INSTITUTO POLITECNICO NACIONAL
CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

CICIMAR-IPN

TROPHIC STRUCTURE, BIOACCUMULATION AND TROPHIC TRANSFER OF TOTAL MERCURY IN THREE RAY SPECIES FROM THE PACIFIC COAST OF BAJA CALIFORNIA SUR

TESIS

QUE PARA OBTENER EL GRADO DE
DOCTOR EN CIENCIAS MARINAS

PRESENTA

DANIELA ALEJANDRA MURILLO CISNEROS

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

SIP-14 BIS

INSTITUTO POLITÉCNICO NACIONAL

SECRETARIA DE INVESTIGACIÓN Y POSGRADO

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"TROPHIC STRUCTURE, BIOACCUMULATION AND TROPHIC TRANSFER OF TOTAL MERCURY IN THREE RAY SPECIES FROM THE PACIFIC COAST OF BAJA CALIFORNIA SUR"

Presentada por el alumno:

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DEDICATORIA

A Fabián, mi apoyo incondicional. Te amo.

A mis padres, por ser los mejores y más comprensivos del mundo.

A mis mejores amigos del "Galván team", ustedes hicieron que este camino fuera inolvidable ¡una de las mejores etapas de mi vida!

AGRADECIMIENTOS

Al Instituto Politécnico Nacional (IPN) y al Centro Interdisciplinario de Ciencias Marinas (CICIMAR) por darme la oportunidad de formar parte de su comunidad estudiantil.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) y la Beca de Estímulo Institucional de Formación de Investigadores (BEIFI) por los apoyos económicos y académicos brindados.

A mi director de tesis, el Dr. Felipe Galván Magaña por su apoyo, por ser mi guía durante esta travesía, por confiar en mí y permitirme formar parte de esta maravillosa familia “Galván Team”.

To my thesis director, Dr. Todd O’Hara for all his support and trust. Thank you for giving me the opportunity to learn from you without knowing me, for opening your home to me and make me feel as part of your family. Carla, Lars, and Ana you made me feel as another member of your beautiful family.

Al comité revisor, Dr. Fernando Elorriaga Verplankcken, Dr. Alberto Sánchez González y Dra. Ana Judith Marmolejo Rodríguez, por aceptar formar parte del comité, por brindarme de su tiempo y conocimientos cada vez que tenía dudas y por sus valiosas observaciones que enriquecieron la tesis. Gracias!

To Dra. Maggie Castellini for receiving me, taking the time to teach me and correct me when I need it. You were so kind to me and I’m very grateful for all your attentions.

A Fabián, mi roca, mi mejor amigo, mi otra mitad, no existen palabras que expresen lo agradecida que estoy por todo tu apoyo a través de este camino llamado Doctorado. Te amo.

A mi hermanita Andrea, que me ha ayudado desde mi maestría a procesar mis muestras, sin ti hubiera sido imposible sacar el trabajo a tiempo. No pueden faltar Juan (polpeta), Chayo, Kenia, Paola y Nallely por ayudarme con una parte del

procesamiento de las muestras, cuando creía que no terminaría en tiempo. Fueron mi salvación. Gracias!!!

A mi familia y al Galvan team, por su gran apoyo y comprensión durante mis ataques nervios y por estar conmigo siempre que los necesite.

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ABSTRACT

The shovelnose guitarfish (*Pseudobatos productus*), bat ray (*Myliobatis californica*) and banded guitarfish (*Zapteryx exasperata*) are among the most abundant elasmobranchs species within the fisheries with gillnets of the Pacific coast of Baja California Sur (PCBCS). Their ecological roles as predators in demersal communities can be key in this ecosystem. Moreover, as predators they can bioaccumulate high concentrations of potentially toxic pollutants like mercury (Hg). Thus, the overall aim of this dissertation was to investigate the trophic ecology and Hg toxicological characteristics of the three ray species in the PCBCS. Total mercury concentrations ([THg]) in muscle and liver significantly increased with length especially in sexually mature organisms with a steeper slope for mature male than mature female. Median muscle [THg] was significantly greater than liver in each ray species. There were individuals with muscle [THg] higher than the advisory thresholds of 0.2 and 0.5 mg kg^{-1}ww (2.4 and 11% of the bat ray; 2.1 and 10% of the shovelnose guitarfish; 12.6 and 45% of the banded guitarfish, respectively). For the stable isotopes analysis we observed high variability in isotopes values, as $\delta^{13}C$ and $\delta^{15}N$ of the shovelnose guitarfish ranged from -18.53 to -12.85‰ and 15.93‰ to 20.37‰, for the banded guitarfish from -18.12‰ to -13.57‰ and 14.41‰ to 19.26‰, and from -17.73‰ to 13.98‰ and 13.97‰ to 18.46‰ for the bat ray, respectively. Isotopic niche analysis using Bayesian ellipses (SEAc) showed that the shovelnose guitarfish occupied the largest isotopic niche compared to the bat ray and banded guitarfish. Banded guitarfish overlap in a 0.50 with the shovelnose guitarfish. The bat ray overlap 0.38 and 0.39 with banded and shovelnose guitarfish. These suggests that the shovelnose and banded guitarfish shared feeding resources and habitat use but both species partitioning resources with the bat ray. We calculated the food web magnification factor (FWMF) that was constituted by zooplankton, three species of fish and five species of invertebrates. The FWMF was equaled 6.38 and was significantly greater than 1.0. This study is the first to describe THg biomagnification in the benthic food web of these three ray species of the PCBCS. This contribution provides important baseline knowledge of the biomagnification dynamics in this environments that represent multiple interacting species.

RESUMEN

Entre las especies más abundantes en las pesquerías de elasmobranquios con redes de enmalle de la costa occidental de Baja California Sur (COBCS) se encuentran la guitarra blanca (*Pseudobatos productus*), guitarra pinta (*Zapteryx exasperata*) and la raya murciélago (*Myliobatis califórnica*). Su rol ecológico como depredadores de las comunidades demersales puede ser clave en este ecosistema. Además, como depredadores pueden bioacumular concentraciones altas de contaminantes potencialmente tóxicos como el mercurio (Hg). Por lo tanto, el objetivo de este trabajo es investigar la ecología trófica y las características toxicológicas del Hg en tres especies de raya de la COBCS. Las concentraciones de mercurio total ([THg]) en músculo e hígado incrementaron significativamente con la talla, especialmente en los organismos sexualmente maduros. La [Hg] media en el músculo fue significativamente mayor que en hígado en cada especie de raya. Hubo algunos individuos con [THg] en músculo mayores que el límite de 0.2 y 0.5 mg kg^{-1}ww (2.4 y 11% raya murciélago; 2.1 y 10% en la guitarra blanca; 12.6 y 45% en la guitarra bandeada, respectivamente). Para el análisis de isótopos estables se observó una alta variabilidad con un intervalo en $\delta^{13}C$ y $\delta^{15}N$ para la guitarra blanca de -18.53 a -12.85‰ y 15.93‰ a 20.37‰, respectivamente; guitarra bandeada de -18.12‰ a -13.57‰ y 14.41‰ a 19.26‰, y raya murciélago de -17.73‰ a 13.98‰ y 13.97‰ a 18.46‰; respectivamente. El análisis de nicho isotópico usando elipses bayesianas (SEAc) mostró que la guitarra blanca tiene el nicho isotópico más grande comparado con la raya murciélago y la guitarra bandeada. La guitarra bandeada se traslapo en un 0.50 con la guitarra blanca. La Raya murciélago traslapo en un 0.38 y 0.39 con la guitarra bandeada y blanca, respectivamente. Esto sugiere que la guitarra blanca y bandeada comparten recursos alimenticios y uso de hábitat pero ambas segregan recursos con la raya murciélago. Se calculó factor de magnificación trófica (FWMF) el cual estuvó constituido por zooplancton, tres especies de peces y cinco especies de inveterados. El FWMF fue de 6.38 y fue significativamente mayor de 1.0. Este estudio es el primero en describir la biomagnificación de THg en la red trófica de estas tres especies de raya del COBCS.

CHAPTER 1. GENERAL INTRODUCTION

1.1. Ecology and biology of the shovelnose guitarfish, banded guitarfish and bat ray

Elasmobranchs are important fishery resources for human consumption in Mexico and worldwide (Domi *et al*. 2005; Ramírez-Amaro *et al*. 2013). Baja California Sur is one of the states within the Mexican Pacific coast with a relatively high catch of elasmobranchs, ranking second in 2013 with 16.20% of the total catch of Pacific (4,711 tons) (SAGARPA 2013). Among the most abundant elasmobranchs species within the fisheries using gillnets are the shovelnose guitarfish (*Pseudobatos productus*), bat ray (*Myliobatis californica*) and banded guitarfish (*Zapteryx exasperata*, Ramirez-Amaro *et al*. 2013).

The shovelnose guitarfish (Fig. 1.1) is a common coastal ray distributed from San Francisco, California (USA) to the Gulf of California (Fig. 1.2; Salazar-Hermoso and Villavicencio-Garayzar, 1999; Farrugia *et al*. 2016). This ray primarily inhabits sandy or muddy shallow waters of bays and estuaries at depths <12 m, but has been recorded at 91.5 m (Márquez-Farías, 2007; Farrugia *et al*. 2016). In the Pacific coast of Baja California Sur, it feeds on benthic invertebrates (crustacean, worms) and fish (Curiel-Godoy *et al*. 2016). This ray is aplacental viviparous (bears live young nourished from a yolk sac) and has a continuous reproductive cycle with a fecundity between 4 to 18 embryos (Downton-Hoffmann, 2007; Farrugia *et al*. 2016). Sexual maturity is typically reached in males and females at a mean of 95.1 and 111.8 cm of total length, respectively (Juaristy-Videgaray, 2016). The maximum total length reported for females is 156 cm and 114 cm for males (Farrugia *et al*. 2016). Maximum longevity observed for this species is 16 years for females and 11 for males (Downton-Hoffmann, 2007).

Figure 1.1. Shovelnose guitarfish. Source: https://www.mexican-fish.com/shovelnose-guitarfish/

Figure 1.2. Map of distribution of the shovelnose guitarfish. Source: Farrugia *et al*. (2016).

The banded guitarfish (Fig. 1.3) is distributed from Newport Beach, California (USA) to Mazatlán (Mexico), including the Gulf of California (Fig. 1.4; Blanco-Parra, *et al*. 2012; Bizarro and Kyne, 2015). This species inhabits shallow rocky reefs and sandy coastal lagoons from the intertidal zone to a depth of 200 m, although primarily at depths between 2.5 and 10 m (Villavicencio-Garayzar 1995; Cervantes-Gutierrez, *et al*. 2018). This ray feeds mainly on fish, and less so on crustaceans (Blanco-Parra, *et al*. 2012). The Banded guitarfish is lecithotrophic viviparous with litter sizes of 2 to

13 young produced annually (Blanco-Parra *et al*. 2009). Sexual maturity is reached at a mean of 69 and 77 cm of total length, for males and females, respectively (Villavicencio-Garayzar, 1995). The maximum size reported for females is 103 cm and 92 cm for males of total length, and maximum estimated age for females and males is 22.6 and 19.6 years (Cervantes-Gutiérrez, *et al*. 2018).

Figure 1.3. Banded guitarfish. Source: https://alchetron.com/Zapteryx

Figure 1.4. Map of distribution of the banded guitarfish. Source: Bizarro and Kyne (2015).

The bat ray (Fig. 1.5) is distributed from Oregon (USA), to Baja California Sur (Mexico), including the Gulf of California (Fig. 1.6; Martin and Cailliet, 1988; Van Hees, *et al*. 2015). This species occurs along the open coast and around islands where it frequents kelp beds and sandy bottoms near rocky reefs and sandy beaches from the intertidal zones to a depth of 108 m but are more common in shallower waters (Van Hees, *et al*. 2015). Along the Pacific coast of Baja California Sur, this

species is a benthic predator that feeds mainly on invertebrates including, crustaceans, gastropods, bivalves and sipunculids (Torres-Garcia, 2015). The reproductive mode of bay Ray is histotrophy viviparous with approximately 12 embryos annually (Van Hees, *et al*. 2015). Females reach maturity at 98.1 cm of disc width and 59.1 cm of disc width for males (Pelamatti, 2015). The maximum size reported for females is 180 cm and 91.5 cm of disc width for males (Pelamatti, 2015), and maximum estimated age for females and males is 24 and 6 years, respectively (Martin and Cailliet, 1988b).

Figure 1.5. Bat ray. Source: https://oehha.ca.gov/fish/species/bat-ray

Figure 1.6. Map of distribution of the bat ray. Source: Van Hees *et al*. (2015).

These three ray species are top predators of their ecosystems (Blanco-Parra, *et al*. 2012, Valenzuela-Quiñonez, *et al*. 2017). Therefore, they will biomagnify some chemicals and via other mechanisms (age) they bioaccumulate high concentrations of potentially toxic pollutants, such as mercury (Hg) (Maz-Courrau *et al*. 2011; Taylor *et al*. 2014).

Bioaccumulation is a non-trophic mechanism such as dependent or lack of loss via reproduction that causes an increase of a chemical concentration in an organism compared to that in its ambient environment through all exposure routes including dietary absorption and transport across body surfaces (Borga *et al*. 2011). Biomagnification is a process where a chemical substances increase in concentration in the tissue of organisms along a series of predator-prey associations, primarily through the mechanism of dietary accumulation, resulting in higher concentrations compared with the source (Gray, 2002; Burkhard *et al*. 2012). This is associated with trophic interactions, e.g., placement in food web and basic feeding as herbivore, omnivore or predator (generalist, specialist, etc.).

1.2. Mercury

Mercury is an environmental contaminant present in marine systems world-wide from both natural (e.g., volcanic activity, erosions and weathering of rock) and anthropogenic sources (e.g., mining, fossil fuels combustion, industrial emissions, direct application of fertilizer and fungicides, disposal of solid waste) (Anković *et al*. 2012). Most mercury in the atmosphere is emitted by anthropogenic activities, and being highly volatile, some forms of Hg can be transported over long distances on local, regional and global scale (Kim and Zoh, 2012). wet and dry atmospheric deposition is the primary source of Hg to the oceans, with smaller contributions from rivers, sediments and hydrothermal vents (Zhang, *et al*. 2014). However, there have been specific point of very high contamination, such as Minamata (Japan), were a chemical plant (Chisso Co. Ltd.) discharge water contained both inorganic and organic (methyl) mercury forms into Minamata bay. This contaminated marine life

and poisoning those who ingested the affected fish and seafood (Davies, 1991; Harada, 1995).

Mercury (Hg) exists in two main groups: inorganic mercury (Hg^0, Hg^+, Hg^{2+}) and organic mercury ($MeHg^+$, Me_2Hg, EtHg; (Kidd *et al.* 2012; Li and Cai, 2013). All forms of Hg are toxic but methylated forms are of most concern due to their lipid solubility and ability of bioaccumulate and biomagnify through the food webs (Kidd and Batchelar, 2012; Hosseini *et al*. 2013; Pham *et al*. 2014; Wang *et al*. 2018). Estuarine and coastal sediments are repositories for Hg, and are known locations for methylation, a bacterial-mediated process (largely sulfate-reducing bacteria) that converts inorganic Hg^{2+} to $MeHg^+$ (Taylor, *et al*. 2014).

Aquatic organisms are exposed to Hg^{2+} and $MeHg^+$ from water and diet (Karen and Kidd; 2012). However, their uptake in marine organisms depends on several biotic, ecological and environmental factors. Dietary intake is the most important pathway for the uptake of $MeHg^+$ in most aquatic organisms, thus structural differences in food webs is a key determinant for mercury distribution in ecosystems (Cai *et al*. 2007). In addition, $MeHg^+$ comprises approximately 90% of total mercury found in muscle of most fish (Kim, *et al*. 2016). Hg tissue residues increase with increasing age or body size of the organisms (bioaccumulation) and increasing trophic position (biomagnification), because elimination rates of $MeHg^+$ are lower than uptake rate for many fish consumers (Pethybridge *et al*. 2010). Efficiency of trophic transfer (biomagnification and bioavailability), results in higher concentrations for organisms that feed on higher trophic levels (Cai *et al*. 2007).

The toxic effects of Hg are well documented. All chemicals forms of Hg have neurotoxic properties (Dietz *et al*. 2013). At high concentrations, inorganic and organic forms of Hg can have negative behavioral, neurochemical, hormonal and reproductive impacts on marine vertebrates such as emaciation, cerebral lesions, and impaired gonadal development and possibly death (Taylor *et al*. 2014; Sandoval-Herrera *et al*. 2016). Inorganic Hg causes loss of equilibrium, inactivity, respiratory distress and death in fish exposed to high concentrations. Mechanisms of toxicity of MeHg are similar to those described for inorganic Hg, with few

exceptions (Kidd and Batchelar, 2012). Likewise, high concentrations of Hg in organisms can lead to a reduction in the abundance and diversity of aquatic species, contributing to stress and degradation of aquatic ecosystems (Naser, 2013). $MeHg^{+}$ exposure in humans have been related with immune deficiencies and neurotoxicity, especially in fetuses (Kim *et al*. 2016). Effects on brain function may manifest as irritability, tremors, changes in vision or hearing, and memory problems. Vomiting, diarrhea increases in blood pressure or heart rate, skin rashes, and eye irritation can occur (Anković, 2012). Thus, agencies such as US Food and Drug Administrations (FDA), World health Organization (WHO) and the Mexican Official Norm (NOM 242-SSA1, 2009) establish $MeHg^{+}$ concentration limits that can be present in fish for human consumption of 1 mg kg^{-1} wet weight (ww) in predatory fish and 0.5 mg kg^{-1} ww for retail fish in Mexico and others countries such as Canada and United states (Canadian Food Inspection Agency, 1998; US Food and Drug Administration, 2007; NOM 242-SSA1, 2009). While the Environmental Protection Agency (EPA) have set a limit of 0.3 mg kg^{-1} and the Alaska Scientific Advisory Committee for Fish Consumption of 0.2 mg kg−1 ww (USEPA, 2001; Hamade, 2014).

In Mexico, studies report the amount of THg in commercially important elasmobranchs, where some exceed the advisory threshold of 1 mg kg^{-1} ww (Table 1.1). Furthermore, most studies focus on sharks with only a few reporting on ray species. Some studies have considered ray species in Pacific Mexican waters from the Gulf of California (García-Hernández *et al*. 2007; Escobar-Sanchez *et al*. 2013; Ruelas-Inzunza *et al*. 2013). Despite the importance of some ray species in Pacific coast of Baja California Sur fisheries (e.g. *P. productus*, *Z. exasperta* and *M. californica*) and that some species of shark have elevated THg concentrations in this area (Escobar-Sánchez *et al*. 2011; Maz-Courrau *et al*. 2011; Barrera-García *et al*. 2012), no studies have been made in rays.

Table 1.1. Mean (± SD), and range of THg concentrations (mg kg^{-1} ww) of muscle tissue for different elasmobranchs species from the Pacific coast of Baja California Sur (PCBCS) and Gulf of California, Mexico.

Species	n	Location	TL or DW* range or SD⁺ (cm)	THg ± SD (range)	Reference
Sphyrna zygaena	37	PCBCS	-	0.16 (0.005-1.93)	Escobar-Sánchez *et al*. 2010
Prionace glauca	38	PCBCS	113-287	1.39±1.58 (0.22-7.63)	Escobar-Sánchez *et al*. 2011
Mobula japónica	3	Gulf of California	134.8-157*	0.14±0.01 (<LD-0.15)	Escobar-Sánchez *et al*. 2013
Mobula munkiana	4	Gulf of California	48.3-99*	0.19±0.03 (0.16-0.22)	Escobar-Sánchez *et al*. 2013
Mobula thurstoni	8	Gulf of California	97.3-144.3*	0.20±0.04 (0.16-0.23)	Escobar-Sánchez *et al*. 2013
Rhinoptera steindachneri	25	Gulf of California	41.9-84.6*	0.37±0.25 (0.04-0.79)	Escobar-Sánchez *et al*. 2013
Carcharhinus falciformis	15	PCBCS	196.6±20.2⁺	3.40±1.42 (1.06-5.84)	Maz-Courrau *et al*. 2011
Prionace glauca	21	PCBCS	206.2±52.8⁺	1.96±1.48 (0.76-6.52)	Maz-Courrau *et al*. 2011
Sphyrna zygaena	31	PCBCS	114±19.2⁺	0.98±0.92 (0.24-2.8)	Maz-Courrau *et al*. 2011
Isurus oxyrinchus	24	PCBCS	127.1±37.9⁺	1.05±0.82 (0.44-4.21)	Maz-Courrau *et al*. 2011
Pseudobatos productus	13	Gulf of California	19-27*	0.31±0.52 (<LD-2.04)	García-Hernández *et al*. 2007
Zapteryx exasperata	7	Gulf of California	30-37*	0.11±0.11 (<LD-0.28)	García-Hernández *et al*. 2007
Myliobatis californica	6	Gulf of California	36-76	0.05±0.06 (<LD-0.15)	García-Hernández *et al*. 2007
Prionace glauca	44	PCBCS	117-269	1.03±0.08	Barrera-García *et al*. 2011

SD: standard deviation; n: sample number; TL: total length; DW: disc width

1.3. Stable isotopes of C and N

A proper knowledge of trophic status of rays is crucial to understand their ecological role in marine ecosystems, identify critical habitat, and their relationship with other species as rays provide a link between apex predators and lower trophic levels (Vaudo and Heithaus, 2011; Barría *et al*. 2017).

Stable isotopes analysis of C ($^{13}C/^{12}C$, reported as $\delta^{13}C$) and N ($^{15}N/^{14}N$, reported as $\delta^{15}N$) is a powerful tool to assess trophic structure of organisms in the food web (Post 2007; Logan and Lutcavage, 2010). $\delta^{13}C$ and $\delta^{15}N$ values track nutrient flow through a food web and take advantage of spatially natural variations in stable isotope ratios at the base of the food web, and the underlying aspects of a species' trophic niche, which the variation reflects, in response to differences in productivity, upwelling and other oceanographic factors (Layman *et al*. 2007; Kim *et al*. 2011; Hammerschlag, *et al*. 2011). Both isotopes are fractionated during metabolic processes, such that heavier isotope (^{13}C and ^{15}N) increase in abundance relative to the lighter isotope (^{12}C and ^{14}N) in the consumer tissues relative to prey (Caut *et al*. 2009; Kim *et al*. 2011). In general, $\delta^{15}N$ of a consumer increases by 3–4‰ per trophic level relative to its diet $\delta^{15}N$ and can estimate the trophic position in the food web. In contrast, $\delta^{13}C$ slightly increases as the trophic level increases (about 1 ‰); and is better used to identify the carbon source at the base of the food web, which vary according to their origin (e.g. inshore vs offshore environments; Post, 2002; Ikemoto *et al*. 2008; Gallagher *et al*. 2017).

Despite high abundance and importance of *P. productus*, *M. californica* and *Z. exasperata* in demersal communities (Ramírez-Amaro *et al*. 2013), very few studies have assessed the trophic ecology of these species in Mexican waters (Downtown-Hoffmann, 2007; Blanco-Parra *et al*. 2012; Vázquez-Moreno, 2015; Torres-García, 2015; Curiel-Godoy *et al*. 2016; Valenzuela-Quiñonez *et al*. 2017). The previous studies have used mainly stomach content and found in general the three ray species fed mainly upon benthic invertebrates such as shrimp and crabs, and include some fish. *P. productus* and *M. californica* were reported as secondary consumers (Torres-García, 2015; Curiel-Godoy *et al*. 2016; Valenzuela-Quiñonez *et*

al. 2017), while *Z. exasperata* as a tertiary consumer (Vazquez-Moreno, 2015). To date, only two studies used stable isotopes analysis to assess trophic ecology of these organisms in the Gulf of California and no study for the PCBCS. Blanco-Parra *et al*. (2012) found no differences by sex for $\delta^{13}C$ and $\delta^{15}N$, suggesting no sexual segregation and observed a relationship between $\delta^{15}N$ and body size related to ontogenetic shift in diet where immature individuals fed at lower trophic levels relative to matures ones. Similarly, Valenzuela-Quiñonez *et al*. (2017) observed in *P. productus* no sexual segregation and an ontogenetic shift in diet for $\delta^{13}C$ and $\delta^{15}N$.

These trophic relationships, in terms of predator-prey interactions, significantly influence community structure and population dynamics (Hussey, *et al*. 2011) as well as Hg concentrations (Croizier *et al*. 2016). Increasingly, $\delta^{13}C$ and $\delta^{15}N$ have been coupled with contaminants analysis to investigate and validate exposure pathways, as well as to quantify the accumulation of the contaminant concentration in the tissue of the predators in the context of trophic position (Domi *et al*. 2005; Ikemoto *et al*. 2008; Lavoie *et al*. 2013). For Mexican waters, there are no studies or information regarding biomagnification of THg in the marine food web of any elasmobranchs. Therefore, this study will be the first to assess this phenomenon for the PCBCS.

1.4. Justification

Evaluating the structure and food web relationship of marine organisms, such as rays, in highly dynamic ecosystems is challenging since most productive food web, trophic relationships are complex, involving numerous species and prey alternatives that shift over time and space. Nonetheless, assessment is fundamental for appropriate conservation and management strategies. In addition, $MeHg^{+}$ is of increasing concern due to toxicity and biomagnification. The number of studies evaluating trace elements bioaccumulation in marine organisms has increased in recent years. This increased effort is mainly focused on fishes and other species

used for human consumption (Domi *et al*. 2005), representing an ecological and human health threat (Zhang *et al*. 2012).

Understanding the mechanisms of mercury bioaccumulation in the food web is critical to predict which pathways are at risk of higher bioaccumulation and biomagnification, which in turn may endanger the health of predators like rays, as well as human consumers of these organisms (Pethybridge, 2010). Likewise, knowing the flow of mercury in the food webs of an ecosystem, could provide information for recommendations on the consumption of species supporting the fishery (Ferris *et al*. 2014) and mechanisms to reduce exposure or promote mitigation.

1.5. Research objectives

Essential to understanding contaminant pathways and influences in any ecosystem is knowledge of trophic relationships. Thus, the overall aim of this dissertation is to investigate the chemical feeding ecology of THg in three ray species of the Pacific coast of Baja California Sur, Mexico, in the context of human health criteria relative to food safety criteria.

1.6. Specific objectives

1. Quantify [THg] in muscle and liver of *P. productus*, *Z. exasperata* and *M. californica* related to size (age), maturity status and sex, and assess the potential implication to human health.

2. Analyze trophic ecology of *P. productus*, *Z. exasperata* and *M. californica* using stable isotopes of C and N.

3. Use stable isotopes of C and N to investigate trophic structure and biomagnification of THg in *P. productus*, *Z. exasperata* and *M. californica*, using primary consumer (zooplankton) and intermediate groups (prey items) to

corroborate and quantify the average rate of biomagnification using Food Web magnification factor (FWMF) and biomagnification factors (BMFs).

1.7. Study area

The study area is located in the Northwest Pacific of Baja California Sur and comprises Bahia Tortugas (27 ° 39'35 "N; 114 ° 52'35" W) and the adjacent area San Sebastian Vizcaíno (28° 30' N; 115° W; Fig. 1.4). This region is located in a transition zone between temperate and subtropical waters and has the influence of the California current, where the dominant northwest winds give place to one of the principal regions of coastal upwelling (Amador-Buenrostro, *et al*. 1995; Martínez-Fuentes *et al*. 2016). The average temperature of the first 50 m depth is of 15.5 °C with an average salinity of 33.6 (Martinez-Fuentes *et al*. 2016). An important feature of this region is the presence of an anticyclonic eddy situated in the western side of San Sebastian Vizcaíno, derived due to the southward advection, the Coriolis Effect on the surface circulation, and the elongated shape of the bay. This eddy has a depth between 60 and 70 m in an area that has a depth of about 150 m. (Amador-Buenrostro *et al*. 1995; Martínez-Fuentes *et al*. 2016). This region is considered as a Centers of Biological Activity (CBA), defined as a relatively small region in the oceans, whose most important feature is the high biological productivity, which contrasts with the surrounding water, which transcends into other ecosystems influenced by physical processes such as eddies and upwellings. This results in significant concentrations of biomass of organisms, many of them of commercial importance. Moreover, these concentrations of biomass usually spread to surrounding ecosystems, thereby generating regions rich in marine fishery resources (Arreguín-Sánchez, 2000).

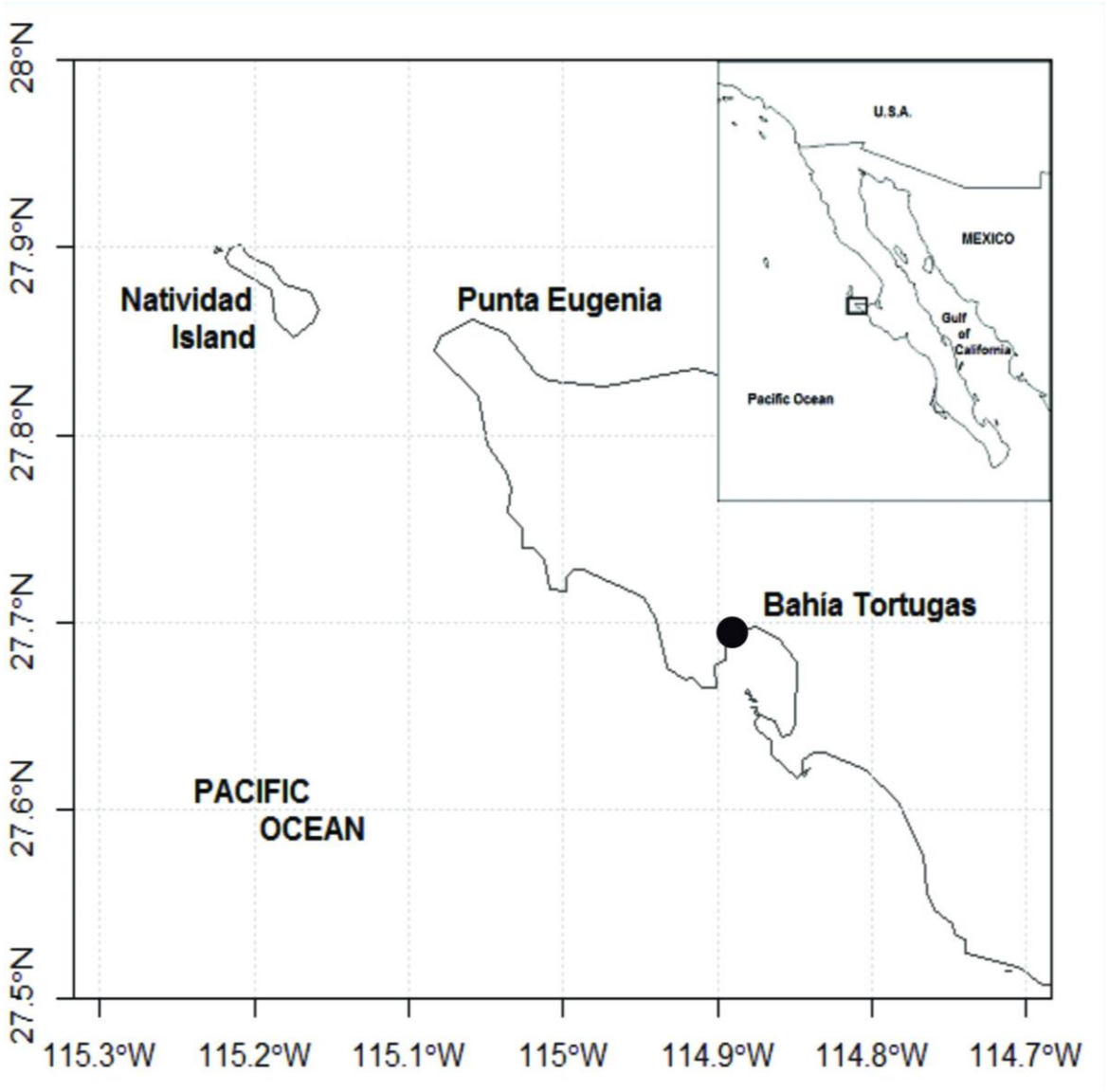

Figure 1.4. Study area. Bahía Tortugas, Baja California Sur, Mexico.

CHAPTER 2. MERCURY CONCENTRATIONS IN THREE RAY SPECIES FROM THE PACIFIC COAST OF BAJA CALIFORNIA SUR, MEXICO: VARIATIONS BY TISSUE TYPE, SEX AND LENGTH

Published in: Marine Pollution Bulletin 126: 77-85

2.1. ABSTRACT

Total mercury concentrations ([THg]) were determined in muscle and liver of the bat ray (*Myliobatis californica*), shovelnose guitarfish (*Pseudobatos productus*) and banded guitarfish (*Zapteryx exasperata*). Generalized linear models (GLM) were used to determine the effects of size and sex in [THg] and showed that both are determinants of [THg] in these species. The [THg] in both tissues significantly increased with length especially in sexually mature organisms with a steeper slope for mature male than mature female. This may relate to elasmobranchs sexual dimorphism driven variation in growth rates. Median muscle [THg] was significantly greater than liver in each ray species but there were some individuals with higher liver [THg] than muscle. There were individuals with muscle [THg] higher than the advisory thresholds of 0.2 and 0.5 mg kg^{-1}ww (2.4 and 11% of the bat ray; 2.1 and 10% of the shovelnose guitarfish; 12.6 and 45% of the banded guitarfish, respectively).

Key words: Mercury, rays, muscle, liver, safety limit.

2.2. INTRODUCTION

Sharks and rays are important fishery resources for human consumption worldwide (Domi *et al*., 2005; Ramírez-Amaro *et al*., 2013) and their meat is rich in nutrients such as protein, omega-3 polyunsaturated fatty acids, vitamins and minerals, (Olmedo *et al*., 2013; Gribble *et al*., 2015; Matos *et al*., 2015). In contrast to their health benefits, some species accumulate high concentrations of mercury

(Hg), due their longevity, size, slow growth, high trophic status and low fecundity (Kim *et al*., 2016).

Monomethyl mercury ($MeHg^+$) is a well-known neurotoxicant that has been subject to intense research, due to its potential adverse effects and ability to bioaccumulate and biomagnify in marine food webs (Horvat *et al.*, 2014; Hosseini *et al.*, 2013). Toxicity depends on the chemical form and bioavailability, $MeHg^+$ comprises up to 90% of total mercury (THg) found in muscle of most fish (Adams and McMichael, 1999; Escobar-Sánchez *et al*., 2010; Hosseini *et al.*, 2013) and between 70% and 100% in rays species (Storelli *et al*., 2002; Horvat *et al*., 2014) that is readily absorbed from the diet and crosses the blood brain barrier and placenta (Brookens *et al*., 2007; Correa *et al*., 2013). As a result, human populations with traditionally high dietary fish intake are exposed to $MeHg^+$ (Olmedo *et al*., 2013; Matos *et al*., 2015).

Most studies assessing THg concentrations ([THg]) in elasmobranchs focused mainly on sharks and to a lesser extent batoids (Escobar-Sanchez *et al*., 2013). Particularly, on the Pacific coast of Baja California Sur (PCBCS), México, elevated [THg] have been reported for some commercially important shark species (e.g. blue shark, *Prionace glauca*; Escobar-Sánchez *et al*., 2011; Maz-Courreau *et al*., 2011; Barrera-García *et al*., 2012). However, there is a lack of information regarding several species of shark, especially batoid species that constitute part of the local diets.

The most important species in the artisanal elasmobranchs gillnet fishery of the PCBCS are the bat ray (*Myliobatis californica*), shovelnose guitarfish (*Pseudobatos productus*) and banded guitarfish (*Zapteryx exasperata*) (Ramírez-Amaro *et al*., 2013). While these species are used for human consumption, especially in coastal areas little is known about the potential human health impacts of the consumption of their meat.

We examined [THg] in muscle and liver tissue of the bat ray, shovelnose guitarfish and banded guitarfish, collected in the PCBCS. Our study analyzes the interactions of tissue type, body size and sex on [THg] for each of the three ray

species and compares concentrations between species for liver and muscle. We evaluated [THg] in muscle related to human exposure (diet) criteria.

2.3. MATERIAL AND METHODS

2.3.1. Sample collection

Elasmobranchs samples were collected in March-April, August-September and November of 2014 in Bahía Tortugas (27 ° 39'35 "N; 114 ° 52'35" W) located on the west coast of Baja California Sur, Mexico (Fig. 2.1). Specimens were captured by local fishermen using gill nets and all individuals collected were commercially sold for human consumption. Size (total length for the shovelnose guitarfish and the banded guitarfish, and disc width for the bat ray) and sex were recorded for each individual. Sexual differentiation was determined by the presence of claspers in males. For each specimen, between 5-30 g of muscle (dorsal side near the head) and liver tissue were collected and placed in plastic bags. Occasionally, organisms were collected from the net lacking internal organs, in which case muscle and liver matched samples were unavailable. All samples were kept on ice in coolers and transported to the laboratory at Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (CICIMAR-IPN, La Paz, BCS, Mexico) and stored frozen (−20 °C).

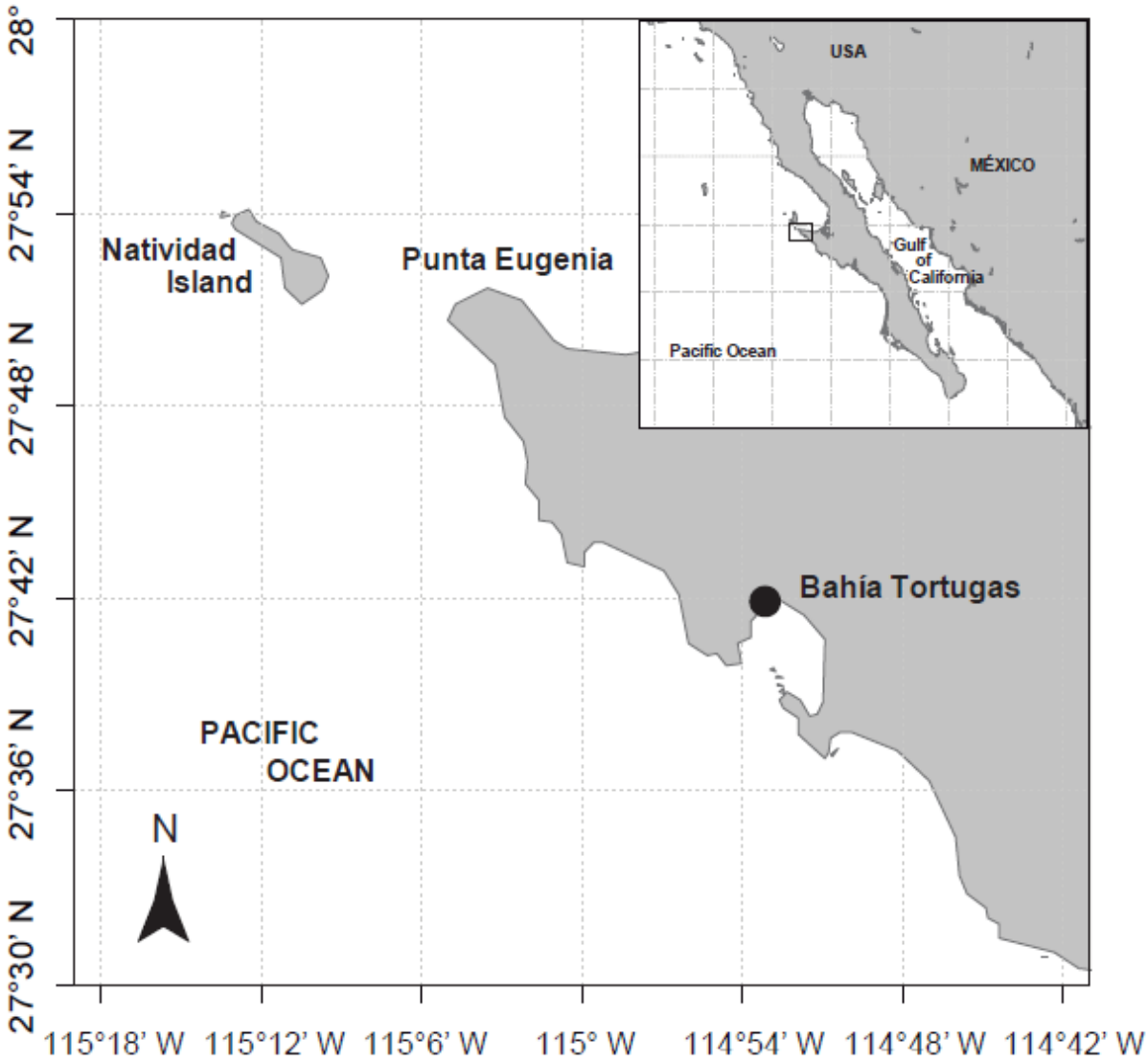

Fig. 2.1. Location of sampling site in Bahía Tortugas, Baja California Sur, Mexico.

In the laboratory, all tissues were sub-sampled (range 2-20 g each) using a clean stainless steel scalpel and stored at −20 °C in acid-washed plastic containers. Samples were freeze-dried (Labcono, FreeZone 2.5 Liter) for 24-48 h as described by Cyr *et al*., (2016) and homogenized using a porcelain mortar and pestle cleaned between samples with HCl acid at 10% and distilled water. Weight of each sample before and after freeze-drying was determined to calculate the percent water in each tissue once a consistent mass was achieved (fully dried).

2.3.2. Total mercury concentration ([THg]) analysis

The [THg] was determined in the Wildlife Toxicology Laboratory (WTL) at the University of Alaska Fairbanks (UAF) USA, using a direct Hg analyzer (DMA-80, Milestone, Shelton, CT, USA; US EPA method 7473) with thermal decomposition, amalgamation and atomic absorption spectrophotometry, in a manner similar to Cyr *et al*., (2016). The instrument was calibrated using a 14-point calibration curve ranging from 0.5 to 400 ng THg. The detection limit was 1 ng THg. Samples were freeze-dried for 24 h again before each run to remove any potential residual moisture. Blanks, aqueous standards (10ng at 0.1 mg kg^{-1}, Perkin-Elmer), and standard reference materials (DORM-4, TORT-2 National Research Council Canada, Ottawa ON, Canada) were analyzed for each analytical run for quality assurance. Measurements of aqueous standards were repeated after every 18 samples. Percent recoveries of standard reference materials and aqueous standards were within 91–109%. All samples were analyzed in triplicate (muscle 16-27 mg, liver 30-41 mg each) and the coefficient of variation for triplicate samples was less than 11%.

2.3.3. Statistical and sexual maturation analysis

Data were grouped by sex and maturity stage for each species of ray as follows: IF= immature female; MF= mature female; IM= immature male; MM= mature male. Maturity stage was assigned according to species morphometric criteria. A disc width for the male of >62 cm and female of >88.1 cm are considered mature for the bat ray (Martin and Caillet, 1988); for the shovelnose guitarfish, a total length for the male >80 cm and a female >100 cm are considered mature (Downton-Hoffmann, 2007); and a total length for the male >69 cm and female >77 cm are deemed mature for the banded guitarfish (Villavicencio-Garayzar, 1995).

Normality and homogeneity of variance were assessed by using Kolmogorov-Smirnov and Bartlett tests. Kruskal-Wallis tests and Mann-Whitney U-test were used to make statistical comparisons between species for each tissue for each

maturation-sex group (e.g., IF of each species). To determine in which species [THg] differed within each group, multiple comparisons of mean ranks for all groups were used. Wilcoxon matched pairs test was used to detect differences in [THg] between muscle and liver for each species in each group (IF, MF, IM, MM), considering only those individuals that had matched samples. In order to detect outliers from each group of data, Grubbs outlier test was performed. Statistical analyses were repeated excluding outliers to establish the potential effect of those individuals on the results. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using Statistica 8.0 (statSoft Inc. Tulsa, OK, USA).

Generalized linear models (GLM) were performed to determine the effects of [THg] as a function of size and sex as well as interactions, in each species for each tissue. This analysis has been utilized in similar studies by McMeans *et al*., (2007) and Bentzen *et al*., (2016). [THg] was log transformed to improve normality and homogeneity of variance. A model validation was systematically applied by checking normality and homogeneity in models' residuals. The selection of the best model was based upon the Akaike Information Criterion (AIC) used in Bentzen *et al*., (2016). We used a forward stepwise approach, by adding one variable at a time. The variables that resulted in a decrease of at least two AIC units were retained in the models (Burnham and Anderson, 2002). R Programming language (RStudio, v. 1.0.44, 2016) was used to create the models.

Finally, the estimated weekly intake (EWI) of [THg] through consumption of each ray species was calculated considering an average adult body weight of 70 kg and consumption of 180 g of fish per week (INEGI, 2014), using the following formula:

$$\mathrm{EWI} = \frac{\text{Amount of fish ingested per week (g/week)} \ * \ \text{[THg] in the ray ingested (µg/g)}}{\text{Kilogram body weight (kg bw)}}$$

The EWI were compared with the provisional tolerable weekly intake (PTWI) set by the joint food and agriculture organization/ world health organization (FAO/WHO), expert committee on food additives (JECFA, 2003). The PTWI is the

amount of a toxic substance that can be ingested without presenting any risk to health over a typical life span. The recommended PTWI for THg is 5 µg kg^{-1} bw per week which correspond to ~350 µg $week^{-1}$ of THg for a 70 kg person (JECFA, 2003; Ordiano-Flores *et al*., 2011). The maximum allowable weekly intake of muscle was calculated dividing the PTWI by the [THg] (Ordiano-Flores *et al*., 2011).

2.4. RESULTS

A total of 267 muscle samples and 229 liver samples from the three ray species were analyzed for [THg]. The biometric data are presented in Table 1. Many, but not all individuals, had matched samples (muscle and liver from the same animal; Table 1). The results for [THg] for each species by each maturation-sex group (IF, MF, IM and MM) is summarized in Table 2.

Table 2.1. Biometric data of muscle and liver tissue of the bat ray, shovelnose guitarfish and banded guitarfish sampled in Bahía Tortugas BCS (Mexico). “Animal matched samples” indicates the number of animals from which both the muscle and liver were analyzed. Total length (TL) for the shovelnose guitarfish and the banded guitarfish and disc width (DW) for the bat ray*.

		Muscle			Liver			
		n	Mean ± SD TL, *DW (cm)	Range TL, *DW (cm)	n	Mean ± SD TL, *DW (cm)	Range TL, *DW (cm)	Animal matched samples
*Bat ray	IF	46	46.21 ± 20	18.20 - 93.40	40	43.86 ± 19.16	18.20 - 93.40	38
	MF	2	-	103 - 113	3	116.67 ±	107 - 130	2
	IM	23	37.96 ± 12.08	21 - 55	17	11.93	21.20 - 55	17
	MM	12	71.40 ± 7.40	63 - 84	11	40.15 ± 12.66	64 - 84	10
						73.42 ± 7.85		
Shovelnose guitarfish	IF	37	89.51 ± 9.35	49 - 99	23	89.85 ± 7.82	69.20 - 99	17
	MF	22	108.23 ± 7.81	100.40 -	19	108.95 ±	100.60 -	18
	IM	4	71.45 ± 8.30	127.20	1	10.54	127.20	1
	MM	34	96.35 ± 9.20	59.80 - 78.40	28	-	78.40	23
				82.20 - 115		97.89 ± 8.85	82.20 - 115	
Banded guitarfish	IF	5	58.76 ± 2.37	56.60 - 61.80	5	58.76 ± 2.37	56.60 - 61.80	5
	MF	18	89.44 ± 7.01	78.20 - 103	16	89.70 ± 7.37	78.20 - 103	15
	IM	11	61.18 ± 4.73	51.60 - 68	12	59.88 ± 6.37	45.60 - 68	11
	MM	53	81.57 ± 5.34	69.20 - 91	54	81.58 ± 5.54	69.20 - 92	46

IF: immature female; MF: mature female; IM: immature male; MM: mature male; n: number of samples and SD: standard deviation

Table 2.2. Mean (±SD), median and range of concentrations of total mercury (THg; mg kg^{-1} ww) in muscle and liver for all animals within a species and by their maturity-sex cohort of bat ray, shovelnose guitarfish and banded guitarfish from Bahía Tortugas BCS.

		Muscle			Liver		
		Mean ± SD	Median	Range	Mean ± SD	Median	Range
Bat ray	IF	0.06±0.04	0.05	0.02-0.20	0.03±0.01	0.02	0.009-0.07
	MF	0.33±0.08	0.33	0.26-0.39	0.08±0.05	0.05	0.04-0.14
	IM	0.04±0.02	0.04	0.01-0.08	0.03±0.02	0.02	0.01-0.09
	MM	0.23±0.19	0.18	0.04-0.65	0.38±0.56	0.10	0.02-1.68
	All	0.09±0.11	0.06	0.02-0.65	0.09±0.25	0.03	0.009-1.68
Shovelnose guitarfish	IF	0.08±0.05	0.06	0.04-0.23	0.04±0.04	0.03	0.01-0.20
	MF	0.11±0.04	0.12	0.06-0.20	0.05±0.04	0.04	0.02-0.21
	IM	0.08±0.05	0.06	0.04-0.17	0.01	0.01	-
	MM	0.14±0.13	0.09	0.04-0.69	0.08±0.17	0.03	0.01-0.94
	All	0.12±0.09	0.08	0.04-0.69	0.07±0.12	0.03	0.01-0.94
Banded guitarfish	IF	0.05±0.01	0.06	0.03-0.06	0.02±0.01	0.02	0.02-0.04
	MF	0.28±0.18	0.24	0.07-0.76	0.11±0.12	0.07	0.02-0.49
	IM	0.08±0.06	0.06	0.03-0.22	0.04±0.04	0.02	0.02-0.17
	MM	0.26±0.19	0.20	0.06-0.84	0.16±0.24	0.07	0.00-1.26
	All	0.24±0.24	0.18	0.03-0.84	0.13±0.20	0.06	0.02-1.26

SD: standard deviation; IF: immature female; MF: mature female; IM: immature male; MM: mature male.

2.4.1. Comparison of [THg] between species and tissues

Significant differences in [THg] in muscle were found among the three species of rays. For IF, the shovelnose guitarfish had higher [THg] than the bat ray ($p=0.02$), while no differences were found compared to the banded guitarfish ($p>0.05$). For MF, significantly higher [THg] for the banded guitarfish were noted, as compared to the shovelnose guitarfish ($p=0.001$). IM banded guitarfish had significantly higher [THg] in muscle than the bat ray ($p=0.04$). For MM, the banded guitarfish had significantly higher [THg] than the shovelnose guitarfish ($p=0.01$) but no differences compared to the bat ray ($p>0.05$), nor between the bat ray and the shovelnose guitarfish ($p>0.05$).

For liver, only MM had significant differences in [THg] between species. The banded guitarfish and the bat ray showed no differences in hepatic [THg], but had significantly higher [THg] compared to the shovelnose guitarfish ($p=0.02$ and $p=0.01$, respectively). When outliers were removed from the data set, the statistical significance noted above did not change for liver and muscle.

The [THg] in muscle tissue was significantly greater than liver for each species and group ($p<0.05$). However, there were a few instances in each species where liver [THg] was higher compared to matched muscle samples. When outliers were removed from the analysis, statistical significance for banded guitarfish IF changed to no significant difference in [THg] between muscle and liver.

2.4.2. Assessing drivers of [THg] muscle and liver

Based on the GLM (Table 3), the best approximating a priori model describing [THg] in the muscle of the bat ray was the interaction between DW and sex, and was 11.86 AIC units from the next best model (DW + sex). The first model carried all the weight ($\sum w_i = 1.0$). The [THg] varied with sex ($F=4.82$, $p = 0.003$), and the interaction between length and sex was significant ($F=6.00$, $p=0.001$, Fig. 2.2.A). However, length by itself was not significant ($F=142.49$, $p>0.05$).

For shovelnose guitarfish muscle, the best approximating a priori model describing variation in [THg] was the interaction between TL and sex; and was 9.21

AIC units from the next best model which was TL (Table 3). These two models carried 1.0 of the AIC model weight. The [THg] increase with length ($F=23.70$, $p<0.001$), and the interaction between length and sex was significant ($F=5.53$, $p<0.001$) with an increase in [THg] mainly in MM compared to other groups (IF, IM and MF; Fig. 2.2.B). However, sex by itself was not significant ($F=1.72$, $p =0.17$).

The interaction between TL and sex was also the best approximating a priori model describing [THg] in banded guitarfish muscle, and was 0.94 AIC units from the next best model, which include TL + sex and was 13.94 AIC units from the next best model, which considers only the TL (Table 3). The top two models carried all the AIC model weight. The [THg] increased with length ($F=152.20$, $p<0.001$), with the increase more marked by sex in MM compared to MF ($F= 1.72$, $p<0.001$; Fig. 2.2.C). Nonetheless, the interaction of TL and sex was not significant ($F= 2.19$, $p=0.09$).

Table 2.3. Models that explain [THg] variability in the muscle of the three ray species from Bahía Tortugas BCS, Mexico. Explanatory variables include length (DW: disc width for the bat ray and TL: total length for the shovelnose guitarfish and the banded guitarfish) and sex. The model with the lowest AIC value is in bold.

	Variable	Coefficients	CI	AIC	W	% Explained deviance
Bat ray	DW	1.19×10^{-2}	1.15×10^{-3}	-9.33	0.00	60.00
	Sex	-	-	17.65	0.00	43.28
	Sex (IF)	-	-	-	-	-
	Sex (IM)	-1.57×10^{-1}	6.63×10^{-2}	-	-	-
	Sex (MF)	7.45×10^{-1}	1.88×10^{-1}	-	-	-
	Sex (MM)	4.59×10^{-1}	8.42×10^{-2}	-	-	-
	DW + sex	-	-	-15.35	0.00	62.79
	DW*sex	-	-	**-27.21**	**1.00**	**70.00**
	DW*sex(IF)	-	-	-	-	-
	DW*sex (IM)	-5.79×10^{-3}	3.71×10^{-3}	-	-	-
	DW*sex (MF)	7.14×10^{-3}	2.75×10^{-2}	-	-	-
	DW*sex (MM)	3.07×10^{-2}	8.03×10^{-3}	-	-	-
Shovelnose guitarfish	TL	8.08×10^{-3}	$1.79x\ 10^{-3}$	-17.12	0.01	17.63
	Sex	-	-	-5.25	0.00	10.67
	Sex (IF)	-	-	-	-	-
	Sex (IM)	-2.21×10^{-2}	1.20×10^{-1}	-	-	-
	Sex (MF)	1.62×10^{-1}	6.15×10^{-2}	-	-	-
	Sex (MM)	1.49×10^{-1}	5.43×10^{-2}	-	-	-
	TL + sex	-	-	-15.75	0.00	21.46
	TL*sex	-	-	**-26.33**	**0.99**	**33.80**
	TL*sex (IF)	-	-	-	-	-
	TL*sex (IM)	-2.65×10^{-2}	1.44×10^{-2}	-	-	-
	TL*sex (MF)	6.05×10^{-3}	6.67×10^{-3}	-	-	-
	TL*sex (MM)	1.71×10^{-2}	5.23×10^{-3}	-	-	-
Banded guitarfish	TL	2.49×10^{-2}	2.26×10^{-3}	-4.61	0.00	58.84
	Sex	-	-	42.89	0.00	32.15
	Sex (IF)	-	-	-	-	-
	Sex (IM)	1.31×10^{-1}	1.61×10^{-1}	-	-	-
	Sex (MF)	6.32×10^{-1}	1.51×10^{-1}	-	-	-
	Sex (MM)	5.94×10^{-1}	1.40×10^{-1}	-	-	-
	TL + sex	-	-	-17.61	0.38	66.92
	TL*sex	-	-	**-18.54**	**0.62**	**69.46**
	TL*sex (IF)	-	-	-	-	-
	TL*sex (IM)	4.07×10^{-2}	4.55×10^{-2}	-	-	-
	TL*sex (MF)	4.49×10^{-2}	4.40×10^{-2}	-	-	-
	TL*sex (MM)	6.23×10^{-2}	4.37×10^{-2}	-	-	-

CI: confidence interval. AIC: Akaike's information criterion. W: AIC weight.

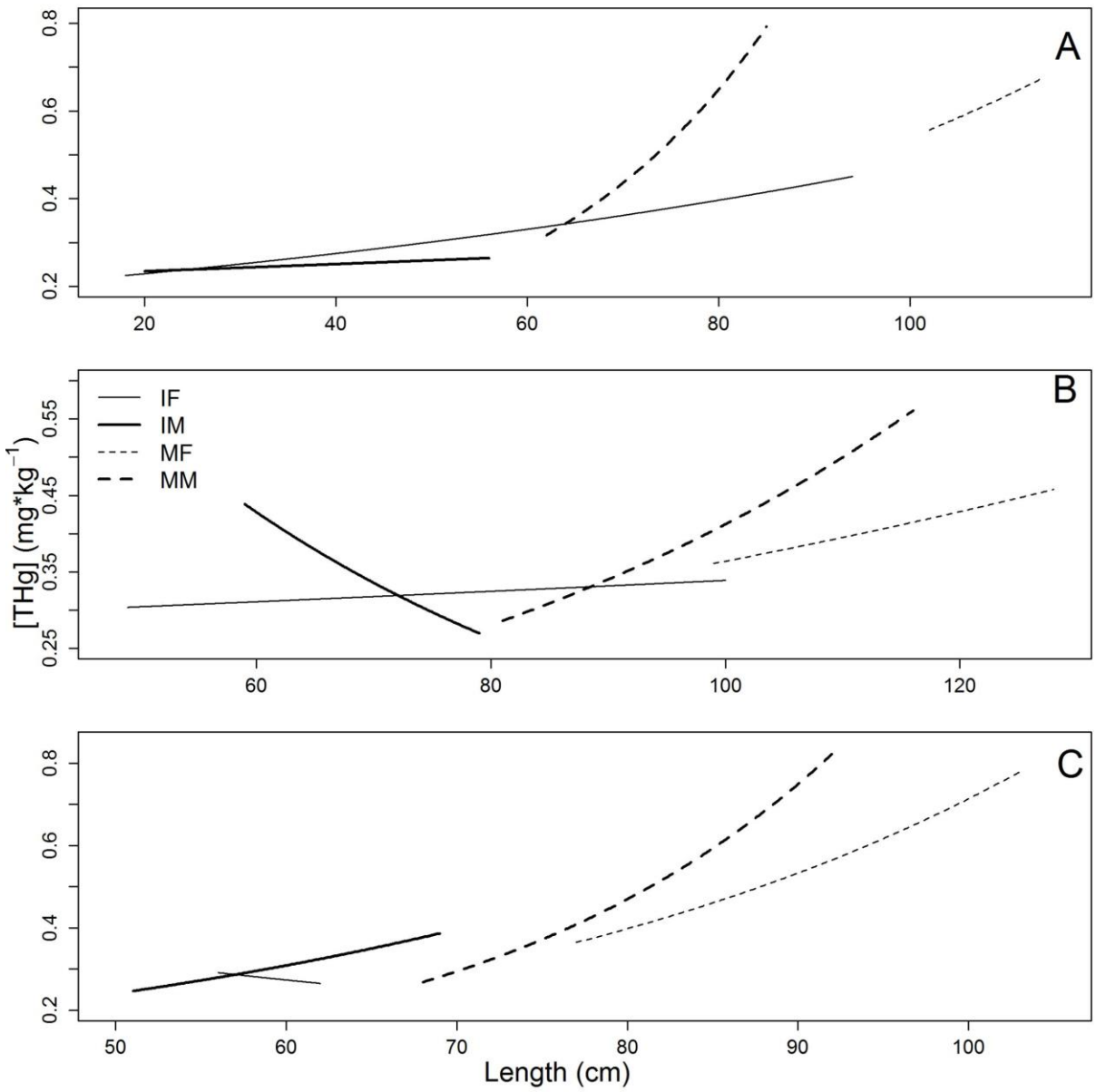

Figure 2.2. [THg] vary with length and sex in muscle from each group (IF, IM, MM and MF) of: A. bat ray, B. shovelnose guitarfish, C. banded guitarfish collected in Bahía Tortugas.

For liver, the best approximating a priori model describing [THg] in the bat ray was DW and sex and was 2.54 units from the next best model, which only considers DW, and this was 4.01 units from the next best model which included DW and sex

interaction (Table 4). These three models carried all the AIC model weight. The [THg] increased with length ($F=84.76$, $p<0.001$) and this increase varied by sex, being higher but not steeper as the slopes appear similar for MM than MF, and for MF than IF and IM ($F=13.04$, $p<0.001$; Fig. 2.3.A). Also, the interaction between DW and sex was significant ($F=12.07$, $p<0.001$).

According to the AIC value, the best model describing [THg] in the shovelnose guitarfish liver included only TL and was two units from the next best model, which included TL and sex and was 3.18 units from the next best model which included the interaction (TL and sex; Table 4). The top two models carried 0.86 of the AIC model weight. The [THg] increased with body size ($F=8.21$, $p=0.006$; Fig. 2.3.B). However, sex ($F=1.28$, $p=0.29$) and the interaction between TL and sex were not significant ($F=1.29$, $p=0.28$).

Finally, the best approximating a priori model describing [THg] in the banded guitarfish liver included the interaction between TL and sex and was 15.09 units from the next best model, which also included TL and sex but without the interaction (Table 4). The top model carried all AIC model weight. The [THg] increased with body length in MF and MM but decreased in IF and IM ($F=59.58$, $p<0.001$; Fig. 2.3.C).

Table 2.4. Models that explain [THg] variability in the liver of the three ray species from Bahía Tortugas BCS, Mexico. Explanatory variables include length (DW: disc width for the bat ray and TL: total length for the shovelnose guitarfish and the banded guitarfish) and sex. The model with the lowest AIC value is in bold.

	Variable	Coefficients	CI	AIC	W	% Explained deviance
Bat ray	DW	7.50×10^{-3}	1.08×10^{-3}	-16.76	0.20	42.21
	Sex	-	-	-1.95	0.00	32.25
	Sex (IF)	-	-	-	-	-
	Sex (IM)	-2.66×10^{-2}	6.61×10^{-2}	-	-	-
	Sex (MF)	4.36×10^{-1}	1.37×10^{-1}	-	-	-
	Sex (MM)	3.94×10^{-1}	8.85×10^{-2}	-	-	-
	DW + sex	-	-	**-19.30**	**0.70**	**49.03**
	DW*sex	-	-	-15.30	0.10	50.51
	DW*sex(IF)	-	-	-	-	-
	DW*sex (IM)	3.27×10^{-3}	4.33×10^{-3}	-	-	-
	DW*sex (MF)	-9.32×10^{-3}	1.21×10^{-2}	-	-	-
	DW*sex (MM)	1.02×10^{-2}	1.32×10^{-2}	-	-	-
Shovelnose guitarfish	**TL**	7.63×10^{-3}	2.69×10^{-3}	**6.97**	**0.63**	**10.86**
	Sex	-	-	15.32	0.01	4.97
	Sex (IF)	-	-	-	-	-
	Sex (IM)	-4.27×10^{-1}	2.65×10^{-1}	-	-	-
	Sex (MF)	5.06×10^{-2}	8.16×10^{-2}	-	-	-
	Sex (MM)	2.29×10^{-2}	7.42×10^{-2}	-	-	-
	TL + sex	-	-	8.97	0.23	15.95
	TL*sex	-	-	10.15	0.13	19.37
	TL*sex (IF)	-	-	-	-	-
	TL*sex (IM)	-	-	-	-	-
	TL*sex (MF)	-8.61×10^{-3}	9.87×10^{-3}	-	-	-
	TL*sex (MM)	6.36×10^{-3}	8.84×10^{-3}	-	-	-
Banded guitarfish	TL	2.29×10^{-2}	3.53×10^{-3}	77.64	0.00	33.40
	Sex	-	-	102.06	0.00	14.51
	Sex (IF)	-	-	-	-	-
	Sex (IM)	2.48×10^{-1}	2.44×10^{-1}	-	-	-
	Sex (MF)	5.94×10^{-1}	2.36×10^{-1}	-	-	-
	Sex (MM)	6.33×10^{-1}	2.19×10^{-1}	-	-	-
	TL + sex	-	-	67.65	0.00	44.69
	TL*sex	-	-	**53.44**	**1.00**	**56.28**
	TL*sex (IF)	-	-	-	-	-
	TL*sex (IM)	-3.36×10^{-3}	7.38×10^{-2}	-	-	-
	TL*sex (MF)	4.83×10^{-2}	7.31×10^{-2}	-	-	-
	TL*sex (MM)	7.10×10^{-2}	7.27×10^{-2}	-	-	-

CI: confidence interval. AIC: Akaike's information criterion. W: AIC weight.

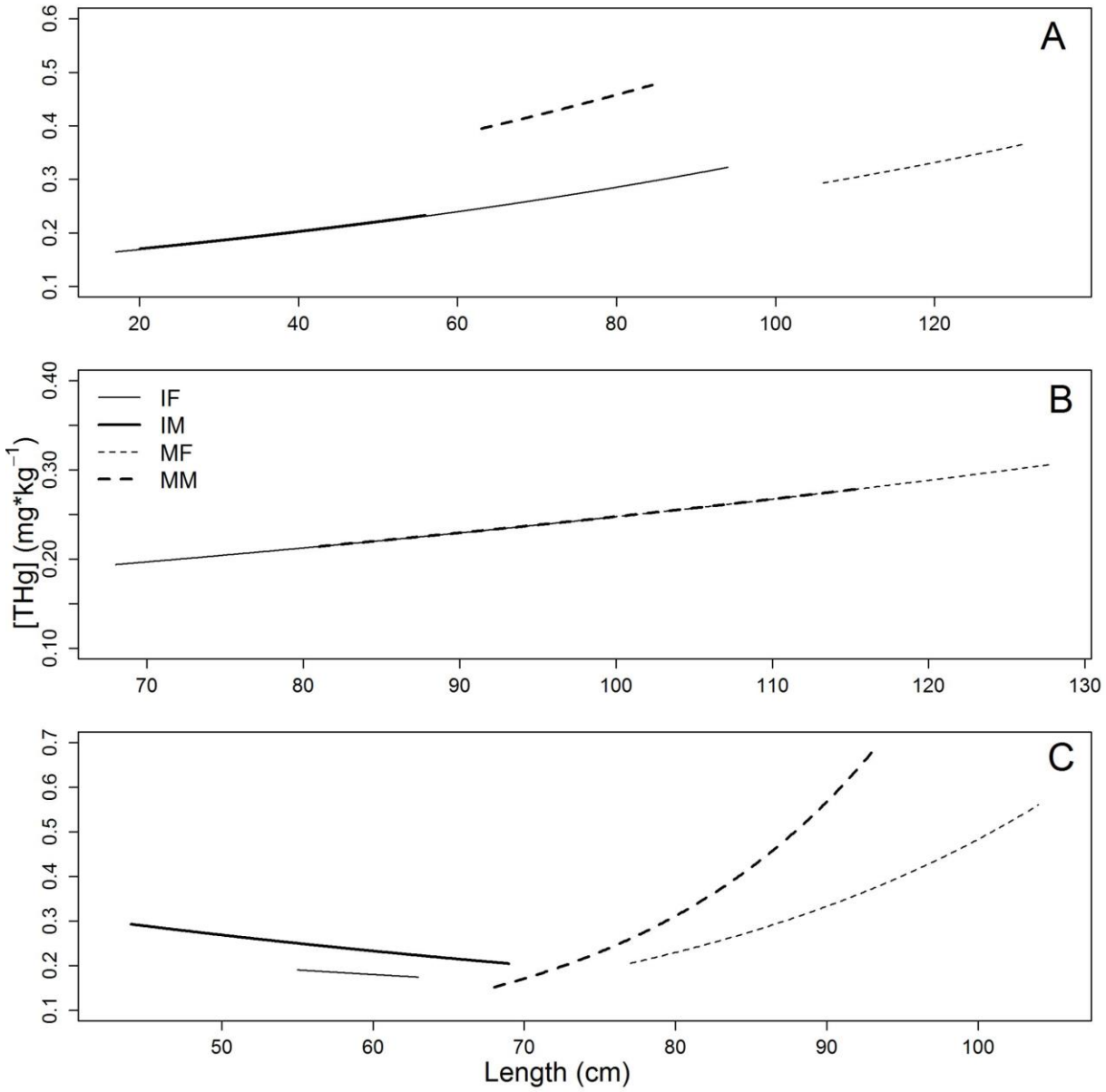

Figure 2.3. [THg] vary with length and sex in liver from each group (IF, IM, MM and MF) of: A. bat ray, B. shovelnose guitarfish, C. banded guitarfish collected in Bahía Tortugas.

2.4.3. Human health risk

All individuals from each species had muscle [THg] below the permissible limit in predatory fish for human consumption (1 mg kg^{-1} ww) set by international agencies,

such as US Food and Drug Administrations (FDA), World health Organization (WHO) and the Mexican Official Norm (NOM 242-SSA1, 2009). Two (2.4%) bat ray, 2 (2.1%) shovelnose guitarfish, and 11 (12.6%) banded guitarfish individuals exceeded the advisory threshold established for the majority of retail fish 0.5 mg kg^{-1} ww in Mexico and others countries such as Canada and US (Canadian Food Inspection Agency 1998; US Food and Drug Administration 2007; NOM 242-SSA1, 2009). Additionally, 8 (11%) bat ray, 10 (10%) shovelnose guitarfish, and 39 (45%) banded guitarfish exceeded the unrestricted consumption threshold set by Alaska Scientific Advisory Committee for Fish Consumption of 0.2 mg kg^{-1} ww (Hamade, 2014). These limits are for $MeHg^{+}$, which comprises 70-100% of the [THg] in the muscle of rays (Storelli *et al*., 2002; Horvat *et al*., 2014); therefore, the measurement of THg provides conservative a conservative estimate on the risk for the $MeHg^{+}$ intake.

The EWI of THg in each species of rays of this study were below the PTWI. The EWI for the bat ray ranged from 0.05 to 1.66 µg kg^{-1} bw (average of 0.23 µg kg^{-1} bw). The shovelnose guitarfish showed a EWI ranged between 0.09 to 0.60 µg kg^{-1} bw (average of 0.29 µg kg^{-1} bw), and a range of 0.09 to 2.18 µg kg^{-1} bw (average of 0.60 µg kg^{-1} bw) for the banded guitarfish. Thus, an adult of 70 kg bw can consume a maximum allowable weekly intake between 0.54 kg to 17.50 kg for the bat ray (average of 3.89 kg), 0.51 kg to 8.75 kg (average of 2.92 kg) for the shovelnose guitarfish and 0.42 kg to 11.67 kg (average of 1.46 kg) for the banded guitarfish.

2.5. DISCUSSION

The three ray species in this study are important cultural and food resources to some human populations, especially in local coastal areas from the PCBCS (Ramirez-Amaro *et al*., 2013). However, we know of only two studies from the Gulf of California that assessed [THg] in the same species in our study (García-Hernández *et al*., 2007; Ruelas-Inzunza *et al*., 2013). Muscle [THg] found in this work for the bat ray and the banded guitarfish were similar to those reported by García-Hernández *et al*., (2007), for cohorts of similar body size (<detection limit to 0.15 mg kg^{-1} ww and <detection limit to 0.28 mg kg^{-1} ww, respectively), but lower than [THg] values for the shovelnose

guitarfish (<detection limit-2.04 mg kg^{-1} ww). Ruelas-Inzunza *et al*., (2013) reported higher muscle and liver [THg] for two individuals of the shovelnose guitarfish and similar [THg] for one individual of the banded guitarfish. Storelli *et al*., (2002) reported higher [THg] in the electric ray (*Torpedo. nobiliana*) and the eagle ray (*Myliobatis aquila*) from the Mediterranean Sea (2.42 mg kg^{-1} ww and 0.83 mg kg^{-1} ww, respectively) than our study. However, these apparent differences must be interpreted carefully as sex, analytical variability, lower sample number than our study, and the amount of bioavailable $MeHg^{+}$ between study areas, could be significant variation factors.

2.5.1. Muscle and liver comparisons

In this study, muscle and liver tissues were selected as they are known to accumulate [THg] making it easy to measure for many fish species. Liver is an important tissue in metal metabolism and often evaluated in many Hg-monitoring studies, while muscle is critical for assessing the risk to human health (Pethybridge *et al*., 2010). Muscle showed significantly higher [THg] than those in the corresponding liver samples for each species of rays analyzed, as evidenced for other elasmobranchs species (Branco *et al*., 2007; Pethybridge *et al*., 2010; Ruelas-Inzunza *et al*., 2013; Horvat *et al*., 2014; Gilbert *et al*., 2015; Nicolaus *et al*., 2016), while other studies in sharks have reported higher concentrations in liver than muscle (Endo *et al*., 2008, 2013, 2015). These results suggest that [THg] significantly varies throughout the body and may be stored in muscle tissue, which constitute a long-term sink for $MeHg^{+}$ with slow depuration rates (Amlund *et al*., 2007; Pethybridge *et al*., 2010). Higher [THg] in muscle are related to the transport of $MeHg^{+}$ bound to thiol ligands of amino acids (e.g. cysteine) that presumably are transported mostly to the muscle tissue for protein synthesis (Leaner and Mason, 2004; Régine *et al*., 2006). In contrast, liver (as well as kidney) has been shown to have much higher depuration rates than muscle (e.g. in the juvenile seabass *Dicentrarchus labrax*; Maulvault *et al*., 2016) in some species. Moreover, elasmobranchs have liver with relatively high lipid content that may affect [Hg] (Endo *et al*., 2015). Endo *et al*., (2013) showed that the greater lipid content observed in the liver of the spiny dogfish (*Squalus acanthias*) may lower [THg]

compared to star-spotted dogfish (*Mustelus manazo*). However, in our study, there were a few MM organisms from the larger sizes in each ray species where [THg] in liver were higher than muscle. This result is similar to the pattern found in the tiger shark *Galeocerdo cuvier* from the coast of Japan, where [THg] were higher in muscle than liver, except for the six larger sharks in which liver had higher [THg] (Endo *et al.*, 2008). The dusky shark (*Carcharhinus obscurus*) and the sandbar shark (*C. plumbeus*) from Australian waters, also showed higher [THg] in muscle than liver. However, when maximum length for both species was reached, liver [THg] was approximately three to four times higher than muscle (Gilbert *et al*., 2015). However, in order to better understand the variation in [THg] in those studies, it is necessary to assess sex-maturation cohorts independently for liver and muscle comparison along with size. These findings related to [THg] possible age and sex dependent differences between muscle and liver may be related to the different energy or nutritional requirements of large sharks, ontogenetic changes in diet or a high turnover and metabolic activity of liver tissue (Endo *et al*., 2008; Gilbert *et al*., 2015).

2.5.2. Sex and size (sexual dimorphism)

Increased body size associated with an increase in [THg] in teleosts and elasmobranchs may include changes in trophic position (Rumbold *et al*., 2014). Furthermore, body size represents an important variable that may reflect ecological, physiological and morphological changes that could affect THg accumulation (e.g. hunting ability, changing energy demands, ontogenetic changes in diet, excretion rates, dilution effect; Trudel and Rasmussen, 2006; Rumbold *et al*., 2014). Sex is reported to influence Hg accumulation in elasmobranchs (Penedo de Pinho *et al*., 2002; Pethybridge *et al*., 2010), probably as a result of the differences in the energetic requirements, maturation condition, growth rates and maternal offloading to embryos between males and females (Pethybridge *et al*., 2010; Endo *et al*., 2013). In this study, body size and sex had a strong effect on [THg] in each ray species. The [THg] increased with body size and this increase was more marked in mature than immature individuals, and more marked in MM than MF. This trend has been previously reported

in the spiny dogfish shark (*Squalus acanthias*), Star spotted dogfish shark (*Mustelus manazo*) and the tiger shark (*Galeocerdo cuvier*). This marked increase could be due to the slowing of growth at maturity, and sex-specific differences in growth rate and ultimate size (Endo *et al*., 2009, 2013, 2015). It is very common that elasmobranchs display sexual dimorphism, where females had higher growth rates and are larger than males, especially in mature stages (Barbosa-Martins *et al*., 2015) so that, males are older than females of the same size. Another possible explanation for this result may be related to maternal transfer to developing embryos, resulting in differences in the accumulation of THg between male and female (Pethybridge *et al*., 2010). In contrast, muscle of the IM shovelnose guitarfish and IF banded guitarfish, as well as, IF and IM of the banded guitarfish liver, [THg] decreased with the increasing body size. It is possible that [THg] undergoes growth dilution (García-Hernández *et al*., 2007). Caution must be taken with interpreting some of these results since some cohorts have low number of samples.

2.5.3. Permissible consumption limit and human health risk considerations

No individuals analyzed had muscle [THg] above the permissible limit of 1 mg kg^{-1} (ww) set by various agencies, such as US Food and Drug Administrations (FDA), World health Organization (WHO) and the Mexican Official Norm (NOM 242-SSA1, 2009). For the threshold established for the majority of retail fish (0.5 mg kg^{-1} ww) in Mexico and other countries (Canadian Food Inspection Agency 1998; US Food and Drug Administration 2007; NOM 242-SSA1, 2009) approximately 2-13% exceeded that level for the three species. For the more conservative advisory threshold (for unrestricted consumption) set by Alaska Scientific Advisory Committee for Fish Consumption of 0.2 mg kg^{-1} ww (Hamade, 2014) a larger range of individual rays by species exceeded this level, approximately 10-45% depending on species. We noted that these findings were related to the apparent propensity for muscle [THg] to be higher than liver. We emphasize that a size and sex based advisory may be required since we note that these drivers alter [THg] and that larger fish may be important to address as

compared to immature fish. Size based consumption advisories are well known, such as in Alaska (Hamade, 2014).

Considering the EWI, based on the amount of edible muscle of rays consumed, the relative level of risk of THg toxicity is low. However, one must take into consideration that specific sectors of the population, such as fisheries communities, include more fish (relative to an overall population average) into their diets, so the level of risk is difficult to assess based on single species. Thus, we emphasize the EWI calculations or for context related to Hg exposure and need for concern. In the present study, some of the larger organisms from each species (mature organisms), especially in the banded guitarfish, displayed the maximum allowable weekly intake of fish that an adult person can consume (approximately half a kg). This is of moderate concern that requires investigation of the fisheries communities to assess how much is consumed. Therefore, more detail of the rate intake of elasmobranchs in these communities is necessary to properly asses the level of risk.

2.5.4. Feeding ecology considerations

It is widely recognized diet is the main pathway for Hg to top predators, and since Hg increases concentration through the aquatic food web (biomagnification), the Hg content of the diet and trophic position of a species are usually considered key factors determining Hg concentrations in tissues and variability (Penedo de Pinho *et al.*, 2002; Pethybridge *et al*., 2010; Horvat *et al.*, 2014; Kim *et al.*, 2016; Sandoval-Herrera *et al*., 2016). This was demonstrated by Horvat *et al*., (2014) who reported higher [THg] in the pelagic stingray, *Dasyatis violacea,* that feeds on pelagic fish, compared to lower [THg] in the eagle ray, *Myliobatis Aquila* and the bull ray, *Pteromylaeus bovinus,* whose diets comprise mostly benthic invertebrates. In our study, bat ray had lower [THg] in muscle for IF, IM and MF (not MM) than the other two rays. These differences could be driven by trophic level in that the bat ray reported trophic position of 3.46 and feed heavily on crustacean such as the mantis shrimp *Hemisquilla ensigera californiensis*, and the crabs *Dynomene spp.* (Torrés-García, 2015). Whereas 1) the shovelnose guitarfish feeds mostly on crustacean like the sand crab *Blepharipoda occidentalis*, the arched

swimming crab *Callinectes arcuatus* and also includes fish in the diet (*Synodus sp*) with a trophic position of 3.83 (Curiel-Godoy *et al*., 2016); and 2) the banded guitarfish showed higher [THg] and feeds at a higher trophic level (4.1) that feeds mainly upon the red crab (*Pleroncodes planipes*) and includes more fish in the diet, such as the plainfin midshipman *Porichtys notatus, Porichtys sp*. and *Synodus sp,* (Vázquez-Moreno, 2015). It is important to note those studies used the same individuals as in our study. These food habits difference could be related to the inter-specific differences in [THg] found in this study since fish is the most significant source of dietary exposure to $MeHg^{+}$ for consumers (Matos *et al*., 2015). However, other specific factors could also play a role in [THg], like inter-species variability in metabolism, physiology and growth rates (Pethybridge *et al*., 2010) that could be sex-based as we shown here for drivers of [Hg] in liver and muscle of the three rays.

2.6. CONCLUSION

Our results demonstrate that length and sex are important factors explaining the variation in [THg] in each species. Our data strongly suggest that sex plays a critical role in determining [THg], therefore, we suggest that sex-maturation cohorts should be assessed independently for liver and muscle in elasmobranchs Hg studies. In this study, here was a wide range of [THg] in each species, with muscle [THg] higher than the relative conservative unrestricted consumption advisory threshold of 0.2 mg kg^{-1} ww (Hamade, 2014) in some individuals. Although, the EWI did not represent a high risk to human health, the maximum allowable weekly intake of some individuals from each species would be only 0.5 kg. This may warrants follow up investigations that could also include the parallel analysis of other elements such as selenium, which has been suggested to have an important ecological role, as an antagonist against the toxicity of Hg forms in aquatic organisms (Branco *et al*., 2012). Additionally, our study highlights that advisories should be based on size and sex since they are critical drivers in the [THg] variations observed in these 3 ray species.

CHAPTER 3. ISOTOPIC NICHE OF THREE SYMPATRIC BAJA CALIFORNIA SUR PACIFIC RAY SPECIES, *PSEUDOBATOS PRODUCTUS*, *ZAPTERYX EXASPERATA* AND *MYLIOBATIS CALIFORNICA*

3.1. ABSTRACT

Along the Pacific coast of Baja California Sur (PCBCS), the banded guitarfish (*Zapteryx exasperata*), shovelnose guitarfish (*Pseudobatos productus*) and bat ray (*Myliobatis californica*) are highly abundant. Their ecological roles as predators in demersal communities can be key in this ecosystem. To better understand their trophic relationship in the PCBCS, stable isotopes analysis of carbon and nitrogen were used. Muscle samples (n=265) were collected from shovelnose guitarfish (n=94), banded guitarfish (n=87) and bat ray (n=84). We observed high variability in isotopes values, $\delta^{13}C$ and $\delta^{15}N$ of shovelnose guitarfish ranged from -18.53 to -12.85‰ and 15.93‰ to 20.37‰, respectively, banded guitarfish from -18.12‰ to -13.57‰ and 14.41‰ to 19.26‰, respectively; and bat ray from -17.73‰ to 13.98‰ and 13.97‰ to 18.46, respectively. Statistical significant Inter-specific differences were found ($p<0.05$) for $\delta^{13}C$ and $\delta^{15}N$, where bat ray showed more depleted values for $\delta^{15}N$ and more enriched for $\delta^{13}C$ than the other species. Isotopic niche analysis using Bayesian ellipses (SEAc) showed that shovelnose guitarfish occupies the highest isotopic niche (TA and SEAc) compared with bat ray and banded guitarfish. Banded guitarfish overlap in a 0.50 with the shovelnose guitarfish. The bat ray overlapped 0.38 and 0.39 with banded and shovelnose guitarfish. These suggests that the shovelnose and banded guitarfish share feeding resources and habitat use but both species partitioning resources with the bat ray.

Key words: carbon, nitrogen, stable isotopes, trophic ecology, niche overlap.

3.2. INTRODUCTION

Information about trophic ecology of a particular species is fundamental to understanding its role in the ecosystem (Yemisken *et al*. 2017). Batoids (rays) are considered essential components of food webs, playing an influential role in the demersal communities as an important link between other food web compartments (Ebert and Bizarro 2007; Bornatowski *et al*. 2014). Therefore, knowledge of their trophic ecology is important for management and conservation strategies (Blanco-Parra *et al*. 2012).

Stable isotopes analysis of carbon ($^{13}C/^{12}C$, reported as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, reported as $\delta^{15}N$) are frequently used to assess trophic ecology and habitat use of elasmobranchs (Fink *et al*. 2012). Stable isotopes values represent assimilated food (C and N), rather than just consumed prey items, that are varyingly time dependent based on tissue type assessed and its turnover rate (Speed *et al*. 2012; MacNeil *et al*. 2005). In general, values of $\delta^{13}C$ slightly increase as trophic level (TL) increases (about 1.0 ‰ per TL), and are used to track sources of primary production in the food web which vary according to origin. For example, inshore environments have more enriched ^{13}C values with respect to the offshore (pelagic) environments (Kinney *et al*. 2011). The $\delta^{15}N$ value predictably increases around 3-5‰ per TL (predator tissue composition relative to prey) providing a powerful tool to calculate trophic positions of organisms (Layman *et al*. 2012).

The banded guitarfish (*Zapteryx exasperata*), shovelnose guitarfish (*Pseudobatos productus*) and bat ray (*Myliobatis californica*) are highly abundant ray species in the PCBCS (Ramirez-Amaro *et al*. 2013) with important predator in demersal communities. Despite high abundance in the fisheries and potential importance to benthic communities, no published studies were found on their trophic relationship in the NWBCS. Therefore, the main goal of this study explores their intra and inter specific variation in trophic ecology by sex and maturation stage in each species of ray using of $\delta^{13}C$ and $\delta^{15}N$ values.

3.3. MATERIAL AND METHODS

3.3.1. Specimen data and sample collection

Muscle samples were collected in March-April, August-September and November of 2014 in Bahía Tortugas (27 ° 39'35 "N; 114 ° 52'35" W) located on the west coast of Baja California Sur, Mexico. Specimens were donated by local fisherman using gill nets to catch various fish species. Size (total length and disc width) and sex were recorded for each individual. Sexual identification was determined by the presence of claspers in males. The four sex and maturation classes are presented in Table 1 for each species. Specific cohorts by species were identified as immature female (IF), mature female (MF), immature male (IM), and mature male (MM) based on Murillo-Cisneros *et al.* (2018). Maturity stage was assigned according to species-specific morphometric criteria. A disc width for the male of >62 cm and female of >88.1 cm are considered mature for the bat ray (Martin and Caillet, 1988). A male individual with a body size >80 cm and female of >100 cm are considered mature for the shovelnose guitarfish (Downton-Hoffmann, 2007). Total length for the male >69 cm and female >77 cm are deemed mature for the banded guitarfish (Villavicencio-Garayzar, 1995). For each specimen, between 5-30 g of muscle (dorsal side near the head) were collected and placed in plastic bags. All samples were kept on ice in coolers and transported to the laboratory at Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (CICIMAR-IPN, La Paz, BCS, Mexico) and stored frozen at −20 °C.

In the laboratory, all tissues were sub-sampled using a clean stainless steel scalpel and stored at −20 °C in 2 ml Eppendorf tubes. Samples were freeze-dried (Labcono, FreeZone 2.5 Liter) for 24-48 h and homogenized using an agate mortar and pestle. 1 mg of each sample was weighed on an analytical microbalance and placed in tin capsules of 3.5 x 5 mm.

3.3.2. Carbon and nitrogen stable isotopes analysis

C and N stable isotopes values were determined in the Mass Spectrometry Isotopic Laboratory (LEsMA) at the Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (CICIMAR-IPN, La Paz, BCS, Mexico) using a mass spectrometer (Delta V Plus Thermo Scientific) with continuous flow coupled to an elemental analyzer (Elemental Combustion System Costech Instruments) in a similar manner to Estupiñán-Montaño *et al*. (2017).

Stable isotopes ratios of the sample and standards were reported in δ notation and expressed as part per thousand (‰) relative to standards and were calculated using the following formula:

$$\delta^{15}N \text{ or } \delta^{13}C = [(R \text{ sample}/R \text{ standard})-1] \times 1000 \ (‰)$$

The standards used were atmospheric N for $\delta^{15}N$ and Pee Dee Belemnite for $\delta^{13}C$ (Hussey *et al*. (2010). The analytical error of the $\delta^{15}N$ and $\delta^{13}C$ values was approximately 0.2‰.

Ten subsamples of each species were lipid and urea extracted with petroleum ether and deionized water following Kim and Koch (2012) method, in order to compare to untreated matched samples.

3.3.3. Statistical analysis

Data were grouped by sex and maturity stage for each species of ray as follows: IF= immature female, MF= mature female, IM= immature male and MM= mature male.

The isotopic result of samples with lipid and urea extracted were compared to untreated tissue using Wilcoxon matched pairs test. Normality and homogeneity of variance were assessed using Kolmogorov-Smirnov and Bartlett tests. Kruskal-Wallis tests was used to make statistical comparisons between each sex-maturation cohort within each species. Differences by species were assessed using Mann-Whitney U-test (all sex-maturation cohort from one species pooled together compared to all sex maturation cohorts of the other species). Differences among species by cohort (e.g.,

MM by species) were analyzed using Student t-test or Mann-Whitney U-test. To assess the relationship between body size and isotopic values ($\delta^{13}C$ and $\delta^{15}N$), non-parametric Spearman linear regression was used. In order to detect outliers from each group of data, Grubbs outlier test was performed. Statistical analyses were repeated excluding outliers to establish the potential effect of those individuals on the results where no effect was detected. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using Statistica 8.0 (statSoft Inc. Tulsa, OK, USA).

3.3.4. Isotopic Niche Width and Trophic Overlap

In order to measure isotopic niche for each species and by each sex-maturation cohort within each species, according to their individual isotopic signatures, the convex hull area (TA) was calculated, which is the total amount of niche space occupied for a given species in a $\delta^{13}C$-$\delta^{15}N$ bi-plot space (Layman *et al*. 2007). We calculated the standard ellipse area as an estimate of isotopic niche width in a bivariate $\delta^{13}C$ and $\delta^{15}N$ space generated with Bayesian inference and corrected in order to reduce bias for small sample size (SEAc). The niche area (SEAc) is defined as the area occupied in bi-plot space in $‰^2$ (Jackson *et al*. 2011). The isotopic niche overlap between species and between each sex-maturation cohort within each species was calculated. These analyses were made using R Programming language (Rstudio, v. 3.4.2, 2017) with the SIBER package.

3.4. RESULTS

The mean $\delta^{13}C$ of -16.74 ± 0.81‰ for the extracted samples was not statistically different from the -16.61 ± 0.90‰ ($p>0.05$). The $\delta^{15}N$ showed a significant difference ($p<0.05$) with an average increase in the $\delta^{15}N$ values of 0.5 ± 0.2‰ following the treatment. As expected, the C:N ratio showed a significant difference between the treatment and untreated samples ($p<0.05$). Mathematical correction to account for urea content was established using a linear model with the three species combined,

with a slope of 0.876 (95% CI [0.808, 0.944]) and an intercepts of 2.6124 (95% CI [1.437, 3.787]).

3.4.1. General stable isotope results

The stable isotopes signatures of the three ray species by each sex-maturation cohort (IF, MF, IM and MM) of this study are presented in Table 1. The $\delta^{13}C$ and $\delta^{15}N$ values of the shovelnose guitarfish showed a wide variability ranging from -18.53 to -12.85‰ and 15.93‰ to 20.37‰, respectively. The banded guitarfish also showed a wide range in $\delta^{13}C$ and $\delta^{15}N$ values, ranging from -18.12‰ to -13.57‰ and 14.41‰ to 19.26‰ but not as high as the shovelnose guitarfish. The ranges of $\delta^{13}C$ and $\delta^{15}N$ were -17.73 to -13.98‰ and 13.97 to 18.46‰, respectively for the bat ray. Furthermore, the bat ray presented two outliers (IF: 13.97‰ and MM: 15.97‰) and banded guitarfish one outlier in the $\delta^{15}N$ values (IF: 14.41‰).

Table 3.1. Mean ± standard deviation (SD), minimum (Min), and maximum (Max) of $\delta^{15}N$ and $\delta^{13}C$ values in muscle for all animals by species and by their maturity-sex cohort of bat ray, shovelnose guitarfish and banded guitarfish from Bahía Tortugas BCS (Mexico).

			$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Species	n	cohort	Min	Max	Min	Max	Mean±SD	Mean±SD
	46	IF	-17.24	-14.13	13.97	18.20	-15.90 ± 0.79	16.98 ± 0.78
	3	MF	-15.21	-14.90	15.91	17.20	-15.10 ± 0.18	16.57 ± 0.64
Bat ray	23	IM	-17.73	-13.98	15.60	17.47	-16.07 ± 0.86	16.69 ± 0.53
	12	MM	-16.99	-14.63	15.97	18.46	-16.00 ± 0.63	17.77 ± 0.68*
	84	ALL	-17.73	-13.98	13.97	18.46	-15.93 ± 0.78[a]	17.00 ± 0.77[a]
	36	IF	-18.53	-13.82	16.21	19.69	-16.75 ± 1.12	17.86 ± 0.78
	21	MF	-17.74	-15.59	16.49	18.43	-16.72 ± 0.54	17.60 ± 0.53
Shovelnose	3	IM	-17.63	-15.97	17.59	18.12	-16.68 ± 0.86	17.90 ± 0.28
guitarfish	34	MM	-17.56	-12.85	15.93	20.37	-16.03 ± 1.18*	17.95 ± 0.99
	94	ALL	-18.53	-12.85	15.93	20.37	-16.48 ± 1.08	17.84 ± 0.81
	6	IF	-17.15	-13.57	14.41	19.01	-15.90 ± 1.56	17.52 ± 1.60
	18	MF	-17.34	-15.26	17.07	19.21	-16.45 ± 0.58	17.98 ± 0.56
Banded	8	IM	-17.54	-15.22	16.30	19.26	-16.39 ± 0.77	17.61 ± 0.96
guitarfish	55	MM	-18.12	-14.61	16.37	18.91	-16.70 ± 0.65	17.77 ± 0.45
	87	ALL	-18.12	-13.57	14.41	19.26	-16.57 ± 0.76	17.78 ± 0.65

IF: immature female; MF: mature female; IM: immature male; MM: mature male; n: number of samples. * denotes intra-specific significant difference, [a] denotes inter-specific significant differences.

3.4.2. $\delta^{15}N$ analysis

No intra-specific differences between the sex-maturation cohorts within the banded and shovelnose guitarfish $\delta^{15}N$ values were found ($p>0.05$). The bat ray showed significant differences between maturations cohorts ($p<0.05$), MM had higher $\delta^{15}N$ values than IF and IM (Table 3.1). Between species, significant differences were observed for $\delta^{15}N$ values ($p<0.05$), the bat ray presented lower values (17.00 ± 0.77‰) relative to the shovelnose guitarfish (17.84 ± 0.81‰) and banded guitarfish (17.78 ± 0.65‰; Table 3.1). The relationship between body size and $\delta^{15}N$ values indicated a significant relationship for shovelnose guitarfish males. However, this relationship was relatively weak and negative ($Rs=-0.36$, $p<0.05$) while females showed no relationship

(Rs=-0.26, p>0.05). Bat ray males showed an increase in their $\delta^{15}N$ values with size (Rs= 0.66, p<0.05) while females did not (Rs= 0.09, p>0.05). The banded guitarfish showed no increase in $\delta^{15}N$ with body size in females (Rs=-0.14, p>0.05) and males (Rs=0.17, p>0.05). When outliers were removed, the statistical significance observed did not change.

3.4.3. $\delta^{13}C$ analysis

The $\delta^{13}C$ values showed significant differences between the sex-maturation cohort in the shovelnose guitarfish (p=0.01), with a less negative value for MM (-16.03 ± 1.18‰) compared to the IF (-16.75 ± 1.12‰) and MF (-16.72 ± 0.54‰; Table 1). The IM cohort was excluded from the analysis due the low sample number. For the bat ray and banded guitarfish, no intra-specific differences were observed for $\delta^{13}C$ values (p>0.05; sex-maturation cohort). Between species, significant differences were found (p<0.05) as the bat ray showed higher $\delta^{13}C$ values (-15.92 ± 0.78‰) than the shovelnose guitarfish (-16.48 ± 1.08‰) and banded guitarfish (-16.57 ± 0.76‰). Furthermore, $\delta^{13}C$ showed no relationship with body size in females and males of each species (bat ray, females: Rs= 0.26, p>0.05; males: Rs= 0.10, p>0.05; shovelnose guitarfish, females: Rs= 0.20, p>0.05; males: Rs= -0.11, p>0.05; banded guitarfish females: Rs= 0.19, p>0.05; males: Rs= 0.09, p>0.05).

3.4.4. Isotopic niche

Overall, the shovelnose guitarfish occupied the largest isotopic niche (TA and SEAc) compared with the bat ray and banded guitarfish (Table 3.2, Fig. 3.1). Furthermore, the standard ellipse area of the banded guitarfish overlapped 0.60 with the standard ellipse area of the shovelnose guitarfish. However, excluding the IF outlier of the banded guitarfish ($\delta^{15}N$= 14.41‰ and $\delta^{13}C$= -13.57‰), the width of the standard ellipse changed and the overlap decreased to 0.46 relative to the standard ellipse of the shovelnose guitarfish. The bat ray overlap was 0.38 and 0.39 with the banded and

the shovelnose guitarfish, respectively. In this case, the result with and without outliers was very similar.

Table 3.2. Isotopic niche (‰^{2}) for the two ray species and their sex-maturation cohort with no bat ray and banded guitarfish outliers. TA: convex area; SEAc: corrected standard ellipse area.

Species		IF	MF	IM	MM	ALL
Bat ray	TA	6.70	0.10	4.60	1.10	8.46
	SEAc	1.62	0.36	1.45	0.60	1.70
Shovelnose guitarfish	TA	7.95	2.67	0.21	9.82	12.33
	SEAc	2.56	0.94	0.76	2.95	2.44
Banded guitarfish	TA	1.12	2.57	3.12	5.15	6.21
	SEAc	1.60	0.95	2.22	0.89	1.08

IF: immature female; MF: mature female; IM: immature male; MM: mature male

The intra-specific analysis by cohort showed immature cohorts of the banded guitarfish (IF and IM) had the highest SEAc values, with 8.41‰^{2} and 2.22‰^{2}, respectively, relative to the mature cohorts. Removing the IF outlier from the analysis, the SEAc value declines to 1.60‰^{2} (Table 3.2; Fig. 3.1). Furthermore, the overlapping of the IF with other cohorts was very low (from 0.12 to 0.26) and increased when removing the IF outlier (from 0.32 to 0.34). This suggests a major effect over the isotopic niche of the banded guitarfish due the outlier contained in the IF cohort. Furthermore, the mature cohorts had the highest overlap in this species, in which the MF overlapped 0.57 with the MM for this species. Regarding the shovelnose guitarfish, the MM and IF were the cohorts with the widest isotopic niche (SEAc: 2.95‰^{2} and 2.56‰^{2,} respectively; Table 3.2, Fig. 3.1.C) which is similar to the value found for the banded guitarfish IM. In this case, we must consider the low sample size of the IM (n=3) of the shovelnose guitarfish under estimating the SEAc value (Jackson *et al*., 2011). In contrast to the banded guitarfish, the shovelnose guitarfish IF presented the highest overlap with the MM (0.60), whereas the rest had a lower overlap (from 0.16 to 0.34).

For the bat ray, IF was the cohort with the widest isotopic niche (SEAc: 1.94‰^{2}) and removing the outlier within this cohort the SEAc value declined to 1.62‰^{2}. Despite this adjustment, this cohort remained with the highest SEAc value (Table 3.2). The MF was the cohort with the lowest SEAc value (0.36‰^{2}). Moreover, immature groups showed the highest overlap (0.60), and the other groups presented a low overlap values (less than 0.26).

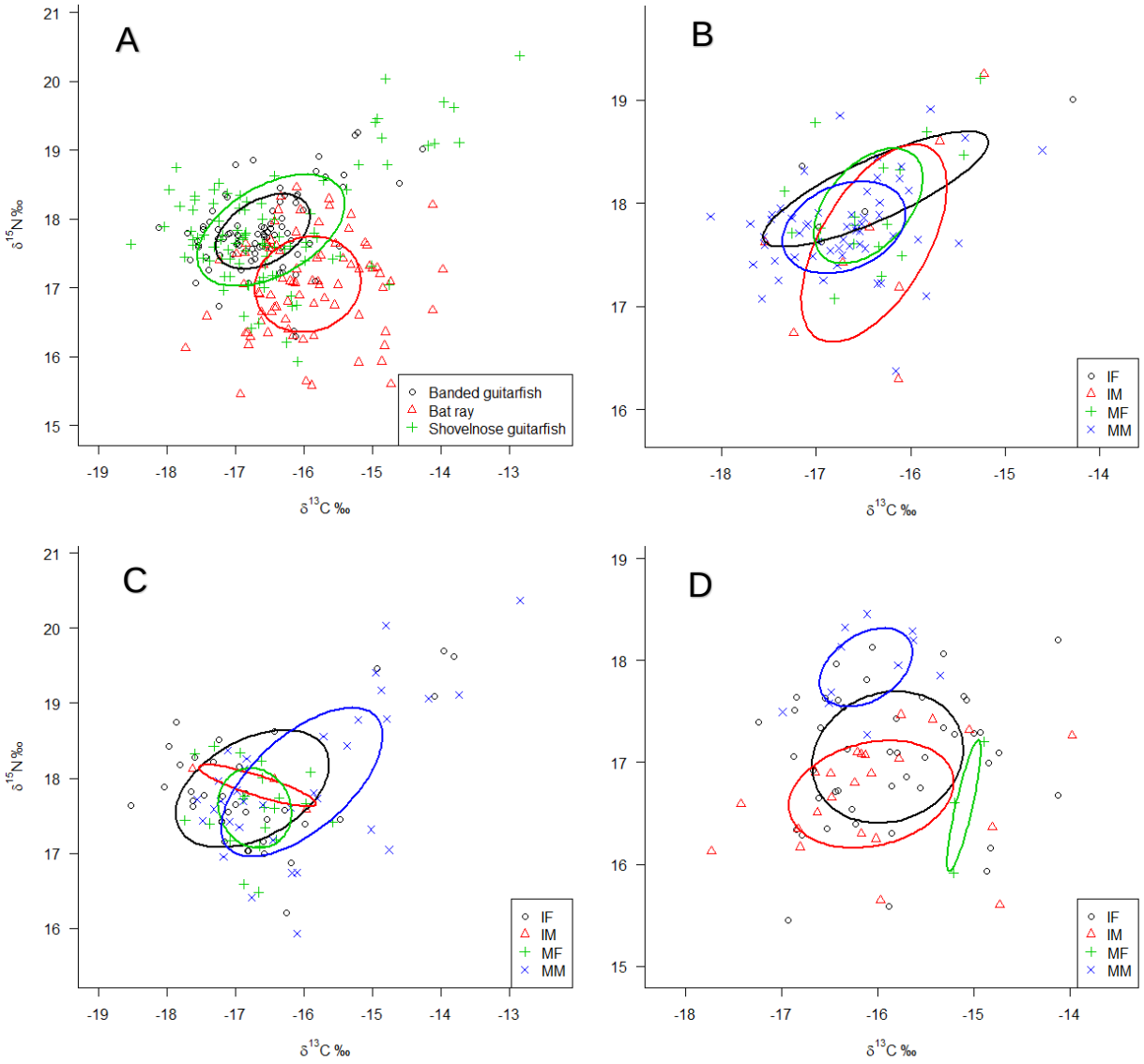

Figure 3.1. $\delta^{13}C$ and $\delta^{15}N$ values with Bayesian ellipses for the three sympatric species from the NBCS. A) by species, B) *Z. exasperata*, C) *P.productus* and D) *M. californica*.

3.5. DISCUSSION

The bat ray, banded and shovelnose guitarfish are among the most abundant species in the Pacific coast of Baja California as well as Gulf of California (Ramirez-Amaro, *et al*. 2011). However, despite that often this three species co-occur, there is a lack of information about their trophic relationships. The analysis of stable isotopes of C and N is an important tool that provides important insights into trophic ecology of vertebrates. Their variability can be the result of environmental conditions, metabolic processes, food quality, or change in behavior, among many others factors (Matich *et al*. 2010).

3.5.1. Interspecific isotopic niche

In this study, the extent of isotopic niche overlap between the banded and shovelnose guitarfish suggest that both species co-occur in the same space and share feeding resources. However, these two species of ray showed a low overlap relative to the bat ray, which suggest a resource segregation. According to stomach contents studies made in the same individuals as our study, the shovelnose and banded guitarfish share one main genus of prey (*Synodus* sp) at different proportions and some other prey that have lower occurrence in their diet (Vazquez-Moreno, 2015; Curiel-Godoy *et al*. 2016). The remaining preys items in each species is different resulting in some degree of resource partitioning between the two species, as a possible strategy to reduce interspecific competition (Wetherbee and Cortés, 2004; Grubbs, 2010). In contrast, bat rays seem to feed on lower trophic prey according to the $\delta^{15}N$ values. This species feed mainly on the crustacean *Hemisquilla californiensis* and other low trophic prey such as filter feeding bivalves and worms *Sipunculus* spp., which could explain the lower $\delta^{15}N$ compared to the other two rays. In addition, the bat ray does not share any of their main prey with the shovelnose and banded guitarfish, but share very few prey of low occurrence in their diet (Torres-García, 2015). This could be related to the relatively low overlap observed in this study between the bat ray with the shovelnose and banded guitarfish. This phenomenon have been previously seen by Vaudo and

Heithaus (2011) in different species of elasmobranchs from Australian waters, where even though varying degrees of diet and isotopic niche overlap was observed, they also found some evidence of resource partitioning. Whereas, Yemisken *et al*. (2017) found that rays *Gymnura altavela*, *Raja asterias* and *Raja clavata* from the Mediterranean Sea, partially segregate their main trophic resources as a mechanism to reduce direct competition in the demersal habitat. However, we highlight studies relying on stomach analysis, as complementary, for the understanding of isotopic results, should be taken with caution, as both approaches involve different time scales (days vs months) and the fact that different prey species could show the same isotopic values, biasing for overlap, even though there is dietary variation (Newsome *et al*. 2007). However, taxonomic evidence based on stomach contents provides basic important information of consumed species, helping understand foraging habitats, when non-taxonomic approaches are applied, such as the stable isotope (Hernández-Aguilar, *et al*. 2016) or the fatty acids analysis (Pethybridge *et al*. 2011).

Species in this study showed a broad range $\delta^{13}C$ and $\delta^{15}N$ values compared to report for the Gulf of California for the banded guitarfish ($\delta^{13}C$: -15.72 to -13.29‰, range 2.43‰; $\delta^{15}N$: 18 to 19.86‰, range 1.86‰; Blanco-Parra, *et al*. 2012) and shovelnose guitarfish ($\delta^{13}C$: -16.03 to -13.59‰, range 2.44‰; $\delta^{15}N$: 18.28 to 21.01‰, range 2.73‰; Valenzuela-Quiñonez, *et al*. 2017). These findings suggest that these species in the Pacific side have a wider habitat use or greater range of movements and feeding resources than the Gulf of California. This variability may be explained by varied feeding strategies as well as high mobility of the individuals to different systems of varying base nitrogen isotope ratios such as coastal and oceanic waters that result in a large degree of individual organism variation in the isotopic values (Tilley *et al*. 2013; Yeakel *et al*. 2016). In addition, our samples came from different fishing camps (Fig 1) that despite their relative proximity, the isotopic composition of primary producers can vary spatially due to biogeochemical processes. We also recognize the complex oceanography of our study area, given the presence of an anticyclonic gyre in the center of San Sebastian Vizcaino bay, as well as the influence of the California Current and upwellings (Amador-Buenrostro *et al*. 1995; Hernández-Rivas *et al*. 2000). The upwellings can be a significant source of anomalously low surface $\delta^{13}C$ values because

of the remineralization of the organic material that sink and is depleted in ^{13}C values relative to surface water (McMahon *et al*. 2013). On the other hand, cyanobacteria dominate the phytoplankton community in this area (Almazán-Becerril *et al*. 2012) which may contribute to denitrification leading to a $\delta^{15}N$ enriched primary production signature (Chen *et al*. 2012). Cyanobacteria are known to fix N_2 lowering the $\delta^{15}N$ values as well.

Furthermore, the shovelnose guitarfish was the species with the widest isotopic niche, which suggest that this species has a wider habitat use or greater range of movements and feeding resources than the other two species.

3.5.2. Intraspecific Assessment

Within a species, differences in diet, trophic position and habitat use can be related to age (size) and sex specific energy requirements, vulnerability to predators and reproduction among others. Such differences affect the structure and dynamics of the populations, communities and ecosystem (Hammerschlag-Peyer *et al*. 2011; Hussey *et al*. 2011; Kiszka *et al*. 2014).

In our study, MM of shovelnose and banded guitarfish showed wider isotopic niches (Table 2; Fig 3.1) along with IF of the shovelnose guitarfish. For the bat ray both immature groups had a wider isotopic niche. This suggest that these cohorts may display larger movements across an isotopically heterogeneous isoscape (broader range of $\delta^{13}C$) and have a more diverse food base with prey interaction on different trophic levels (broader range $\delta^{15}N$). Increasing body length allows individuals to undertake large-scale movements and rapidly expand home range with size (Hussey *et al*. 2011) in part to meet energy requirements for the MM. Immature groups of the bat ray have a wider trophic spectrum than matures, which indicate a more diverse prey base (Torres-García, 2015). Thus, immature animals probably have a greater range of movements or they tend to be more generalist, feeding on available resources. Similarly, juveniles of the small spotted catshark *Scyliorhinus canicula* from the northwestern Mediterranean Sea, had a wider isotopic niche than adults, maybe due a

greater range of movements or a generalist diet, whereas adults of both sexes probably stay in the same areas for reproduction (Barría *et al*. 2017). In contrast, MF of shovelnose and banded guitarfish had smaller isotopic niche width relative to the other groups (Table 3.2; Fig. 3.1). Some authors suggest females congregate in a preferred temperature range due to higher energetic demands to maintain a larger body size (female reach sexual maturity at a size greater than males), the reproductive cost of yolk eggs, or to meet nutritional demands of pups during gestation (Schlaff *et al*. 2014).

All sex-maturation cohorts in each species presented different extents of overlap indicating shared resources and habitat use and partitioning others, likely in order to support their co-occurrence (Shipley *et al*. 2018). Mature cohorts were those with the highest overlap probably related to reproduction, since both sexes are known to migrate to shallow waters for mating after which they segregate like many other elasmobranchs (Bizarro and Kyne, 2015; Farrugia *et al*. 2016). We could not see this pattern in the bat ray because their low sample number for the MF. However, IF and IM of this species showed a high overlap. High values of dietary overlap within a guild of sympatric predators would suggest that prey are not a limiting factor in the environment (Vaudo and Heithaus, 2011).

3.5.3. Body size, and $\delta^{13}C$ and $\delta^{15}N$

Ontogenetic shift in diet is frequently observed in elasmobranchs, with consumption of larger and higher trophic level prey attributable to metabolic requirements of larger individuals and changes in foraging ability due to increased gape and swimming speed (Bizarro *et al*. 2007; Grubbs, 2010; Hussey *et al*. 2012). In our study, we found low Rs and no significance in the relationship between $\delta^{13}C$ and $\delta^{15}N$ with body length in the shovelnose and banded guitarfish. In contrast, in the Gulf of California, these species showed an ontogenetic shift in diet according to stomach content and $\delta^{13}C$ and $\delta^{15}N$ analysis, where juvenile stages feeds mainly upon crustacean while adult individuals incorporate more fish into their diet, as well as a positive relationship between body size and $\delta^{15}N$ (Blanco-Parra *et al*. 2012; Valenzuela-Quiñones *et al*. 2017). Our results suggest that regardless of size,

individuals feed at similar trophic levels. However, we not discard that given the limited sampling of smaller individuals (below the size of sexual maturity) for each sex of the banded and shovelnose guitarfish could influence our result. In contrast, the bat ray did show a relationship between $\delta^{15}N$ and body size suggesting an ontogenetic shift in diet which is in agreement with several species of elasmobranchs (Grubbs, 2010, Blanco-Parra *et al*. 2012; Valenzuela-Quiñonez *et al*. 2017).

3.6. CONCLUSION

Our study indicates that the shovelnose and banded guitarfish share feeding resources and habitat use with these species partitioning resources with the bat ray. The three ray species as predators in benthic communities interact within multiple components of the marine system and probably display large movements between different isoscapes. The shovelnose guitarfish with widest isotopic niche. However, further investigations are needed to corroborate this conclusion such as combining telemetry and isoscapes measures to fully understand habitat use and inherent movements (Bearshop *et al*. 2005; Hussey *et al*. 2011). The high isotopic overlap for shovelnose and banded guitarfish may suggest prey are not currently limiting factors in this ecosystem.

CHAPTER 4. TROPHIC STRUCTURE AND BIOMAGNIFICATION OF TOTAL MERCURY IN RAY SPECIES WITHIN A BENTHIC FOOD WEB

4.1. ABSTRACT

Stable isotopes of C ($\delta^{13}C$) and N ($\delta^{15}N$) were used to explore the trophic structure and evaluate mercury (Hg) biomagnification in the food web of three commercially important ray species from the Pacific coast of Baja California Sur (PCBCS), the shovelnose guitarfish (*Pseudobatos* productus), banded guitarfish (*Zapteryx* exasperata) and bat ray (*Myliobatis californ*ia). The food web of these ray species predominately consisted of zooplankton, three species of fish and five species of invertebrates. Mean $\delta^{15}N$ values in all species ranged from 10.54 ± 0.18‰ in zooplankton to 17.84 ± 0.81‰ in the shovelnose guitarfish. Mean $\delta^{13}C$ values ranged from -22.05 ± 0.75‰ in the red crab to -15.93 ± 0.78‰ in the bat ray. Mean total Hg concentration ([THg]) in all species ranged from 0.0009 ± 0.0002 mg kg^{-1} ww in zooplankton to 0.24 ± 0.19 mg kg^{-1} ww in the banded guitarfish. We calculated the food web magnification factor (FWMF) that equaled 6.38 and was significantly greater than 1. This study is the first to describe THg biomagnification in the benthic food web of these three ray species of the PCBCS. This provide an important baseline knowledge of the biomagnification dynamics in this environment that represent multiple interacting species.

Key words: mercury, biomagnification, stable isotopes, food web, rays.

4.2. INTRODUCTION

There is increasing awareness of mercury (Hg) bioaccumulation in aquatic systems worldwide (Dang and Wang, 2010). Some forms of Hg represent highly neurotoxic environmental contaminants present in marine systems (Pethybridge *et al*. 2010) that are introduced into the environment by natural and anthropogenic processes, such as volcanic emissions, soil erosion, mining, agriculture and burning of fossil fuels (Ordiano-Flores *et al*. 2011; Hurtado-Banda *et al*. 2012).

Hg bioavailability, bioaccumulation and biomagnification is influenced by the chemical form, environmental (water chemistry) and biological (e.g. trophic level, dietary structure, body size) factors (Dang and Wang *et al*. 2012; Hosseini *et al*. 2013). Dietary intake of mostly organic forms of Hg contributes more than 90% of the total uptake of mercury in most fishes, thus structural differences in food webs influences pathways for bioavailable Hg through aquatic systems. Efficiency of trophic transfer (biomagnification and bioavailability), results in higher tissue concentrations for fish that feed at higher trophic levels (Cai *et al*. 2007; Willacker *et al*. 2013). Therefore, information of trophic ecology of marine consumers allows relative quantitative assessment of mercury concentrations across food webs to directly measure biomagnification (Cai *et al*. 2007; Ferris *et al*. 2014).

Traditionally, estimation of biomagnification of contaminants through a food web compared contaminants concentrations in organisms of specific trophic levels; with published aquatic food-web models and data on feeding behavior and stomach contents (Domi *et al*. 2005; Ikemoto *et al*. 2008). Recently, there has been an increase in use of ratios of stable isotopes of carbon and nitrogen in biomagnification studies (Rigét *et al*. 2007; Pethybridge *et al*. 2012). In general, $^{15}N/^{14}N$ ($\delta^{15}N$) is on average 3-5‰ higher in a predator relative to its prey. $\delta^{15}N$ can be used to assign or calculate the trophic position of organisms in the food web. In contrast, $\delta^{13}C$ slightly increase as the trophic level increases (about 1‰); and can be used to identify foraging location due differences between inshore and offshore baseline carbon contributions (Ikemoto *et al*. 2008; Pethybridge *et al*. 2012).

The Pacific coast of Baja California Sur (PCBCS) supports a number of commercially important species of elasmobranchs, with the shovelnose guitarfish *(Pseudobatos* productus), banded guitarfish (*Zapteryx* exasperata) and bat ray (*Myliobatis californica*) among the most abundant ray species in the fisheries using gillnets (Ramírez-Amaro *et al*. 2013). Despite their ecological importance in demersal communities, knowledge of their trophic ecology is limited with one study of stomach content analysis in the shovelnose guitarfish (Downtown-Hoffmann, 2007) and there is only one study that assessed total mercury concentrations ([THg]) in these three

species (Murillo-Cisneros *et al*. 2018). However, no studies have rigorously explored food web biomagnification of these ray species from the PCBCS.

In this study, we used $\delta^{13}C$ and $\delta^{15}N$ to quantify the trophic structure and biomagnification of THg in the bat ray, banded guitarfish and shovelnose guitarfish occurring in the PCBCS. Specifically, we use a regression model to estimate the food web magnification factor (FWMF). In addition, we calculated simple biomagnification factors (BMFs; [THg] predator/[THg] prey > 1.0), and trophic level (TL) normalized BMFs between specific predator-prey pairs to determine if mercury is increasing with increasing TL, or not. These data provide a greater quantitative understanding of the trophic transfer and bioavailability of Hg in this ecosystem, and important baseline information for subsequent comparative studies on these and other batoids species, worldwide.

4.3. MATERIAL AND METHODS

4.3.1. Specimen data and sample collection

Muscle samples were collected in March-April, August-September and November of 2014 in Bahia Tortugas (27 ° 39'35 "N; 114 ° 52'35" W) located on the west coast of Baja California Sur, Mexico. Specimens were captured by local fisherman using gill nets. Size (total length and disc width) and sex were recorded for each individual. For each specimen, the stomach and between 5-30 g of muscle (dorsal side near the head) were collected and placed in plastic bags. We obtained relatively abundant, commonly caught fish (*Caulolatilus princeps* and *Paralabrax nebulifer*) and lobster (*Panulirus* interruptus) of the study area from local fishermen. Zooplankton hauls at the surface for 10 to 15 minutes at 1.5-2 knots using a conventional plankton net (60 µm mesh, 60 cm mouth diameter and 2 m in length) was conducted seven times. The zooplankton samples were placed in plastic bottles of one liter. All samples were placed in ice in coolers and transported 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).

Stomach contents were assessed by Torres-García (2015), Vázquez-Moreno (2015) and Curiel-Godoy *et al*. (2016) to determine feeding habits of each ray species. The prey items in the lowest stage of digestion (intact or nearly intact) were placed in acid-washed plastic containers. These samples were analyzed for [THg] and stable isotopes of C and N. Muscle of the three ray species were sub-sampled (range 2-20 g each) using a clean stainless steel scalpel and stored at −20 °C in acid-washed plastic containers. All samples were freeze-dried (Labcono, FreeZone 2.5 Liter) for 24-48 h as described by Cyr *et al*. (2016) and homogenized using a porcelain mortar and pestle cleaned between samples with 10% HCl acid at and distilled water. Weight of each sample before and after freeze-drying was determined to calculate percent water in each tissue once a consistent mass was achieved (fully dried).

For stable isotopes analysis, all samples were homogenized using an agate mortar and pestle. 1 mg of each sample was weighed on an analytical microbalance and placed in tin capsules of 3.5 x 5 mm.

4.3.2. Carbon and nitrogen stable isotopes analysis

C and N stable isotopes values were determined in the Mass Spectrometry Isotopic Laboratory (LEsMA) at the Centro Interdisciplinario de Ciencias Marinas del Instituto Politécnico Nacional (CICIMAR-IPN, La Paz, BCS, Mexico) using a mass spectrometer (Delta V Plus Thermo Scientific) with continuous flow coupled to an elemental analyzer (Elemental Combustion System Costech Instruments).

Stable isotopes ratios of the sample and standards were reported in δ notation and expressed as part per thousand (‰) relative to standards and were calculated using the following formula:

$$\delta^{15}N \text{ or } \delta^{13}C = [(R \text{ sample}/R \text{ standard})-1] \times 1000 \text{ (‰)}$$

The standards used were atmospheric N for $\delta^{15}N$ and Pee Dee Belemnite for $\delta^{13}C$ (Hussey *et al*. (2010). The analytical error of the $\delta^{15}N$ and $\delta^{13}C$ values was approximately 0.2‰.

Trophic levels (TL) of the prey species and consumers of the food web were determined relative to baseline $\delta^{15}N$ (assume sampled zooplankton occupy a TL= 2) using the following formula:

$$TL_{consumer} = \frac{(\delta^{15}N_{consumer} - \delta^{15}N_{Baseline})}{3.4} + 2$$

Where $\delta^{15}N_{consumer}$ is the average $\delta^{15}N$ signature value of the predator; $\delta^{15}N_{Baseline}$ is the $\delta^{15}N$ signature of TL= 2 which in this case is represented by the sampled zooplankton. We have taken 3.4‰ as the mean nitrogen fractionation between two trophic positions or an increase in 1.0 TL (Minagawa and Wada, 1984; Post; 2002).

4.3.3. Total mercury concentration ([THg]) analysis

The [THg] was determined in the Wildlife Toxicology Laboratory (WTL) at the University of Alaska Fairbanks (UAF) USA, using a direct Hg analyzer (DMA-80, Milestone, Shelton, CT, USA; US EPA method 7473) with thermal decomposition, amalgamation and atomic absorption spectrophotometry, in a manner similar to Cyr *et al*., (2016). The instrument was calibrated using a 14-point calibration curve ranging from 0.5 to 400 ng THg. The detection limit was 1 ng THg. Samples were freeze-dried for 24 h again before each run to remove any potential residual moisture. Blanks, aqueous standards (10ng at 0.1 mg kg^{-1}, Perkin-Elmer), and standard reference materials (DORM-4, TORT-2 National Research Council Canada, Ottawa ON, Canada) were analyzed for each analytical run for quality assurance. Measurements of aqueous standards were repeated after every 18 samples. Percent recoveries of standard reference materials and aqueous standards were within 91–109%. All samples were analyzed in triplicate (muscle 16-27 mg, liver 30-41 mg each) and the coefficient of

variation for triplicate samples was less than 11%. Therefore, the mean of each triplicate was calculated and used for the analysis.

4.3.4. Biomagnification factor calculations

To examine THg biomagnification, we used published THg data by Murillo-Cisneros *et al*. (2018). Three types of biomagnification factor were calculated. The simplest measures are the biomagnification factors (BMF), which describe the ratio of the chemical concentration of the predator (numerator) relative to the prey (denominator); in this case, we present this basic ratio and a TL adjusted ratio as noted below (Gobas *et al*. 2009):

$$\mathrm{BMF} = \frac{[\mathrm{THg}]_{\mathrm{Predator}}}{[\mathrm{THg}]_{\mathrm{Prey}}}$$

We also calculated the BMF normalized to trophic position (BMF_{TL}) as follows:

$$\mathrm{BMF}_{\mathrm{TL}} = \frac{[\mathrm{THg}]_{\mathrm{Predator}}/[\mathrm{THg}]_{\mathrm{Prey}}}{\mathrm{TL}_{\mathrm{Predator}}/\mathrm{TL}_{\mathrm{Prey}}}$$

A simple linear regression analysis describes the relationship between calculated TL and logarithmic [THg] to quantitatively assess food web biomagnification, or the Food Web Magnification Factor (FWMF) for THg. The FWMF is calculated as antilog of the regression slope with base 10 (Fisk, *et al*. 2001; Borga, *et al*. 2011). If values are statistically greater than 1.0 (via the t-test) this indicates magnification in the food web or predator to prey (for BMFs), while values statistically less than 1.0 represent biodilution suggesting active elimination or interrupted trophic transfer (Dehn *et al*. 2006). Values not different from 1.0 will be considered inconclusive or that concentrations do change for that specific predator and prey representation.

We used a boostrapping approach to assess if the observed value of FWMF was significantly greater than 1.0 or not. Bootstrapping methods provide convenient means of estimating the standard errors of a parameter (Gonçalves and White, 2005). To do

this, we built a programming loop with 1000 cycles that, for each cycle, extracted 80% of the observed data (with replacement) and fitted a linear regression model (Fig. 4.2A). We then used a t-test to calculate 95% confidence intervals and to determinate if the mean of the boostrapped FWMF values was statistically different from 1.0 at a 95% confidence level (Fig. 4.2B).

4.4. RESULTS

4.4.1. Trophic structure

Stable isotope values of the species from our food web are presented in table 1. In general, mean $\delta^{15}N$ values in all species ranged from 10.54 ± 0.18‰ in zooplankton to 17.84 ± 0.81‰ in the shovelnose guitarfish. As expected, finfish were more enriched in ^{15}N than invertebrates. The lowest mean $\delta^{15}N$ value in invertebrates was 13.17‰ in *P. planipes* with the highest of 17.27‰ in *H. californiensis*. Whereas the lowest mean in $\delta^{15}N$ value in finfish was of 15.65‰ in *P. nebulifer* and the highest of 16.71‰ in *C. princeps*. The three ray species had enriched $\delta^{15}N$ values in this food web (Table 4.1; Fig. 4.1).

Mean $\delta^{13}C$ values ranged from -22.05 ± 0.75‰ in the red crab to -15.93 ± 0.78‰ in the bat ray. The $\delta^{13}C$ showed lower values in invertebrates than finfish and rays species. *P. planipes* had the lowest mean $\delta^{13}C$ values (-22.05‰) and *P. interruptus* the highest (-16.42‰). In finfish, *P. notatus* showed the lowest mean $\delta^{13}C$ value (-18.38‰) and *P. nebulifer* the highest value (-17.11‰). The ray species had more enriched values relative to the others species of invertebrates and finfish (Table 4.1). A significant relationship was displayed between mean $\delta^{13}C$ and $\delta^{15}N$ values in all species of the food guild (Fig. 4.1).

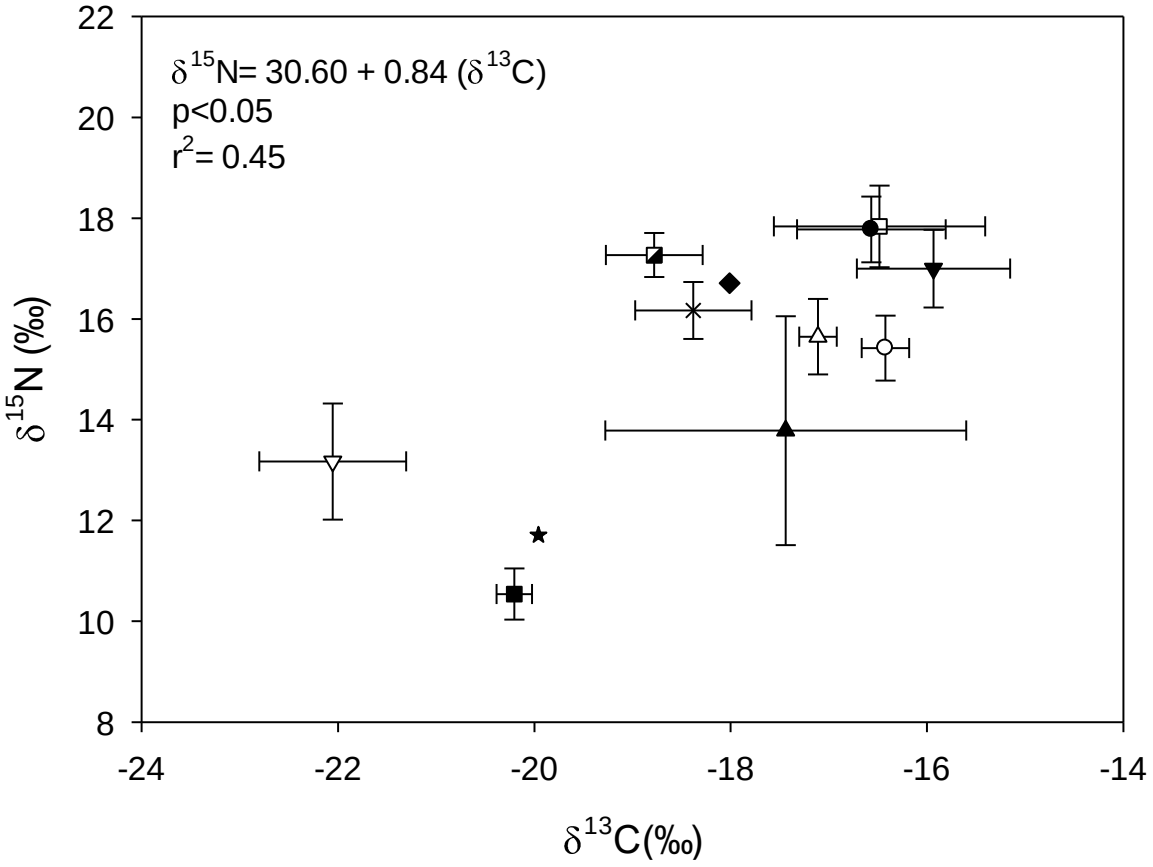

Figure 4.1. Relationship between δ13C and δ15N of selected trophic guild groups of demersal invertebrates, finfish and ray species from Bahía Tortugas: (■) zooplankton, (▲) *B. occidentalis*, (★) *C. arcuatus*, (▽) *P. planipes*, (○) *P. interruptus*, (◪) *H. californiensis*, (Δ) *P. nebulifer*, (x) *P. notatus*, (♦) *C. princeps*, (▼) *M. californica*, (□) *P. productus*, (●) *Z. exasperata*.

4.4.2. THg concentration

Mean [THg] for the three ray species are as reported in Murillo-Cisneros *et al*. (2018). The [THg] were determinated in samples of the food web as described in the method section and reported in Table 4.1. Mean [THg] in all species ranged from 0.0009 ± 0.0002 mg kg^{-1} ww in zooplankton to 0.24 ± 0.19 mg kg^{-1} ww in the banded guitarfish (Table 4.1). Mean [THg] in invertebrate species ranged from 0.01 mg kg^{-1} ww in *C. arcuatus* to 0.09 ± 0.06 mg kg^{-1} ww in *P. interruptus*. In finfish, mean concentration

ranged from 0.03 mg kg^{-1} ww in *C. princeps* to 0.06 ± 0.01 mg kg^{-1} ww in *P. notatus* (Table 4.1).

Table 4.1. Summary of mean ± standard deviation of [THg], $\delta^{13}C$, $\delta^{15}N$ and estimated trophic level (TL) of selected trophic guild groups of demersal invertebrates, finfish and ray species from Bahía Tortugas. Sample number is in parentheses.

	$\delta^{13}C$ (‰)	**$\delta^{15}N$ (‰)**	**TL**	**[THg] (mg kg^{-1} ww)**
Zooplankton	-20.21 ± 0.18 (5)	10.54 ± 0.51 (5)	2.00 ± 0.15	0.0009 ± 0.0002 (3)
Pleuroncodes planipes	-22.05 ± 0.75 (3)	13.17 ± 1.15 (3)	2.77 ± 0.34	0.02 ± 0.01 (3)
Blepharipoda occidentalis	-17.44 ± 1.84 (3)	13.78 ± 2.27 (3)	2.95 ± 0.67	0.01 (1)
Callinectes arcuatus	-19.96 (1)	11.71 (1)	2.34	0.01 (1)
Panulirus interruptus	-16.42 ± 0.24 (3)	15.42 ± 0.65 (3)	3.43 ± 0.19	0.09 ± 0.06 (3)
Hemisquilla californiensis	-18.78 ± 0.49 (6)	17.27 ± 0.44 (6)	3.98 ± 0.13	0.04 ± 0.01 (6)
Porichthys notatus	-18.38 ± 0.59 (2)	16.17 ± 0.57 (2)	3.66 ± 0.17	0.06 ± 0.01 (2)
Paralabrax nebulifer	-17.11 ± 0.19 (3)	15.65 ± 0.75 (3)	3.50 ± 0.22	0.05 ± 0.02 (3)
Caulolatilus princeps	-18.01 (1)	16.71 (1)	3.81	0.03 (1)
Bat ray	-15.93 ± 0.78	17.00 ± 0.77	3.90 ± 0.23	0.09 ± 0.11
Banded guitarfish	-16.57 ± 0.76	17.78 ± 0.65	4.13 ± 0.19	0.24 ± 0.19
Shovelnose guitarfish	-16.48 ± 1.08	17.84 ± 0.81	4.15 ± 0.24	0.12 ± 0.09

Bat ray: *M. californica*; banded guitarfish: *Z. exasperata*; shovelnose guitarfish: *P. productus*.

4.4.3. Biomagnification

In this study, increases, or not, of [Hg] from prey to predator (BMF and BMF_{TL}) varied according to specific predator-prey scenarios (Table 4.2). The highest BMF and BMF_{TL} value was observed from *C. arcuatus* and *B. occidentalis* relative to banded guitarfish and lowest from *P. notatus* relative to the bat ray and shovelnose guitarfish BMF and BMF_{TL} were significantly >1.0 for *H. californiensis* to each ray species, indicating a generalized biomagnification of THg from prey to predator. Whereas the

rest of the scenarios were no statistically different from 1.0 which could be related to the low sample number of the preys.

Table 4.2. Biomagnification factors (BMF) and biomagnification factors normalized to trophic level (BMF_{TL}) of [THg] from Bahía Tortugas food web.

	Bat ray		Banded guitarfish		Shovelnose guitarfish	
	BMF	BMF_{TL}	BMF	BMF_{TL}	BMF	BMF_{TL}
P. planipes	4.46	3.17	11.88	7.98	5.94	3.98
C. arcuatus	8.60	6.22	22.92	15.67	11.46	7.81
H. californiensis	2.26	2.30	6.01	5.80	3.01	2.89
B. occidentalis	6.29	4.76	16.77	12.00	8.39	5.97
P. notatus	1.44	1.35	3.84	3.40	1.92	1.69
P. nebulifer	1.93	1.73	5.14	4.36	2.57	2.17

Bat ray: *M. californica*; banded guitarfish: *Z. exasperata*; shovelnose guitarfish: *P. productus*.

Log_{10}[THg] increased significantly with increasing $\delta^{15}N$, that was represented as the calculated TL, in the food web (R^2=0.32; $p<0.05$) indicating [THg] increases with TL. The FWMF was determined to be 6.38. The mean of the bootstrapped FWMF values was 6.35 (95% C.I. [6.27, 6.43]), and was statistically different from 1.0 (t_{999}=138.9, $p<0.05$; Fig. 4.2B), indicating THg statistically significant biomagnification of THg in the food web of Bahía Tortugas suggesting diet as the major exposure route for fish and some invertebrates (Fig. 4.3).

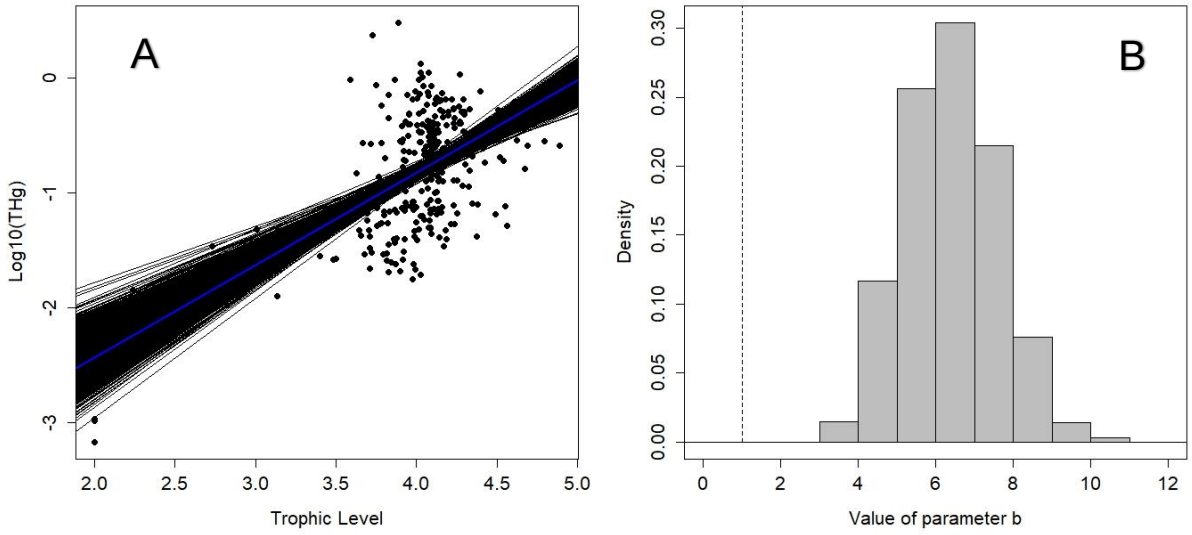

Figure 4.2. Bootstrapping (A) Relationship between trophic level and Log10[THg], the black lines are the 1000 adjustment of the aleatory models; the blue line is the mean regression. (B) Histogram of the parameter values, the dotted line is the expected value of 1.0.

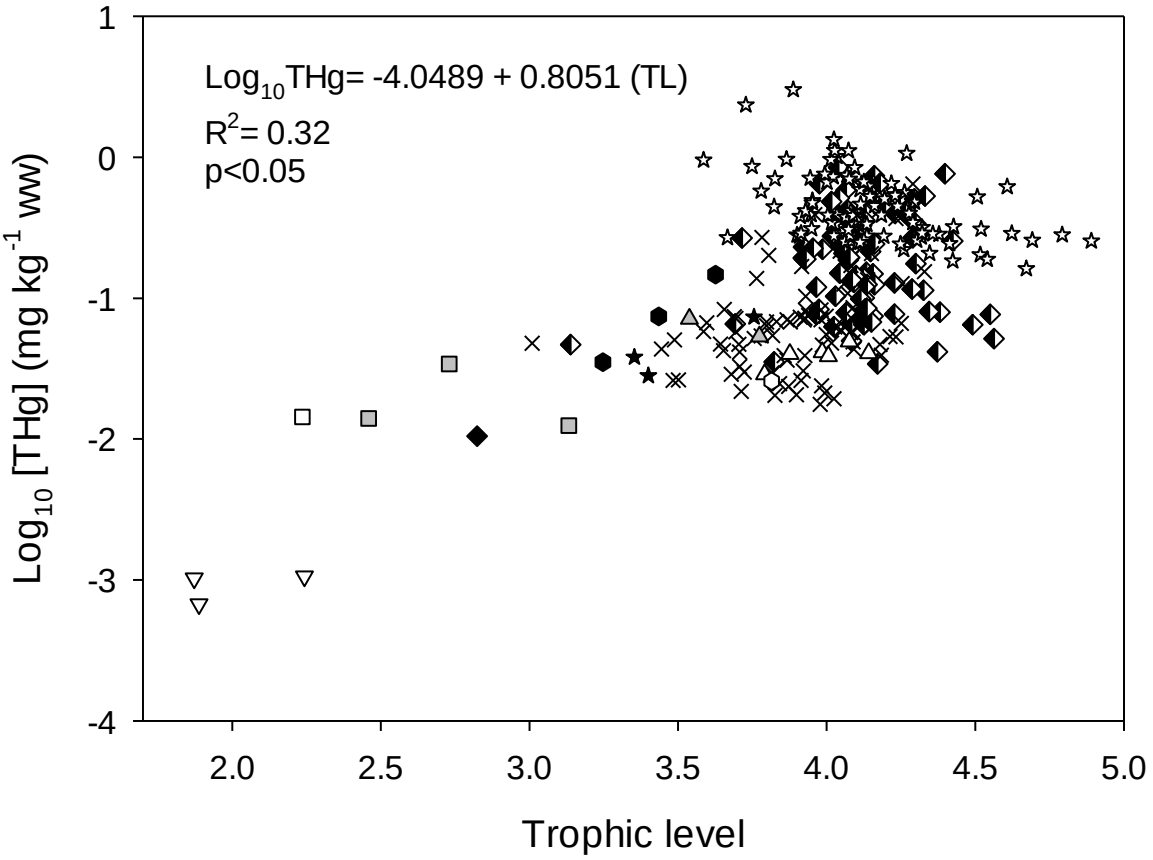

Figure 4.3. Relationship between log10[THg] and TL of selected trophic guild groups of demersal invertebrates, finfish and ray species from Bahía Tortugas: (▽) zooplankton, (□) B. occidentalis, (◆) C. arcuatus, (■) P. planipes, (●) P. interruptus, (△) H. californiensis, (★) P. nebulifer, (▲) P. notatus, (○) C. princeps, (x) M. californica, (☆) P. productus, (◐) Z. exasperata.

4.5. DISCUSSION

This study is the first to quantitatively assess trophic transfer of Hg and the degree of THg biomagnification in ray species, and associated species, of a subtropical ecosystem from the PCBCS. This provides important baseline knowledge of the biomagnification in this environment with strong quantitative and statistical support.

4.5.1. Trophic structure

The food web components studied consist of twelve selected species (zooplankton included as TL= 2 representatives of primary consumers). As expected, the $\delta^{15}N$ showed an increase from primary consumers (TL= 2) of the food web to predators (ray species; Fig. 4.2). Whereas $\delta^{13}C$ displayed a similar pattern observed in others food webs, with an increase in the $\delta^{13}C$ with the increase in $\delta^{15}N$ (Campbell, *et al*. 2005; Dehn, *et al*. 2006).

P. planipes was the species with lower $\delta^{13}C$ values, which is related to the fact that larvae and juveniles are fully pelagic (Aurioles-Gamboa, 1992). In addition, *P. planipes* had low $\delta^{15}N$ values relative to other groups, likely due grazing on phytoplankton (Aurioles-Gamboa and Pérez-Flores, 1997). *H. californiensis* and *P. interruptus* were invertebrates with higher $\delta^{15}N$ values. *H. californiensis* had a wide food spectrum (deVries, *et al*. 2015) and *P. interruptus* is considered an omnivore (Castañeda-Fernández-De-Lara, *et al*. 2010), both feeding on benthic organisms associated with higher bacterial activity leading to ^{15}N enrichment (Chen, *et al*. 2012). Finfish species showed higher $\delta^{13}C$ and $\delta^{15}N$ relative to invertebrates (with exception of *H. californiensis*). All finfish from this study are carnivorous feeders of benthic invertebrates (Lall-Arora, 1948; Elorduy-Garay and Peláez-Mendoza, 1996; Smith-Vaniz, *et al*. 2010). The three ray species had the highest $\delta^{13}C$ and $\delta 1^{5}N$ values. These species forage in coastal environments (Blanco-Parra, *et al*. 2012; Valenzuela-Quiñonez, *et al*. 2017) and among the main prey items are the invertebrates and finfish studied (Torres-García, 2015; Vazquez-Moreno, 2015; Curiel-Godoy, *et al*. 2016).

4.5.2. [THg]

Lower trophic organisms, such as zooplankton, are one of the entry points for transfer of Hg to predators that can lead to significant magnification of [THg] in top predators (Foster, *et al*. 2012). In our study, zooplankton displayed relatively low [THg] (mean 0.009 mg kg^{-1} dry weight bases) compared to other regions, such as Baltic Sea (range mean 0.07 to 0.08 mg kg^{-1} dw; Beldowska, *et al*. 2017) and Hudson Bay,

Canada (range from 0.002 to 0.03 mg kg^{-1} dw; Foster, *et al*. 2012). Those studies analyzed each taxonomic group separately, while in our study we analyzed composite zooplankton samples, without any separation according to size classes or taxonomic groups. This may lead to the observed differences between each area due to species sample composition differences in the concentration of THg (Foster, *et al*. 2012). In addition, Hg bioavailability may be different as a result of the physical and chemical characteristics of each ecosystem (Lavoie, *et al*. 2013). Also, our study area is characterized for having a high primary production (Almazán-Becerril *et al*. 2012) which can reduce the uptake of Hg by higher trophic level organisms such as zooplankton because the pool of Hg is diluted by a large amount of biomass, therefore reducing concentrations predators (Lavoie, *et al*. 2013).

The *P. planipes* [THg] were within the values previously reported for the PCBCS (Escobar-Sánchez *et al*. 2011; Maz-Courrau *et al*. 2011). Finfish species from this study were found with lower [THg] compared to fish from other studies from different locations. For example, Ruelas-Inzunza *et al*. (2008) in the coast of Sinaloa (Gulf of California) found [THg] in *C. princeps* of 0. 57 mg kg-1 dw (~0.14 mg kg^{-1} ww) likely related to the waste effluent from the intensive agriculture where some Hg compounds are used as fungicides (Ruelas-Inzunza *et al*. 2008). In addition, *P. nebulifer* presented lower [THg] than those found in different sites of southern California (mean range between 0.1 to 0.36 mg kg^{-1} ww; Phillips *et al*. 1997). Bahía Tortugas is an area considered as relatively pristine with limited anthropogenic activities. Nonetheless, we take into consideration that we had a low sample size for the finfish species so these values need further investigation, and the three ray species are very well represented.

4.5.3. Biomagnification of THg

It is well known that [THg] can increase with trophic level, a phenomenon called biomagnification (Ikemoto *et al*. 2008; Thera and Rumbold, 2013; Pethybridge *et al*. 2011). The FWMF is considered a reliable and quantitative tool to assess and better understand contaminant biomagnification (Borga, *et al*. 2011; Pethybridge, *et al*. 2011)

and can represent the increase or decrease (or no change) in contaminant concentrations relative to trophic position (Riget, *et al*. 2007).

This study demonstrated statistically and biologically significant biomagnification of THg in the benthic food web from zooplankton (primary consumers) to three ray species (abundant predator) of the PCBCS. This is similar to other studies documenting biomagnification in marine food webs (Table 4.3). However, the degree of biomagnification in those studies is different than the values found in our study. These variations may largely be a result of differences in the amount of Hg entering the base of the food web (Rigét *et al*. 2007; Thera and Rumbold 2013), as well as differences in food web structure and complexity (Pethybridge *et al*. 2011; Thera and Rumbold, 2013), since some of those studies have analyzed marine mammals (Atwell, *et al*. 1998; Lemos-Bisi, *et al*. 2012; Kehrig, *et al*. 2017) and seabirds (Jaeger, *et al*. 2009). The degree of generation of bioavailable Hg can also vary by location. The only other study that included elasmobranchs (to the authors' knowledge) is from Pethybridge *et al*. (2012) in Australian waters in a food web that included deep water elasmobranchs (>600 m). The FWMF that those authors found is almost double (13.4) the value found in our study (6.38). Vertical differences in foraging behavior are likely to be directly responsible for these differences. This is because, low oxygen deeper intermediate waters are sites for enhanced Hg methylation that is transferred to organisms living at depth and predators foraging at depth (Choy, *et al*. 2009), which could explain the differences between both ecosystems.

The two methods of biomagnification (predator-prey) used are BMF and BMF_{TL} as one accounts for trophic level and the other does not. Thus, providing slightly different insights into assessments of THg concentrations among predators and prey. The results indicate lower values for the BMF_{TL} than BMF in each scenario (Table 4.2). These fluctuations appears to be driven by the effect of the magnitude resulting from differences in trophic level. In addition, these factors assumed that the selected comparison is representative of simple predator-prey relationship (Dehn *et al*. 2006). However, we take into consideration that these species have diverse diets (Torres-

García, 2015; Vazquez-Moreno, 2015; Curiel-Godoy *et al*. 2016) and we do not disregard that this could change with season.

As expected, according to our BMF and BMF_{TL} results, the species with the lowest [THg] such as *B. occidentalis, C. arcuatus* and *P. planipes* presented the highest values of biomagnification in the three ray species compared to *H. californiensis* and *P. notatus*. Elevated concentrations of THg in prey may in fact reduce the transfer of Hg to predators by intracellular competitive uptake kinetics and regulation mechanisms (DeForest *et al*. 2007; Lavoie *et al*. 2013).

Table 4.3. FWMF of [THg] in different marine food webs around the world.

THg vs $\delta^{15}N$ and TL	**Tissue**		**Area**	**FWMF**	**Reference**
Log_{10}(THg) vs TL	Whole body/muscle	ww	PCBCS	6.38	This study
Log_{10}(THg) vs $\delta^{15}N$	Whole body/muscle	dw	Lancaster Sound, Canada	1.60	Atwell, *et al*. (1998)
Log(THg) vs TL	Whole body/muscle	ww	Norwegian Arctic	4.87	Jaeger, *et al*. (2009)
Log(THg) vs TL	Whole body/muscle	dw	Southern Brazil (Atlantic)	6.84	Di Beneditto, *et al*. (2012)
Log(THg) vs TL	Whole body/muscle	ww	Florida	5.05	Thera and Rumbold (2013)
Log(THg) vs $\delta^{15}N$	Whole body/muscle	dw	Gulf of Lions (Western Mediterranean)	1.7	Harmelin-Vivien, *et al*. (2012)
Log_{10}(THg) vs $\delta^{15}N$	muscle	dw	three tropical coastal food webs from Brazilian coast	Range 1.17-1.67	Lemos-Bisi, *et al*. (2012)
Log(THg) vs TL	Whole body/muscle	ww	southeastern Australia	13.4	Pethybridge, *et al*. (2012)
Log(THg) vs TL	muscle	dw	Brazilian southeastern coast	7.44	Kehrig, *et al*. (2017)

TL: trophic level; ww: wet weigth; dw: dry weigth; PCBCS: Pacific Coast of Baja California Sur.

4.6. CONCLUSION

This study confirms and quantitatively describes THg biomagnification in the benthic food web from zooplankton to ray species of the PCBCS. Our findings provide an important baseline knowledge of the degree of biomagnification and trophic structure that can be used for improved environmental management of this ecosystem. However, a larger sample size in the prey of predators and the inclusion of more diverse prey taxa could provide a better picture of the biomagnification phenomenon in this ecosystem.

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