseudobatos productus*, *Zapteryx exasperata*, *Isurus oxyrinchus*, *Galeorhinus galeus*, *Heterodontus francisci*, *Heterodontus mexicanus*, *Cephaloscyllium ventriosum* and *Squatina californica* (Arriaga *et al.,* 2000; Carabias-Lillo *et al.,* 2000; Ramirez-Amaro *et al.,* 2013).

SVB is also an important aggregation point for marine mammals like the California sea lions (*Zalophus californianus*), the common seal or harbor seal (*Phoca vitulina*) and the northern elephant seals (*Mirounga angustirostris*), which are distributed in different part of the bay. The California sea lion are found in Isla Natividad, Bahía Asunción, Isla Cedros and Archipelago San Benito, the harbor seal in Isla Natividad and the northern elephant seals mainly aggregate in Isla Cedros and the Archipelago San Benito (Gallo-Reynoso *et al.,* 1984; Elorriaga-Verplancken *et al.,* 2015).

The more important points inside SVB to the develop of this research project were Laguna Manuela (28° 14' 52" N 114° 04' 09.7" W), Bahía Tortugas (27° 41′ 30″ N and 114° 53′ 00” W) and Isla Cedros (28° 10′ 58″ N 115° 13′ 04″ W; Fig. 1), because they represent three important fishery camps were sharks are landed and they are also some of the most developed urban communities, after Guerrero Negro and Santa Rosalia (Arriaga *et al.,* 2000).

2.1 Laguna Manuela (LM)

Laguna Manuela is a permanent fishery camp located within SVB at 30 km to the border with Baja California Sur (BCS). This camp has been identified as one of the most important fishing spots in Baja California (BC) because of the significant diversity of fishery resources that are landed in this area (Cartamil *et al.,* 2011; Castillo-Géniz *et al.,* 2016). The fishing camp is formed by a sandy spit, located at the entrance to the Laguna Manuela (LM) estuary. It does not have permanent structures, boat launch ramps, or electricity, and its access roads are unpaved and poorly maintained, in fact, most fishermen usually lived l in the nearby villages of Ejido Morelos and Villa de Jesús María (7 and 10km inland) and move to LM each morning to fish in SVB. Here the fishery activities are developed with pangas, which are typical Mexican boat (smaller

vessels of approximately 5-8 m in length). Normally in Laguna Manuela camp they use 25 pangas, which operate with longlines and/or gillnets, depending on target species (Cartamil *et al.,* 2011; Castillo-Geniz *et al.,* 2016). However, the main fishery effort directed to elasmobranch is developed with the longlines (Castillo-Géniz *et al.,* 2016), meanwhile in the majority of the Baja fishermen do not use longlines anymore, and they changed the equipment to gill-nets, because of their higher catch rates.

Monthly monitoring, developed by the Programa Tiburón of the Centro Regional de Investigación Pesquera de Ensenada (Crip-Ensenada), documented a catch rate of I. oxyrinchus being the 36% of total elasmobranch catches. This species is normally target in longlines, but it is still affected by by-catches mortality in with gill-nets.
Other important data is that in this area were reported incidental captures of some individuals of white sharks in bottom gill nets (Castillo-Géniz *et al.,* 2016).

2.2 Bahía Tortugas and Isla Cedros

Bahía Tortugas is a small village with a permanent fishery camps and more facilities than Laguna Manula, which make of this locality a very important point for sharks landing and fishery in general. Bahia Tortugas is located southern then Punta Eugenia, so it is not strictly inside SVB, however fishermen organized their fishery trips inside SVB, setting out from the different permanent fishery camps normally used for lobster fishery (Malarrimo, Campito, Queen and Chester; Fig. 1B). In this way, artisanal fishermen can take advantaged from the high trophic chain and the high fishery resource offered by SVB and the one offered by Bahia Tortugas bay, differentiating the fishery efforts and target species. In addition, Bahía Tortugas received the teleost fishes and elasmobranchs captured in Isla Cedros, because the island needs a point on the peninsula to send the fishery product for its consequently sales in fishery markets. All the fishery products from the artisanal fishery were exported to United States or sold to the central states of Mexico.

Inside SVB, around Bahía Tortuga and Isla Cedros the fishery is developed with bottom gill nets and the loglines are almost dismissed. The gill nets have different mesh sizes, depending on the target species, and they modify the depth of the gill nets based on their empirical knowledge of the biology of the different shark species, to maximize

their captures. Normally fishermen positioned the gill-nets between 1 and 10 miles from the coastal lines and usually between 20 and 80 fathoms of depth, which means 36-150 m. In most cases fishermen, do not have a very good equipment, so they often raise the gill nets by hands, however recently they start to update their fishery equipment with bobbins to raise the nets (fishermen personal communication).

CHAPTER 2 - ISOTOPIC NICHE AND RESOURCE SHARING AMONG YOUNG WHITE SHARKS (*Carcharodon carcharias*) AND SHORTFIN MAKO SHARKS (*Isurus oxyrinchus*) IN BAJA CALIFORNIA, MEXICO

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Note: The references style can be different from the format used in the other chapter of thesis, because the MEPS required its specific style for the references in the text and for the reference's list.

Abstract

White sharks (*Carcharodon carcharias*) and shortfin mako sharks (*Isurus oxyrinchus)* are globally distributed apex predators and keystone species. However, regional information regarding juvenile biology, such as habitat preferences and trophic ecology, is lacking. This study investigates habitat use and feeding ecology of juvenile shortfin mako and white sharks in an aggregation site with high catch of these species by artisanal fisheries in Sebastian Vizcaino Bay (SVB; Baja California, Mexico) using stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). During 2015 and 2016 we collected muscle samples from newborn, young of the year (YOY), and juvenile shortfin mako (n=156) and white (n=11) sharks from individuals with similar body size, as well as local prey, to develop a conceptual foraging framework based on stable isotope analysis. We found a positive relationship between shortfin mako length and $\delta^{15}N$ values, indicating ontogenetic changes in diet based on prey or locality. Bayesian isotopic mixing models (MixSIR) using prey from different regions in the eastern North Pacific suggested a diet shift in shortfin makos from offshore, northern habitats to inshore habitats of southern Baja (e.g. SVB), while analysis of white sharks reflected use of inshore habitats of both southern California, northern Baja, and SVB. Our results suggest shared resource use between these shark species and potentially high consumption of prey from SVB and other similar coastal regions in southern Baja. This study characterizes high use of inshore regions for juvenile shortfin mako and white sharks, which accounts for the increased interactions with coastal artisanal fisheries and has important implications for management and conservation practices.

2.1. INTRODUCTION

Habitat use information for juvenile stages of large, migratory shark species are generally sparse (Dahlgren *et al.,* 2006, Heithaus, 2007, Huepel *et al.,* 2007). In general, sharks play a vital role in marine ecosystems (Dulvy *et al.,* 2014) and while coastal nursery grounds and juvenile aggregation areas have been observed, further details are not well characterized (Vélez-Marín et al, 2009; Oñate-González *et al.,* 2017), especially for juvenile white (*Carcharodon carcharias*) and shortfin mako sharks (*Isurus oxyrinchus*). Previous studies suggest that juvenile habitat selection is driven by high food availability and predator avoidance (Krausman, 1999, Heithaus, 2007). For juvenile white sharks (*Carcharodon carcharias*) and shortfin mako sharks (*Isurus oxyrinchus*), understanding ecological niche, habitat preference, and resource use has direct implications for development of optimal management and conservation strategies (Bethea *et al.,* 2009, Kinney & Simpfendorfer*,* 2009).

Both white and shortfin mako sharks are globally distributed and found in tropical and temperate oceans (Compagno, 2002), and are classified as Vulnerable based on the International Union for Conservation of Nature (IUCN) (Fergusson *et al.,* 2009, Cailliet *et al.,* 2009). Mexican regulations prohibit fisheries from targeting white sharks (NOM-029-PESC-2006), but allow commercial catch of shortfin mako sharks. Some artisanal fisheries directly target juvenile shortfin mako sharks, but capture juvenile white sharks as by-catch (Cartamil *et al.,* 2011, Ramírez-Amaro *et al.,* 2013, Lyons *et al.,* 2013, Castillo-Géniz *et al.,* 2016, Oñate-González *et al.,* 2017). The long lifespans and low fecundity rates of both species (Castro, 1993, Compagno, 2002) mean that juvenile survival rates strongly influence population growth rates (Castro, 1993, Simpfendorfer & Heupel, 2004).

Adult white and shortfin mako sharks largely utilize different marine habitats. Adult white sharks aggregate near pinniped colonies in California (Klimley *et al.,* 2001, Le Boeuf, 2004, Weng *et al.,* 2007), Australia (Bruce, 1992), South Africa (Martin *et al.,* 2005), and Mexico (Hoyos-Padilla, 2016), while seasonally making long-distance, offshore migrations (Boustany *et al.,* 2002, Bonfil *et al.,* 2005). Adult shortfin mako sharks are primarily oceanic and epipelagic in the Pacific Ocean (Holts & Bedford*,* 1993, Abascal *et al.,* 2011, Sippel *et al.,* 2004). However, there is evidence of high

habitat overlap for juvenile nursery areas of these two species. Tagging studies in the eastern Pacific Ocean suggest that young-of-the-year (YOY) and juveniles of both species are distributed close to shore, utilizing surface waters off the California and Baja California coast (Holts & Bedford, 1993, Dewar *et al.,* 2004; Weng *et al.,* 2007, Weng *et al.,* 2012, Medina-Trujillo 2013). Previous studies document overlapping nursery areas for young sharks of different species and underscore the importance of these sites as essential habitats for shark development and population growth (Kinney & Simpfendorfer, 2009). Nursery or juvenile aggregation areas require a confluence of biological and physical attributes, such as highly productive coastal regions with shallow waters (<50–100m) that offer high food availability and protection (Dahlgren *et al.,* 2006, Heithaus, 2007). Researchers hypothesize that YOY and juvenile white and shortfin mako sharks share common prey resource and metabolic needs (Ezcurra *et al.,* 2012, Semmens *et al.,* 2013) that could contribute to co-occurring nursery areas. However, the hypothesis of spatial and resource overlap among YOY and juvenile white and shortfin mako sharks lacks quantitative evidence.

A proposed aggregation area for YOY and juvenile white and shortfin mako sharks, based on relatively high artisanal fisheries catch of both species, is Sebastian Vizcaino Bay (SVB) in northern Baja California Sur, Mexico (Cartamil *et al.,* 2011, Santana-Morales *et al.,* 2012, Medina-Trujillo 2013, Oñate-González *et al.,* 2017, Conventional Tagging program of INAPESCA, unpubl. data). This bay is an area of high productivity due to its coastal topography, winds, strong upwelling, and consequently high chlorophyll concentrations. The confluence of currents with the flow of the California Current (CC) and the bathymetric configuration of the bay create a restricted, productive region with an anti-cyclonic gyre present in its central area (Amador-Buenrostro *et al.,* 1995, Hernández-Rivas *et al.,* 2000). These conditions support a productive ecosystem (Hernández-Rivas *et al.,* 2000, Martínez-Fuentes *et al.,* 2016), which is intensively harvested by an artisanal fishery that targets bony fishes, elasmobranchs (sharks and rays), lobsters, and mollusks (Hernández-Rivas *et al.,* 2000, Cartamil *et al.,* 2011, Ramírez-Amaro *et al.,* 2013). This fishery captures juvenile mako and white sharks, but the residency and resource use of these species in SVB and surrounding areas is unknown.

Diet characterization and trophic level are traditional components used to describe shark resource use. Traditionally, shark diet characterization was based on stomach content analysis (SCA), which provides a snapshot of a shark's diet over the timescale of one or several days (Shiffman *et al.,* 2012). These studies suggest that young white and shortfin mako sharks feed primarily on fish, squid, and small elasmobranchs, then may expand their diet to larger prey items with growth (Tricas and McCosker, 1984, Le Boeuf, 2004, Dewar *et al.,* 2004, Weng *et al.,* 2007, Mucientes-Sandoval & Saborido-Rey*,* 2008, Preti *et al.,* 2012, Carlisle *et al.,* 2012, Santana Morales *et al.,* 2012, Lyons *et al.,* 2015). However, SCA provides a short snapshot of diet and requires large sample sizes to quantify long-term feeding patterns (Wetherbee & Cortés, 2004), so ontogenetic shifts are difficult to assess over large spatial and temporal scales. More recent shark diet studies use stable isotope analysis (SIA), a technique based on natural tracers of carbon and nitrogen isotope values ($\delta^{13}C$ and $\delta^{15}N$) to track resource flow within and across ecosystems. Individuals acquire the isotopic composition of consumed prey, which varies depending on environmental conditions (i.e., productivity regimes) and trophic level (Post 2002, Graham *et al.,* 2010). The $\delta^{13}C$ patterns in marine ecosystems are largely influenced by primary production and in the eastern Pacific, higher $\delta^{13}C$ values are typically related to coastal regions (upwelling zones) while lower $\delta^{13}C$ values are more frequent in less productive, offshore regions (Niño-Torres *et al.,* 2006, Graham *et al.,* 2010, Layman *et al.,* 2012). Baseline $\delta^{15}N$ values are dictated by different nitrogen sources in aquatic systems (e.g., nitrate, ammonium, N_2, etc.), higher $\delta^{15}N$ values are typical of regions where denitrification processes recycle nitrates (high upwelling regions) while lower $\delta^{15}N$ values are generated from N_2-fixation processes from cyanobacteria (oligotrophic regions) (Vanderklift & Ponsard*,* 2003, Graham *et al.,* 2010). In addition to these baseline differences, biochemical reactions during metabolism cause fractionation in isotope composition, causing systematic increases in $\delta^{15}N$ and $\delta^{13}C$ from prey to predator, defined as trophic enrichment factors (TEFs). Generally, trophic level estimates use $\delta^{15}N$ values because TEFs are generally 3-4‰ compared to $\delta^{13}C$ TEFs of 0-1‰ (Post, 2002, Martínez del Rio *et al.,* 2009). Further quantitative analyses using Bayesian statistics on stable isotope data are available to describe niche width (e.g.

Newsome, 2007, Parnell *et al.,* 2008, Jackson *et al.,* 2011) and estimate relative contribution of prey source inputs (e.g. Moore & Semmens 2008, Parnell *et al.,* 2013). Overall, SIA has become a powerful tool to compare movements and resource use among shark species and across size classes, as isotopic differences reflect habitats and/or trophic differences integrated over a period of time (Peterson & Fry, 1987; Hobson, 1999; Post, 2002; Graham *et al.,* 2010; Bird *et al.,* 2018).

This study uses SIA to characterize and compare habitat and resource use in shortfin mako and white sharks in the potential nursery and juvenile aggregation area of SVB. We compare SIA values of both species to evaluate resource partitioning, as has been previously reported (Malpica-Cruz *et al.,* 2013). We apply Bayesian mixing models to stable isotope data collected from both shark species and their potential prey to assess ontogenetic changes in habitat use.

2.2 MATERIALS AND METHODS

2.2.1. Study area and sample collection

Samples were collected in 2015 and 2016 from SVB (28°14'52" N, 114°04' 09.7" W to 27°41’30” N, 114°53’00’’ W), which is a semicircular bight (100 km × 200 km) with a large, shallow continental shelf (20 km wide), with a mean depth of 25-30 m and with a maximum depth of 200 m near Isla Cedros (Amador-Buenrostro *et al.,* 1995, Hernández-Rivas *et al.,* 2000) (Fig. 3). SVB is a biological hotspot with high primary productivity, high chlorophyll-a concentrations, an anticyclonic gyre in the center of the bay and a large phytoplankton community, mainly formed of cyanobacteria (Amador-Buenrostro *et al.,* 1995, Palacio-Hernández *et al.,* 1996, Hernández-Rivas *et al.,* 2000).

Shark samples were provided by longline or gillnet artisanal fisheries, which operate mainly in the central part of the bay and close to Isla Cedros (28° 10′ 58″ N 115° 13′ 04″ W; Fig. 3). All sharks were landed at fishing camps at Laguna Manuela (28° 14' 52" N 114° 04' 09.7" W) and Bahía Tortugas (27° 41′ 30″ N and 114° 53′ 00” W) from August to November, when young white and shortfin mako sharks are caught (Conde-Moreno & Galván-Magaña*,* 2006, Castillo-Géniz *et al.,* 2016, Oñate-González *et al.,* 2017). For all sampled sharks, the following data were recorded: total length (TL), fork length (FL), pre-caudal length (PCL), sex, maturity stage, site of capture, and

fishery methods (longline or gillnets). We collected ~10 mg of muscle tissue from the dorsal region of the body behind the head and stored samples at -20°C in the field with transport on ice to the laboratory. Samples were collected with support of trained technical staff from Programa Tiburón from National Fisheries Institute of Mexico in Ensenada (INAPESCA) and the Fishery Ecology Laboratory of Centro Interdisciplinario de Ciencias Marinas (CICIMAR) of the Instituto Politécnico Nacional (IPN).

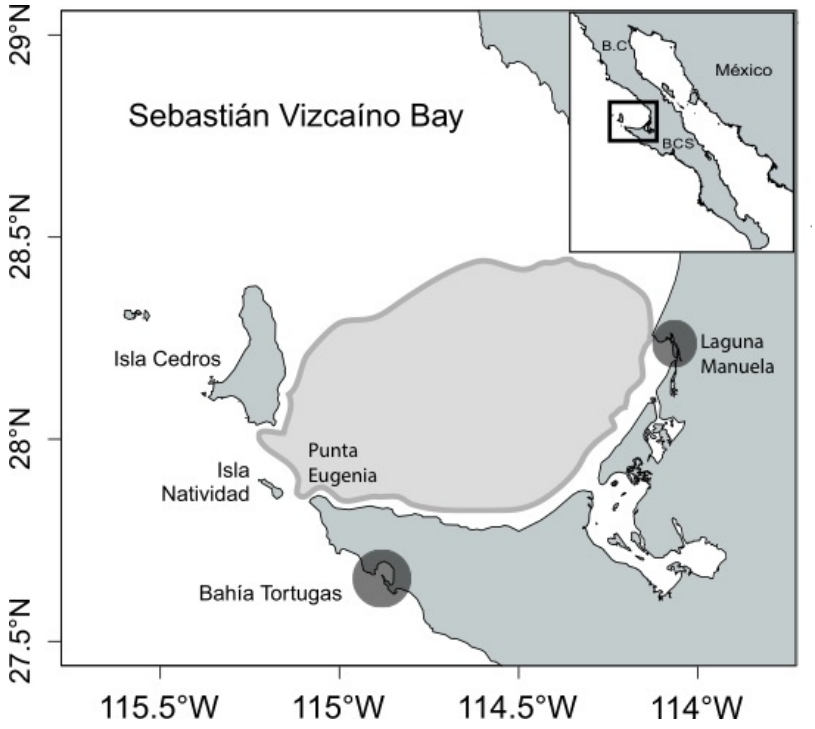

Figure 3. Map of the study area in Sebastian Vizcaino Bay (SVB), Baja California Sur, Mexico. Small dark shaded circles are the landing locations. The grey shaded area is the main area in which longline and gillnet fisheries operate.

Since some local fishermen remove the head of white sharks at sea, the total length (TL) could not be measured. For these individuals, we used the trunk length (measured from the cut of the head to the pre-caudal fin) or alternative length (measured from the first dorsal fin to the pre-caudal fin) to estimate TL (see Fig. 5a). We established measured the body proportion, for some specimens, between trunk or alternative length andvs. TL using photographs of newborn white sharks (n=5) (Lowe *et al.,* unpubl. data, Castillo-Géniz personal communication) with the program Sigma Scan Pro 5 (Copyright © 2017 Systat Software, Inc). Then, we established a linear regression between the trunk or alternative length vs. TL and we used this relationship

to estimate TL for sharks with only trunk or alternative length (Fig. 5b; p-value <0.05, r^2 = 1 for both equations). This method was necessary for some white sharks in this study (n=7; indicated with * in Fig. 5).

Each sampled individual was classified into newborn, YOY, juvenile, or adult age groups based on species-specific reports of birth and maturity sizes. For shortfin mako sharks: birth TL 70–74 cm (Mollet *et al.,* 2000, Joung *et al.,* 2005) and size-at-maturity TL 180–210 cm for males and 256–278 cm for females (Cailliet *et al.,* 1983, Joung *et al.,* 2005, Semba *et al.,* 2011). The threshold between YOY (<102 cm TL) and juvenile (>102 cm TL) individuals was determined using shortfin mako parameters from von Bertalanffy equations (Ribot-Carballal *et al.,* 2005) as there is no previously reported size threshold for YOY shortfin mako sharks. For white sharks: birth TL 120-150 cm, YOY TL <175 cm (Bruce & Bradford, 2012), and minimum size-at-maturity TL 350 cm for males and 480 cm for females (Francis, 1996, Uchida *et al.,* 1996, Bruce & Bradford, 2012).

We collected muscle samples from consumed prey (from shortfin mako shark stomachs) and potential prey for both shark species. For shortfin makos, stomach contents were collected from newborn and juveniles and contained fishes and invertebrates, which were identified to the lowest taxonomic level possible (e.g., *Tylosurus* spp., *Prionotus* spp., *Coryphaena* spp., *Ophidion* spp., *Lophiodes* spp*., Synodus lucioceps*, *Scomber japonicus*, *Pleuroncondes planipes*) and sampled for isotopic analysis (n = 21). For white sharks, we collected potential prey samples (n = 54) of different species (i.e., *Mustelus californicus*, *Mustelus lunulatus*, *Mustelus henlei*, *Myliobatis californica*, *Galeorhinus galeus*, *Cynoscion* spp., *Scorpaena* spp., *Cynoscion albus*), based on white shark diet described in previous studies (Weng *et al.,* 2007, Santana-Morales 2012) and availability of taxa within the study area.

2.2.2. Sample preparation

Shark and prey tissue samples were prepared for isotopic analysis at CICIMAR. Samples were freeze-dried (LABCONCO) for 48 hours, then a subsample (~5mg) was homogenized in an agate mortar and pestle to a fine powder. Approximately 0.5 mg of

muscle powder was weighed with an analytical microbalance (precision of 0.001 mg) into an 8 × 5 mm tin capsule. Results are expressed in delta notation as follows:

$$\delta X = (R_{sample}/R_{standard} - 1) \times 1000 \quad \text{(Eqn. 1)}$$

Where X is ^{13}C or ^{15}N, R_{sample} and $R_{standard}$ are the isotopic ratios ($^{13}C/^{12}C$ or $^{15}N/^{14}N$) of the sample and the standard, respectively, and units are parts per thousand (‰). The standards used were Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen (AIR) for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Species or regional prey stable isotope data are reported as mean ± SD in the results and discussion.

Untreated, bulk samples were analyzed for SIA at the CICIMAR-IPN Laboratory of Mass Spectrometry (LEsMa) in La Paz, Baja California Sur, Mexico, on a Costech 4010 elemental analyzer interfaced with a Delta V Plus isotope ratio mass spectrometer (IRMS; Thermo-Electron) via a Conflo IV. Samples were analyzed in three runs, each with 70 samples. The average instrumental precision for $\delta^{13}C$ and $\delta^{15}N$ values is $\pm$ 0.3‰ based on reference materials from each run (IAEA-NO^{-3} n = 16; IAEA-N^{-1} n = 16; USGS-40 n=16; USGS-63 n = 16). Urea extracted samples were run at the Stable Isotope Lab at the University of California, Merced were also analyzed on a Costech 4010 Elemental Analyzer coupled to a Delta V Plus IRMS with a Conflo IV. Again, samples were analyzed in three runs with 70 samples each. The average instrumental precision for both isotopes values is $\pm$ 0.3‰ based on reference materials from each run (acetanilide n=21; USGS-40 n=21; USGS-41a n = 21).

2.2.3. Treatment of urea and lipid effects on isotope values

Several studies recommend lipid and urea extraction for shark muscle samples due to isotopic effects of these compounds (Kim & Koch 2012, Li *et al.,* 2016, Carlisle *et al.,* 2016). Lipids are ^{13}C-depleted relative to proteins and different lipid content can bias $\delta^{13}C$ values (Newsome *et al.,* 2010, Li *et al.,* 2016, Carlisle *et al.,* 2016). Urea ($(NH_2)_2CO$) and trimethylamine oxide (TMAO, C_3H_9NO) are produced by sharks as waste and used for osmoregulation. Urea is ^{15}N-depleted, which can bias $\delta^{15}N$ values

(Logan *et al.,* 2008, Kim & Koch 2012, Churchill *et al.,* 2015, Li *et al.,* 2016, Carlisle *et al.,* 2016). TMAO also contains carbon, which may bias $\delta^{13}C$ values (Kim & Koch 2012, Li *et al.,* 2016, Carlisle *et al.,* 2016). While urea extraction has been deemed necessary for reliable SIA values (Kim & Koch 2012, Li *et al.,* 2016, Carlisle *et al.,* 2016), chemical lipid extraction methods can also introduce error, particularly for $\delta^{15}N$ values (Post *et al.,* 2007; Carlisle *et al.,* 2016). Mathematical correction algorithms have been proposed as equal or better treatment of lipid content for correction of $\delta^{13}C$ and assume strong importance comparing isotope values across species (Shipley *et al.,* 2017), particularly in lean muscle from species with low lipid content (Post *et al.,* 2007, Logan *et al.,* 2008).

We extracted urea and TMAO from shark muscle samples using 3 rounds of 15 min of sonication in DI water according to methods reported in Kim & Koch (2012). We then used mathematical correction algorithms for $\delta^{13}C$ values reported in Carlisle *et al.,* (2016). For white sharks we used a white shark-specific algorithm, and for makos used a multi-species algorithm (including shortfin mako, salmon sharks, leopard sharks, white sharks and blue sharks), as reported in Carlisle *et al.,* (2016):

$$\Delta^{13}C = \beta_0 + \beta_1 Ln\,(C{:}N_U) \qquad \text{(Eqn. 2)}$$

Where β0 + β1 are species-specific coefficients determined by the model (β 0= -7.69 ± 0.82 and β1= 6.74 ± 0.66 for mako and β0= -7.80 ± 0.61 β1= 6.90 ± 0.48 for white sharks) and $C{:}N_U$ is the C:N ratio of the sample after urea extraction (Carlisle et la., 2016). We report carbon isotope values for untreated and corrected $\delta^{13}C$ values with subscripts “raw” and “corr.”

We did not extract lipids from prey samples because C:N ratio was lower than 3.5 in all prey samples (Post 2007) and there is evidence that apex predators with lipid-rich diets may use this substrate for tissue synthesis, causing lipid removal to confound predator-prey isotopic comparison, including isotopic mixing model results (Newsome *et al.,* 2010).

2.2.4. Quantification of isotopic niche

Ecological niche is defined as an n-dimensional hyper-volume (Hutchinson, 1978), which dimensions can be quantified with SIA and referred to as "isotopic niche" (Newsome, 2007). We quantified isotopic niche for white and shortfin mako sharks using SIBER (Stable Isotope Bayesian Ellipses in R) in SIAR (Stable Isotope Analysis in R; Parnell *et al.,* 2008, Jackson *et al.,* 2011) with R (R Development Core Team, 2008). SIBER creates a convex hull that encompasses all isotopic data, then fixes an ellipse to represent the "core isotopic niche" of consumers (Jackson *et al.,* 2011). This ellipse is generated with a Bayesian approach and is corrected using posteriori randomly replicated sequences (SEA_C = standard ellipse area correction, Jackson *et al.,* 2011). This ellipse is more robust as it is less sensitive to extreme values and small sample sizes (Jackson *et al.,* 2011) and represents isotopic niche width and allows quantification of consumer niche overlap (Bearhop *et al.,* 2004, Newsome, 2007).

2.2.5. Isotopic variation over ontogeny

We estimated incorporation rate of muscle based on the observed natural diet "switch" of juvenile shortfin mako sharks. Large migratory sharks are difficult to keep in captivity, and feeding experiments have generally used smaller, less active elasmobranch species (i.e, stingrays [Fisk *et al.,* 2009] and leopard sharks [Malpica Cruz *et al.,* 2012, Kim *et al.,* 2012a]). The data here allow for opportunistic quantification of isotopic incorporation rates in large, wild, highly active sharks due to the apparent natural diet switch from resources before entering SVB. We used the exponential growth model used for captive diet switching experiments (Tieszen *et al.,* 1983) and fit parameters with the nls function in R (R Development Core Team, 2008):

$$\delta^h X_t = \delta^h X_\infty - (\delta^h X_\infty - \delta^h X_0)\, e^{-\lambda t} \qquad \text{(Eqn. 3)}$$

where $\delta^h X_t$ is the isotopic value at time t, $\delta^h X_\infty$ is the isotopic value after steady state was reached with the new diet, $\delta^h X_0$ is the initial isotopic value, and λ is the fractional turnover rate (Tieszen *et al.,* 1983).

2.2.6. Bayesian mixing models and estimates of habitat use

To estimate regional prey inputs into shortfin mako and white shark diet, we characterized the isotopic composition of prey from four broad regions known to be used by juvenile shortfin mako and white sharks (Sippel *et al.,* 2004, Weng *et al.,* 2007, Oñate-González *et al.,* 2017, Nasby-Lucas *et al.,* in revision). Since juvenile shortfin mako and white sharks are known to use waters of southern California (e.g., Southern California Bight[SCB]/northern Baja) as well as southern and central Baja (includes SVB and referred to as "southern Baja" throughout results and discussion), we collected prey data from these two regions. We then split the northern area (SCB/northern Baja) and southern Baja into inshore and offshore areas because reported prey $\delta^{13}C$ and $\delta^{15}N$ values are different between inshore vs. offshore regions (higher $\delta^{13}C$ and $\delta^{15}N$ inshore; Madigan *et al.,* 2018). Inshore and offshore regions were distinguished by coastal vs. pelagic based on oceanographic characteristics as in Madigan et al (2018). The four characterized regions considered in this study are: SCB/northern Baja offshore, SCB/northern Baja inshore, southern Baja inshore, and southern Baja offshore. We used stable isotope values of taxa known to be prey for both species (Tricas & McCosker 1984, Preti *et al.,* 2012), which included foraged fish, (i.e. species that serve as primary food sources for marine predators; Pikitch *et al.,* 2012), cephalopods, and crustaceans, and generated overall mean ± SD for each region. Prey isotope values for northern regions were taken from published values (Madigan *et al.,* 2012a, 2018) and included fish (e.g., scombrids, sardine *Sardinops sagax*, anchovy *Engraulis mordax*), squids (e.g. jumbo squid *Dosidicus gigas* and mesopelagic species), and pelagic red crab *Pleuroncodes planipes* (see Table 2 for prey species and SIA values). Southern Baja offshore prey were taken from pelagic waters off Magdalena Bay, Mexico and southern Baja inshore prey were taken from SVB (as described above) as well as inshore waters of Magdalena Bay, another semi-circular bay in BCS, Mexico. Isotopic differences between regions were assessed graphically and statistically (Mann-Whitney U-Tests) to assess appropriateness of these groupings.

For mixing model analysis over ontogeny, shortfin mako sharks and white sharks were grouped into size classes (embryo, <80 cm, 80-100 cm, 100-120cm, 120-

140cm, 140-160cm, 160-180cm, and larger individuals >180cm FL). The four estimated regional prey means, as described above, were used as source inputs. Shark specific TEFs from Kim *et al.,* (2012a; $\Delta^{13}C$ = 1.7±0.5; $\Delta^{15}N$ = 3.7±0.4) were applied to the data. We used the Bayesian isotopic mixing model MixSIR (Moore & Semmens 2008) with uninformative priors and 10^6 iterations. Reported proportion of diet (%) are median estimate values from mixing model runs.

2.3. RESULTS

2.3.1. Biological sampling

We obtained muscle tissue from 165 shortfin mako sharks, which included 89 females (♀) and 76 males (♂) with the following age classes: 15 embryos or newborns (5 ♀, 10 ♂), 34 YOY (70-100 cm TL, 22 ♀, 12 ♂), and 116 samples of juveniles (102-196 cm TL, 62 ♀, 54 ♂). We also sampled one adult pregnant female, from which we collected muscle tissue and two embryos (2 ♂). A second set of 9 embryos (3 ♀, 6 ♂) came from an adult female shortfin mako (captured at N 26° 38' 43.8" W -116° 58' 45.8"), though muscle was not available from the pregnant female. We collected and analyzed 11 white shark muscle samples (7 ♀, 4 ♂). Age classes were 5 newborns (130-155 TL; 3 ♀, 2 ♂), 3 YOY (175-186 cm TL; 2 ♀, 1 ♂), and 3 juveniles (208-293 cm TL; 2 ♀, 1 ♂).

Prey species from shortfin mako stomachs (n = 22) included corvina *Cynoscion* spp (n = 4), sea robins *Prionotus* spp (n = 3), needlefish *Tylosurus pacificus* (n = 2), Pacific mackerel *Scomber japonicus* (n = 2), pelagic red crab, unidentified squid, and other demersal and pelagic species. Inshore species from Magdalena Bay included small black skipjack (n = 3), gonatid squid (n = 4) and pelagic red crab (n = 4), and these SIA values from these species collectively comprised the southern Baja region. Offshore species sampled from southern Baja included Pacific saury *Cololabis saira*, jack mackerel *Trachurus symmetricus*, Pacific mackerel, pelagic red crab, and cephalopods including *Dosidicus gigas, Argonauta* spp., and pelagic octopus. Analysis of $\delta^{13}C$ and $\delta^{15}N$ values in these species revealed strong differences between inshore and offshore southern/central Baja (described below; see Table 2).

2.3.2. Stable isotope results

To assess the effects of urea, we compared muscle tissue from both shark species with no urea extraction (n=159) and only urea extraction (no lipid extraction: n=159). The average difference between untreated and urea-extracted values was low for $\delta^{13}C$ values (0.3 ± 0.6‰) and high for $\delta^{15}N$ values (1.3 ± 0.6‰). The mean C:N ratio for untreated tissues was 3.0 ± 0.2 and for urea-extracted tissues was 3.3 ± 0.1, which is similar to protein values (Post *et al.,* 2007), indicating that mathematical correction for lipid content for $\delta^{13}C$ values was of minimal importance. We applied the mathematical lipid correction based on C/N values, which slight increased $\delta^{13}C$ values for both species. For mako sharks, the urea extracted mean $\delta^{13}C$ values was -17.8 ±0.6 ‰ and $\delta^{13}C_{corr}$ values was -17.4 ± 0.5‰; for white sharks the urea extracted $\delta^{13}C$ values was -17.1 ± 0.1 ‰ and $\delta^{13}C_{corr}$ values was -16.5 ± 0.7‰.

Shortfin mako $\delta^{13}C_{corr}$ values ranged from -18.3 to -15.4‰ (-17.4 ± 0.5‰) and $\delta^{15}N$ values ranged from 14.3 to 21.3‰ (19.1 ± 1.4‰) (Table 1). The isotopic composition of shortfin mako sharks did not vary by sex (Fig. 5; Wilcoxon signed-rank test, $\delta^{15}N$ values: W = 3697.5, p = 0.2 and $\delta^{13}C$ values: W = 3384, p = 0.9), but there was variation based on size class. The $\delta^{15}N$ values increased with TL among embryo, YOY, and juvenile shortfin mako sharks as described by a logarithmic regression (Fig. 5; n = 165, $p < 0.05$, $r^2 = 0.7$). The isotopic incorporation rate model for muscle estimated the residence time from initial (i.e., newborn) to final (i.e., juvenile) diet as 0.7 years (~255 d; $\delta^h X_\infty = 20$, SE = 0.1, t = 138.2, Pr(>|t|) = $<2 \times 10^{-16}$; $\delta^h X_\infty - \delta^h X_0 = 2.9$, SE = 0.2, t = 15.8, Pr(>|t|) = $<2 \times 10^{-16}$; $1/\lambda = 0.7$, SE = 0.1, t = 4.7, Pr(>|t|) = 4.01×10^{-06}; Residual SE = 0.8, DF = 162). The one mature, pregnant female in our sampled population was much larger than the other individuals and had a $\delta^{15}N$ value of 17.3‰, which was incongruous with the logarithmic ontogenetic trend for immature shortfin mako sharks. The $\delta^{15}N$ value of the mature, pregnant female was more similar to the embryos (including those not sampled from her; TL < 70 cm; $\delta^{15}N$ values range 16.1 to 17.9‰, mean 16.8 ± 0.6‰).

White shark $\delta^{13}C_{corr}$ values ranged from -17.9 to -15.6‰ (-16.5 ± 0.7‰) and $\delta^{15}N$ values from 17.7 to 20.4‰ (18.6 ± 0.7‰) (Table 1). White shark isotopic values did not vary by sex (Fig. 5; Wilcoxon signed-rank test, $\delta^{15}N$ values: W = 7, p = 0.2 and

$\delta^{13}C$ values: W = 1, p = 0.4) nor $\delta^{15}N$ values by TL (Fig. 5; linear regression fit: p = 0.5, r^2 = -0.05). Five individuals from Laguna Manuela, the most northern site, had $\delta^{13}C_{corr}$ values that ranged from -16.6 to -15.6‰ and $\delta^{15}N$ values from 18.2 to 18.6‰, which included the two largest juveniles (TL = 208 and 272 cm) with $\delta^{13}C_{corr}$ values of -16.3 and -16.0‰ as well as one newborn and one YOY with the highest $\delta^{13}C_{corr}$ values (-15.7 and -15.6‰). However, the $\delta^{15}N$ values of all Laguna Manuela white sharks were similar to others sampled from the central part of Sebastian Vizcaino and Cedros Island (n = 6), which had $\delta^{13}C_{corr}$ values of -17.9 to -16.1‰ and $\delta^{15}N$ values of 17.7 to 20.4‰.

Table 1. Isotopic values for size classes of mako and white sharks.

	Size Classes	TL (cm)	$\delta^{13}C$ (‰)				$\delta^{15}N$ (‰)			
			Min	Max	Mean	SD	Min	Max	Mean	SD
Mako sharks	All (n=165)	64.5-302	-18.3	-15.4	-17.4	0.5	14.3	21.3	19.1	1.4
	Embryos (n=15)	< 70	-18.2	-16.6	-17.3	0.5	16.1	17.9	16.8	0.6
	YOY (n=34)	70-100	-18	-15.4	-17.1	0.6	14.3	20.1	17.4	1.2
	Juveniles (n=116)	102-196	-18.3	-16	-17.5	0.4	17.1	21.3	19.8	0.8
White sharks	All (n=11)	130-293	-17.9	-15.6	-16.5	0.7	17.7	20.4	18.6	0.7
	Newborns (n=5)	130-155	-17.1	-15.7	-16.4	0.5	18.4	18.6	18.5	0.1
	YOY(n=3)	175-186	-17.9	-15.6	-16.8	1.1	18.3	20.4	19.3	1
	Juveniles (n=3)	208-293	-17.9	-16	-16.4	0.4	17.7	18.6	18.2	0.4

Shortfin mako and white sharks had significant differences in $\delta^{13}C_{corr}$ values (Wilcoxon signed-rank test, W = 255.5, p < 0.0001), but not in $\delta^{15}N$ values (Wilcoxon signed-rank test, W = 1189.5, p = 0.08). Shortfin mako and white sharks with similar TL had similar $\delta^{15}N$ values (Fig. 4). We quantified the similarity in shortfin mako and white shark stable isotope values using the isotopic niche analysis in SIBER. The isotopic niche of shortfin mako sharks (black ellipse in Fig. 4; SEA_c = 2.1) and white sharks (red ellipse in Fig. 4; SEA_c = 1.5) yielded partially overlapping ellipse areas with an estimated mathematical overlap of 0.2 and a Bayesian mean overlap of 0.3. When the two smallest white shark individuals with the highest $\delta^{13}C_{corr}$ values were removed,

SIBER ellipses had small increase in inter-species overlap (mathematical overlap = 0.4, Bayesian mean overlap = 0.3; shown as the red ellipse in Fig.5; SEA_c =1.5).

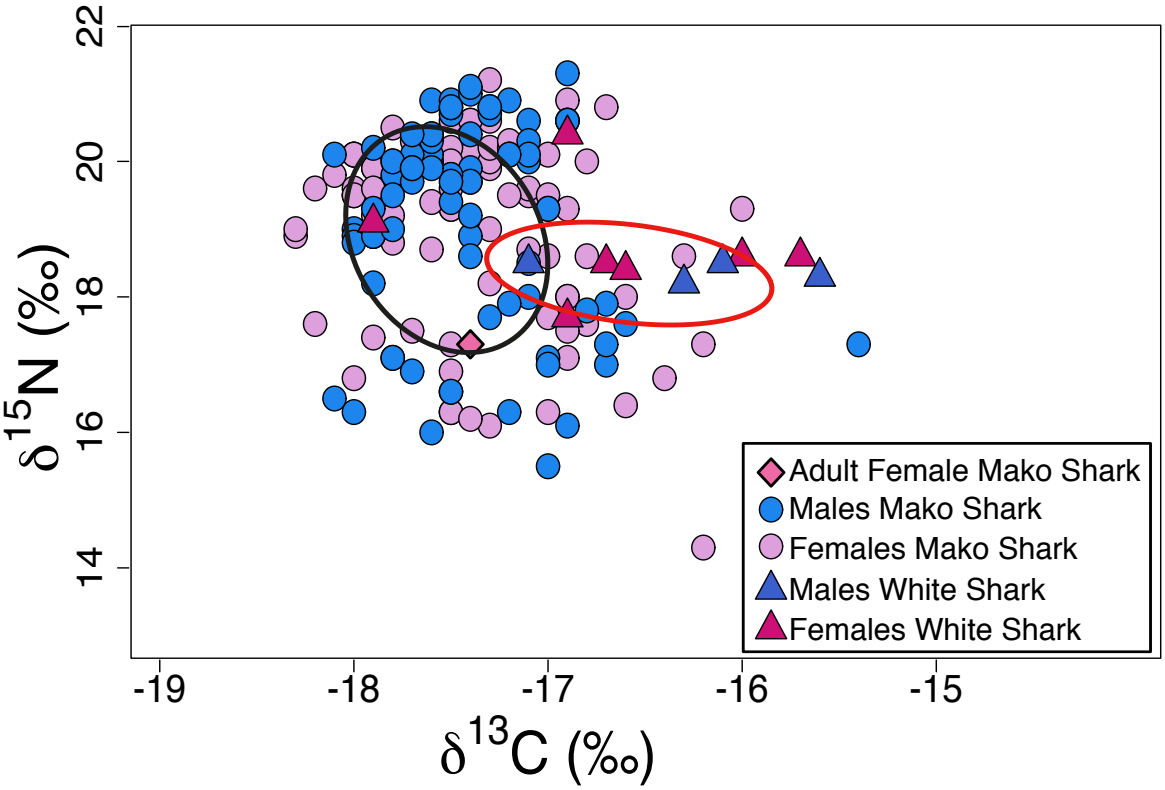

Figure 4. δ15N and δ13C values for both species and by sex. The black ellipse is the isotopic niche (SIBER analysis) for shortfin mako sharks (n = 165). The red ellipse is the isotopic niche of all white sharks analyzed (n = 11). The overlap between the isotopic niches of shortfin mako and small white sharks suggest resource sharing at certain body sizes.

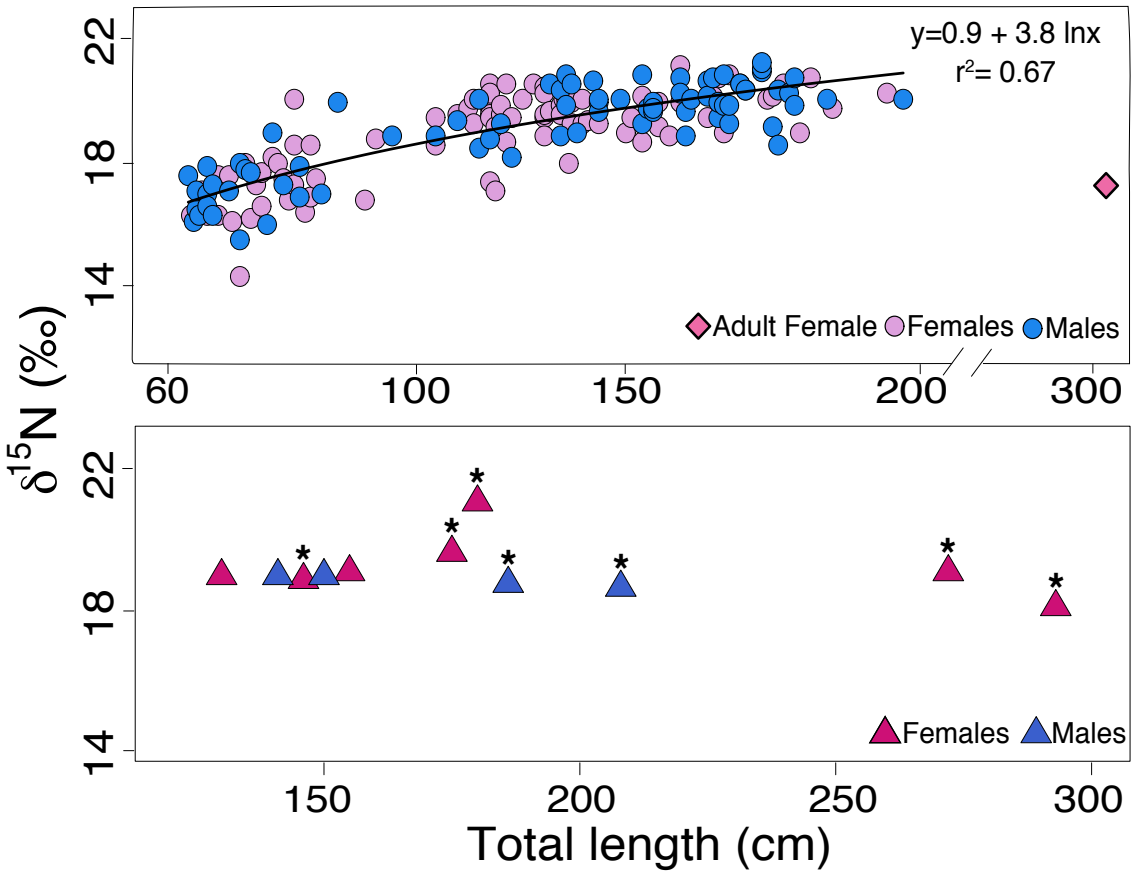

Figure 5. δ15N values related to the total length for shortfin mako and white sharks. δ15N values increased with TL for shortfin mako but not for white sharks. For shortfin mako sharks this relationship was best described by a logarithmic regression, with equation and r^2 values shown.

Regional prey groupings (as described in the methods) revealed significant differences in isotope composition (Fig. 6a and Table 2). Regional prey values were: SCB/northern Baja offshore ($\delta^{15}N$ = 13.8 ± 1.2‰, $\delta^{13}C$ = -18.9 ± 0.7‰; n = 122); SCB/northern Baja inshore ($\delta^{15}N$ = 14.5 ± 0.9‰, $\delta^{13}C$ = -17.1 ± 0.7‰; n = 30); southern Baja offshore ($\delta^{15}N$ = 12.1 ± 1.5‰, $\delta^{13}C$ = -19.3 ± 0.7‰; n = 65); and southern Baja inshore ($\delta^{15}N$ = 15.5±1.9‰, $\delta^{13}C$ = -17.6±1.1‰; n = 33) (Fig. 6a). The $\delta^{15}N$ values in the southern Baja inshore region were significantly higher than all other regions (Mann-Whitney U-test; $p < 0.01$ for all pairwise regional comparisons). These high $\delta^{15}N$ values for prey have overlap with TEF-corrected $\delta^{15}N$ values of juvenile shortfin mako sharks, some of which had high $\delta^{15}N$ values (Fig. 6a).

Bayesian mixing models indicated large differences in regional prey use and changes with ontogeny from the four regions analyzed (SCB/Northern Baja inshore,

SCB Northern Baja offshore, southern Baja inshore, and southern Baja offshore) for shortfin mako and white sharks. Shortfin mako embryos reflected offshore values from both southern Baja (46%) and northern Baja/SCB (31%) with likely maternal influence (see Discussion). YOY shortfin makos (TL 80-100 cm) reflected SCB/northern Baja offshore prey inputs (75%) with minimal inputs (>12%) from other regions (Fig. 6b), then shortfin makos exhibit a shift to southern Baja inshore prey inputs (e.g. SVB) increasing to 52% at 100-120 cm and >80% at 160-180 cm (Fig 5b). The remainder of shortfin mako sharks in these size classes comprised of offshore prey from SCB/northern Baja (15-40%) with minimal inputs from the other two regions (Fig 5b). White sharks showed high inputs from southern Baja inshore (25 - 59%) across sizes analyzed (120 cm to >180cm), but also relatively high input from inshore SCB/northern Baja at some size ranges (6 to 40%). Across the white shark size classes analyzed, mixing model results indicated some foraging on prey from SCB/northern Baja offshore (17-25%) and southern Baja offshore (8-17%), but in low proportions (Fig. 6b; see supplementary material for the density distributions of consumers).

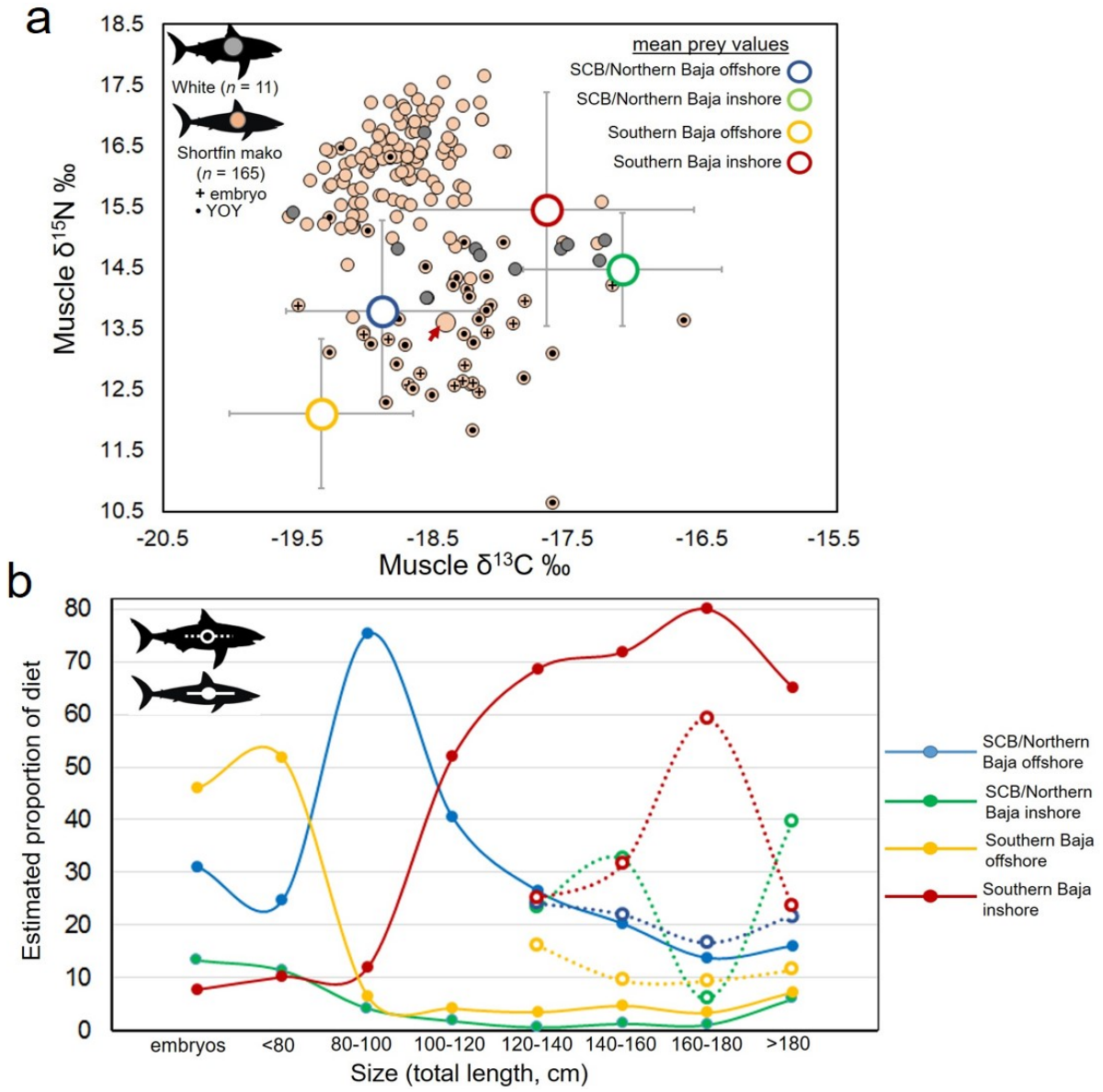

Figure 6. $\delta^{13}C$ and $\delta^{15}N$ values in shortfin mako and white sharks compared to prey of known foraging habitats for both species. (a) Biplot of all sharks analyzed, as well as mean (±SD) values of prey from four regions: southern California/northern Baja offshore, southern California/northern Baja inshore, southern Baja offshore, and southern Baja inshore (including SVB). Shark values are TEF-corrected based on Kim *et al.,* 2012a. Red arrow indicates the single large pregnant female analyzed (302 cm TL). **(b)** Results of Bayesian mixing model showing habitat use across ontogeny. Smaller shortfin makos reflected offshore, northern Baja waters and then the SVB region, while white sharks used a mix of inshore waters in northern and southern Baja California including SVB.

2.4. DISCUSSION

Our findings suggest high use of the southern Baja inshore region (including SVB) across much of the juvenile size ranges analyzed, with differences in ontogenetic isotope dynamics between shortfin mako and white sharks at the SVB aggregation site

in BCS, Mexico. Results for shortfin makos indicate significant and consistent isotopic changes throughout ontogeny. This shift in isotopic values is likely due to movements from offshore regions with lower $\delta^{15}N$ values to SVB (similar local environments) with high $\delta^{15}N$ values, but could also represent a shift from a maternal isotopic signal to local foraging (discussed below). The similarity in isotopic niche between shortfin mako and white sharks in this sampled population indicates shared resource use in SVB and/or surrounding habitats in southern Baja California, an inference supported by known movements from fisheries capture and conventional/electronic tagging studies as well as similarly high $\delta^{15}N$ values among both species, which reflects the regional baseline. This study reinforces the assertion that SVB, and potentially similar areas in the region, are aggregation sites with resource sharing among newborn, YOY, and juveniles of these two species. Furthermore, the consistent increase in shortfin mako $\delta^{15}N$ values from newborns to larger juveniles, which reached apparent steady-state of $\delta^{15}N$ values, presented a natural diet switch "experiment" that provided the first estimated muscle tissue incorporation rate (~255 days) in shortfin mako sharks.

2.4.1. Resource use across size classes of shortfin shortfin mako and white sharks

Juvenile and YOY stages of different shark species are known to frequent the same nursery areas for several months after birth (Huepel *et al.,* 2007, Conventional Tagging program of INAPESCA, unpubl. data, Castillo-Géniz, pers. comm.), but the extent of resource sharing can be difficult to discern. The similarity between $\delta^{13}C$ and $\delta^{15}N$ values in juvenile shortfin mako sharks and YOY white sharks with similar TL (shortfin makos sharks > 102 cm TL and white sharks < 186 cm TL) suggested similar habitat and resource use in SVB. Our results indicate that isotope values of juvenile shortfin mako sharks and YOY white sharks are ^{13}C and ^{15}N-enriched compared to other age classes for both species. It is notable that the shortfin makos analyzed in this study had significantly higher $\delta^{15}N$ values (mean 19.8‰, with many >20‰) than all white sharks analyzed here (Fig. 6a) and any juvenile shortfin makos analyzed in the SCB in a previous study (16.4 ± 0.8‰; Madigan *et al.,* 2012a). This is likely driven by the higher $\delta^{15}N$ values of prey in the Southern Baja region than that in the

SCB/Northern Baja region. Prey in SVB also had higher isotope values than any prey analyzed from SCB (Fig. 6a); these high prey $\delta^{15}N$ values (fish species $\delta^{13}C$ = -18.2 ± 1.4 ‰ and $\delta^{15}N$ = 16.2 ± 1.3; invertebrates $\delta^{13}C$ = -20.1 ± 1.1 ‰ and $\delta^{15}N$ = 10.6 ± 2.7 ‰) result in differentiation of sharks consuming the prey. Hence, SVB shortfin makos foraging on SVB prey differ from those sampled in northern areas.

Isotopic mixing model results quantified the use of prey resources in SVB and other coastal southern Baja prey and suggest that juvenile shortfin mako and young white sharks consume these local SVB prey or those with similar isotopic composition (Fig. 6). These results are also supported by the alignment between the isotopic result of juvenile shortfin mako and YOY white sharks and the isotopic values of fish species ($\delta^{13}C$ = -18.2 ± 1.4 ‰ and $\delta^{15}N$ = 16.2 ± 1.3 ‰) found in the stomach contents of shortfin mako sharks caught in SVB, after the application of shark specific muscle trophic enrichment factors (Kim *et al.,* 2012a). However, the mixing model results should be interpreted with precaution because the low sample size of white sharks and the TEF used, which was developed for a smaller and less active species (*Triakis semifasciata*) and may differ for the species and the life stages considered in this study. In addition, it should be noted that the prey and error demonstrated in Figure 5a are means ± 1 **?** and the entire prey variation is not captured in this depiction. However, we would like to outline External sources of variation, like high levels of nitrogen in resource consumed, can affect the TEF and the general accuracy of the model (Parnell *et al.,* 2010; Parnell *et al.,* 2013). This external variation in high nitrogen sources, like the ones of SVB bay, can explain the high levels of nitrogen in juveniles mako sharks and the apparently lack of inclusion of these samples into the isoescape. A general accepted assumption explanations for when consumers lie outside of the resource polygon: 1) misrepresentation of potential food sources or 2) consumer trophic enrichment factor is misrepresented (Brett 2014). However, recent studies demonstrate the “point-in-polygon” assumption is not substantiated because the Bayesian approach recognizes that source data are distributions and assumes mixing polygons are from a probability function, not average values. This distinction affects the simulation produced from Bayesian mixing models and consequently, interpretation of the polygon geometry (Smith *et al.,* 2013). Although we make

assumptions regarding TEF, isotope source variability, and metabolic characteristics of young sharks, our results provide preliminary insight to the pre-adult ecology of two large sharks species that are difficult to study.

The extent of isotopic overlap among species, qualitatively and based on SIBER-generated isotopic niches (Fig. 4), indicated some resource overlap at certain sizes. This partial similarity in diet and habitat for juvenile shortfin mako and YOY white sharks parallels resource sharing in other co-existing elasmobranch species with similar sizes and morphologies across different life stages (Ellis *et al.,* 1996, Bethea *et al.,* 2004, Tilley *et al.,* 2013). Isotope-inferred resource use overlap in SVB by juvenile shortfin mako and YOY white sharks complements other examples of little resource partitioning in co-occurring and resident elasmobranch species in communal nursery areas where prey availability is not limited (i.e., Dale *et al.,* 2011, Kinney *et al.,* 2011, Tilley *et al.,* 2013).

Previous studies suggest resource partitioning between juvenile shortfin mako sharks and YOY white sharks in SVB (Weng *et al.,* 2007, Malpica-Cruz *et al.,* 2013). Malpica-Cruz *et al.,* (2013) sampled shortfin mako and white sharks with similar body sizes to our specimens and found $\delta^{13}C$ and $\delta^{15}N$ values comparable to those in this study. Malpica-Cruz *et al.,* (2013) showed an increase of $\delta^{15}N$ values with body size for both species, while in our study white sharks showed no change over the sizes analyzed. Our results indicated similar $\delta^{13}C$ values in juvenile shortfin mako and YOY white sharks, though white sharks showed slightly higher $\delta^{13}C$ values. For shortfin mako sharks, results here indicated an inflection, or change from increasing to steady-state values, of $\delta^{15}N$ values over ontogeny at 100 cm (compared to 85 cm in Malpica-Cruz *et al.,* 2013). The change observed here corresponded more closely with the juvenile TL size threshold, and this difference was likely driven by our larger dataset (n = 165) compared to that in Malpica-Cruz *et al.,* 2013 (n = 23). Furthermore, Malpica-Cruz *et al.,* (2013) attributed high $\delta^{13}C$ values of YOY white sharks to consumption of benthic fishes inside this bay, which differs from the pelagic feeding strategy of shortfin mako sharks. We compared $\delta^{13}C$ and $\delta^{15}N$ values for benthic preys we collected in SVB ($\delta^{13}C$ = -16.8 ± 1.0‰ and $\delta^{15}N$ = 15.6 ± 1.9‰) with our YOY and newborn white shark muscle tissue and did not find alignment between these isotope compositions.

At certain sizes in our study, white and shortfin mako sharks $\delta^{13}C$ values were dissimilar, with higher $\delta^{13}C$ values in the two smallest white sharks; this could be interpreted as more benthic foraging in white sharks (e.g., Malpica-Cruz *et al.,* 2013) but additional support is needed to support this inference. Overall, the alignment of isotope values of both species at certain sizes may demonstrate more similar feeding patterns in the area than previously thought.

The high $\delta^{15}N$ values for the juvenile shortfin mako sharks in this study indicated an ontogenetic change in diet, which could be attributed to local foraging shifts or to incorporation of isotope values from distinct ecoregions during different life-stages. Malpica-Cruz *et al.,* (2013) related the high $\delta^{15}N$ values of shortfin mako sharks to a dietary change from the incorporation of exogenous prey after birth. Based on regional prey characterizations and mixing model results here, we suggest that high $\delta^{15}N$ values of juvenile shortfin mako sharks here could be due to the same process (a shift from offshore prey inputs from maternal signature) or foraging shifts from offshore regions to southern Baja California. The offshore isotope signal is apparent in embryo and YOY shortfin mako sharks, as well as the large pregnant female (Fig. 6a), which may be due to direct maternal transference during gestation rather than direct foraging. However, future studies could use retrospective methods to reconstruct shark life history (e.g. vertebral analysis; Kim *et al.,* 2012b; Carlisle *et al.,* 2015) to clarify maternal signal versus migratory shifts and elucidate birthing region of the sharks in SVB.

The dynamics of isotopic change from neonates to larger juveniles (Fig. 5) is similar to patterns attributed to diet shifts in other pelagic predators in both captive (Kim *et al.,* 2012a, Madigan *et al.,* 2012b) and wild (Graham *et al.,* 2007) conditions. This signal allows for estimates of turnover or incorporation rate (λ) in YOY shortfin makos. We estimated a residence time ($1/\lambda$) of ~255 days, which is faster than the estimated rate of 475 days reported in leopard sharks (Malpica-Cruz *et al.,* 2012). This difference is probably due to the different resting metabolic rate of leopard sharks, which are less active and have lower metabolic demands than partially endothermic, RAM-ventilating shortfin mako sharks. In addition, juvenile shortfin makos have higher relative growth rates than adults, which means faster incorporation of prey isotopic signal in muscle tissue (Reich *et al.,* 2008, Newsome *et al.,* 2009). Isotopic turnover rates based on

allometric relationships in ectothermic fish (Weidel *et al.,* 2011) estimate 229–350 days for shortfin mako sharks with TL 100–196 cm (based on weight estimates of 7.6 – 63 kg from relationships of TL in Kohler *et al.,* [1996]), a range inclusive of our ~255 day estimate. Although young, endothermic sharks have higher relative growth and metabolic rates (Bernal *et al.,* 2001a, Carlson *et al.,* 2004, Ezcurra *et al.,* 2012), the Weidel *et al.,* (2011) relationship produces similar incorporation rates as estimated in this study. We note that our results, based on wild data, are preliminary and can only be confirmed by captive studies on juvenile mako or white sharks.

2.4.2. Do trophic or baseline shifts explain the variation in isotopic values?

The ontogenetic shift in $\delta^{15}N$ values in juvenile shortfin mako sharks and the high $\delta^{15}N$ values of YOY and newborn white sharks can be caused by a change in localized prey or changes in prey isotopic baseline due to migration. Ontogenetic shifts in prey consumption are well supported in sharks, where larger sharks forage on larger prey due to hunting strategy (Klimley, 1985, LeBoeuf, 2004) or physiological capability (Gerritsen, 1984). However, it is also possible that the isotopic composition of SVB prey is different from maternal or primary nursery areas and consequently reflects migration from other ecoregions.

Ontogenetic diet shifts are well described for white sharks (Tricas and McCosker, 1984, Klimley 1985, Le Boeuf, 2004, Dewar *et al.,* 2004, Kim *et al.,* 2012b) and to a lesser degree for shortfin mako sharks (Velasco-Tarelo, 2005, Malpica-Cruz *et al.,* 2013). However, the individuals in this study had smaller body sizes than those reported in the literature for ontogenetic dietary changes to larger prey (Tricas and McCosker, 1984, Klimley 1985, Le Boeuf, 2004). Our mixing model results suggest that the isotopic change in shortfin mako muscle reflects a shift from offshore areas in northern Baja and southern California, likely due to maternal foraging, to inshore regions of central-southern Baja. A recent study of 113 shortfin makos tagged in the SCB revealed movements of some juveniles to SVB, and retention in the area for weeks to months (Nasby-Lucas *et al.,* in revision). It is thus possible that the ontogenetic change, particularly for $\delta^{15}N$ values, reflects a dietary shift associated with migration from the SCB to inshore southern regions of Baja California.

Meanwhile, white sharks reflect movements between inshore regions in both northern and southern Baja (Figure 5). The inference of migration driven isotopic changes aligns with tagging studies for both species. However, this conclusion could be reinforced both by expanding diet studies of both species within SVB, complementary tagging techniques (e.g. acoustic arrays and tagging within SVB), and chemical tracer techniques that discern trophic and baseline effects (e.g. amino acid-compound specific isotope analysis; Madigan *et al.,* 2014).

To further contextualize our results within predator-prey isotopic dynamics in SVB, we can compare shark SIA values to known resident predators in SVB. Adult California sea lions (*Zalophus californicus*) from San Benito Archipelago have $\delta^{13}C$ = -16.8 ± 0.4 ‰ and $\delta^{15}N$ = 19.3 ± 0.4‰, and Isla Cedros sea lions have $\delta^{13}C$ values = -16.3 ± 0.4‰ and $\delta^{15}N$ values = 20 ± 0.5‰ (values adjusted from pups; Elorriaga-Verplancken *et al.,* 2016, unpubl. data). California sea lions are local residents and make short, coastal foraging trips (Kuhn & Costa*,* 2014), which in this case would be near their rookeries and inside SVB. The similar isotopic composition between California sea lions, juvenile shortfin mako sharks and YOY white sharks supports the hypothesis that these sharks reside and forage in SVB and/or similar inshore regions along Baja California for extended timeframes.

Newborn and YOY white sharks are present at Sebastian Vizcaino Bay every year during summer months (Oñate-González *et al.,* 2017), which coincides with their parturition season (Francis, 1996, Uchida *et al.,* 1996, Domeier & Nasby-Lucas, 2013). Parturition sites for white sharks have remained enigmatic, but given the small individuals caught in SVB, it is possible that some white sharks are born and reside inside the bay. Juvenile white sharks have been commonly reported to be born in the SCB and then move to SVB when they are larger (Weng *et al.,* 2007; Weng *et al.,* 2012; Oñate-González *et al.,* 2017); our mixing model results suggest a mix of southern California and SVB isotopic signals in the smallest white sharks analyzed (Fig. 6b). It is thus possible that parturition sites occur on both regions, however the small sample size for white sharks suggest precaution in the interpretation of results.

2.4.3. Potential maternal transference of isotope values

Embryo and neonate shortfin mako sharks showed considerable isotopic variability (Fig. 6a). Past studies have reported significantly higher $\delta^{15}N$ values in neonate muscle than their mothers (Vaudo *et al.,* 2010, Olin *et al.,* 2011, Frankel *et al.,* 2012, Pilgrim, 2007, Matich *et al.,* 2015). The increased $\delta^{15}N$ value of neonates in some species is thought to be from ^{15}N-enriched yolk (McMeans *et al.,* 2009, Vaudo *et al.,* 2010, Olin *et al.,* 2011) or from trophic enrichment in placentatrophic viviparous sharks (Hussey *et al.,* 2010, Vaudo *et al.,* 2010, Matich *et al.,* 2015). However, neonate sharks here did not show $\delta^{15}N$ values higher than the mother. We found embryo isotope values ($\delta^{13}C$: -18.2 to -16.6‰ and $\delta^{15}N$: 16.1 to 17.9‰) to be similar to the adult, pregnant female shortfin mako ($\delta^{13}C$= -17.4‰ and $\delta^{15}N$ = 17.3‰,) (Fig. 6a), suggesting direct maternal transference of nutrients and feeding resources without substantial fractionation to neonate shortfin makos (Jenkins *et al.,* 2001). In contrast to species in some previous studies, shortfin mako and white sharks are aplacental viviparous with oophagy (ingestion of unfertilized eggs) (Francis, 1996, Uchida *et al.,* 1996, Joung *et al.,* 2005), such that embryos rely solely on yolk and ingestion of unfertilized eggs. We note that the $\delta^{13}C$ values in neonate shortfin mako sharks are lower than the adult female, possibly due to reliance on lipid-rich, ^{13}C-depleted yolk (Murchie & Power, 2004), which could also explain previous observations of SIA in neonate white sharks (Hussey *et al.,* 2012). Our results suggest that yolk ingestion does not cause substantial nitrogen isotope fractionation or that yolk nutrients are ^{15}N-depleted from maternal sources, due to similarity between maternal and neonate $\delta^{15}N$ values. Additional studies of sharks with different reproduction modes and comparison of maternal, yolk, and neonate isotopic composition are needed to yield additional insight to the physiological and biochemical processes related to maternal transference. Further confirmation of isotopic similarity between embryo, newborn, and YOY shortfin mako sharks and pregnant females could help establish the preferred habitat of mature adult females, as well as parturition habitat, which remains elusive.

2.5. CONCLUSIONS

We used SIA to investigate habitat use of YOY and juveniles of two vulnerable shark species in an aggregation area in southern Baja California. At young life stages, shortfin mako and white sharks couple long distance migrations with long-term residency in nursery areas. Our results suggest that some YOY and juvenile shortfin mako and white sharks may migrate to SVB from other regions, where they then forage and share prey resources. The ontogenetic shift in shortfin mako sharks with increasing $\delta^{15}N$ values with size can be explained by long-term use of prey resources within SVB and potentially other isotopically similar, surrounding areas. The large dataset we present for shortfin mako sharks, including embryos, YOY, juveniles, and adult allowed us to provide a new estimate for isotopic incorporation rate for shortfin mako sharks (~255 days). However, our sampled population included few white sharks and therefore these interpretations are preliminary. This study indicates the importance of this region as a nursery, independent of the high artisanal catch of YOY and juvenile white and shortfin mako sharks. Identifying such regions is crucial to protect these life stages and has implications for conservation and management. Our conclusions could be strengthened by future, complementary studies; for example, SVB is an ideal region to install acoustic receivers and tag juveniles to assess residency while other chemical tracer techniques could be employed to evaluate residency in the region. Management policies that focus on conservation of these vulnerable species should strive to conclusively identify SVB and other regions as shared aggregation area for young shortfin mako and white sharks, and can thus employ management measures that support sustained or increased population growth of these species.

Table 2. Isotopic values for prey species of the different regions, used in the mixing model.

Region	prey type	species	$\delta^{13}C$ (SD)	$\delta^{15}N$ (SD)	ref
Southern Baja inshore	Pacific mackerel	*S. japonicus*	-17.8 (0.6)	15.7 (0.9)	[this study]
	Black skipjack	*E. lineatus*	-16.8 (0.4)	18.3 (0.8)	
	Corvinas	*Cynoscion spp.*	-15.7 (0.8)	16.4 (1.0)	
	Lizardfish	*S. lucioceps*	-19.1 (–)	16.8 (–)	
	Needlefish	*Tylosurus spp.*	-17.1 (0.2)	18.3 (0.6)	
	Scorpionfish	*Scorpaena spp.*	-17.9 (0.2)	15.9 (0.6)	
	Goosefish	*Lophiodes spp.*	-17.8 (–)	15.8 (–)	
	Sea robins	*Prionotus spp.*	-18.0 (1.5)	15.5 (0.8)	
	Dolphinfishes	*Coryphaena spp.*	-18.4 (–)	13.4 (–)	
	Cusk eels	*Ophidion spp.*	-17.1 (–)	16.7 (–)	
	Pelagic red crab	*P. planipes*	-18.4 (0.5)	13.4 (1.3)	
	Armhook squid	*Gonatidae spp.*	-18.4 (0.1)	14.6 (0.3)	
	Squid	*Unid. spp.*	-17.7 (1.3)	12.7 (2.5)	
	MEAN		**-17.6 (1.1)**	**15.5 (1.9)**	
Southern Baja offshore	Pacific saury	*C. saira*	-19.7 (0.5)	10.7 (1.5)	[this study]
	Lanternfish	*Myctophidae spp.*	-20.1 (0.5)	12.2 (0.7)	
	Pacific mackerel	*S. japonicus*	-18.6 (0.1)	14.6 (0.2)	
	Jack mackerel	*T. symmetricus*	-19.4 (–)	12.2 (–)	
	Halfbeak	*H. naos*	-19.2 (0.2)	8.6 (0.3)	
	Pelagic triggerfish	*Canthidermis spp.*	-20.1 (–)	11.1 (–)	
	Flyingfish	*Exocoetidae spp.*	-20.1 (–)	12.4 (–)	
	Humboldt squid	*D. gigas*	-19.4 (0.2)	13.1 (0.6)	
	Armhook squid	*Gonatidae spp.*	-19.5 (0.3)	12.6 (1.2)	
	Pelagic octopus	*O. tuberculata*	-19.3 (0.9)	13.7 (1.9)	
	Cephalopod	*Unid. spp.*	-18.7 (0.5)	12.1 (0.5)	
	Pelagic red crab	*P. planipes*	-19.0 (0.9)	11.6 (0.9)	
	Large krill	*Euphausidae spp.*	-18.7 (0.4)	13.0 (0.5)	
	MEAN		**-19.3 (0.7)**	**12.1 (1.5)**	
Northern Baja/	Sardine	*S. sagax*	-16.9 (0.4)	13.9 (0.5)	[1]
SCB inshore	Jack mackerel	*T. symmetricus*	-18.2 (0.8)	14.2 (0.9)	[1]
	Pacific mackerel	*S. japonicus*	-17.6 (0.9)	15.1 (0.9)	[1]

References: [1] Madigan *et al.,* (2018) [2] Madigan *et al.,* (2012a)

CHAPTER 3 – ISOTOPIC VARIATION IN BLOOD COMPONENTS OF JUVENILES SHARKS INDICATE THE INFLUENCE OF BLOOD BIOCHEMESTRY.

3.1. INTRODUCTION

Shortfin mako sharks (*Isurus oxyrinchus*) and white (*Carcharodon carcharias)* are categorized as keystone species in marine ecosystems (Murphy 1996, Kacev, 2003). They are globally distributed and mainly found in tropical and temperate oceans (Compagno, 2002) and both are classified as vulnerable based on the International Union for Conservation of Nature (IUCN) (Fergusson *et al.,* 2009, Cailliet *et al.,* 2009). The trophic ecology, habitat use and aggregation areas are generally well-described for the adult stages. These preferably aggregate near pinniped colonies throughout the world (Bruce, 1992: Klimley *et al.,* 2001, Le Boeuf, 2004; Martin *et al.,* 2005; Weng *et al.,* 2007; Hoyos-Padilla, 2016) with seasonally offshore migrations (Boustany *et al.,* 2002, Bonfil *et al.,* 2005). Adult shortfin mako sharks are primarily oceanic and epipelagic in the Pacific Ocean (Holts & Bedford*,* 1993, Abascal *et al.,* 2011, Sippel *et al.,* 2004).

However, the habitat use information for juvenile stages is scares worldwide and while they have been observed frequenting coastal and surface waters off the California and Baja California coast (Weng *et al.,* 2007; Medina-Trujillo 2013), further details like habitat overlap of juvenile shortfin mako and white sharks' and sharing of food sources are still lacking (Dahlgren *et al.,* 2006; Heithaus, 2007; Huepel *et al.,* 2007; Vélez-Marín et al, 2009). One shared aggregation area for YOY and juvenile white and shortfin mako sharks is Sebastian Vizcaino Bay (SVB) in northern Baja California Sur, Mexico, which is a confirmed nursery area for white sharks and a probable aggregation site for mako sharks, based on capture data from artisanal fishery and telemetry studies (Weng *et al.,* 2007; Cartamil *et al.,* 2011; Santana-Morales *et al.,* 2012; Medina-Trujillo 2013; Oñate-González *et al.,* 2017; Conventional Tagging program of INAPESCA, unpubl. data).

In Mexican waters the young size classes of both species are captured in the artisanal fisheries, the mako as target species and the white shark as bycatch

(Santana-Morales *et al.,* 2012; Ramírez-Amaro *et al.,* 2013). The biological characteristics of lamnoid sharks, such as long-life span and low fecundity rates (Castro, 1993; Smith *et al.,* 1998; Compagno, 2002) have as consequence that juvenile stages assume an ecological relevance in the population growth (Castro, 1993; Simpfendorfer & Heupel, 2004), implicating the importance of focus research efforts on juvenile classes.

The habitat preferences of young and juvenile sharks are mainly leaded by high food availability and protection from predators (Krausman, 1999; Heithaus, 2007). So, to know the trophic ecology of shortfin mako and white shark juvenile stages is critical to better understand their habitat preference, which has implications for the optimal conservation strategies (Bethea *et al.,* 2009; Kinney *et al.,* 2011).

Stable isotope analyses (SIA) have been used to study foraging habits and habitat use of marine vertebrates (Estrada *et al.,* 2003; Shiffman *et al.,* 2012). This approach is based on the stable isotopic ratio of nitrogen (15N/14N, expressed as δ15N) and carbon of preys and sources (13C/12C, expressed as $\delta^{13}C$) are reflected in predictable manners in consumer tissues. Values of δ13C in the consumers indicate the original source of carbon at the base of the food web, which allows to make inferences regarding habitat use (Kelly 2000; Graham *et al.,* 2010). Values of $\delta^{15}N$ increase in each trophic level and they are influenced by the routing of dietary proteins and tissues synthesis (Fuller *et al.,* 2005; Robbins *et al.,* 2005). The isotopic values of a predator's tissues show an integration of their prey's isotopic composition within a specific time frame, however elements might not be assimilated at the same rate because their incorporation is influenced by difference in metabolic rates among tissues (Bearhop *et al.,* 2002; Martinez del Rio *et al.,* 2009). In fact, the isotopic signature of a specific tissue represents and depends by integrated signatures of various biochemical fractions (protein, lipid, and carbohydrate) (Bearhop *et al.,* 2002; Martinez del Rio *et al.,* 2009; Wolf *et al.,* 2009), which are influenced by anabolic, catabolic and assimilation reactions specific for each tissue types (Martinez del Rio *et al.,* 2009; Wolf *et al.,* 2009; Kim *et al.,* 2012). Consequently, both stable isotopes are influenced by several factors including tissue biochemistry, tissue type (active or inert), body growth rates, fat percentage and dietary protein contents (Bearhop *et al.,* 2002;

Robbins *et al.,* 2005; Kurle 2009; Vanderklift & Ponsard 2003; Fuller *et al.,* 2005; Martínez del Rio *et al.,* 2009; Olson *et al.,* 2010). The final stable isotopic composition measured in predator tissues is the resulting of the combined effects of metabolism (the turnover of existing tissue), growth (the addition of new tissue), isotopic routing (mixing of diet components and their canalization to individual tissues), tissue protein composition, however feeding resources and their composition are primary determinant of $\delta^{13}C$ and $\delta^{15}N$ values (MacNeil *et al.,* 2005). The perform of this analysis in multiple tissues with different turn-over rate and biochemistry, and consequently differences in integration of dietary sources provides information about foraging habits during different time scales and allow to infer if isotopic variations are more related to dietary sources used or to the tissue biochemistry features (Vander Zanden *et al.,* 2015).

Active metabolic tissues with high rates of biochemical turnover, such as whole blood and plasma, provide dietary information assimilated from recent feeding, while tissues with lower turnover rates, such as red blood cells (RBCs), provide feeding information with more time, from several months to almost one year (Hobson, 1996; Rosenblatt *et al.*, 2013).

The use of blood components in the stable isotope analysis requires centrifugation after collection, which can be a logistic challenge working in remote areas. So, to prevent coagulation and better perform centrifugation some blood preservatives are used, but their effects on stable isotope values are still poor investigated and most of the researches have focused on the effect of preservative on blood of birds and mammals (Hobson & Clark*,* 1993; Bugoni *et al.,* 2008; Lemons *et al.,* 2012). So, establishing the efficacy preservation methods for SIA is a research priority.

The objectives are: 1) to test the possible differences between two preservation methods, which use distinct anticoagulant coating, 2) to investigate if the isotopic results reflected by predators are related to tissues' biochemical structure, and 3) to investigate the resource sharing among age classes of shortfin mako and white sharks in different time scales, using different blood components with different turn-over rates (whole blood, RBCs and plasma).

3.3. MATERIAL AND METHODS

3.3.1. Study area and sample collection

The shortfin mako sharks and the white sharks were captured during August-November of 2015 and 2016 in SVB (28° 14' 52" N 114° 04' 09.7" W to 27° 41’ 30” N and 114° 53’ 00’’ W), a semicircular bight 100 km wide by 200 km long. There is a, shallow continental shelf that is 20 km wide, mean depth of 25-30 m, and a connection with the Pacific Ocean (Amador-Buenrostro *et al.,* 1995; Hernández-Rivas *et al.,* 2000). The SVB is characterized by a high primary productivity and an anticyclonic gyre (Palacios-Hernández *et al.,* 1996; Hernández-Rivas *et al.,* 2000) in the bay center, which causes water stratification and nutrient recycling processes. Also, the bay has a large phytoplankton community, formed by cyanobacteria (Almazán-Becerril *et al.,* 2012).

The individuals were opportunistically sampled, because they were caught by longline and gillnet artisanal fisheries, which took place in the center area of SVB and close to Isla Cedros (28° 10′ 58″ N 115° 13′ 04″ W; Fig. 7). Sharks were landed onshore at the fishing camps of Laguna Manuela (28° 14' 52" N 114° 04' 09.7" W) and Bahía Tortugas (27° 41′ 30″ N and 114° 53′ 00” W), where they were sampled. For each specimen, the biological data were recorded: total length (TL), fork length (FL), pre-caudal length (PCL), sex, maturity stages, site of capture, and fishery methods (longline or gillnets).

For data analysis, we used the total length (TL) reported for the birth and maturity sizes to discriminate between the young of the year (YOY) and juvenile age classes for both species. We also used the model of von Bertalanffy (Ribot-Carballal *et al.,* 2005) to discriminate between age classes, particularly for shortfin mako sharks because there are no reported TL for YOY for this species. Shortfin mako sharks have a birth length of 70-74 cm (Mollet *et al.,* 2000; Joung *et al.,* 2005) and maturity is reached at TL 180-210 cm for males and 256-278 cm for females (Cailliet *et al.,* 1983; Joung *et al.,* 2005; Semba *et al.,* 2011). The species-specific model of von Bertalanffy (Ribot-Carballal *et al.,* 2005) allows to determine YOY (< 102 cm TL) and juvenile (>102 cm TL) stages.

The white sharks have a birth length of 120-150 cm and previous studies classified individuals < 175cm as YOY (Bruce & Bradford, 2012). The minimum TL for mature white sharks is 350 cm for males and 480 cm for females (Francis, 1996; Uchida *et al.,* 1996; Bruce & Bradford, 2012).

Methodological tests- In the present study, we test the effects of two commonly blood preservatives (K2-EDTA and lithium heparin) on stable carbon (δ13C) and stable nitrogen (δ15N) values in whole blood (WHB), RBCs, and plasma of different shark species (*Carcharhinus galapagensis*, *Sphyrna zygaena* and *Isurus oxyrinchus*) collected in the fishery camp of Punta Lobos, close to La Paz, Baja California Sur (Fig.8). To collected blood samples, we used a sterilized syringe of 10 ml (NIPRO Medical Corporation) with sterilized caterer needles (Ambiderm S.A. de C.V.) and blood samples were stored in 4ml BD vacutainer with ~7.2mg of lithium heparin coating (n=22) (Becton Dickinson and Company © 2018 BD) and with ~7.2mg of K2-EDTA coating (n=17).

The blood samples were split into red blood cells and plasma using the centrifugation process with a portable centrifuge (Porta-Spin Portable Fixed Speed Centrifuge Unico C826H) between the 3-5 h after the samples collection. The red blood cells (RBC) obtained were stored in the same vacutainer, meanwhile the plasma was transferred in an eppendorf tube, both were stored at -20°C in the field and laboratory for the successively transport to the laboratory in La Paz, Mexico. During the return trip to the laboratory samples remained frozen in coolers with ice during the 15 h.

Shortfin mako and white sharks blood collection-We used a procedure similar to the one mentioned above. In this case, we collected blood via two vacutainers for each shark, following the same step described above and using the same instruments. One vacutainer was centrifuged to split the blood into red blood cells and plasma and the other one was stored frozen to use the whole blood. The centrifugation was developed following the same procedures and using the same centrifuge mentioned before. However, just the samples collected during the 2016 were centrifuged, due to the possibility to use the centrifuge in the fishery camps. The red blood cells (RBC) and the plasma were stored in the same way described before for the methodological test, meanwhile the second blood vacutainer collected was conserved without

centrifugation to analyze the whole blood and was stored using the vacutainer. Both vacutainers were stored at -20°C in the field and laboratory, but they were transported frozen in coolers with ice during the 15 h return trip to the laboratory in La Paz, Mexico.

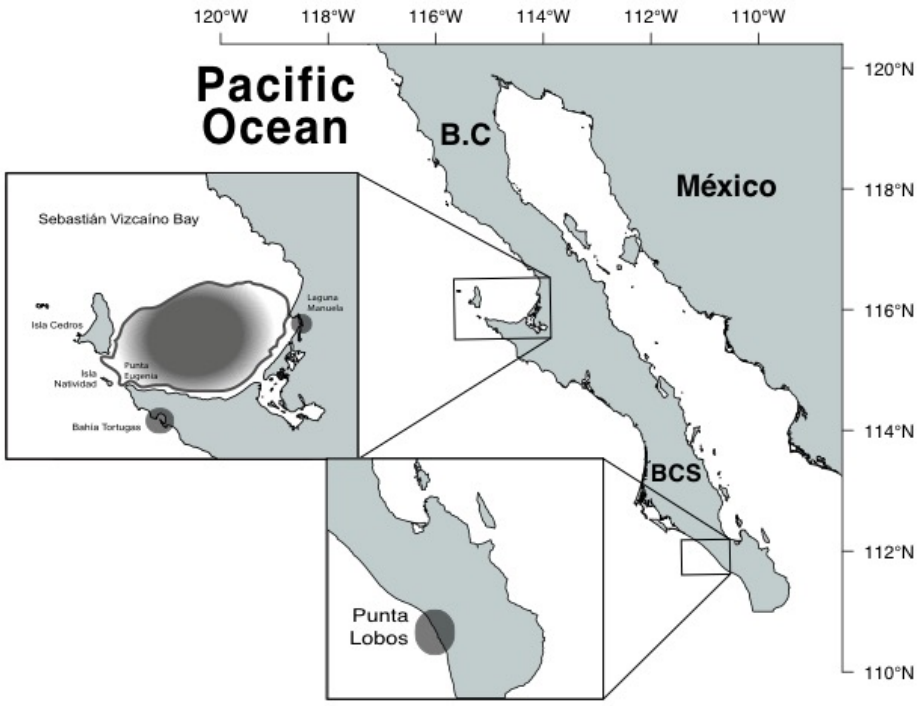

Figure 7. Map of the studies area in Baja California Sur, Mexico. The small dark shaded circle areas are the sharks landing locations in SVB and Punta Lobos, where we collected samples. The black contoured region in SVB s the mainly fishery area inside the bay.

3.3.2 Sample preparation

All the whole blood samples, RBCs and plasma were prepared for isotopic analysis at CICIMAR. First, samples were freeze-dried (LABCONCO) for 48 h, then a subsample (~5mg) was homogenized in an agate mortar and pestle to a fine powder. Approximately 0.5 mg of muscle powder was weighed with an analytical microbalance (precision of 0.001 mg) into an 8 × 5 mm tin capsule. Samples were analyzed at the CICIMAR-IPN Laboratory of Mass Spectrometry (LEsMa) in La Paz, Baja California Sur, Mexico. Results are expressed in delta notation following Eqn. 1 in chapter 2, and all the samples were analyzed using the same instrumental precision for $\delta^{13}C$ and $\delta^{15}N$

and described previously in Chapter 2. All summarized stable isotope data are reported as mean ± 1 standard deviation in the results and discussion.

3.3.3. Preservation methods and effects on isotope values

For the stable isotope analysis on blood, there is no consensus on the best method for preservation methodology. Some studies suggest that different preservation methods could change stable isotope ratios; whereas other studies failed to detect differences between different preservation methodology (Bugoni *et al.,* 2008). The_Lithium heparin coating tubes are reported to not introduce error in isotopic analysis, because their results are isotopically like to one of blood collected in tubes without anti-coagulant (Kim *et al.,* 2012). On the other hand, the K2-EDTA coating was found to alter the isotopic results: red blood cells and plasma were ^{15}N depleted (Lemons *et al.,* 2012). In order to assess if different anti-coagulants can alter the isotopic results on sharks' blood components, we test the differences between blood samples stored with Lithium heparin coating (n=22) and samples with K2-EDTA coating (n=17). Both sets of blood samples were split into RBCs and plasma using a centrifugation process, to evaluate the anticoagulant effect in the different blood components (Lemons *et al.,* 2012). We evaluated the statistical differences between these two sets of sharks' blood component with a Wilcox rank sum test for nonparametric data for the $\delta^{13}C$ and $\delta^{15}N$ values because of their uneven distribution and because nonparametric are more efficient for small dataset (Nahm, 2016). In the rest of the blood dataset we used a Wilcox rank sum or Student t-test depending from the data distribution.

3.3.4. Isotopic incorporation rates in different blood components

We used an exponential growth model used for captive diet switching experiments (Tieszen *et al.,* 1983) and fit parameters with the nls function in R (R Development Core Team, 2008) previously described in Chapter 2 (Eqn. 1).

3.4. RESULTS

3.4.1. Methodological test results

We tested a set of blood components (RBCs and plasma) obtained from blood samples preserved with Lithium heparin vacutainer (n=22) and samples with K2-EDTA vacutainer (n=17). In the case of the RBCs with Lithium heparin coating the mean values were -16.4 ± 1.1‰ for $\delta^{13}C$ and 16.9 ± 0.9‰ for $\delta^{15}N$ values, meanwhile the plasma had mean values of -16.2 ± 1.8‰ for $\delta^{13}C$ and 16.6 ± 1.3‰ for $\delta^{15}N$. The RBCs preserved with K2-EDTA coating showed mean values of -16.4 ± 1.1 ‰ for $\delta^{13}C$ and 17.02 ± 1.1‰ for $\delta^{15}N$ values, meanwhile the plasma had mean values of -16.4 ± 1.7‰ for $\delta^{13}C$ and 16.6 ± 1.3‰ for $\delta^{15}N$ (Table 3).

We did not found statistics differences for the RBCs results between both set ($\delta^{13}C$: Wilcoxon signed-rank test W = 36.5, p-value = 1; $\delta^{15}N$; Wilcoxon signed-rank test W = 34, p-value = 0.9). In the same way, there were not significant differences between the plasma samples preserved with Lithium heparin and the samples preserved with K2-EDTA for both isotopes ($\delta^{13}C$: Wilcoxon signed-rank test W = 51, p-value = 0.6; $\delta^{15}N$: Wilcoxon signed-rank test W = 48.5, p-value = 0.5). The mean for the C:N ratio was lower than 3.5, such for plasma such for RBCs in both data sets (Table 3). Because of the accepted C:N ratio for protein without lipid effects is < 3.5 and > 2.3 without urea effects (Kim *et al.,* 2012), we decided not to extract lipid and urea from all the samples collected.

Table 3. Isotopic values between blood components preserved with Lithium heparin and K2-EDTA.

		$\delta^{13}C$				$\delta^{15}N$				C:N			
		Min	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**
Lithium heparin set	RBC	-17.6	-15.1	-16.4	1.1	15.4	18.32	16.9	0.9	2.9	3.5	3.3	0.2
	Plasma	-18.3	-13.5	-16.2	1.8	13.5	17.8	16.6	1.3	1.6	3.3	2.1	0.3
K2-EDTA set	RBC	-18.2	-15.2	-16.4	1.1	15.0	18.5	17.0	1.1	2.97	3.8	3.3	0.3
	Plasma	-18.7	-14.6	-16.4	1.7	13.5	18.2	16.6	1.3	1.69	2.5	2.1	0.3

3.4.2. Biological sampling and stable isotope results

We collected 147 whole blood samples for the shortfin mako sharks, including 67 males (♂) and 80 females (♀). All these samples were collected and stored in vacutainers with K2-EDTA and included 49 YOY (63.5-100 cm TL) and 98 from juveniles (102-196 cm TL) stages based on the TL classification. For RBCs we obtained 62 samples (32 ♂ and 30 females ♀) including 44 YOY and 65 juveniles' shortfin mako. In the case of plasma, we collected 70 samples for the mako sharks (34 ♂ and 36 ♀), which included 15 YOY and 55 juveniles. The RBCs and plasma sampled did not exactly correspond with the number of whole blood collected because it was not always possible to centrifuge the samples.

We collected 6 samples of whole blood for white sharks (4 ♂ and 2 ♀), which came from 2 newborns (146-150 cm of TL), 3 YOY (175-180-186 cm of TL) and 1 juvenile (272 cm of TL). For RBCs we collected 2 samples (1 ♂ and 1♀), which were 1 newborn (150 cm of TL) and 1 YOY (180 cm of TL). In the same way, for plasma we count with 2 samples from 1 male and 1 female, including 1 newborn (150 cm of TL) and 1 YOY (180 cm of TL).

3.4.2.1. Stable isotope results of shortfin mako sharks

The isotopic values for the whole blood of shortfin mako sharks in all the data set ranged from -23. 2‰ to -16.2 ‰ (mean -17.4 ± 0.9‰) for $\delta^{13}C$ and from 11.7 ‰ to 19.6 ‰ for $\delta^{15}N$ (mean 16.5 ± 1.5 ‰). For RBCs the $\delta^{13}C$ values ranged from -19.5 ‰ to -16.5 ‰ (mean -17.5 ± 0.7 ‰) and for $\delta^{15}N$ from 13.5 ‰ to 18.7 ‰ (mean 16.5 ± 1.2

‰). The plasma showed isotopic values ranged from -19.9 ‰ to -16 ‰ (mean -17.6 ± 0.9 ‰) for $\delta^{13}C$ and from 14 ‰ to 20.7 ‰ (mean 17.2 ± 1.3‰) $\delta^{15}N$ (Table 4). The isotopic composition of shortfin mako sharks did not vary by sex in all the blood components of shortfin mako sharks (whole blood $\delta^{13}C$ values: Wilcoxon signed-rank test W = 2378, p-value = 0.24 and whole blood $\delta^{15}N$ values: Wilcoxon signed-rank test W = 545.5, p-value = 0.3. RBCs $\delta^{13}C$ values: Wilcoxon signed-rank test W = 545.5, p-value = 0.35 and RBCs $\delta^{15}N$ values: Student test t= 0.7, p-value = 0.5. Plasma $\delta^{13}C$ values: Wilcoxon signed-rank test W = 664.5, p-value = 0.5 for and Plasma $\delta^{15}N$ values: Student test t = 0.5, p-value = 0.6 for δ15N).

The $\delta^{15}N$ values increased with TL among embryo, YOY, and juvenile shortfin mako sharks in all the three blood components, however this increment is quite well described by a logarithmic regression just in case of the whole blood (Fig. 8; n = 147, $p < 0.05$, $r^2 = 0.5$), but not for RBC (n = 62, $p < 0.05$, $r^2 = 0.2$) and plasma (n = 70, $p < 0.05$, $r^2 = 0.3$). The $\delta^{13}C$ values increased with TL only between some embryos and juveniles shortfin mako sharks for the whole blood, however a logarithmic regression could not well describe this increase (Fig. 8; n = 147, $p < 0.05$, $r^2 = 0.06$).

The C:N ratio in whole blood of shortfin mako sharks ranged from 1.4 to 9.1 (mean 2.4 ± 0.7 ‰), in RBCs ranged from 2 to 3.7 (2.8 ± 0.2 ‰) and in plasma ranged from 1.3 to 2.8 (2 ± 0.4 ‰). For whole blood we found that the relationship between the C:N ratio and the $\delta^{13}C$values was described by a linear regression (n=147, p-value < 0.05, r2=0.4). For RBCs and plasma the relationship between the C:N ratio and the $\delta^{13}C$ values was described by a linear regression (RBCs: n=62 p-value < 0.05, r2=0.1; plasma: n=62 p-value=0.5, r2=-0.009)). However, both models cannot well interpreted the data because of wide range in C:N ratio values in all the blood components. The mean values for all the blood components are minors than the accepted value for protein without lipid effects (< 3.5) (Kim *et al.,* 2012). However, for plasma they are similar to the value accepted for the absence of urea effect (> 2.3) (Kim *et al.,* 2012) and for whole blood and RBCs the mean values are greater compared to the reference value for urea absence (> 2.3) (Kim *et al.,* 2012).

Table 4. Isotopic values for shortfin mako and white sharks by age classes and blood components.

	Size Classes	TL (cm)	$\delta^{13}C$								
			Whole Blood			RBCs			Plasma		
			Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Mako sharks	All	64.5-196	-23.2	-16.2	-17.4 ± 0.9	-19.5	-16.5	-17.5 ± 0.6	-19.9	-16	-17.6 ± 0.9
	YOY	<102	-23.2	-16.3	17.7 ± 1.3	-18.8	-16.5	-17.4 ± 0.7	-19.5	-16.2	-18.3 ± 0.9
	Juveniles	>102	-19.8	-16.2	-17.3 ± 0.6	-19.5	-16.5	-17.6 ± 0.6	-19.9	-16	-17.4 ± 0.8
White sharks	All	146-272	-18.3	-14.2	-16.2 ± 1.5	-16.3	-15.4	-15.8 ± 0.7	-16	-15.9	-16 ± 0
	Newborns	146-150	-18.3	-15.9	-17 ± 1.7	-16.3	-15.4	-15.8 ± 0.7	-16	-15.9	-16 ± 0
	YOY	175-186	-17.5	-15.2	-16.3 ± 1.2						
	Juveniles	272	-	-	-14.2 ± 0	-	-	-	-	-	-
	Size Classes	**TL (cm)**	$\delta^{15}N$								
			Whole Blood			RBCs			Plasma		
			Min	Max	Mean ± SD	Min	Max	Mean ± SD	Min	Max	Mean ± SD
Mako sharks	All	64.5-196	11.7	19.6	16.5 ± 1.5	13.5	18.7	16.5 ± 1.2	14	20.7	17.2 ± 1.3
	YOY	<102	11.7	18.2	14.9 ± 1.2	14.3	17.4	15.6 ± 1.1	14	19.1	15.9 ± 1.4
	Juveniles	>102	13.3	19.6	17.3 ± 1	13	18.7	16.8 ± 1	15.2	20.7	17.6 ± 1
White sharks	All	146-272	16.4	17.1	16.8 ± 0.3	16	17.1	16.5 ± 0.8	17.4	17.5	17.4 ± 0
	Newborns	146-150	16.4	16.5	16.4 ± 0	16	17.1	16.5 ± 0.8	17.4	17.5	17.4 ± 0
	YOY	175-186	16.6	17.1	16.9 ± 0.2						
	Juveniles	272	-	-	17.1 ± 0	-	-	-	-	-	-

The isotopic incorporation rate model developed for nitrogen values estimated the residence time from initial (i.e., newborn) to final (i.e., juvenile) diet as 1.3 years, for whole blood (~ 474.5 d; δ^hX_∞ = 17.4, SE = 0.1, t-value = 121.7, Pr(>|t|) = $<2e^{-16}$; δ^hX_∞-δ^hX_0 = 2.4, SE = 0.2, t-value = 11.7, Pr(>|t|) = $<2e^{-16}$; $1/\lambda$ = 1.3, SE = 0.5, t-value = 2.4, Pr(>|t|) = 0.01. Residual SE = 1, DF = 144). The same estimation for RBCs estimated the incorporation rates as 0.17 years (~ 62 d; δ^hX_∞ = 18.2, SE = 2.5, t-value = 7, Pr(>|t|) = $2.27e^{-09}$; δ^hX_∞-δ^hX_0 = 2.6, SE = 2.5, t-value = 1, Pr(>|t|) = 0.3; $1/\lambda$ = 0.17, SE = 0.3, t-value = 0.6, Pr(>|t|) = 0.5. Residual SE = 1, DF = 59). In the case of plasma the incorporation rate model calculated the incorporation rate as 0.02 years (~7.3 d; δ^hX_∞ = 37.2, SE = 198.7, t-value = 0.2, Pr(>|t|) = 0.8; δ^hX_∞-δ^hX_0 = 21.2, SE = 198.6, t-

value =0.1, Pr(>|t|) = 0.9; 1/λ = 0.02, SE = 0.2, t-value = 0.1, Pr(>|t|) = 0.9. Residual SE = 1.1, DF = 67).

We found differences in the isotopic results comparing the blood components of the shortfin mako sharks: whole blood diverged from plasma for both isotopes ($\delta^{13}C$ values: Wilcoxon signed-rank test W = 6174.5, p-value = 0.02 $\delta^{15}N$ values: Wilcoxon signed-rank test W = 3910, p-value = 0.004) and the RBCs diverged from plasma just for $\delta^{15}N$ values (Student test t = -3.0898, p-value = 0.002). On the other hand, isotopic composition of whole blood and RBCs did not show statistic differences ($\delta^{13}C$value: Wilcoxon signed-rank test W = 5292.5, p-value = 0.06 and $\delta^{15}N$ values: Wilcoxon signed-rank test W = 4635.5, p-value = 0.8).

We evaluated if these differences between blood components of shortfin mako sharks could be related with one size classes (YOYs and juveniles) more than other. We compared the isotopic values of YOY and juveniles mako sharks for whole blood and plasma, then the isotopic results among size classes for whole blood and RBCs, and finally the isotopic values of YOY and juveniles for RBCs and plasma. Comparing whole blood vs. plasma, and then whole blood vs RBCs of YOYs and juveniles shortfin mako sharks we found two opposite tendencies, both for $\delta^{13}C$ and $\delta^{15}N$ values: in the comparison of whole blood and plasma we found statistic differences in YOYs' stages ($\delta^{13}C$ values: Wilcoxon signed-rank test W = 544, p-value = 0.005 and $\delta^{15}N$ values: W = 223, p-value = 0.02), but confronting whole blood and RBCs we found differences in juveniles stages ($\delta^{13}C$ values Wilcoxon signed-rank W = 2948.5, p-value = 0.006 and $\delta^{15}N$ values Wilcoxon signed-rank W = 2855, p-value = 0.02). Finally, considering RBCs and plasma, we found differences in the $\delta^{15}N$ results of juvenile shortfin mako sharks ($\delta^{15}N$ values: Wilcoxon signed-rank W = 1773, p-value = 0.001), but not for carbon ($\delta^{13}C$ values: Wilcoxon signed-rank test W = 1410, p-value = 0.43). On the contrary carbon values of YOY classes showed differences, but nitrogen values did not ($\delta^{13}C$ values: Wilcoxon signed-rank test W = 44, p-value = 0.004; $\delta^{15}N$ values: Wilcoxon signed-rank W = 124, p-value = 0.6).

3.4.2.2. Stable isotope results of white sharks

The isotopic values for the whole blood of white sharks in all the data set ranged from -18. 3‰ to -14.2 ‰ (mean -16.2 ± 1.5 ‰) for $\delta^{13}C$ and from 16.4 ‰ to 17.1 ‰ (mean 16.8 ± 0.3 ‰) for $\delta^{15}N$. For RBCs the $\delta^{13}C$ values ranged from -16.3 ‰ to -15.4 ‰ (mean -15.8 ± 0.7 ‰) and for $\delta^{15}N$ from 16 ‰ to 17.1 ‰ (mean 16.5 ± 0.8 ‰). The plasma showed isotopic values ranged from -16 ‰ to -15.9 ‰ for $\delta^{13}C$ (mean -16 ± 0‰) and from 17.4 ‰ to 17.5 ‰ $\delta^{15}N$ (mean 17.4 ± 0 ‰) (Table 4). For white sharks, we could not apply any statistic test to find differences between sex and size classes in the isotopic composition of blood components due to the low sample size. The $\delta^{15}N$ values increased with TL among newborns, YOY and juveniles of white sharks and this increase is quite well described by a logarithmic regression in case of the whole blood (Fig. 8; $n = 6$, $p < 0.05$, $r^2 = 0.5$), for which we have a higher number of samples compare with RBC and plasma, however it still limited. In the same way, the $\delta^{13}C$ values increased with TL among newborns, YOY and juveniles of white sharks for the whole blood, as described by a logarithmic regression (Fig. 8; $n = 147$, $p < 0.05$, $r^2 = 0.06$).

The C:N ratio in whole blood of white shark samples ranged from 1.8 to 2.7 (mean 2.2 ± 0.3 ‰), in RBCs ranged from 2.6 to 2.9 (2.8 ± 0.2 ‰) and in plasma ranged from 1.4 to 2.5 (2 ± 0.7 ‰). In this case, we can apply a linear regression only to the whole blood of white sharks, due to the low sample sizes of RBCs and plasma. However, this model ($n=6$, p-value > 0.05, $r^2= -0.07$) cannot well interpreted the data because the wide range in C:N ratio values.

The mean values for all the blood components are lower than the accepted value for protein without lipid effects (< 3.5) (Kim & Koch, 2012). However, for whole blood they are similar the value accepted for the absence of urea effect (> 2.3) (Kim & Koch (2012) and for plasma and RBCs they are greater compared to the reference value for urea absence (> 2.3) (Kim & Koch, 2012).

3.4.2.3. Shortfin mako vs white sharks

We did not found significant differences in $\delta^{13}C$ values of whole blood between shortfin mako and white sharks (Wilcoxon signed-rank test W = 228, p-value = 0.045)

and for δ15N values (Wilcoxon signed-rank test W = 420.5, p-value = 0.8). We cannot apply any statistic test to find differences between RBCs and plasma between shortfin mako and white sharks due to the low sample size of white sharks.

Shortfin mako and white sharks with similar TL showed a similar pattern of $\delta^{15}N$ values for whole blood, RBCs and plasma (Fig. 8B). Juvenile shortfin mako sharks (>102) showed similar nitrogen values to newborns (146-150 cm of TL) and YOY white sharks (175-186 cm of TL) in the three blood components (Table 4). In the case of whole blood the range of nitrogen values seem different between juvenile mako sharks and newborn and YOY white sharks, however this is due to an outlier value ($\delta^{15}N$ value=13.3 ‰) that we identified using the Grubb's test (Barnett and Lewis, 1994). Removing this value, the isotopic values for juvenile mako sharks (> 102 cm TL) ranged from -19.8 ‰ to -16.2 ‰ (mean= -17.3 ± 0.6 ‰) for carbon and from 14.5 ‰ to 19.6 ‰ (17.3 ± 0.9 ‰) for nitrogen, which are closer to those of white sharks, particularly to YOYs.

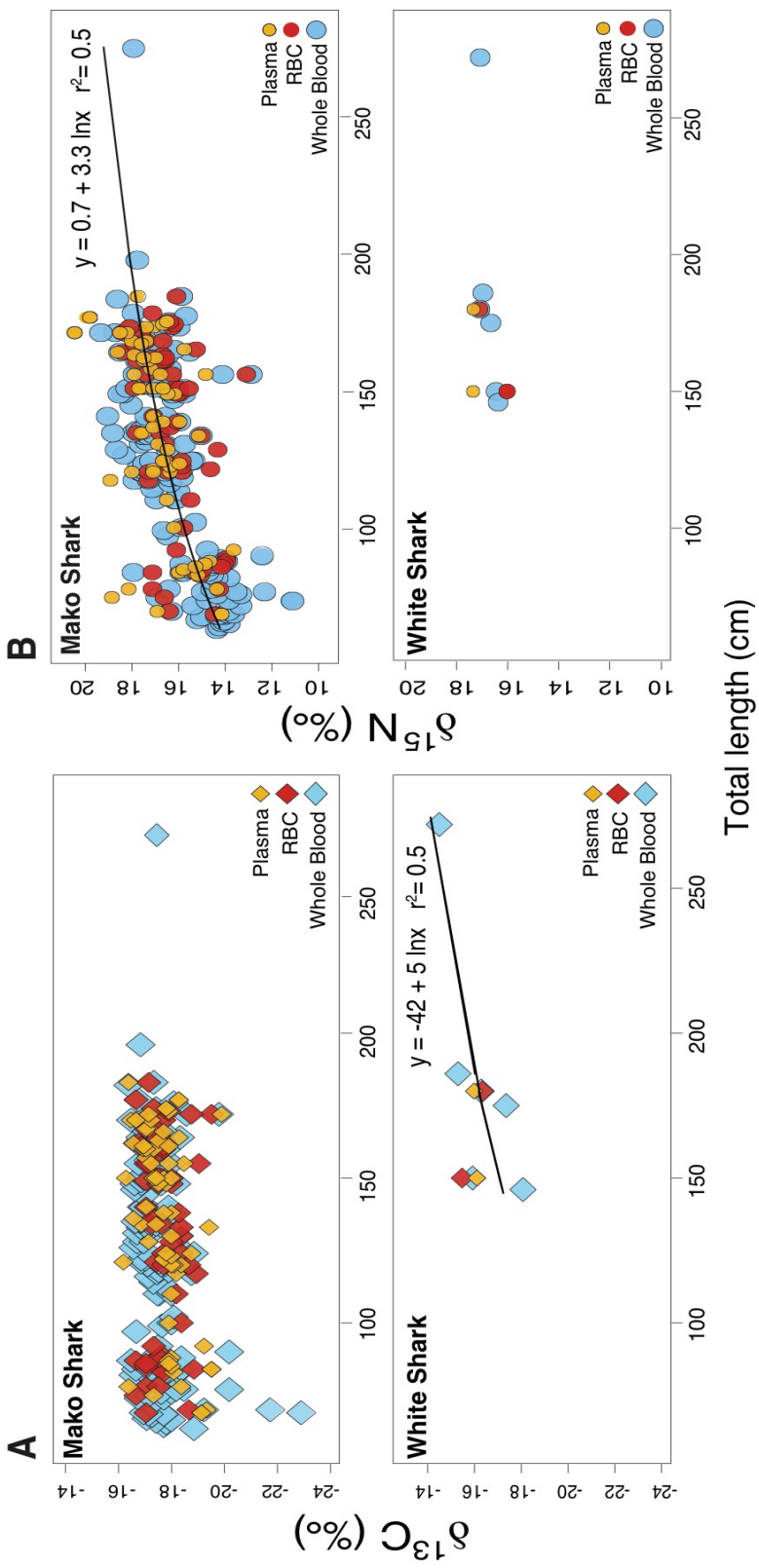

Figure 8. Isotopic values of whole blood, RBCs and plasma for both species related to their total length. (A) $\delta^{13}C$ values for shortfin mako and white sharks and **(B)** $\delta^{15}N$ values for shortfin mako and white sharks.

3.5. DISCUSSION

Our findings suggest that there are no differences in the isotopic results for shark blood between the two preservation methods tested (K2-EDTA vs lithium heparin coating). The differences in the isotopic composition among blood components (whole blood, RBCs and plasma) and size classes of shortfin mako sharks are more related to tissue biochemistry than an incorporation of different dietary sources. Our estimation of incorporation rate supports the hypothesis that the different biochemical features of blood components can influence the turn-over rates and consequently the isotopic composition reflected during different time frames. The consistent increase in shortfin mako $\delta^{15}N$ values from newborns to larger juveniles in the different tissues allow a first estimation of incorporation rate among various blood components (whole blood: ~ 474.5 days, RBCs: ~ 62 days, plasma: ~7.3 days). In addition, the similarity in the $\delta^{13}C$ and $\delta^{15}N$ values for all blood components between shortfin mako and white sharks with comparable body size, may indicate incorporation of similar resources in SVB and/or surrounding environments throughout time.

3.5.1. Preservation methods

The lack of information on preservative methods and standardized protocols to collect and store blood samples make difficult to establish right sampling procedures and complicates the comparisons across studies, because different anticoagulants can introduce errors in isotopic results. So, it is important to investigate which preservation methods can be used without an influence on the isotopic results. Different researches showed that depending on the anticoagulant used, the $\delta^{13}C$ and $\delta^{15}N$ values of blood components can be depleted or enriched (Bugoni *et al.*, 2008; Lemons *et al.*, 2012). Lemons *et al.,* (2012), demonstrated that $\delta^{15}N$ values of RBCs and plasma in sea turtles (*Chelonia mydas*) are ^{15}N-depleted when preserved with EDTA-K2, compared to the controls (Lemons *et al.*, 2012). The results obtained in our study for shortfin mako sharks and white sharks were in contrast with the ones of Lemons *et al.,* (2012) and showed that carbon and nitrogen values were very similar between blood preserved with Li heparin and K2-EDTA. Previous researches demonstrated that lithium heparin anticoagulant did not affect the isotopic signature of blood (Kim & Koch,

2012) and the lack of differences between our results with the Li heparin preservation methods and the K2-EDTA, suggest that neither K2-EDTA influenced the isotopic results of RBCs and plasma. So, the K2-EDTA represents an effective and reliable method to preserved blood for lamnoid, carcharhinidae and sphyrnidae sharks. Basing on these findings we decided to use the isotopic data obtained from all the other blood samples stored with EDTA-K2 coating. Our experiment represents the first demonstration of an alternative preservation method, compared to lithium heparin, for blood samples of different shark groups.

3.5.2. Isotopic differences among blood components are related with biochemistry characteristics

Previous results in different species of elasmobranchs and reptiles reported different turn-over rates of blood components. The whole blood was reported with a turn-over rates of 7 months in the case of $\delta^{15}N$ and 2–4 months in the case of $\delta^{13}C$ values in leopard sharks (*Triakis semifasciata*; Malpica-Cruz et al., 2012), about 265 days in *Potamotrygon motoro* for $\delta^{15}N$ (MacNeil et a., 2006), about 7 months for $\delta^{15}N$ in the whole blood of (*Carcharhinus plumbeus*) (Logan & Lutcavage, 2010).

The turn-over rates of RBCs were reported about 141.5 days for $\delta^{13}C$ and 277.3 for $\delta^{15}N$ in the american alligator (*Alligator mississippiensis*; Rosenblatt *et al.*, 2013) and for both isotopes in leopard sharks (*Triakis semifasciata*) the turn-over rates of RBCs were reported about 135-200 days (MacNeil *et al.*, 2006; Matich *et al.*, 2015) and about 300 days in the same specie (Kim *et al.,* 2012).

The turn-over rates of plasma was reported about 22 days for $\delta^{13}C$ and 33 days for $\delta^{15}N$ in the blacktip reef shark (*Carcharhinus melanopterus)*, the sicklefin lemon shark (*Negaprion acutidens)* and the bullsharks (*Carcharhinus leucas;* Matich *et al.*, 2015). Other authors reported the turn-over rates of plasma around 39 days for $\delta^{15}N$ in catsharks (*Scyliorhinus stellaris and Scyliorhinus canicula*; Caut *et al.*, 2013), 63 days for $\delta^{13}C$ and 62.4 for $\delta^{15}N$ in american alligator (*Alligator mississippiensis*; Rosenblatt *et al.,* 2013) and lastly around 100 days for both isotopes in leopard sharks (*Triakis semifasciata;* Kim *et al.,* 2012).

These changes in the turn-over rates are related to biochemistry characteristics of the different blood components, which influenced their renovation tissue rates and the incorporation rates isotopic signatures. We estimated faster incorporation rates of $\delta^{15}N$ values for plasma (~7.3 d) and RBCs (~ 62 d) compared to literature results (MacNeil *et al.*, 2006; Malpica-cruz, *et al.,* 2013; Rosenblatt *et al.,* 2013; Matich *et al.,* 2015). This difference is probably due to the relative higher growth, metabolic growth and physiology characteristics (i.g. endothermy) of juvenile shortfin makos, which generate faster incorporation of source isotopic signal in tissue (Reich *et al.,* 2008, Newsome *et al.*, 2009). Our estimation for isotopic incorporation rate indicate that whole blood has a longer turn-over compared to plasma, probably because this tissue is composed by different kind of components, with different turn-over rates: plasma is a short-lived tissue compared to red and white blood cells (Vander Zanden *et al.,* 2015). Furthermore, the recycling of some essential components (iron, hemoglobin, etc) during the renovation process, the difference in cell types and in their life spans determine that whole blood is really complex mixture of a variety of cells (Walsh *et al.,* 2004). As consequence the isotopic signature of whole blood is derived by the partial isotopic signatures incorporated by the different components during time. Consequently, our model could not discriminate between the different cellular components and considered the whole blood as a unique tissue with an isotopic signature resulting from different isotopic signatures of its cell components. The large turn-over rates obtained is probably the result of the sub-estimation of the partial turn-over rate of each blood component and their contributions to the isotopic signature of whole blood. All these results, particularly for whole blood, are preliminary and can only be confirmed by captive studies on large sharks.

We found tissue-specific differences comparing blood components of shortfin mako sharks with considerable variation in turn-over rates like whole blood and plasma, and RBCs and plasma. The comparison between whole blood and plasma showed higher $\delta^{15}N$ values for plasma in all the data set, which is coherent with other studies (Caut *et al.,* 2013). Previously researchers reported that plasma contains the free amino acid pool (Hughes *et al.*, 2017), with a high protein content (serum albumin like-proteins, fibrinogen and clotting-factor proteins) (Lehninger, 1982), and that its

metabolic activity influences its $\delta^{15}N$ composition more than variation in dietary nitrogen balance (Hughes *et al.,* 2017). The high metabolic activity of plasma and its consequently fast turnover rates generate a constant replacement of proteins, so its $\delta^{15}N$ composition depend from the isotopic signature of dietary protein content recently incorporated by different nitrogen sources (Hughes *et al.,* 2017). Our high $\delta^{15}N$ values in plasma, compared to whole blood, are probably due to an incorporation of ^{15}N-enriched proteins with diet, more than a dietary shift during time. In fact, mako sharks are known to spend several months after birth inside Sebastian Vizcaino (Unpublished data from the conventional sharks tagging program of the National Fisheries and Aquaculture Institute between 2010 and 2015) and feed in this ecosystem (Malpica-Cruz *et al.,* 2013; Tamburin *et al.,* 2019). The isotopic composition of Sebastian Vizcaino baseline is ^{15}N-enriched by an intense Cyanobacterian activity, which produces ^{15}N-enriched residual nitrate that influences the region´s baseline and the protein features in the system. To test if plasma values are influenced by ^{15}N-enriched protein sources we compared the prey values of shortfin mako sharks and our $\delta^{15}N$ results obtained in plasma. The isotopic composition of fish (i.e., *Tylosurus sp*, *Prionotus spp*, *Coryphaena spp*, *Ophidion spp*, *Lophiodes spp*., *Synodus lucioceps*, *Scomber japonicas*; $\delta^{15}N$ = 16.2 ± 1.3‰ and $\delta^{13}C$= -18.2 ± 1.4‰) found in shortfin mako shark stomachs are close to the plasma values of shortfin mako sharks, suggesting a direct incorporation of this dietary sources in plasma, reflecting the protein amount incorporated without high fractionating process (Newsome *et al.,* 2009). Our results demonstrate that the high $\delta^{15}N$ levels in shortfin mako sharks' plasma are influenced by its biochemistry features, which determined a preferential incorporation of ^{15}N-enriched nitrogen sources used by consumers. In addition, the high values of $\delta^{15}N$ values in plasma of shortfin mako sharks, which are aligned with the prey sources of Sebastian Vizcaino Bay confirm that juveniles mako sharks feed in this system during long-time scales, which is reflected in a tissue representing the last dietary time frames before their capture.

Comparing whole blood, RBCs and plasma the mean $\delta^{13}C$ values are lower in plasma, however the total range of the whole blood have minimum values more negatives. These low $\delta^{13}C$ values in plasma of shortfin mako sharks are explained by

the high lipid content in this tissue, which depends by plasma biochemical characteristics. In fact, sharks' plasma contains the serum albumin-like proteins (Andreeva *et al.,* 2010) which are the principal carriers of fatty acids in vertebrate blood, suggesting that the total blood lipid amounts are concentrated in the plasma portion than RBCs (Lehninger 1982), because they bound these albumin-like proteins in plasma. Lipid are ^{12}C-enriched and decrease $\delta^{13}C$ values, which explained our lower mean $\delta^{13}C$ result in plasma, compared to whole blood and RBCs, for its biochemical characteristics. Nevertheless, $\delta^{13}C$ values in whole blood showed a wider range with minim values lower than the plasma's values. These minimum values are associated with the YOYs shortfin mako sharks and are related to the biochemistry features of whole blood and the metabolism associated to this life stage. Whole blood is constituted mainly by RBCs and plasma, which mean that its isotopic composition is derivate by the isotopic signature of plasma and broken RBCs. However, RBCs have not high lipid contents, determining that the lipid amount in whole blood is mainly provided by plasma (Lehninger, 1982). Plasma have a high lipid content which decrease the carbon values, because ^{12}C-enriched and this influence in whole blood biochemistry can justify our low $\delta^{13}C$ levels in whole blood. During gestation, there is a direct maternal transference of lipid nutrients (Cherel *et al.,* 2005), causing an evident decrease in δ13C levels of early life-stages like YOYs of shortfin mako sharks. An analternative explanation for these low $\delta^{13}C$ levels in whole blood and particularly in plasma is reresent by a possible lipids effect and also a small urea effect. Previously studies reported that elasmobranch urea could potentially affect $\delta^{13}C$ and that the expected $\delta^{13}C$ values could be lower than body proteins (Carlisle *et al.,* 2016). In fact, urea contains carbon and it is preferentially concentrated in deaminases and transaminases (Gannes *et al.,* 1998), which are proteins and consequently are more abundant in plasma. This will determine a high amount of urea in plasma, and consequently in whole blood compared to RBCS, determining a decrease in $\delta^{13}C$ values. However, our results of C:N ratio in all blood components of shortfin mako sharks are close or greater the accepeted values for the absence of lipid and urea, suggesting that the $\delta^{13}C$ results are more related to biochemical composition of blood components, than to lipid and urea effects. In facts, we did not extract lipids and urea

because more researches are needed to assess accurate methodologies to chemical extract lipid and urea. Kim *et al.,* (2012), reported that it could affect the blood biochemistry with biochemical modifications, like the remoting of free AAs in addition to urea, which are abundant in plasma and can consequently bias our conclusions.

Our results of shortfin mako sharks showed that the differences for both $\delta^{13}C$ and $\delta^{15}N$ values between size classes (YOYs vs juveniles) comparing blood components of shortfin mako sharks are effective related with one size classes more than other. In the case of whole blood vs. plasma were determined by YOYs' shortfin mako sharks. These differences were caused by lower carbon values and higher nitrogen values of YOYs' plasma, compared to YOYs' whole blood. Previously studies reported that in animals with high growth rates, such YOYs' shortfin mako sharks, there is a decrease in the fractionating process caused by a fast protein accretion to sustain intense grow rates. These processes provoke a direct incorporation of the food resources' isotopic composition and a consequently direct synthesis of muscle tissues from the dietary proteins (Newsome *et al.,* 2009). This lack of fractionating process explains the low $\delta^{13}C$ values and the high $\delta^{15}N$ values in plasma of YOYs, because they directly incorporated the isotopic composition of diet. In fact, the food resources and consequently their isotopic signature are direct transferred from mothers to embryos through yolk during the gestation (Cherel *et al.,* 2005). Yolk, uterine fluid and encased nutrient eggs used by embryos for nutrition during embryonic development, are mainly formed by lipids and proteins (Lutton *et al.,* 2005; Sato *et al.,* 2016), representing a source of ^{13}C-depleted nutrients, and amino acids. This explain the low $\delta^{13}C$ values in plasma of YOYs' shortfin mako sharks as a consequence of the incorporation and reflection in their plasma of ^{13}C-depleted dietary sources (Cherel *et al.,* 2005). In fact, the ^{13}C-depleted values are more evident in YOYs than in juveniles shortfin mako sharks, because these life stages used yolk uterine fluid and encased nutrient eggs as nutrition sources during gestation, meanwhile juvenile shortfin mako sharks shifted their diet feed from yolk to exogenous food resources of Sebastian Vizcaino bay. The $\delta^{15}N$ values in plasma of YOYs' shortfin mako sharks were similar to nitrogen values found in muscle of other large individuals sampling in the Pacific coast ($\delta^{13}C$ −16.3‰ and $\delta^{15}N$ 17.1‰; Lyons *et al.,* 2015), including an adult pregnant

female of shortfin mako sampled near Isla Cedros ($\delta^{13}C$ = -17.6 ‰ and $\delta^{15}N$=15.3‰; Tamburin *et al.,* 2019) during this study. These large animals are likely foraging outside the bay in oligotrophic pelagic environment (Gilmore *et al.,* 2005), so the similarity between their isotopic composition in long-term tissues, such muscles, the isotopic composition of short-term tissues, such plasma, in embryos suggest the direct transference of nutrients and feeding resources during gestation without substantial fractionation between mothers and neonate shortfin makos.

In the case of RBCs vs. plasma, the differences were determined by juvenile shortfin mako sharks and they were just for $\delta^{15}N$ values, showing an inverse pattern with the anterior comparison between blood components. The faster turnover of plasma (~ 1-2 moths; Matich *et al.*, 2015; Caut *et al.,* 2013), compared to RBCs, and its biochemistry characteristics (high metabolic activity, constant replacement of proteins, representation of free amminoacidic pool, the reflection of the isotopic signature from the last food sources; Kurle, 2002; Hughes *et al.,* 2017) make of this tissue a perfect proxy for the isotopic signature of recent consumers' diet (Tieszen *et al.,* 1983). This time frame corresponds to the moths reported for juveniles shortfin mako sharks to frequent Sebastian Vizcaino bay and feed in local preys (Unpublished data from the conventional sharks tagging program of the National Fisheries and Aquaculture Institute between 2010 and 2015; Malpica-Cruz *et al.,* 2013; Tamburin *et al.,* 2019) and suggest that juveniles shortfin mako sharks feed on Sebastian Vizcaino trophic chain during the last months before be caught and incorporating its amino acids sources ^{15}N-enriched, which are reflected in the high $\delta^{15}N$ values of plasma. In fact, it is remarkable that shortfin makos analyzed in this study had higher $\delta^{15}N$ values, particularly for plasma (mean 17.6 ± 1 ‰) (Table 4) compared with muscle tissues of juvenile shortfin makos analyzed in the SCB in a previous study (16.4 ± 0.8‰; Madigan *et al.,* 2012a). This is likely due to the higher $\delta^{15}N$ values of preys of Sebastian Vizcaino bay and the incorporation of their isotopic signature in short-term tissue, like plasma.

3.5.3. Differences in nitrogen sources are reflected in similar size classes of shortfin shortfin mako and white sharks.

Our results evidenced that shortfin mako and white sharks with similar TL have same $\delta^{15}N$ levels in the three different blood components. In whole blood, the juvenile mako sharks (>102 cm TL) showed $\delta^{15}N$ values similar to YOY white sharks (175-180-186 cm TL), but higher compared to those of newborn white sharks (146-150 cm TL). In RBCs, the nitrogen levels between juveniles mako sharks, newborn (146-150 cm TL) and YOY (175-180-186 cm TL) white sharks are comparable. In the same way, $\delta^{15}N$ results of juveniles mako sharks (>102 cm TL) are similar to those of newborn (146-150 cm TL) and YOY white sharks (175-180-186 cm TL), comparing plasma.

Sharks of both species with similar total length are previously reported to share resources inside Sebastian Vizcaino bay, using similar preys (Tamburin *et al.,* 2019) and reflecting similar nitrogen values in muscle tissues. Our results showed that also for blood components the sharks with comparable size showed similar nitrogen values, indicating the use of similar nitrogen sources. In addition, the similarity of these results for blood components that, based on our results, were estimated to have long and fast incorporation rates (whole blood, RBCs and plasma respectively) suggest that species with similar TL are sharing local resources of Sebastian Vizcaino Bay during long-time scales, which is reflected in tissues representing dietary time scales going from more than a year and ending to few days before sharks' capture.

The nitrogen composition of consumers depends from the different amino acids proportions in dietary proteins (Hobson *et al.,* 2014; Hughes *et al.,* 2017). So, the similarity in our $\delta^{15}N$ values between sharks with similar TL of both species indicated the sharing of resources with similar amino acids composition. In fact, $\delta^{15}N$ values in whole blood are similar between juvenile mako sharks and YOY white sharks, because they are sharing nitrogen sources on the Sebastian Vizcaino trophic chain (Tamburin *et al.,* 2019), which is ^{15}N-enriched. On the other hand, the $\delta^{15}N$ results between juvenile mako and newborn white sharks are not similar. Newborn white sharks start recently to feed in Sebastian Vizcaino trophic chain, so long-rate tissue like whole blood do not reflect the ^{15}N-enriched isotopic composition of the bay; meanwhile juvenile mako sharks have spent more time in this area, incorporating Sebastian Vizcaino signature and reflecting it in blood. In addition, the whole blood of YOY reflect

the isotopic signature of the prey resources used by the adult female white sharks in offshore areas (e.g. Guadalupe Island) and direct transferred to the YOY white sharks during gestation. To support this hypothesis and further contextualize our results, we compare the isotopic composition of whole blood in YOY white sharks in with the principal prey items used by adult white sharks from offshore regions like Guadalupe Island (Tunas: $\delta^{13}C$=17.8 ± 0.1± ‰ and $\delta^{15}N$ = 15.3 ± 0.2 ‰) (Jaime *et al.,* unpub.), because this region is suggested as aggregation area for the adult females genetically related to the young sharks considered in this study. The similarity in their isotopic composition suggest that YOY white sharks reflected in their tissue composition, particularly for the long-term matrices such blood, the isotopic signature of the feeding sources used by mothers and offload during gestation.

The plasma $\delta^{15}N$ values of shortfin mako and white sharks with analogous body sizes are similar and our results are comparable to the ones reported by Malpica-Cruz *et al.,* (2013). These authors attributed the high nitrogen values found in plasma of both species to an incorporation of exogenous food and an ontogenetic shift. In fact, they mentioned that the isotopic composition of young sharks changes rapidly from the newborn stages, as a function of size, due to an increase in the trophic level of consumed preys, with a consequently increase in nitrogen values which can be explained as an ontogenetic change in diet. Nevertheless, ontogenetic shifts on bigger prey are well supported in large sharks as a consequence of improvements in hunting strategy (Klimley, 1985; LeBoeuf, 2004) or physiological capability (Gerritsen, 1984), but the individuals of both species considered in this study were smaller than those reported in the literature for ontogenetic dietary changes to larger prey (Tricas and McCosker, 1984; Klimley 1985; Le Boeuf, 2004; Velasco-Tarelo, 2005; Malpica-Cruz *et al.,* 2013). So, we suggest that high $\delta^{15}N$ values for shortfin mako and white sharks with similar TL, particularly in plasma, indicated an ontogenetic change in diet, but that this change is more related to a local foraging with ^{15}N-enriched exogenous sources Sebastian Vizcaino bay, than a foraging on larger preys.

Previously researches reported plasma as a tissue which faster turnover, compared to whole blood and RBCs, and with high metabolic activity which cause the retention of the free amminoacid pool incorporated from the protein food sources

(Kurle, 2002) and the of the isotopic signature from the last food used by consumers (Hughes *et al.,* 2017). In addition, its biochemistry characteristics, like the high metabolic activity, causes a constant replacement of its proteins and make the plasma a proxy for diet composition (Tieszen *et al.,* 1983) influencing its $\delta^{15}N$ composition more than variation in dietary protein content or balance in nitrogen compounds (e.g. urea and TMAO) (Hughes *et al.,* 2017). These biochemical processes increased the protein amminoacid amount retained in plasma (Schier *et al.,* 1996), which also explain our $\delta^{15}N$ results.

We found similar $\delta^{15}N$ values in RBCs of shortfin mako and white sharks with similar TL, but in minor scale compared with plasma. We expected higher nitrogen values in RBCs, because they are a less metabolically active tissue compared to plasma and exhibit a lower rate of protein replacement during normal physiological maintenance (Arneson & MacAvoy 2005; Kurle 2002). These biochemistry characteristics cause increase in nitrogen fractionating process during the catabolism, increasing the recycling of endogenous proteins and a consequently increment of ^{15}N-enriched amino acids retained and used in the body in tissues (Hobson & Clark, 1993). However, our results showed a different pattern: $\delta^{15}N$ values in RBCs are not particularly high and they are comparable to the nitrogen levels in whole blood, but lower compare to plasma results.

Previously researches estimated the turnover of RBCs from 135 to 300 days (MacNeil et al., 2006; Kim *et al.,* 2012; Malpica-Cruz et al., 2013; Rosenblatt *et al.,* 2013; Matich *et al.,* 2015). This larger turn-over rate of RBCs, compared to plasma, reflect the isotopic signature used and incorporated by young sharks during this timeframe produced variations in the isotopic signature reflected by juvenile mako sharks and newborn and YOY white sharks, based on the different sources' isotopic composition used and incorporate during this timeframe by these age classes.

Adult shortfin mako and white sharks generally developed transoceanic migration (Bonfil *et al.,* 2005) and they can incorporate the isotopic signature from food sources consumed in offshore areas. This isotopic signature is directly transferred to embryos during gestation and incorporated in early life stages, such it is previously reported for both specie (Tamburin *et al.,* 2019), and it can be incorporated in long

turnover rate tissues like RBCs. In this case, the isotopic signature reflected in RBCs of young shortfin mako and white sharks is a combination of the isotopic composition transferred by adult stages and coming from off shore areas with the ^{15}N-enriched signature of Sebastian Vizcaino Bay. This direct transference could explain our nitrogen results in RBCs and the lack of high the expected high nitrogen values in RBCs juveniles shortfin mako, despite they were feeding in a 15N-enriched trophic chain.

3.6. CONCLUSIONS

The use of blood in SIA studies is challenging for the sampling collection into the field and for the lack of information to interpret results. In our experiment, we assess that two different preservatives methods are equally effective to conserve blood samples without any influences in isotopic values. The differences found among tissues with different turnover rates in shortfin mako sharks, indicate that the tissue biochemistry has a key role in the incorporation of different isotopic signatures. Nevertheless, the similarity between the isotopic values of whole blood, RBCs and plasma between the shortfin mako and white sharks with similar TL, suggest a use of similar sources during different time periods, which is critical to better understand their ecology and generate more knowledge about young life stages of lamnoid sharks. These results obtained with SIA are important to understand that the use with long and fast incorporation estimated rates (whole blood, RBCs and plasma respectively) suggest that they are sharing local resources of Sebastian Vizcaino bay during long-time periods. Our results suggest that a multiple-tissue approach is important understand the habitat preferences and the residency of young sharks in nursery areas, like SVB, however more studies are needed, particularly for the influence of biochemical characteristics on isotopic incorporation for metabolic active tissues.

CHAPTER 4 – NEW NURSERY AREA FOR WHITE SHARKS (*Carcharodon carcharias*) IN THE EASTERN PACIFIC OCEAN

Note: The references style can be different from the format used in the other chapter of thesis, because the *Turkish Journal of FISHERIES and AQUATIC SCIENCES* required its specific style for the references in the text and for the reference's list.

Abstract

The white shark is a worldwide protected species and nevertheless coastal nursery grounds and juvenile aggregation areas have been reported, the actual information about birth or nursery areas for this species is still lacking. Then, is necessary to focus research effort to identify new aggregation site, especially for young stages. the During 2015 to 2017 we obtained data of 12 neonates and juveniles around Isla Cedros, in the western coast of Baja California, Mexico suggesting this island as important nursery area for white sharks. This information will help in management plans for conservation in aggregation habitats of young white sharks.

Keywords: Neonates, reproduction, habitat preferences, critical hot spot

4.2. INTRODUCTION

White Sharks (*Carcharodon carcharias*) are distributed globally in subtropical and temperate waters and positioned as apex predators. This species of shark is known to have a long-life span, low reproductive potential and fecundity rate (Compagno, 2002; Bruce, 2008) and are listed in the Appendix II of the 2004 Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and the International Union for Conservation of Nature (IUCN) (Fergusson, Compagno & Marks, 2009). Furthermore, in Mexican waters the white shark is protected by a permanent fishing ban prohibiting its capture (NOM-029-PESC-2006). Therefore, the early stages are ecologically important for understanding the species populations and generating adequate management plans for species conservation (Castro, 1993; Simpfendorfer & Heupel, 2004). Both juvenile and adult white sharks are caught as by-catch in commercial and sports fisheries over the world using gillnets, longlines and handlines. In the coastal waters of Baja California, white sharks are reported to be incidentally caught in the artisanal fisheries (Cartamil *et al.,* 2011; Santana-Morales *et al.,* 2012; Ramírez-Amaro *et al.,* 2013; Oñate-González *et al.,* 2017), which may be due to the intensity of fisheries activities in coastal areas that overlap nursery grounds (Dahlgren *et al.,* 2006; Heithaus, 2007).

The aim of this information is to assess the presence of young of the year (YOYs) and juvenile white sharks in Isla Cedros, using capture records from artisanal fisheries in the area.

Our study area of Isla Cedros in the western coast of Baja California have depths of up to 200 m and has been previously documented to support a strong upwelling of cool waters, which sustain a complex trophic chain (Hernández-Rivas, Jiménez-Rosenberg, Funes-Rodríguez & Saldierna-Martínez, 2000). These productive waters allow the development of a rich marine community, incorporating a range of elasmobranchs species (*Mustelus* spp., *Squatina californica*, *Triakis semifasciata*, *Pseudobatos productus*, *Zapterix exasperata*), targeted by artisanal fisheries (Des Lauriers, 2009).

4.3. MATERIALS AND METHODS

The white shark captures were recorded from local fishermen, when they fish around the Isla Cedros (28º 10′ 58″ N; 115º 13′ 04″ W, Fig. 9). Local fishermen were previously trained by members from the Fish Ecology Laboratory of Centro Interdisciplinario de Ciencias Marinas (CICIMAR) to identify different shark species. The training of fishermen consisted to teach them to use morphological features (teeth shape, body shape, skin color, fin position and fin shape), identification guides and photographic material to identify white sharks and distinguish them from other species. This training was necessary because the majority of artisanal fishermen in Baja California do not distinguish the white sharks from other species, like shortfin mako sharks (*Isurus oxyrinchus*) or porbeagle (*Lamna nasus*) especially in their juvenile stages. Furthermore, the artisanal fishery of Baja California commonly classify the elasmobranchs in: “tiburón” (TL>150 cm), “cazón” (TL<150 cm) and rays (Ramírez-Amaro *et al.,* 2013). We also trained them to record biological data such as total length (TL) and sex by the presence or absence of the reproductive organs (claspers) (Compagno 2002). Fishermen recorded capture date with GPS position and biological data for each shark capture or observation. However, in some cases, they were not able to estimate the TL, so an estimation of the total weight (TW) from captured sharks was registered. Then TL was obtained by using the converter length-weight relationship of NEFSC Apex Predators Program from the National Oceanic and Atmospheric Administration (NOAA) web site. (https://www.nefsc.noaa.gov/nefsc/Narragansett/sharks/calc.html), allowing the conversion of shark weight to fork length (FL) and TL (Kohler, Casey, & Turner, 1996).

The different age classes, such as neonates, YOYs and juveniles, were determined based on birth and maturity sizes reported in literature (Francis, 1996; Uchida, Toda, Teshima, & Yano, 1996; Bruce & Bradford 2012). The reported range for birth length for white sharks is from 120 to 150 cm TL, and previous studies classified individuals < 175cm as YOY (Bruce & Bradford 2012). The TL for mature white sharks is 350 cm for males and 480 cm for females (Francis 1996; Uchida *et al.,* 1996; Bruce & Bradford 2012). We estimated white shark´s age using the inverse of

the von Bertalanffy model, with species-specific parameters (Cailliet *et al.*, 1985), to classify white sharks as newborns, YOYs, juveniles and adults, based on their TL.

4.4. RESULTS

During 2015 to 2017 we obtained twelve white shark´s records (Table 5). In most cases, sharks were captured in gillnets and they were still alive, so fishermen were able to release them after data collection. Captured white sharks: three females, three males and six undetermined individuals (Table 5) ranged in size from 110 to 500 cm TL. Based on the birth and maturity sizes, the sharks were classified as: 6 neonates, 3 YOYS, 2 juveniles and 1 adult. All captured individuals were reported during March to November, with the peak of captures during the summer months.

Table 5. White sharks recorded, including their biological data and capture dates.

Symbol*	WT (kg)	LT (cm)	Sex	Age Class	Date	Kind of Record
+	400	330	?	Juveniles	3/5/15	captured
◆	18	139	?	Neonate	8/21/15	captured
▼	28	110	F	Neonate	4/19/16	captured
●	60	190	F	Young of the Year (YOY)	5/17/16	captured
●	-	130	F	Neonate	Middle of June 2016	captured
⊠	-	300	M	Juveniles	Middle of June 2016	captured
▲	-	150	M	Neonate	10/19/16	captured
×	-	150	M	Neonate	10/11/16	captured
✳	-	180	?	Young of the Year (YOY)	First week of July 2017	captured
△	-	500	?	Adult	7/29/18	observed by free diving
◇	-	180	?	Young of the Year (YOY)	8/1/17	captured
○	-	140	?	Neonate	7/22/17	captured

* The different symbols correspond to each shark recorded and location, which are shown in Figure 1.

4.5. DISCUSSION

All age classes of white sharks are known to occur in the western coast of Baja California, particularly around Sebastian Vizcaino Bay, with the highest frequency of neonates and YOY white sharks recorded during the summer months (Weng *et al.,* 2007; Santana-Morales *et al.,* 2012; Weng *et al.,* 2012; Oñate-González *et al.,* 2017) because this season is possibly related to parturition and births of this species (Francis, 1996; Uchida *et al.,* 1996).

High rates of by-catch, mainly in juvenile white sharks are common in Laguna Ojo de Liebre, which is considered the core area of this nursery region (Santana-Morales *et al.,* 2012). In fact, Sebastian Vizcaino bay was previously reported as nursery area for this species by Oñate-González *et al.,* (2017), basing on white shark incidental catch records. Santana-Morales *et al.,* (2012) reported the highest records of young white sharks in Laguna Ojo de Liebre, describing 111 white sharks over 11 years, with 80% of the YOY captured in this core of the nursery area. Meanwhile Oñate-González *et al.,* (2017) reported a total of 390 white shark incidentals catch records along the western coast of Baja California, mentioning that the highest incidental catch rates of newborn and YOY sharks were inside the Sebastian Vizcaino bay, without related them to a specific area.

Nevertheless, Sebastian Vizcaino was defined a nursery area for white sharks, no records or data about juvenile and YOY white sharks have been reported for the outer region of Sebastian Vizcaino bay and Isla Cedros till the date (Fig. 9). Our capture records are mainly close to Isla Cedros, which would be a parturition area for white shark, as previously hypothesized from Domeier & Nasby-Lucas (2013). We were able to collect data from such remote area, compared with Ojo de Liebre lagoon, because we trained artisanal fishermen to identify white sharks to specie-specific level, developing a teaching methodology based on their empirical experience. This kind of training and collection of biological data with the fishermen is a methodology to obtain data across large time periods, taking advantaged that artisanal fishermen spend a lot of time into the sea as previously documented (De la Parra-Venegas *et al.*, 2011).

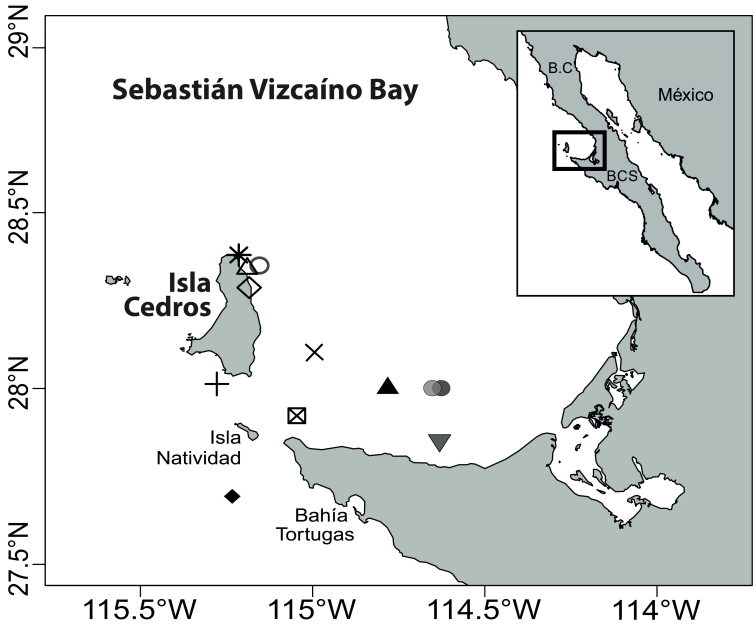

Figure 9. Map of the study area in Baja California Sur, Mexico to research the importance of Cedros Island, Mexico as pupping ground for young white sharks (*Carcharodon carcharias*). Symbols represent location of white shark records.

Our results showed that not only the YOYs are caught by fisheries, because also neonates are the most affected age category. The aggregation of juvenile and YOY white sharks around Isla Cedros provides an indication of the ecological importance of this region for WS conservation. In fact, they could use this area for birth purposes, once they reached the sexual maturity.

Until now the coastal water of Southern California Bight (USA), the Gulf of California and Sebastian Vizcaino bay are reported as nursery area (Lowe et al., 2014; Santana-Morales et al., 2012; Lyons et al., 2013; Ramirez-Amaro et al., 2013; Oñate-González et al., 2017, however there are not specific conservative measures ongoing on those regions, because of white shark is already protected in Mexico and US. The common strategy to manage a nursery area is to establish marine protected areas. Carefully identification of the nursery ground or critical biological hotspots for a species will improve our ability to manage and conserve nearshore habitats (Dahlgren *et al.,* 2006; Heupel, Carlson, & Simpfendorfer, 2007), which are also critical for supporting fisheries and provide human livehood. Our findings highlight that also the offshore and outer regions are important, because young white sharks can aggregate in shallow

coastal water around islands like Isla Cedros, which offer a protected site in open water. This research represents the first data of a new nursery area from the outer regions Sebastian Vizcaino bay, providing evidence that suggests that waters off Isla Cedros, could be an important habitat for young white sharks and their reproduction early stages. This kind of data can will provide more valuable information for shark biology, critical habitat and fisheries managers. In conclusion, we need to continue doing research of the frequency of juvenile and YOY white sharks in Isla Cedros to better understand the role of this region in the ecology of white sharks.

CHAPTER 5 – MULTI-TISSUE STABLE ISOTOPES ANALYSIS IDENTIFY COMMON HABITAT USE AND TROPHIC INTERACTIONS IN JUVENILES LAMNOID ACROSS DIFFERENT TIMES FRAMES

5.1. INTRODUCTION

Stable isotope analysis (SIA) has become a practical tool for evaluating trophic relationships in aquatic systems and the multiple tissues approach has become a popular method to investigate feeding habits during time, to detect eventual ontogenetic shifts in diet, to quantify diet specialization and to infer habitat use through large time frames (Shiffman *et al.,* 2012; Bond *et al.,* 2016). The comparison of isotopic signatures between metabolically slow or "inert" tissues and metabolically faster tissues, with large differences in turnover rates allow to detect changes in predators' diet and resources used over time (Shiffman *et al.,* 2012). The dietary sources are not immediately incorporated into animal's tissues and this delay in time, which is related with the tissue-specific turn-over rates, provide information about temporal variability in diet by comparing the isotopic values of multiple tissues (Bearhop *et al.,* 2004). This isotopic routing in tissues can be influenced by age, growth rates, animals' metabolism and biochemical structure of dietary components (Martinez del Rio *et al.,* 2009; Hussey *et al.,* 2010a). Then, the use of multiple tissues with different turn-over rates in SIA provide more precise dietary information and a greater trophic resolution than the use of one tissue, allowing to monitor trophic dynamics in sharks, because the differences in metabolism and biochemistry among tissues can be used to more precisely characterize the trophic ecology of sharks (MacNeil *et al.,* 2005).

In juveniles sharks the access to abundant prey to feed, survive, and growth to reach an optimum size to be competitive with other depredators is one of the main factor driving their habitat selection (Heithaus, 2007), which determines the importance of understanding their trophic dynamic during times to better know their habitat use and ecology. In fact, assays of SIA with multiple tissue types (e.g. blood, muscle) can be used to track elasmobranchs' movement patterns on different time scales (i.e. short- vs long-term movements). The use of short-turnover tissues, like plasma, contrasted with longer-turnover tissues, like muscle or RBC, will identify the individuals not in

isotopic equilibrium with their current isotopic environment (e.g. new arrivals to that area, or influencing from previous isotopic signatures, like maternal foraging) (Shiffman *et al.,* 2012) or will identify the individuals aligned with their local preys or the local environmental isotopic signatures (i.e., local primary producer isotopic composition or organic matter) (Graham *et al.,* 2010). In practice, an animal's isotopic composition can be used as a natural "tag" to track their movements through isotopically distinct habitats or to confirm their residency in an area across time. This method is particularly effective for juvenile stages of large marine vertebrates, like sharks, which are sometimes complicated to sample and offer a valid alternative to the expensive electronic tagging technologies (Graham *et al.,* 2010).

Sharks are often considered key stone species and apex predators playing a critical role in marine ecosystems (Dulvy *et al.,* 2014), particularly for the predators and consumers dynamics and the top-down ecological controls (Baum *et al.,* 2009). Trophic relationships of sympatric elasmobranch species are characterized by high level of dietary overlap, but normally these studies do not consider the effect of ontogenetic change on trophic levels (Sommerville *et al.,* 2011; Navia *et al.,* 2017). Ontogenetic dietary changes can be the results of differences in the amount of prey consumption, changes in prey consumption or changes in the feeding habitat used through different maturity stages (Wetherbee & Cortés, 2004; Dale *et al.,* 2011). This variability determines changes in the trophic groups of the specie throughout life, and therefore perform different trophic roles at these maturity stages (e.g neonates, juveniles and adults; Navia *et al.,* 2017). So, the estimation of the TP and trophic relationships for the different maturity stages, particularly poor studied stages like juveniles, is important to better understand the relation between predators and consumers inside the ecosystems (Cortes, 1999) and the corrected measures of TP are critical for understanding food web interactions and trophic structure in marine ecosystem (Hussey *et al.,* 2014).

We compared SIA in short-turnover and longer-turnover tissues of juvenile shortfin mako and white sharks inhabit the same nursery area to quantifying diet specialization and habitat use through large time periods. We also estimated the TP for both species in the system, to provide more information about their trophic role

during this earlier life stage.

5.2. MATERIALS AND METHODS

5.2.1. Sample collection and sample preparation

The study area and the samples collection were developed in the same locations and following the procedures descripted in Chapter 2 for muscle tissues and in Chapter 3 for blood, RBC and plasma for both species.

The samples were prepared to obtain $\delta^{13}C$ values and $\delta^{15}N$ values following the methodologies described in Chapter 2 for muscle tissues, including the urea treatment, and in Chapter 3 for blood, RBC and plasma for both species

The zooplankton samples were collected using hauls at the surface for 10 to 15 minutes at 1.5-2 knots using a conventional plankton net (500 µm mesh, 60 cm mouth diameter and 2 m in length) was conducted seven times each. The zooplankton samples were stored in plastic bottles of one liter and maintained in ice in coolers during the transport to the laboratory at Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (CICIMAR-IPN, La Paz, BCS, Mexico) and stored frozen (−20 °C).

In the laboratory, zooplankton samples were filtered with 0.05 mm filter with a portion of the sample deposited in acid-washed plastic containers, and the remaining samples was storage at frozen (-20 °C). Zooplancton samples were freeze-dried (LABCONCO) for 48 hours, then a subsample (~5mg) was homogenized in an agate mortar and pestle to a fine powder. Approximately 0.5 mg of this powder was weighed with an analytical microbalance (precision of 0.001 mg) into an 8 × 5 mm tin capsule. Results are expressed in delta notation following Eqn. 1 in chapter 2.

5.2.2. Quantification of isotopic niche

We quantified isotopic niche for white and shortfin mako sharks using SIBER (Stable Isotope Bayesian Ellipses in R) in SIAR (Stable Isotope Analysis in R; Parnell *et al.,* 2008, Jackson *et al.,* 2011) with R (R Development Core Team, 2008). We quantified the isotopic niche for white and shortfin mako sharks for muscle and whole

blood, to compare results. In the case of shortfin mako the samples size allows the quantification of isotopic niche for whole blood, RBC and plasma (Fig. 11), but for the white sharks was not possible to develop this analysis due to the low sample size.
Trophic position (TP) of both species in the trophic chain of SVB were calculated for muscle, RBC and plasma considered in this study, to evaluate if different tissues reflecting different time frames can provide different information and to evaluate eventually shifts in the trophic level of both predators inside the ecosystem of Sebastian Vizcaino bay. We did not calculate the TL using whole blood because actually the discrimination factor for this tissue is unknown.

The TP of both species in all the tissues were determined relative to baseline $\delta^{15}N$ sampled inside the study area using the formula proposed by Post (2002) and the relative discrimination factors proposed by Kim *et al.,* (2012):

$$TP_{consumer} = \lambda + \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{Baseline})}{\Delta^{15}N}$$

Where $\delta^{15}N$consumer is the average $\delta^{15}N$ value of predators; $\delta^{15}N$Baseline is the $\delta^{15}N$ average value of the sampled zooplankton, λ is the TP of the baseline which is the sampled zooplankton, with an assumed TP=2, and $\Delta^{15}N$ is the discrimination factor tissues-specific. In the case of muscle use 3.7‰ as discrimination factors, for RBC we used 2.4‰ and 2.2‰ for plasma.

5.3. RESULTS

The biologic and isotopic results are reported in Chapter 2 for the muscle tissues and in Chapter 3 for the blood components and resumed in Table 6.

We did not found significant differences in $\delta^{13}C$ values of whole blood between shortfin mako and white sharks (Wilcoxon signed-rank test W = 228, p-value = 0.045), in fact their isotopic values are similar. In the same way, we did not find significant differences $\delta^{15}N$ values of whole blood between shortfin mako and white sharks (Wilcoxon signed-rank test W = 420.5, p-value = 0.8) and their isotopic values are similar among all blood components. We cannot apply any statistic test to find

differences between RBCs and plasma between shortfin mako and white sharks due to the low sample size of white sharks (Table 6).

Table 6. isotopic values for shortfin mako and white sharks among different tissues divided by age classes.

	Size Classes	TL(cm)	$\delta^{13}C$			
			Muscle	Whole Blood	RBCs	Plasma
			Mean±SD	Mean±SD	Mean±SD	Mean±SD
Mako sharks	All	64.5-196	-17.4 ± 0.5	-17.4 ± 0.9	-17.5 ± 0.6	-17.6 ± 0.9
	Embryos	< 70	-17.3 ± 0.5	-	-	-
	YOY	<102	-17.1 ± 0.6	17.7 ± 1.3	-17.4 ± 0.7	-18.3 ± 0.9
	Juveniles	>102	-17.5 ± 0.4	-17.3 ± 0.6	-17.6 ± 0.6	-17.4 ± 0.8
White sharks	All	146-272	-16.5 ± 0.7	-16.2 ± 1.5	-15.8 ± 0.7	-16 ± 0
	Newborns	146-150	-16.4 ± 0.5	-17 ± 1.7	-15.8 ± 0.7	-16 ± 0
	YOY	175-186	-16.8 ± 1.1	-16.3 ± 1.2	-	-
	Juveniles	272	-16.4 ± 0.4	-14.2 ± 0	-	-
			$\delta^{15}N$			
			Muscle	Whole Blood	RBCs	Plasma
			Mean±SD	Mean±SD	Mean±SD	Mean±SD
Mako sharks	All	64.5-196	19.1 ± 1.4	16.5 ± 1.5	16.5 ± 1.2	17.2 ± 1.3
	Embryos	< 70	16.8 ± 06	-	-	-
	YOY	<102	17.4 ± 1.2	14.9 ± 1.2	15.6 ± 1.1	15.9 ± 1.4
	Juveniles	>102	19.8 ± 0.8	17.3 ± 1	16.8 ± 1	17.6 ± 1
White sharks	All	146-272	18.6 ± 0.7	16.8 ± 0.3	16.5 ± 0.8	17.4 ± 0
	Newborns	146-150	18.5 ± 0.1	16.4 ± 0	16.5 ± 0.8	17.4 ± 0
	YOY	175-186	19.3 ± 1	16.9 ± 0.2	-	
	Juveniles	272	18.2 ± 1.4	17.1 ± 0	-	-

We compared the muscle tissues of white shark's vs the blood of shortfin mako for $\delta^{13}C$ values and we found statistic differences (Wilcoxon signed-rank test W = 1321, p-value < 0.05). In the same way we found differences for $\delta^{15}N$ values between muscle tissues white sharks and blood of shortfin mako (Wilcoxon signed-rank test W = 1482, p-value < 0.05)

Shortfin mako and white sharks with similar TL showed a similar pattern of $\delta^{15}N$ values for muscle, whole blood, RBCs and plasma (Fig. 10). It is notable that muscle and plasma of shortfin mako sharks showed a similar range of nitrogen values, however the mean values are higher in muscle tissues (plasma: 14 ‰ to 20.7 ‰, mean 17.2 ± 1.3‰; muscle: 14.3 to 21.3‰ mean 19.1 ± 1.4‰).

A remarkable aspect is that the $\delta^{13}C$ and $\delta^{15}N$ values of shortfin mako sharks and white sharks showed similar values and data distribution patterns among the four tissues *(*muscle, whole blood, RBCs and plasma) considered for the sharks with similar TL (Fig. 10). Particularly for muscle tissues and whole blood, for which we have a higher sample size compared to RBC and plasma, the increment of $\delta^{15}N$ values with TL among embryo, YOY, and juvenile are well described by logarithmic regression, as previously reported in Chapter 2 for muscle tissues (Fig. 5) and in Chapter 3 for whole blood (Fig. 8).

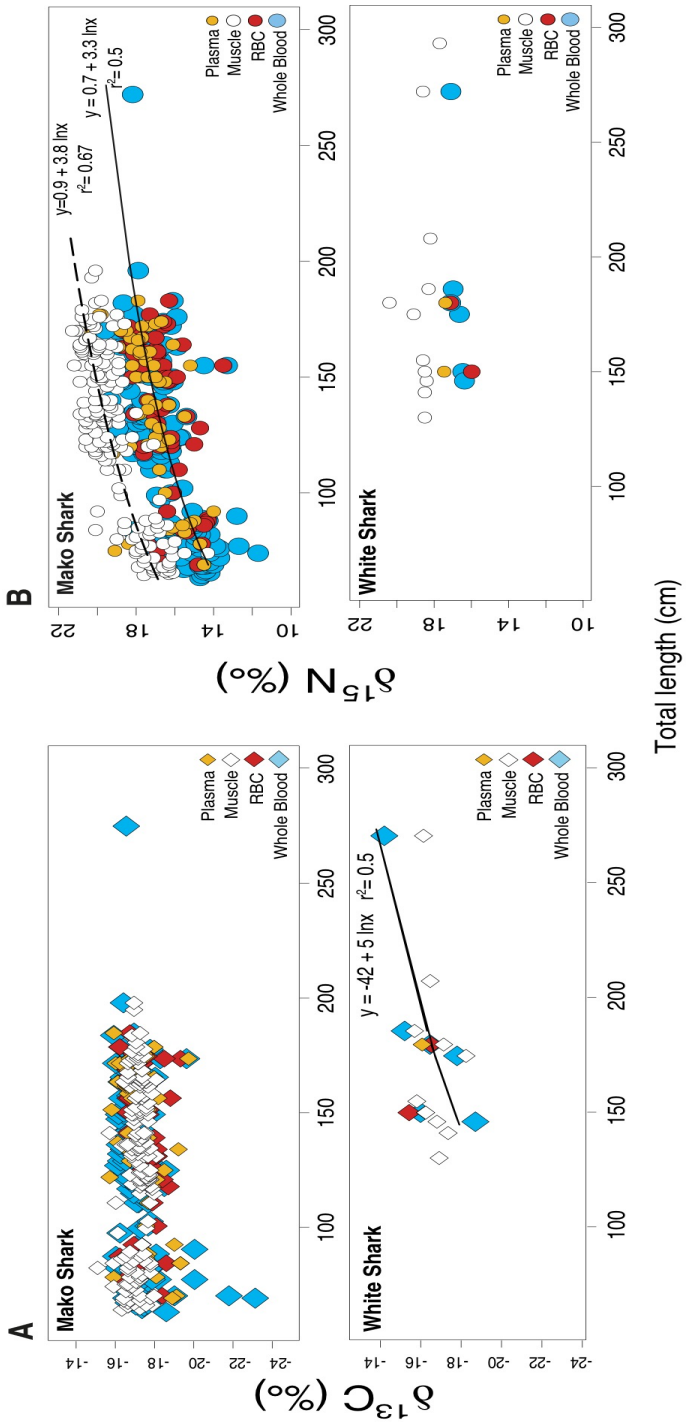

Figure 10. $\delta^{15}N$ values related to the total length for shortfin mako and white sharks among the four tissues (muscle, whole blood, RBCs and plasma) considered in this study. $\delta^{15}N$ values of muscle and whole blood increased with TL for shortfin mako and this relationship was best described by a logarithmic regression, with equation and r^2 values shown. For the white sharks the increase is low for $\delta^{15}N$ values of muscle and whole blood increased with TL. Meanwhile, the $\delta^{13}C$ values increase with TL for white sharks, well described logarithmic regression, with equation and r^2 values shown.

We quantified the similarity in shortfin mako and white shark stable isotope values between muscle and blood using the isotopic niche analysis in SIBER.

The isotopic niche of muscle tissue of shortfin mako sharks (blue ellipse in Fig. 11A; SEA_c = 2.1) and white sharks (red ellipse in Fig. 11A; SEA_c = 1.5) yielded partially overlapping ellipse areas with an estimated mathematical overlap of 0.2 and a Bayesian mean overlap of 0.3. The isotopic niche of blood of shortfin mako sharks (green ellipse in Fig. 11A and black in Fig. 11B; SEA_c = 4.2) and white sharks (black ellipse in Fig. 11A; SEA_c = 1.2) showed partial overlapping ellipse areas with an estimated mathematical overlap of 0.5 and a Bayesian mean overlap of 0.2 (Table 7).

If we consider the different tissues for each specie, like muscle tissue and whole blood of shortfin mako, we obtained the estimated mathematical overlap for the isotopic niche of 0.15 and the Bayesian mean overlap of 0.3. Considering muscle tissue and whole blood of white sharks the estimated mathematical overlap of 4.4×10^{-17} Bayesian mean overlap of 0.07.

The partial overlap decreased when we consider the combined tissues of both species: estimated mathematical overlap of muscle tissue of shortfin mako sharks and blood of white sharks is $3.3x10^{-18}$ and the Bayesian mean overlap is 0.1. The SIBER also calculated the reverse combination which is quite similar: estimated mathematical overlap of muscle tissue of white sharks with the blood of shortfin mako sharks is $6.3x10^{-17}$ and the Bayesian mean overlap is 0.2. (Table. 7).

Table 7. Isotopic niche (SIBER) values for muscle and blood tissues in shortfin mako and white sharks, and their respectively mathematical and Bayesian overlap values estimated.

	SEAc
Muscle Mako	**2.1**
Blood Mako	**4.2**
Muscle White	1.5
Blood White	1.2

	Mathematical Overlap	**Bayesian Overlap Mean**
Muscle White vs Muscle Mako	0.2	0.3
Muscle White vs Blood Mako	6.3×10^{-17}	0.2
Muscle White vs Blood White	4.4×10^{-17}	0.07
Muscle Mako vs Blood Mako	0.15	0.3
Muscle Mako vs Blood White	3.3×10^{-18}	0.1
Blood White vs Blood Mako	0.5	0.2

We were able to quantify the similarity in stable isotope values for among the four tissues of shortfin mako sharks, because for this species we have a large dataset. The isotopic niche of muscle tissue of shortfin mako sharks (red ellipse in Fig. 11; SEA_c = 2.1), whole blood (black ellipse in Fig. 11; SEA_c = 4.2), RBC (blue ellipse in Fig. 11; SEA_c = 2.5) and plasma (green ellipse in Fig. 11; SEA_c = 3.2) showed a notable overlap in the ellipse areas for whole blood vs. RBC with an estimated mathematical overlap of 2.4 and a Bayesian mean overlap of 0.5, for whole blood vs plasma of shortfin mako with an estimated mathematical overlap of 2.1 and a Bayesian mean overlap of 0.7, and for RBC vs plasma with an estimated mathematical overlap of 1.5 and a Bayesian mean overlap of 0.5 (Table 8).

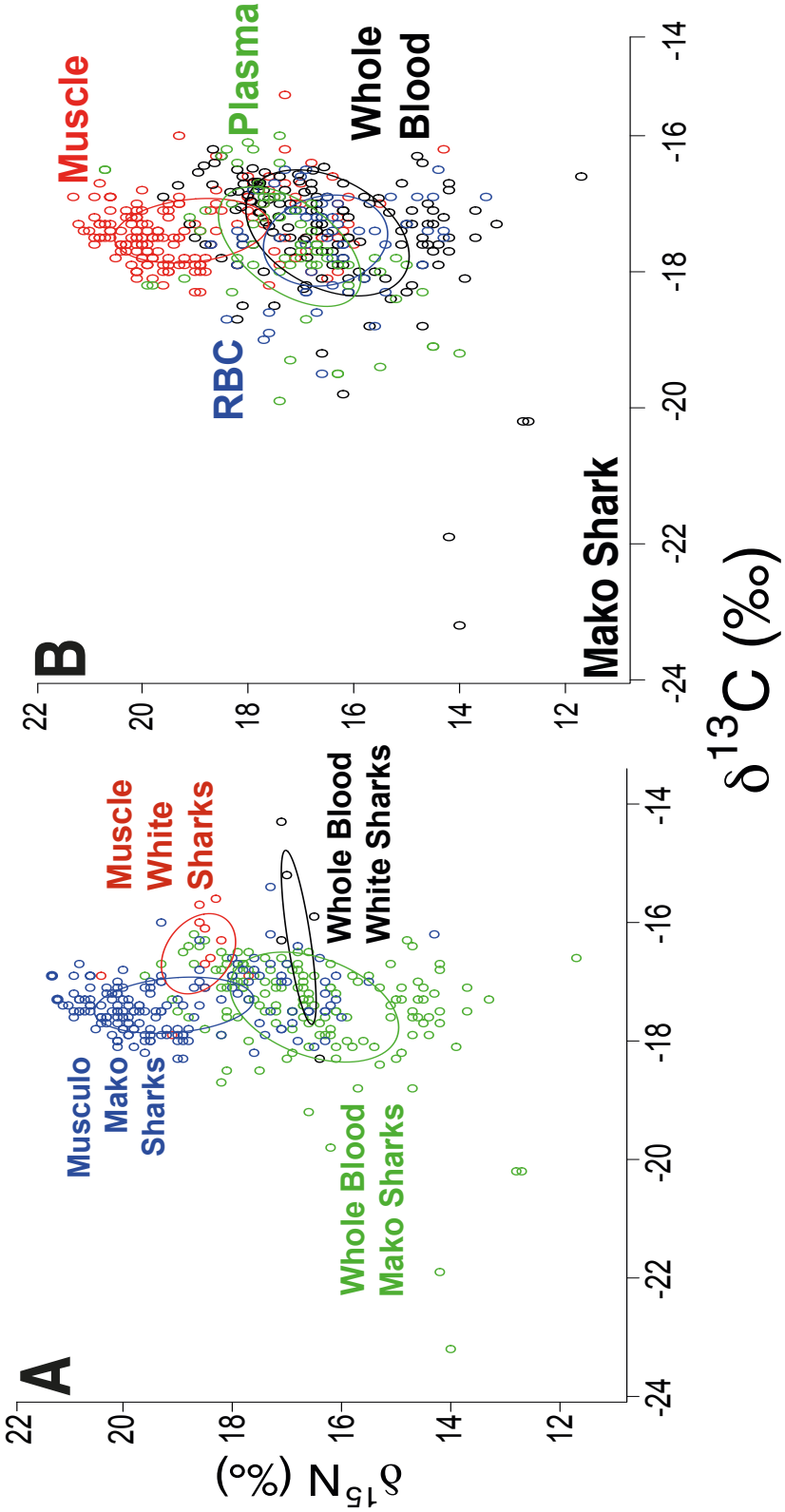

Figure 11. Isotopic niche (SIBER analysis) simulations. **(A)** isotopic niche (SIBER analysis) simulations for muscle tissues and whole blood tissues for shortfin mako sharks and white sharks. **(B)** isotopic niche (SIBER analysis) simulations for blood components for shortfin mako sharks.

All these overlaps are between the blood components of shortfin mako sharks, however we want to highlight that for muscle tissues vs. plasma of shortfin mako sharks showed a mathematical overlap of 0.5 and a Bayesian mean overlap of 0.4, which are higher than the mathematical overlap of 0.15 and the Bayesian mean overlap of 0.3 of muscle tissues vs whole blood of this specie.

Table 8. Isotopic niche (SIBER) values for blood components in shortfin mako, and their respectively mathematical and Bayesian overlap values estimated.

		SEAc
Muscle Mako		**2.1**
Blood Mako		**4.2**
RBC Mako		2.5
Plasma Mako		3.2
	Mathematical Overlap	**Bayesian Overlap Mean**
Muscle Mako vs Blood Mako	0.15	0.3
Blood Mako vs RBC Mako	2.4	0.5
RBC Mako vs Plasma Mako	1.5	0.5
Muscle Mako vs Plasma Mako	0.5	0.4
Muscle Mako vs RBC Mako	2.9×10^{-07}	0.4
Blood Mako vs Plasma Mako	2.1	0.7

The estimated trophic position using muscle tissues were TP=4.4 for shortfin mako shark and TP=4.2 for white sharks, using RBC we obtained TP=3.5 for shortfin mako shark and TP=4.2 for white sharks, and using plasma the values estimated were TP= 3.6 for shortfin mako shark and TP= 4.4 for white sharks.

5.4. DISCUSSION

Our finding in Chapter 2 and 3 suggest a similarity between $\delta^{13}C$ and $\delta^{15}N$ values in shortfin mako sharks and white sharks, with an increase of $\delta^{15}N$ values with sharks' size, indicating dietary shift from a maternal isotopic signal to ^{15}N-enriched baseline like the local isotopic signature of SVB. The differences in the isotopic composition among blood components (whole blood, RBCs and plasma) and size classes of shortfin mako sharks are more related to tissue biochemistry than an incorporation of different diet sources, in fact blood components also reflect the regional isotopic signature of the SVB.

This similarity in the $\delta^{13}C$ and $\delta^{15}N$ values among multiple tissues between shortfin mako and white sharks indicated the incorporation of similar resources in SVB and the sharing of feeding resources and habitat throughout time. In particular, our finding about muscle tissues reported in Chapter 2 suggest shared resource use in SVB and/or surrounding habitats in southern Baja California and isotopic changes throughout ontogeny, particularly for shortfin mako sharks, which is due likely due a change in the habitat use. The ontogenetic change in shortfin mako sharks $\delta^{15}N$ values reflects a dietary shift associated with migration from the southern California to inshore southern regions of Baja California, shifting from a maternal isotopic signal to local foraging inside SVB and the incorporation of its regional baseline. The results reported in Chapter 3 for all blood components between shortfin mako and white sharks, still indicated the use of similar resources in SVB and/or surrounding environs with incorporation in the tissues of the isotopic baseline signature of this region throughout "short" time frames

The similarity between the isotopic result of shortfin mako and white sharks with similar TL for tissues with different turn-over rate like muscle, whole blood, RBCs and plasma suggest a common habitat use throughout large period of times. In fact, our estimation of the turn-over rates highlight that the muscle and whole blood are "long-term" tissues, meanwhile the RBC and plasma reflected "shorter" time frames. The results of the residence time ($1/\lambda$) for some tissues are different from previous literature, like muscle (~255 days), and probably due to the faster metabolism and the high growth rates of the juvenile sharks considered in this research. The residency

time for whole blood (~ 474.5) is worthy of consideration higher than the one estimated for another long-time tissue, like muscle. This longer residency time is probably due to the biochemical characteristics of blood and its biochemistry: blood is formed by a mixture of a variety of cells and essential elements (e.g: iron, hemoglobin, etc) with different biochemical properties (Walsh *et al.,* 2004)., different turn-over rates and consequently different partial isotopic signatures, which influenced its final isotopic composition. These characteristics determine that the whole blood is formed by cellular components and elements which are continually renovating, probably contributing to the final large turn-over rates, also because the blood never replace itself for complete because a complete tissue replacement will cause serious damaged in the organism. The larger turn-over rate estimated for the whole blood could also be due a limitation of our model: it could not discriminate between the different cellular components and their partial isotopic signatures, because it cannot include the biochemical characteristic of this matrix and considered the whole blood as a complex mixture tissue with an isotopic signature resulting from different isotopic signatures of its cell components.

These results are preliminary, but we considered them important because it is the first estimation for residency time of whole blood, which can be useful for further researches.

Our outcomes point out that muscle and blood are long term tissues and the isotopic results for each tissue compared between shortfin mako and white sharks are similar. We discussed in Chapter 2 that the muscle results suggest shared sources and incorporating the isotopic signature of the trophic chain of Sebastian Vizcaino, so the similarity of the isotopic results between muscular tissue and blood allow to extend those conclusions to this tissue. The comparison between both matrix and the similarity in the $\delta^{13}C$ and $\delta^{15}N$ values obtained, confirm that that both species are feeding inside the bay, incorporating its isotopic composition and sharing the habitat use.

We found difference in $\delta^{13}C$ and $\delta^{15}N$ values when we developed crosswise comparison between muscle of white sharks vs. the blood of shortfin mako, which we attributed more to the tissues specific biochemistry than to changes in dietary sources, as previously discussed in Chapter 3 for the differences between cellular components

with different biochemical properties. The tissues specific biochemistry seems to have more influences in the incorporation of isotopic signature than dietary shfits, because comparing all the four tissues (muscle, whole blood, RBC and plasma) for the two species we can observed similarity in results, particularly for $\delta^{15}N$ values which increased in shortfin mako and white sharks with similar TL. We discussed that these high levels in nitrogen values in shark tissues are likely due to the incorporation of the 15N-enriched signature of the preys inside Sebastian Vizcaino bay and the reflection of these high levels among tissues reflecting different time frames suggest that this incorporation took place during extended period of times. In fact, we found similar results of nitrogen values in long term tissues and short term tissues, demonstrating that shortfin mako sharks and white sharks feeding in the trophic chain of Sebastian Vizcaino bay for long period of time. Furthermore, we found high nitrogen values in plasma than in muscle tissues for both species and plasma is a proxy for the isotopic signature of the recent dietary protein sources used (Tieszen *et al.,* 1983; Hughes *et al.,* 2017), which imply that the plasma reflected the isotopic signature of the preys used by shortfin mako and white sharks the months before they had been caught in fishery. Juveniles shortfin mako sharks are reported to frequent Sebastian Vizcaino bay for several months after birds and feed inside the bay (Unpublished data from the conventional sharks tagging program of the National Fisheries and Aquaculture Institute between 2010 and 2015; Malpica-Cruz *et al.,* 2013; Tamburin *et al.,* 2019

), and our results found in the four tissues used in this study, particularly comparing nitrogen levels muscle vs plasma, confirmed that this species frequent the bay for long period of time active foraging on its local trophic chain.

Furthermore, the similarity between the results for shortfin mako sharks and for white sharks suggest that also the white sharks frequent this bay as previously reported (Oñate-González *et al.,* 2017), residing close to it for long periods and active feeding on its preys during juvenile stages

The extent of isotopic overlap among the long-term tissues (muscle and blood) among species qualitatively and based on SIBER-generated isotopic niches (Fig. 11), indicated some resource overlap at certain sizes and confirm that in this sampled population there is a sharing of resource use in SVB throughout large periods of times.

This similarity in isotopic niche of juvenile shortfin mako and young white sharks demonstrated dietary and habitat sharing across different life stages, which is previously reported for co-existing elasmobranch species with similar sizes (Ellis *et al.,* 1996, Bethea *et al.,* 2004, Tilley *et al.,* 2013).

The results for the estimation of the TP are coherent with the previously reported for Cortes (1999), particularly for muscle tissues and RBC. Nevertheless, the values reported by Cortes (1999) showed a slightly increase, compared to our results. These higher values of TP are probably due the size of the sharks used in Cortes (1999) study: its samples came from larger sharks compared to our work, which normally feed on prey with high trophic levels, high protein components and consequently high nitrogen levels, which increase the TP estimation.

However, the individuals used in this study had smaller body sizes than those reported in the literature foraging on larger prey (Tricas and McCosker; 1984, Klimley 1985; Le Boeuf, 2004; Velasco-Tarelo, 2005; Malpica-Cruz *et al.,* 2013), so it is unlike that these sharks have high TP due to the use of high trophic level preys. Our TP estimation is similar to the one reported by Cortes (1999) and are probably due to the high nitrogen levels in the SVB baseline and preys, which increase the TP calculated for both species. Furthermore, our results showed TP similar to the values reported in Cortes (1999) for muscle of shortfin mako and white sharks, and for plasma of white sharks. This similarity and the high nitrogen levels in these tissues are probably due to the maternal transference: in fact the mother signature is reflected in tissues with a long turn-over rate, like muscles, and in the case of plasma of white sharks it is still reflected not for the long turn-over rates, because plasma reflect a short period of time, but because the individuals are newborns. So, tissues like plasma with a turn-over rates of days reflected the isotopic signature transferred by the mother during gestation. Adult white sharks are well known to forage on prey with high trophic levels and nitrogen values, and this signature with high nitrogen levels can be transferred of the newborns during embryonic development. This transference is reflected by newborns of white sharks used in our study and cause an increase in our TP estimation.

Our results estimated higher trophic levels for juvenile sharks compared to the

TP reported by Navia *et al.,* 2017, which determined juvenile sharks of different species as secondary consumers (TL< 4) with a diet based on fishes. On the other hand, Hussey *et al.,* (2014 and 2015) reported higher TP (=4-6) for shortfin mako and white sharks, estimating them as tertiary consumers with a diet based mainly based on elasmobranchs. However, the specimens used in this study are larger than the sharks sampled in our research, for which is realistic that they occupied higher TP compared to our results and it became reasonable that our specimens are secondary consumers, supporting also the results reported by Hussey *et al.,* (2014 and 2015) for adult shortfin mako and white sharks as tertiary consumers. Our results suggest that the age classes and the biologic characteristics of different species, like embryonic development, and the changes in the isotopic baseline signature could cause affected the TP. So, these factors should be considerate during this kind of estimations because can affect the conclusion about the sharks' role in aquatic food webs and their influence the aquatic communities.

The changes in the TP estimation results in this study based in the tissue used confirming that ontogeny dietary and habitat use changes can influence the estimation of TP, implying that elasmobranchs participate to different trophic groups and play different trophic roles throughout its life, not only depending from their diet during different life stages, but also from their life history characteristics and habitat used (Navarro-González *et al.,* 2012; Hussey *et al.,* 2015; Navia *et al.,* 2017). Our TP results indicates that juvenile mako sharks and white sharks are top predators and secondary consumers in the system of SVB, which will have important implications for future management. In fact, previous studies reported that top predators are the species with the highest connectivity and lowest topological redundancy in food webs, which means that a reduction in their population caused strong structural effect in the food web studied and a possible indirect trophic effect known as 'trophic cascade' (Navia *et al.,* 2016; Myers *et al.,* 2007; Ferretti *et al.,* 2010).

5.5. GENERAL CONCLUSIONS

The use of SIA in multiple tissue allows to elucidate the dietary habits of juvenile mako sharks and white sharks, their sharing of resources, their common habitat use and their residency inside SVB. We were also able to confirm the use of Sebastian

Vizcaino bay for extended period of time for both species using the multiple tissue approach, confirming that SIA are a useful tool to investigate the habitat use of sharks.

We combined the SIA in multiple tissues with other data, like the incidental capture record of young white sharks and their direct observation to elucidate the habitat use of this specie inside its nursery area. The results of these techniques, discussed in Chapter 4, suggest that the nursery area can be more extended that what previously reported, because the high concentration of records around Isla Cedros, which technically is part of SVB but far away from the core area of the nursery area previously reported by Oñate-González *et al.,* (2017). Our data provided evidences that waters off Isla Cedros, can be critical habitat for young white sharks and suggest that the nursery area could be more extended that what previously hypothesized.

In conclusion, ours results highlight the necessity to developed more studies about the possible influence of the tissue biochemistry on the stable isotopic values and the needed to develop more researches and experiments using whole blood, which could be an alternative long-term tissues to muscle. Our research, highlight how SVB is an important area for young life stages of vulnerable species like shortfin mako and white sharks, meaning that SVB is good area to develop future and complementary studies, for examples telemetry studies and more researches with chemical tracers (e.g. AA and isoescapes) to better define residency in the region. We also need to improve the research effort to monitored frequency of juvenile and YOY white sharks in Isla Cedros, because this habitat play an important role in the ecology and reproductive cycle of white sharks, and the results showed in this study represent a first approximation that the nursery area of SVB can be really more extended that what is currently reported.

◊ The results in muscle tissues suggest that some YOY and juvenile shortfin mako and white sharks may migrate to SVB from other regions, where they then forage and share prey resources.

  ◊ The increase in $\delta^{15}N$ values with shark size suggest ontogenetic shift from maternal source to a long-term use of prey resources within SVB, particularly for shortfin

mako sharks because our sampled population for white sharks is small and therefore these interpretations are preliminary.

- ◊ The nitrogen values in embryos and newborns shortfin mako sharks indicate direct maternal transference of nutrients and feeding resources to neonate shortfin makos, without high fractionation. This outcome has an important and practical application: it would be possible to take advance from the juvenile sharks caught in fisheries and opportunistically sampled to infer the trophic ecology of adults shortfin makos, which are very difficult to sampling.
- ◊ We assessed that two different preservatives methods are equally effective to conserve blood samples without any influences in isotopic values, which will have important implication for future studies and methodologies.
- ◊ The differences found blood components, particularly in shortfin mako sharks, are more likely due to biochemical structure of this matrix and its components that dietary changes, indicating that the tissue biochemistry has a key role in the incorporation of different isotopic signatures. This will need to be considered in further tissues on elasmobranch blood.
- ◊ Shortfin mako sharks and white sharks with similar TL (shortfin makos sharks > 102 cm TL and white sharks < 186 cm TL) reflected in their tissues (muscle, blood, RBC and plasma) the regional baseline 15-N enriched isotopic signature of SVB. These results suggest that SVB have a unique isotopic signature (isoscape), which is trackable in the animals that feed for long times in its trophic chain and that both species showed similar habitat in SVB. In addition, these conclusions suggested that the sharks can be indicator of the system and its regional biological processes.
- ◊ The similarity in isotopic niche and isotopic composition between shortfin mako and white sharks in multiple tissues reflecting large and short time periods suggest shared resource and common habitat use inside SVB throughout extended times confirming their residency in this area during their earlier life stages.
- ◊ The large dataset we presented for shortfin mako sharks, including embryos, YOY, juveniles, and adult allowed us to provide a new estimate for isotopic incorporation rate for shortfin mako sharks muscle tissues, (~255 days), whole blood (~ 474.5 d), RBC (~ 62 d) and plasma (~7.3). These results are the first estimation based on

tissue of free-living large sharks as shortfin mako, for juveniles lamnoid sharks, and for the blood tissue.

- ◊ TP confirm that juvenile mako sharks and white sharks are top predators and secondary consumers in the system of SVB, nevertheless their life-stages.
- ◊ This research represents the first data of a new nursery area from the outer regions SVB for the young white sharks. In addition, the unique record of the adult female pregnant of shortfin mako in the same area, pointed out that the region of Isla Cedros is a critical habitat and possible pupping ground for both species.
- ◊ Finally, this study elucidated the feeding habits and habitat use of shortfin mako and white sharks, highlighting some important conclusions for these species, which could be useful to interpret further results about shortfin mako and white sharks, but also about juveniles lamnoid sharks in general. During researches also came out some importance aspects and results about SIA in different tissues and the importance of system like SVB as critical habitat.

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