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Abstract: A small and genetically isolated bottlenose dolphin (*Tursiops truncatus*) population that resides year-round in the Bocas del Toro Archipelago-Panama (BDT). Photo-identification and genetic data showed that this dolphin population is highly phylopatric and is formed exclusively by individuals of the "inshore form". This study aimed to investigate the trophic ecology and mercury concentrations of bottlenose dolphin in BDT to assess their coastal habits. We collected muscle samples (n=175) of 11 potential fish prey species, and skin samples from free-ranging dolphins in BDT (n=37) and La Guajira-Colombia (n=7) to compare isotopic niche width. Results showed that BDT dolphins have a coastal feeding habit, belong to the "inshore form" ($\delta^{13}C$= -13.05±1.89‰), and have low mercury concentrations (mean=1,637±1,387 ng g^{-1}dw). However, this element is biomagnified in the BDT food chain, showing a marginal dolphins health risk (*RQ*=1.00). These results call for preventive dolphin conservation measures and continue monitoring the pollutant levels.

Keywords: *Tursiops truncatus*, inshore form, Bocas del Toro, bioaccumulation, stable isotopes.

1. Introduction

Bottlenose dolphins have a global distribution including tropical, subtropical and temperate marine waters and are opportunistic predators that inhabit a wide range of ecosystems from semi-open coastal environments to the open sea (Reeves et al., 2008), in which factors such as habitat topography and prey availability are known to influence their feeding preferences and foraging strategies (e.g., Smolker et al., 1997; Allen et al., 2001; Blanco et al., 2001; Ingram and Rogan, 2002; Duffy-Echevarría et al., 2008; Allen et al., 2011; Jiménez and Alava, 2013; Browning et al., 2014; Rossman et al., 2015). Given that bottlenose dolphins are opportunistic predator and have global distribution they are considered top predators that can shape the structure of aquatic communities, particularly in coastal ecosystems (Bowen, 1997). Bottlenose dolphin populations around the world that inhabit coastal marine habitats consequently overlaps with human activities that results detrimental for dolphins. This is particularly concerning since coastal dolphin populations are generally small (less than 100 individuals) and show high site philopatry (e.g. Fruet et al., 2014; Vermeulen and Bräger, 2015; Barragán-Barrera et al., 2017; Bayas-Rea et al., 2018), which makes them more vulnerable to human activities than pelagic dolphins populations. For instance, unusual mortality events in coastal dolphins have been associated to exposure of high pollutant concentrations in the Gulf of Mexico (Damseaux et al., 2017). Many coastal bottlenose dolphin populations show genetic isolation and are exposed to pollutants among other threats, and given their global conservation status 'least concern', it is difficult to establish conservation strategies that can effectively mitigate human threats (e.g. Sellas et al., 2005; Parsons et al., 2006; Segura et al., 2006; Fruet et al., 2014; Vermeulen and Bräger, 2015; Barragán-Barrera et al., 2017; Bayas-Rea et al., 2018).

Collecting information about the diet and the foraging habits of bottlenose dolphin's populations is critical to establish conservation and management plans (Hernandez-Milian et al. 2015). The diet of dolphins has been studied primarily via stomach contents, fatty-acid and stable isotope analyses (e.g. Ford et al., 1998; Hooker et al., 2001; Das et al., 2003; Riccialdelli et al., 2010; Milmann et al., 2016). Dietary estimation indicates that coastal bottlenose dolphins prey on cephalopods and a variety of demersal and pelagic fish (Mead

and Potter, 1990; Van Waerebeek et al., 1990; Blanco et al., 2001; Gannon and Waples, 2004; De Pierrepont et al., 2005; Spitz et al., 2006; Milmann et al., 2016; Moura et al., 2016). However, bottlenose dolphin diet varied in relation to locations and habitats (coastal – oceanic) (e.g. Mead and Potter, 1990; 1995; Walker et al., 1999; Gannon and Waples, 2004; Santos et al., 2007; Hernandez-Milian et al., 2015; Milmann et al., 2016), seasons (Gannon and Waples, 2004), sex and reproductive status (e.g. lactating female – pregnant female), and age class (juveniles – adults) (e.g. Blanco et al., 2001; Gannon and Waples, 2004; Santos et al., 2007; Fernández et al., 2011; Hernandez-Milian et al., 2015).

The application of stable isotope analysis (SIA) is a powerful tool that can determine habitat use, trophic position and niche width in marine mammals (Newsome et al., 2006; 2010; Graham et al., 2010). This approach allows for comparisons among tissues, individuals and species, so it has been widely used to assess the trophic ecology of marine top predators such as dolphins, killer whales, sperm whales, leopard seals, sharks, tunas, marlins and swordfish (e.g. Mendes et al., 2007; Méndez-Fernandez et al., 2012; Polo-Silva et al., 2012; 2013; Bisi et al., 2013; Loor-Andrade et al., 2015; Chouvelon et al., 2017; Samarra et al., 2017; Botta et al., 2018; Acosta-Pachón and Ortega-García, 2019).

For example, the isotopic values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) provide an overall insight of an organism diet as it reflects the isotopic composition of prey and foraging habitat (DeNiro and Epstein, 1978; 1981; Newsome et al., 2006; 2010). Due to fractionation, consumers will likely be enriched approximately by 0-1% in ^{13}C and 2-5% in ^{15}N relative to its prey (Peterson and Fry, 1987). Consequently, values of $\delta^{13}C$ provide information regarding primarily production and diet composition, whereas values of $\delta^{15}N$ indicate trophic position. In marine top predators' less depleted values of ^{13}C are associated with feeding coastal areas, and more depleted ^{13}C values correspond to offshore areas. Likewise, high values of $\delta^{15}N$ indicate that the consumer is positioned at high levels of the local food web (Graham et al., 2010; Newsome et al., 2010).

Top predators such as bottlenose dolphins have shown high mercury (Hg) concentrations as the result of bioaccumulation with individual age and biomagnification processes in the food

webs (Fossi et al., 2012). Therefore, Hg can be used as ecological tracer to assess the trophic position in the food chain, since Hg variations have been associated to age, individual size, and trophic level (Boush and Thieleke, 1983; Power et al., 2002; Cai et al., 2007; Newsome et al., 2010; Kehrig et al., 2013; 2017; Marrugo-Negrete et al., 2018). Hg concentration also varies with location, depth, and distance to the shore (Rivers et al., 1972; Colaco et al., 2006, Choy et al., 2009), with higher Hg values found in coastal ecosystems largely due to the influence of freshwater inputs and coastal development (Strom and Graves, 2001).

The bottlenose dolphin coastal population in the Archipelago of Bocas del Toro (BDT) in Panama is small (around 80 individuals) and shows high levels of genetic isolation (May-Collado et al., 2015; Barragán-Barrera et al., 2017). This dolphin population share a unique mitochondrial haplotype not found in other Caribbean bottlenose dolphin populations suggesting high philopatry and site fidelity (Barragán-Barrera et al., 2017). Given the high philopatry of BDT's dolphins in the Archipelago, they could be used a sentinel species to monitor the spatial and temporal contaminants trends, like Hg (Wells et al., 2004; Reif et al., 2015). This element has high level of toxicity and may increase the vulnerability of marine organisms to parasites and infectious agents (Wiener et al., 2003). High Hg concentrations detected in tissues of dolphins (e.g. Leonzio et al., 1992; Frodello et al., 2002; Roditi-Elasar et al., 2003; Aubail et al., 2013; Borrel et al., 2014) have been used to monitor long-term pollution in waters around the Mediterranean Sea (Fossi et al., 2012; Borrel et al., 2014; Shoham-Frider et al., 2016). Although Hg negative effects have not been measured directly on individuals, declines in the Mediterranean Sea bottlenose dolphin population have been attributed to pollution, so this population has considered as 'vulnerable' by the IUCN (Bearzi et al., 2012). Particularly in BDT, studies on coral and sediment quality have found moderate Hg levels (Guzmán and Jiménez, 1992; Guzmán and García, 2002; Berry et al., 2013). Given the potential negative effects of Hg on the small and highly philopatric bottlenose dolphin population in BDT, the main aim of this research was to study the foraging ecology and to determine the degree of contamination in dolphins. We also aim to test the hypothesis of whether bottlenose dolphin coastal habits are reflected in their isotopic signatures and THg concentrations. In order to test this hypothesis, we aimed to achieve the following objectives: a) to measure the total Hg (THg) concentration, b) stable isotope carbon ($\delta^{13}C$ values) and

nitrogen ($\delta^{15}N$ values) in muscle of prey fish species from BDT and the skin of bottlenose dolphins in BDT and Guajira (Colombia), and c) to compare the isotopic niche width between these dolphin populations. Assessing coastal habits and monitoring of Hg in these coastal and vulnerable bottlenose dolphins as top predators in BDT represents important precautionary measures to determine their potential risk health and to facilitate future monitoring.

2. Materials and Methods

2.1 Study area

The Bocas del Toro Archipelago (BDT) is located on the Northwest Caribbean coast of Panama (Fig. 1). The Archipelago is home to several marine ecosystems including, seagrass, sandy bottom, coral reef, and mangrove forest (Guzmán and García, 2002; D'Croz et al., 2005). Sections of the Archipelago seem to be influenced by open ocean conditions due to water interchange through channels between sand cays and islands (D'Croz et al., 2005). The inner portion of the Archipelago, the Almirante Bay, is a semi-enclosed lagoon that appears to have considerable influence by oceanic conditions via the Bocas del Drago channel, located at the northern end of the Archipelago (Guzmán et al., 2005; D'Croz et al., 2005; Berry et al., 2013). At the southern end of Almirante Bay, is the Bocastorito Bay (also called Dolphin Bay), which is a semi-closed lagoon surrounded by mangrove forest and is considered to be an important nursery ground for the local bottlenose dolphin population and is not influenced by oceanic waters (May-Collado et al., 2012). Southern portions of the Archipelago are influenced by oceanic conditions, via water exchange through the Crawl Cay channel (Fig. 1). The climatic conditions of the Archipelago are highly influenced in terms of rainfall, so dry and rainy seasons are not clearly defined (Guzmán and Guevara, 1998; D'Croz et al., 2005; Guzmán et al., 2005).

There are two major human settlements in the region that are located in Colon Island and Almirante Bay; and other small settlements are scattered throughout the Archipelago. Colon

Island and Almirante Bay are the main source of chemical and noise pollution in BDT. Human impacts in the Archipelago include: overfishing, pollution, sedimentation, and vessel traffic (Seemann et al., 2013). During the months of high precipitation, rivers like the Changuinola River discharge large amounts of sediment to the semi-closed lagoon of Almirante Bay, which transports sediments from urban areas and banana plantations. Indeed, although mercury also has a natural origin in the Archipelago (Guzmán and Jiménez, 1992; Guzmán and García, 2002), the main industrial source is due to agricultural run-off bananas plantations and vessel traffic associated to their exportation in the Almirante Port (Berry et al., 2013).

2.2 Tissue samples collection permits

Tissue samples from fish and bottlenose dolphins from BDT were collected with permission from the Autoridad Nacional del Ambiente in Panama (ANAM; permits SC/A-11-12, SC/A-43-12, SC/A-17-14, SE/A-101-16). Methods for remote skin biopsy collection were approved by the Smithsonian Tropical Research Institute IACUC (Institutional Animal Care and Use Committee; permits number 2011-1125-2014-06 and 2016-0203-2019-A2 to Dr. May-Collado). In addition, we included bottlenose dolphin tissue samples collected at La Guajira, located on the northeast portion of the Caribbean coast of Colombia (Fig. 1), to conduct niche trophic comparisons between dolphin populations. The tissue samples were collected under Resolution 1177 Permit for Specimen Collection of Wildlife Biodiversity Non-Commercial Purposes of Scientific Research; authorization granted by National Authority for Environmental Licenses – ANLA in Colombia to Universidad de los Andes.

2.2.1. Dolphin skin samples collection

Skin samples from free ranging bottlenose dolphins were collected using a 0.22 caliber modified rifle PAXARMS that fire remote biopsy darts with adjustable pressure (Krützen et al., 2002). Biopsy darts were shot from an approximate distance of 7–10 m from the research boat to the dolphins (Weller et al., 1997; Krützen et al., 2002; Fruet et al., 2016). This system allows the penetration of the dolphins epidermis leaving behind a small wound (Tezanos-

Pinto and Baker, 2011), and the effect on dolphins is expected to be low because the polycarbonate body of the dart spreading the impact over a wide area reducing the risk of injury when penetrating the skin (Krützen et al., 2002; Tezanos-Pinto and Baker, 2011; Fruet et al., 2016). Biopsy darting is a useful methodology to sample tissue of bottlenose dolphins from BDT because these dolphins are very evasive to vessels, and this system allows to collect remote samples and identify the individuals simultaneously (Fruet et al., 2016). Therefore, skin biopsies were collected only if the individual dolphin was photo-identified in order to avoid re sampling the same individual dolphin (Krützen et al., 2002). When dolphins were sighted, a skin sample, photograph, and the location of the sighting were recorded. Skin samples were preserved in alcohol 70% and stored at -20 ºC (Amos and Hoelzel, 1991) for subsequent laboratory analysis.

2.2.2. Fish muscle samples collection

Fish samples were collected from Almirante Bay, Dolphin Bay, and one 'Outermost' area located at the southern part of the Archipelago, Cayo Coral, which is influenced by oceanic conditions (D'Croz et al., 2005; Guzmán et al., 2005; Berry et al., 2013). In Almirante Bay and Dolphin Bay fish traps were used to capture live fish, and samples from the 'Outermost' area were collected by local fishermen using fish nets. Captured live fish were euthanized by immersing them in ice-cold water bath (-4ºC) following Barker et al. (2002) and Blessing et al. (2010) protocols. The samples were later transported to the Bocas del Toro Smithsonian Research Station for further processing. Approximately 400 mg of dorsal white muscle was removed from each specimen and preserved in alcohol 70% and stored at -20 ºC for subsequent laboratory analyses.

2.3. DNA extraction and molecular sexing

To determine the sex for each individual dolphin for which a skin sample was collected, we extracted DNA from skin samples using the DNeasy kit (QIAGEN, Valencia, CA, USA). Samples were sexed following the protocol proposed by Gilson et al. (1998), conducting multiplex PCR to amplify the male-specific SRY gene and ZFY/ZFX genes of males and

females as positive controls. Electrophoresis was performed to observe the bands pattern and determine the sex of each individual.

2.4. Stable Isotope analyses

Dolphin and fish tissue samples were left on a bench to let alcohol evaporated, then were homogenized, and freeze-dried. Previous to isotopic analyses, 100 mg of sample was washed with 4 ml of cyclohexane for lipids removal (De Niro and Epstein, 1978; Méndez-Fernandez et al., 2012). The sample was maintained at constant agitation by 10 min and then centrifuged at 4,500 rpm for 5 min to discard supernatant containing lipids (Méndez-Fernandez et al., 2012). The process was repeated three times. Next the samples were dried at 45°C in an oven for 48 h. A small portion of the lipid-free sample (0.2 – 0.4 mg) was weighted in a tin cup to conduct stable isotope analyses with a continuous flow mass spectrometer (ThermoScientific Delta V Advantage) coupled to an elemental analyser (Thermo Scientific Flash EA 1112). The isotopic measurements were expressed in parts per thousand (‰), using the delta (δ) unit relative to the deviation from international standard values PeeDee Belemnite Carbonate for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$ (Méndez-Fernandez et al., 2012; Marrugo-Negrete et al., 2018). Based on replicate measurements of internal laboratory standards, experimental precision (SD) was < 0.09 for $\delta^{13}C$ and < 0.36 for $\delta^{15}N$.

2.5. Average Trophic Level Calculation

Following Hobson et al. (2002), the trophic level (*TL*) for each fish species was calculated according this equation:

$$\boldsymbol{TL} = TL\ \text{Reference primary consumer} + \frac{\delta 15\text{N Consumer} - \delta 15\text{N Reference primary consumer}}{\delta 15\text{N Enrichment between } TL},$$

where '*TL* Reference primary consumer' represents the trophic level of the reference primary consumer which was assumed as two (*TL* = 2). The '$\delta^{15}N$ Consumer' is the mean of nitrogen isotope ratio (‰) of the consumer of interest and the '$\delta^{15}N$ Reference primary consumer'

represents the mean of nitrogen isotope ratio of the reference primary consumer. The 'δ^{15}N Enrichment between *TL*' is the enrichment of δ^{15}N between trophic levels, which was assumed as 3.4‰ because this value represents the most frequent δ^{15}N enrichment factor in aquatic food webs (Lavoie et al., 2013).

2.6. Isotopic niche width and Bayesian stable isotope mixing model

To analyze stable isotope data in the context of isotopic niche width, we adopted the metrics based in a Bayesian framework (Stable Isotope Bayesian Ellipses in R: SIBER; Jackson et al., 2011) to compare bottlenose dolphins in BDT and La Guajira. To test the SIBER assumption of a multivariate normal distribution for each group, the R package mvnormtest' was used (Jarek, 2015). The area of the standard ellipse (SEAC), which is an ellipse obtained by Bayesian inference that contains 40% of the data regardless of sample size and corrected for small sample sizes, was adopted to compare niche width between groups. A Bayesian estimate of the standard ellipse and its area (SEAB) was used to test whether a group ellipse is smaller or larger than the other. The convex hull area (TA), although much more sensitive to sample size, was also employed to compare among groups and their overlap (Layman et al., 2007). All these metrics were calculated using the functions implemented in the package Stable Isotope Bayesian Ellipses (SIBER model) in R v. 3.4.3.

To estimate the proportional contribution of potential local prey sources to the bottlenose dolphin diet in BDT, we conducted a Bayesian stable isotope mixing model (Parnell et al., 2013) (MixSIAR model) using the isotopic ratios of nitrogen and carbon from dolphin's skin samples (consumer tissue) and fish muscle samples (dietary items). Only fish species with three or more specimens collected were considered in the analysis. We used as enrichment factors for δ^{13}C and δ^{15}N the values 1.5 ± 0.5‰ and 3 ± 0.3‰, respectively, which were calculated in a previous study by Fernández et al. (2011) for skin bottlenose dolphin tissue. The Bayesian stable isotope mixing model was run with a Markov Chain Monte Carlo method (chain length 1,000,000) using the MixSIAR package in R v. 3.4.3. (Stock and Semmens, 2016).

2.7. Mercury analyses

Total mercury (THg) concentrations of tissues were determined using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254 from Altec©) as described in Bustamante et al. (2006). The THg determination process in this spectrometer is conducted in three phases: 1) a drying phase (10 s) at 90°c to remove water from the freeze-dried samples; 2) a decomposition phase (150 s) in which a heating process at 750°C is carried out to release Hg from the samples, producing Hg vapor which is transported toward the surface of a gold amalgamator; and 3) a measuring phase in which the amalgamator is heated to 800°C to release the collected Hg to the spectrophotometer, allowing Hg detection by atomic absorption. Duplicates of dried, homogenized tissue samples (ranging from 1 to 10 mg) were analyzed without a chemical pre-treatment to assess mean THg concentration and SD for each (Aubail et al., 2013; Angel et al., 2018). Measurements were repeated at least two times until haying analytical differences below 10%. Following Bustamante et al. (2006), the analytical quality of the THg measurements in the AMA-254 was controlled using blanks at the beginning of each analytical session, and running analyses of certified reference material (CRM) TORT-2 (Lobster hepatopancreas, National Research Council of Canada) at the beginning and every ten analyses. The measured concentration for the CRM was 0.264 ± 0.013 ng mg^{-1} (n = 50) and the recovery was thus 98%. The THg measurements were showed in ng g^{-1} on a dry weight basis (dw) and the detection limit was 0.059 ng.

2.6. Calculation of trophic magnification factors

Hg biomagnification was evaluated assuming a trophic chain in which bottlenose dolphins are at the top of the food chain, based on the relationship between THg concentrations with $\delta^{15}N$ ratios on both dolphins and fish. We calculated the mean isotopic and THg composition and its standard deviation in skin samples of bottlenose dolphins, and in muscle samples of potential prey (11 fish species). To evaluate significant correlations between THg with $\delta^{15}N$ ratios as evidence of trophic magnification, Shapiro–Wilk test of normality and Bartlett test of homogeneity of variances were used to test the assumptions of parametric tests on data. A log-transformation of THg data was used to meet these assumptions. Then, Pearson

correlation was performed to assess this relationship, and linear regression analysis was conducted to quantify trophic Hg magnification (Lavoie et al., 2013). Results for statistical analyses were carried out in R v. 3.4.3. and considered significant at *p*-value level of < 0.05. Trophic magnification factor (*TMF*) was assessed using the slope (β) of the regression of log-THg, which represents the change in THg concentration per unit change in $\delta^{15}N$ through the food chain (Fey et al., 2019), in the following equation:

$$TMF = 10^{\beta}$$

According this model, when *TMF* value is > 1 there is evidence for THg accumulation (Nfon et al., 2009). We also evaluated the 'Bioaccumulation factor' (*BAF*) to assess Hg accumulation between prey and dolphins, as the ratio of Hg concentration in consumer (dolphin) to the Hg concentration prey (fish) (Arnot and Gobas, 2006). This relation was also used to calculate the 'Biotransference factor' (*BTF*) to evaluate trophic magnification, which provides information on Hg transference from dwarf round herring (prey) in the *TL* = 2 to fish in the *TL* = 3 (according to their trophic level calculated here), and to bottlenose dolphin (consumer) as the last trophic level (Strandberg et al., 1998; Wang, 2002; Barwick and Maher, 2003), as follows:

$$BTF = \frac{\text{Hg concentration in consumer (dolphin)}}{\text{Hg concentration in prey (fish)}}$$

According this model, when the *BTF* value is > 1 there is evidence for positive biomagnification, which means Hg increases at least three trophic positions in the food web (Strandberg et al., 1998; Barwick and Maher, 2003; Kehrig et al., 2013).

2.7. Risk assessment

Dolphin health risk to THg exposure via fish consumption was assessed using a Maximum Allowable Concentration (*MAC*) analysis based on the reference dose (*RfD*, ng g^{-1} dw), and then a risk quotient (*RQ*) based on this MAC_{Rfd} calculated. The *RfD* provides a conservative risk assessment data as it is derived from toxicity and adjusted values from the no observable

adverse effect level (NOAEL) values obtained in mammals, and therefore is generally used for assessing risk to human health from exposure to trace elements (Hung et al., 2006). MAC_{Rfd} approach allows assessing the maximum Hg concentration that dolphins can tolerate in their prey without negative health effects, and *RQ* approach allows to assess the levels of Hg risk for dolphins (Hung et al., 2004; 2006).

To calculate dolphins *Hg-intake*, fish Hg concentrations were converted from dry weight (dw) to wet weight (ww) using the following equation:

$$Cw = Cd\ (100 - \%H),$$

where *Cw* and *Cd* represents dw and ww respectively, and %H is the humidity percentage which ranges around 80% for several fish species (Murray and Burt, 1969; Hislop et al., 1991; Payne et al., 1999; Alonso-Fernández and Saborido-Rey, 2012). Next and following Hung et al. (2004; 2006), dolphins *Hg-intake* was calculated as follows:

$$Hg\text{-}Intake\ (\text{ng g}^{-1}\ \text{day}^{-1}) = \frac{CF \times IR \times EF \times ED}{BW \times AT},$$

where *CF* represents Hg concentrations in all fish together (ng g^{-1} ww) which was calculated taking into account the contribution of each item to the dolphin diet according to SIAR model results. The *IR* is the ingestion rate per day reported for Atlantic bottlenose dolphins (kg day^{-1}) based on an average annual food consumption of around 2,000 kg of fish for adult dolphins (Kastelein et al., 2002). *EF* is the exposure frequency per day for one year (day $year^{-1}$), represented as 365 days assuming BDT dolphins are exposed to Hg every day for one year (Hung et al., 2004). *ED* is the exposure duration (years), and assuming life-time Hg exposure, we used 45 years as estimated life-time for bottlenose dolphins (Jefferson et al., 2002). *BW* represents the body weight (kg), which is 260 kg according to the weight of Atlantic bottlenose dolphin individuals that showed a mean annual food consumption of 2,000 kg (Kastelein et al., 2002). Finally, *AT* is the average time (days) and represents the period in days in which dolphins are exposed to Hg during life-time.

According to Hung et al. (2006), when *Hg-Intake* is equal to *RfD*, the *CF* is equal to MAC_{RfD}, as follows:

$$RfD = \frac{MAC_{RfD} \text{ x } IR \text{ x } EF \text{ x } ED}{BW \text{ x } AT}$$

Thus, the *RfD* calculated in the previous model is used in the following equation to calculate the MAC_{RfD}, as follows:

$$MAC_{RfD} = \frac{RfD \text{ x BW x AT}}{IR \; x \; EF \; x \; ED}$$

To assess *RQ*, we integrated results of exposure and dose-response assessments, using the following equation:

$$RQ = \frac{\text{Hg concentration in all prey}}{MAC_{RfD}}$$

2.8. Statistical comparisons

The existence of significant differences between isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and THg measurements in fish among the three main locations within BDT (Almirante Bay, Dolphin Bay, and 'Outermost' area), was evaluated posteriorly using Analysis of variance (ANOVA) and Tukey multi-comparison tests, considering significant at *p*-value level of < 0.05. Similarly, significant differences at *p*-value level of < 0.05 in the isotopic values and THg concentration with dolphins sexes was assessed using ANOVA or Kruskal-Wallis tests separately for each element. All these statistical analyses were performed in R v. 3.4.3. after test assumptions of parametric data using Shapiro–Wilk test of normality and Bartlett test of homogeneity of variances.

3. Results

A total of 175 fish and 37 dolphin tissue samples were analyzed in this study. Figure 1 shows the locations of collection for the 37 dolphin samples. Overall, there were 18 females (16 adults, two juveniles) and 19 males (18 adults, one juvenile). A total of 21 species of fish were collected (N = 223), but only 11 species had at minimum of three sub-samples to be included in the analysis (N = 175; Table 1). Of these 11 species, only dwarf round herring (*Jenkinsia lamprotaenia*) was found in all sampled main locations: Almirante Bay (n = 19), Dolphin Bay (n = 15), and the 'Outermost' area (n = 16).

3.1. Mercury and stable isotope of bottlenose dolphins

The comparison of carbon and nitrogen isotopic signatures between tissue samples of dolphins revealed differences in isotopic niche between populations from BDT and La Guajira (Fig. 2). BDT dolphins showed a broader isotopic signature range than La Guajira dolphins (Fig. 2). The $\delta^{13}C$ values for BDT dolphins ranged between -16.40‰ and -9.17‰ (mean = -13.11‰ ± 1.95), and the $\delta^{15}N$ ranged between 5.76‰ and 12.77‰ (mean = 10.24‰ ± 1.48) (Fig. 2). In contrast, La Guajira dolphins had $\delta^{13}C$ values in a shorter range, between -16.44‰ and -15.26‰ (mean = -15.71‰ ± 0.41), and the $\delta^{15}N$ also had a shorter range between 10.90‰ and 14.85‰ (mean = 12.30‰ ± 1.26) (Fig. 2). Regarding THg values, BDT dolphin values ranged between 94 and 4,627 ng g^{-1} dw (mean = 1,637 ± 1,387 ng g^{-1} dw) and La Guajira dolphin values ranged between 2,720 and 10,590 ng g^{-1} dw (mean = 5,526 ± 3,209 ng g^{-1} dw) (Fig. 3). Finally, BDT dolphins did not show significant differences in THg or isotopic signatures between males and females (Kruskal-Wallis $\delta^{13}C$ H = 0.01, ANOVA $\delta^{15}N$ F = 0.38, Log-THg F = 0.004, all p > 0.50), which means both sexes feed on prey of the same or similar trophic level within the Archipelago. Because dolphins move during the day around the Archipelago, and dolphin were biopsied at various times of the day, we could not assume that the site of sampling was the site where the animals foraged. Therefore, comparisons among the three main locations within BDT were rendered not informative.

The results of the Bayesian stable isotope mixing model (MixSIAR) diet estimated suggested a high mean contribution of 40% by striped parrotfish (*Scarus iseri*) and 24% by the dwarf round herring (*J. lamprotaenia*) to the diet of BDT dolphins (Fig. 4). Other prey fish species contributed to less than 4% to their diet, and the blue runner (*Caranx crysos*), lane snapper (*Lutjanus synagris*), and the northern red snapper (*Lutjanus campechanus*) were the third, fourth and fifth prey items that contributed to their diet (4, 3 and 3%, respectively). Remaining fish prey species contributed to 2% to dolphins' diet (Fig. 4).

3.2. THg, $\delta^{13}C$ and $\delta^{15}N$ stable isotope values in fish

The results showed a wide variation of THg, $\delta^{13}C$ and $\delta^{15}N$ values among the fish prey collected (see Table 1). The striped parrot fish showed the lowest THg and $\delta^{15}N$ values (mean = 77 ± 14 ng g^{-1} dw; and mean = 7.35 ± 1.59‰, respectively) while the dwarf round herring showed the lowest $\delta^{13}C$ values (-16.85‰, ± 1.58). Compared to fish, the dolphins showed the highest THg concentration (mean = 1,637 ± 1,387 ng g^{-1} dw) as well the highest $\delta^{15}N$ value (mean = 10.25‰ ± 1.48).

There were significant differences in the relationship between THg and isotopic signatures of carbon and nitrogen between locations where dwarf round herring were collected (ANOVA, $\delta^{13}C$ F = 165.40, $\delta^{15}N$ F = 26.98, Log-THg F = 9.58, all $p < 0.05$; Fig. 5B–C). The relationship between $\delta^{13}C$ and $\delta^{15}N$ appears to indicate that the diet of dwarf round herring in Almirante Bay and Dolphin Bay is based on oceanic and lower trophic level food items ($\delta^{13}C$ mean = -17.91‰ ± 0.42, -17.78‰ ± 0.57, respectively; $\delta^{15}N$ mean = 7.90‰ ± 0.38 and 8.45‰ ± 0.44, respectively), while in 'Outermost' area it is based on coastal but higher trophic level food items ($\delta^{13}C$ mean = -14.73‰ ± 0.71; $\delta^{15}N$ mean = 8.88‰ ± 0.36; Fig. 5A). THg showed significant and lower values in dwarf round herring collected in Dolphin Bay in relation to 'Outermost' area and Dolphin Bay (mean = 83 ± 42 ng g^{-1} dw, 153 ± 58 ng g^{-1} dw, and 196 ± 193 ng g^{-1} dw, respectively; $p < 0.05$, Tukey's test; Fig. 5B–C).

Trophic relationship, biomagnification between dolphins, and prey and risk assessment of adverse effects

The estimated relative *TL* was also assessed for bottlenose dolphins and the fish species described in Table 1. We used the redtail parrotfish (*Sparisoma chrysopterum*, n = 2) as the reference primary consumer despite we only collected two specimens, because it showed the lowest $\delta^{15}N$ values ($\delta^{15}N$ = 5.45‰) due to their herbivorous habits (Luna and Torres, 2018). Bottlenose dolphins and the French grunt (*Haemulon flavolineatum*) showed the highest *TL* (3.40) while the striped parrotfish (*S. iseri*), the yellow fin mojarra *(G. cinereus*), and the dwarf round herring showed the lowest *TL* values (2.56, 2.79, and 2.86, respectively).

Figure 6 shows the relationship between THg with $\delta^{15}N$ for bottlenose dolphins and all fish expected to form the BDT' dolphin food chain. The Pearson's correlation coefficients showed significant positive correlations between THg and $\delta^{15}N$ (r = 0.54; $p < 0.05$), and the β coefficient from linear regression showed a magnitude of 0.60. Using this β coefficient in the *TMF* equation, result showed a value of 3.98 (*TMF* > 1).

Figure 7 represents a hypothesized trophic chain for BDT dolphins, with dolphins as top predators in the Archipelago, and potential prey located in two trophic positions (*TL* 2 and 3) according to the *TL* calculated in Table 1. The Figure 7 shows *BAF* values between members of the trophic chain; values higher than one, are represented by black arrows. To evaluate the biomagnification among three trophic levels (*BTF*), we conducted comparisons between the dwarf round herring in *TL* = 2 (*TL* = 2.86), other fish including the foureye butterflyfish (*Chaetodon capistratus*), the northern red snapper (*L. campechanus*), blue runner (*C. crysos*), the white grunt (*Haemulon plumieri*), and the French grunt (*H. flavolineatum*) as *TL* = 3 (*TL* = 3.14-3.41), and dolphins as next trophic level (top predator). The dwarf round herring was used as a reference primary consumer in this trophic chain because it is widely distributed in the Archipelago (it was present in the three main locations within BDT) and is known to be part of the diet of several pelagic fish species in the Caribbean (Friedlander and Beets, 1997). The *BTF* calculated between the dwarf round herring and fish from third *TL* are presented in blue arrows on Figure 7; all these *BTF* also showed values higher than one.

Table 2 summarizes the values used into equations to calculate the risk assessment of bottlenose dolphins in BDT. The table also includes the *RfD* and MAC_{RfD} values calculated here. *RQ* analyses based on MAC_{RfD} showed a value of one (*RQ* = 1.00).

4. Discussion

This study provides the first description of feeding ecology using stable isotopes and THg biomagnification process of bottlenose dolphins from the Archipelago of Bocas del Toro (BDT, Panama). Our results support previous findings based on genetic evidence that this bottlenose dolphin population is an 'inshore form' with significant geographical isolation (Barragán-Barrera et al., 2017), and highlights the vulnerability of this population to human activities (May-Collado et al., 2015; Sitar et al., 2016).

4.1. Stable isotope composition of bottlenose dolphins

The carbon isotope ratio ($\delta^{13}C$) is an indicator of the feeding habitat of an organism (Newsome et al., 2006; 2010; Graham et al., 2010). In BDT, the range of $\delta^{13}C$ values (-16.40 to -9.17‰) indicated that bottlenose dolphins have a wide feeding niche. By contrast, dolphins in La Guajira (Colombia) showed a much narrower range of carbon values and with lower values of $\delta^{13}C$ (-16.44 to -15.26‰) (Fig. 2). The differences in isotopic niche wide indicated that dolphin prey species are acquiring carbon by sources unique to each location. La Guajira coast is characterized by considerable seasonal variation in productivity which affects prey availability, and consequently dolphin presence (Gordon, 1967; Müller-Karger et al., 1989; Andrade et al., 2003; Andrade and Barton, 2005; Lonin et al., 2010; Paramo et al., 2011; Farías-Curtidor and Barragán-Barrera et al., 2017; Barragán-Barrera et al., 2019). Bottlenose dolphins in La Guajira do not appear to be resident to the area, and recent genetic findings identify them as 'worldwide distributed form' (Duarte et al., 2018), which do not show residency to inshore areas (Tezanos-Pinto et al., 2009).

Differences between coastal and oceanic habitat are reflected in their $\delta^{13}C$ values, with higher values representing coastal habitats and lower values in oceanic habitat (Schell et al., 1998; Burton and Koch, 1999; Das et al., 2003; Newsome et al., 2010). The values of dolphin skin samples at La Guajira were more depleted in ^{13}C than those reported for the BDT dolphins, but were similar to values reported from other offshore bottlenose dolphins (e.g. Díaz-Gamboa, 2003; Segura et al., 2006). This corroborates that dolphins at La Guajira prefer to forage in oceanic habitats, which is consistent with their genetic designation of the 'worldwide distributed form'. In contrast, the BDT bottlenose dolphins showed $\delta^{13}C$ values similar to other coastal populations (e.g. Segura et al., 2006; Barros et al., 2010), indicating that they consume prey from coastal areas. The skin stable isotope $\delta^{13}C$ values also indicate that the BDT dolphins fed on prey with several carbon sources. This region consists of several productive coastal ecosystems including coral reefs, mangroves, and seagrass beds (Guzmán and García, 2002; D'Croz et al., 2005; Guzmán et al., 2005). Coastal primary producers such as macroalgae and seagrasses show elevated $\delta^{13}C$ values (Peterson and Fry, 1987). Consequently, the mutton snapper (*Lutjanus analis*) and the yellow fin mojarra (*G. cinereus*), which can be found in shallow coastal ecosystem (such as coral reefs, mangroves, and seagrass beds), had the highest mean $\delta^{13}C$ values (-11.84‰ and -11.85‰, respectively) (Allen, 1985; Bussing, 1995). In contrast, the dwarf round herring, which is one of the most common and more widely distributed fish in the Archipelago, showed the lowest $\delta^{13}C$ value (mean = -16.85‰). This result may be explained by their nocturnal feeding behavior, in which dwarf round herring usually inhabits inshore areas during the day (Friedlander and Beets, 1997), but feed on zooplankton during the night in offshore areas (Radakov and Silva, 1974; Friedlander and Beets, 1997). Dwarf round herrings consume a ^{13}C-depleted diet, since offshore primary producers (phytoplankton) have low $\delta^{13}C$ values, and consequently provide a low carbon oceanic food sources for their coastal predator such a bottlenose dolphins. Additionally, it has been suggested that nutrient inputs via river plume can increase carbon sources within BDT (Seemann et al., 2013). A previous work showed the lowest $\delta^{13}C$ isotope ratios of the particulate organic carbon in Almirante Bay (mean = -20.7‰ ± 0.5) (Seemann et al., 2013), and the dwarf round herring also showed more depleted ^{13}C values in Almirante Bay than in Dolphin Bay and the 'Outermost' area. Since Almirante Bay is a semi-enclosed lagoon with low nutrient influence from the Changuinola River runoff, and because

productive marine ecosystems in this area have been affected by human activities (Seemann et al., 2013), low primary production may reflect low $\delta^{13}C$ values.

The present study revealed that the dwarf round herring is one of the most important prey in the diet of BDT bottlenose dolphins despite that the Archipelago offers a broad array of potential prey. These findings agree with the stomach content of one dead female found in Dolphin Bay, while this study was conducted on July 27, 2012. The female contained a large number of unidentified sardines. Besides the dwarf round herring, the other species that more contribute to the diet of these dolphins is the striped parrotfish. The stable isotope $\delta^{15}N$ indicated that the BDT bottlenose dolphins feed on prey at a low trophic level. The dwarf round herring and the striped parrotfish are species that are located at 2.86 and 2.56 trophic level, respectively. In contrast, the $\delta^{15}N$ values of La Guajira bottlenose dolphins indicate that they feed on prey at a higher trophic level, suggesting their diet consist primarily of pelagic species.

The great availability of herrings in the Archipelago appears to be an important reliable food source for females and males dolphins in BDT, since no significant differences between isotopic signatures and sex were detected. This predictable prey item may explain why dolphins remain in the area, despite the intensity of interactions with dolphin-watching boats. A study by Kassamali-Fox et al. (2015) found that boats affected the dolphin foraging and social behavior, by switching to avoidance behaviors. The number of boats during interacting predicted the likelihood of these dolphins to return to these behaviors. However, given the availability of herring in the area and despite the presence of vessels, it is likely that dolphins are able to forage on their favorite prey.

4.2. Hg concentrations

The BDT dolphins showed lower concentrations of THg in comparison to the dolphins at La Guajira and other bottlenose dolphin populations, such as the Indian River Lagoon and the Everglades in Florida, USA (THg mean = 7,000 and THg mean = 9,314 ng g^{-1} dw, respectively) (Stavros et al., 2007; Damseaux et al., 2017), and the Mediterranean Sea (THg

= 5,700 ng g^{-1} dw) (Aubail et al., 2013), where also other delphinid species show higher THg concentrations in their skin (e.g. Striped dolphin, *Stenella coeruleoalba*, THg mean = 3,000 ng g^{-1} dw; Aubail et al., 2013). Nevertheless, THg levels reported here were similar to concentrations reported for bottlenose dolphins in Charleston in South Carolina, USA (THg mean = 1,700 ng g^{-1} dw) (Stavros et al., 2007), and for other delphinid species in the Mediterranean Sea (e.g. Common dolphin, *Delphinus delphis*, THg mean = 1,700 ng g^{-1} dw; Harbour porpoise, *Phocoena phocoena*, THg mean = 1,600 ng g^{-1} dw; Aubail et al., 2013) and in Sub-Antarctic waters (e.g. Commerson's dolphin, *Cephalorhynchus commersonii*, THg mean = 1,380 ng g^{-1} dw; Cáceres-Saez et al., 2015) (see Table 3). Many coastal areas are influenced by several anthropogenic activities that may increment the Hg concentrations in these marine environments and thus on dolphin's prey and subsequently in their tissues (Evans and Crumley, 2005). However, the influence of prey may not be necessarily a reflection of the level of anthropogenic contamination, but differences in prey sources. For example, pelagic fish Hg concentrations have been shown to increase with increasing median depth of occurrence (Monteiro et al., 1996; Choy et al., 2009), so pelagic prey can reflect higher Hg concentrations than none polluted coastal areas. Following this, La Guajira dolphins appear to feed on a narrow prey selection items of pelagic habits, resulting in higher concentrations of THg than the coastal bottlenose dolphins of BDT.

Dolphins in BDT feed primarily on prey at low trophic levels with low concentration of Hg. The Hg low concentrations could be also related to the dominance of juvenile fish in the Archipelago, which reflect lower Hg concentrations compare to bigger ones as a consequence of bioaccumulation of Hg with age (Boush and Thieleke, 1983; Marrugo-Negrete et al., 2018). The abundance of juvenile fish in areas such as Almirante Bay is a result of dramatic decrease of larger predatory fish due to overfishing (Seemann et al., 2013). Previous works have reported Hg concentrations in corals and sediments in the Archipelago (Guzmán and Jiménez, 1992; Guzmán and García, 2002; Berry et al., 2013; Seemann et al., 2013). Because of their fish diet, dolphins may be bioaccumulating and magnificating THg through their prey, as suggested by the positive significant correlation between Hg concentrations with $\delta^{15}N$. Figure 7 shows the predicted Hg biotransference from low trophic level prey to the top of the chain where dolphins have the highest THg concentrations among all collected

specimens. Hg biomagnification processes have been reported in other dolphin populations, for example in the coastal waters of Brazil, the Atlantic spotted dolphin (*Stenella frontalis*) and the Guiana dolphin (*Sotalia guanensis*) (Kehrig et al., 2013; 2017). A potential concern about Hg exposure is that it can be quickly assimilated by their diet and accumulated in dolphin tissues throughout their life (Kehrig et al., 2013) resulting in long-term health risks for dolphins. Dolphin health can be used a proxy of the health of the marine environment and thus as a wakeup call of Hg exposure to humans (Reif et al., 2015).

4.2. Health risk

The results from the *RQ* based on the MAC_{RfD} indicate there is a potential health risk for both females and males dolphins in BDT due to Hg bioaccumulation through their diet. According to Hung et al. (2006), an $RQ < 1$ do not represent health risk. However, our analysis resulted in a value of $RQ = 1$, which indicates a marginal health risk (Hung et al., 2004). Given the conservation status of this population, this result should be taken as an important factor affecting the long-term health condition of this population. As described before, dolphins in BDT show high site fidelity and Hg bioaccumulates through an animal's lifespan. Considering this, Hg could be a major health threat for calves, taking account that reproductive females can pass Hg from their tissues during gestation and through their milk (Frodello et al., 2002), exposing calves to premature mortality (Romero et al., 2016). Furthermore, the Archipelago and particularly Dolphin Bay, where dwarf round herring showed the highest THg concentrations, is surrounded by mangrove forest, an ecosystem that is known to retain Hg making it available as methyl-Hg in water sediments to aquatic environments (Miskimmin et al., 1992; Barkay et al., 1997; Kannan et al., 1998; Evans et al., 2001; Silva et al., 2003; Bergamaschi et al., 2012; Dameaux et al., 2017). The high Hg concentrations in dolphins living in South Florida, for example, have been attributed to this intrinsic characteristic of the mangrove forest (Dameaux et al., 2017).

This study provides evidence that the BDT dolphin population is at risk. In addition to being genetically isolated (Barragán-Barrera et al., 2017) and under significant pressure due to boat traffic (May-Collado and Wartzok, 2008, May-Collado and Quiñones-Lebrón, 2014; May-

Collado et al., 2015), this dolphin population is showing signs of Hg bioaccumulation. The stable isotope analysis confirmed that this population belongs to the 'inshore form', they consume a wide selection coastal prey items, similar to what has been reported in other coastal dolphin populations (e.g. Segura et al., 2006; Barros et al., 2010). The majority of the dolphin coastal prey items in the BDT are at low trophic level or are juvenile stage, and thus showed low concentrations of Hg. The risk assessment, trophic magnification and biotransference models showed that the Hg magnification process could be a cause of concern. Hg accumulates fast in tissues of species of high trophic level, and once in high concentrations, it is toxic, and it may causes cancer (Béland et al., 1993; Martineau et al., 1994), immunotoxicity (Desforges et al., 2016), neurotoxicity (Krey et al., 2015), reduction in antibody concentration (Reif et al., 2015), and damage on the endocrine, hematopoietic, hepatic and renal systems (Bossart, 2011; Schwacke et al., 2002; Correa et al., 2014; Reif et al., 2015). Given the high site fidelity of dolphin to BDT, it is likely that lactating females may be transferring Hg through their milk to their newborns, and potentially affecting the calf development and survival. Further studies are needed to continue monitoring the Hg exposure and transfer from mother to calf in this inshore dolphin population, and monitoring Hg in biota in order to provide some insights on temporal trends in BDT as well.

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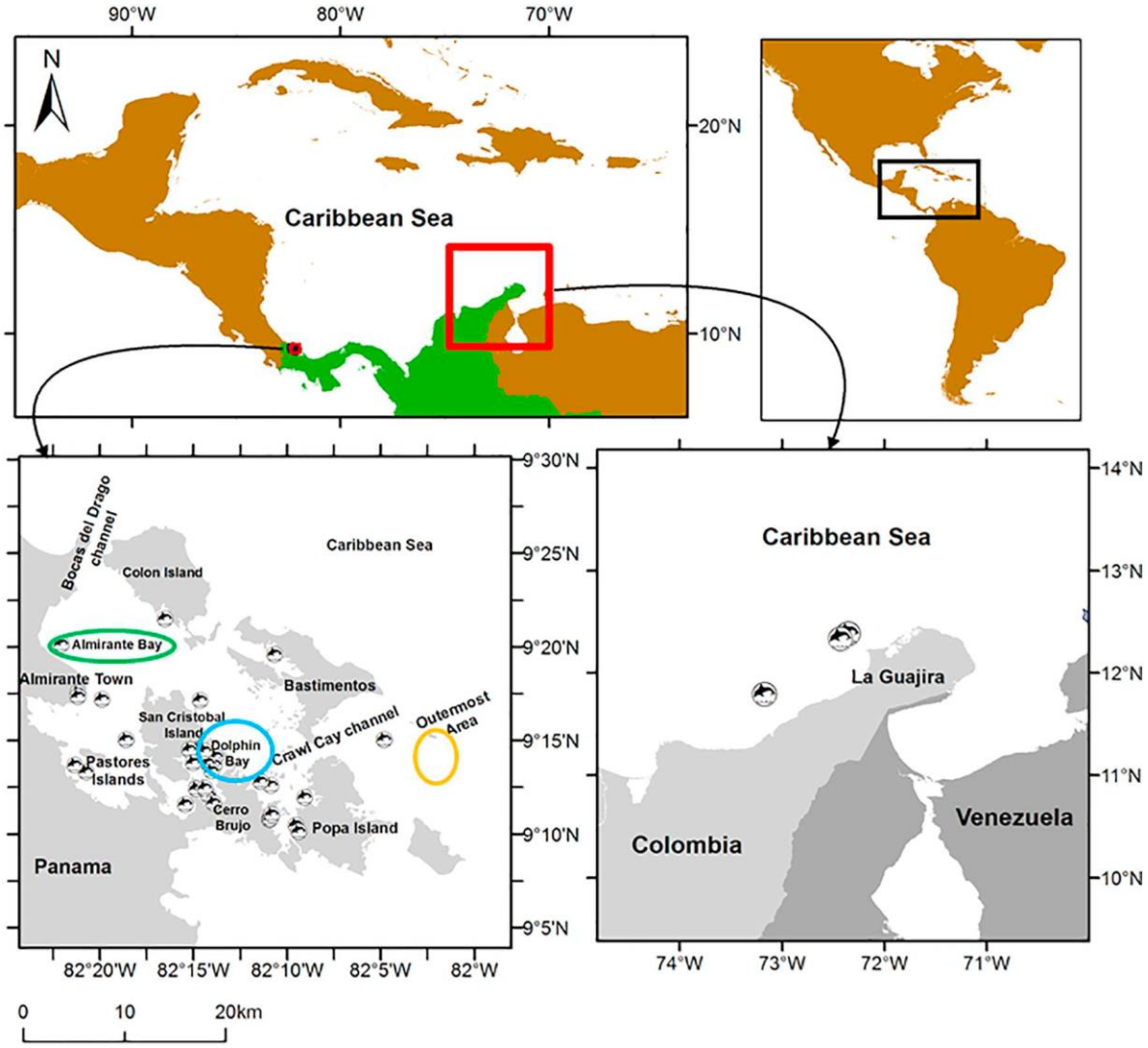

Fig. 1. Location of common bottlenose dolphins (*Tursiops truncatus*) sampled in Bocas del Toro, Panama and La Guajira, Colombia in the Caribbean. The map of Bocas del Toro bottom left shows the three main locations where fish were collected, including Almirante Bay (green), Dolphin Bay (blue), and the 'Outermost' area (yellow).

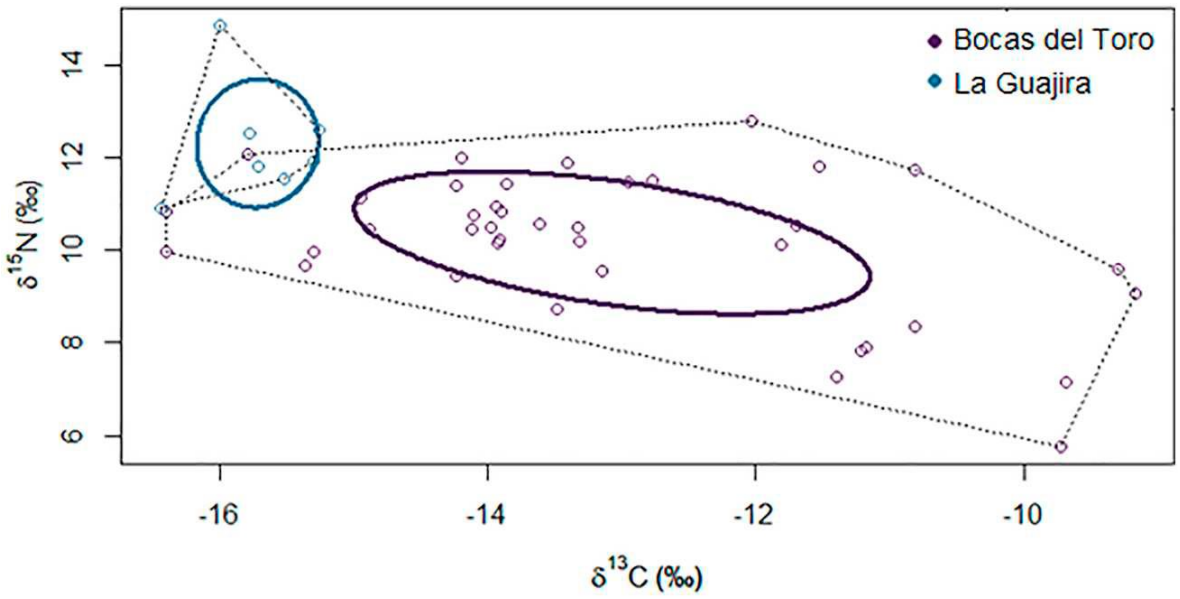

Fig. 2. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values (‰) for Bocas del Toro, Panama (purple) and La Guajira, Colombia (blue) bottlenose dolphins (*Tursiops truncatus*) representing the niche trophic widths. Solid lines indicate standard ellipses areas corrected for small sample sizes (SEAc) and the convex hull area (TA) by dotted line.

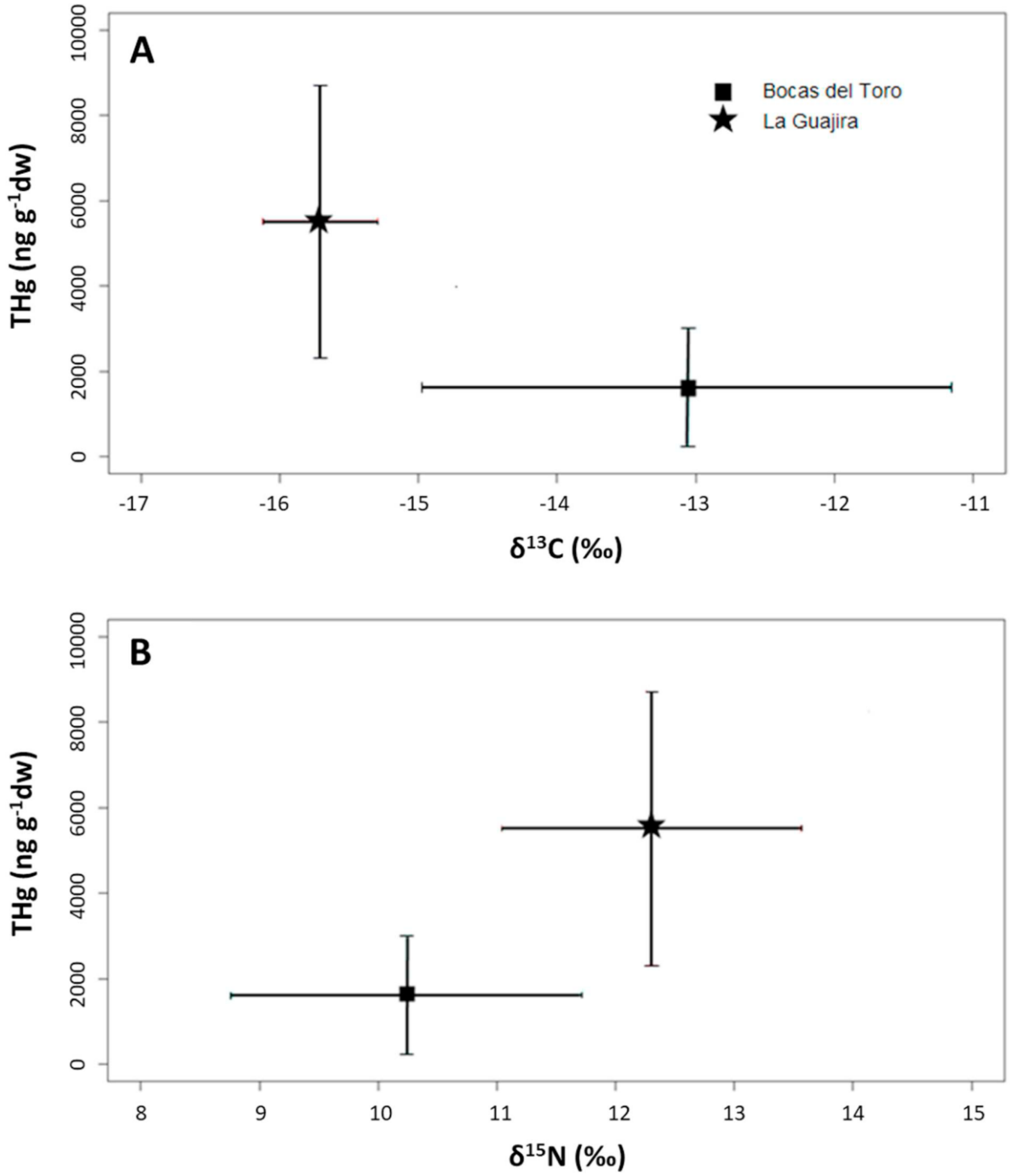

Fig. 3. Stable isotope values (‰) in relation to total mercury THg (ng g^{-1} dw) of bottlenose dolphins (*Tursiops truncatus*) in Bocas del Toro, Panama (star) and La Guajira, Colombia (square). Carbon (A, $\delta^{13}C$) and nitrogen (B, $\delta^{15}N$).

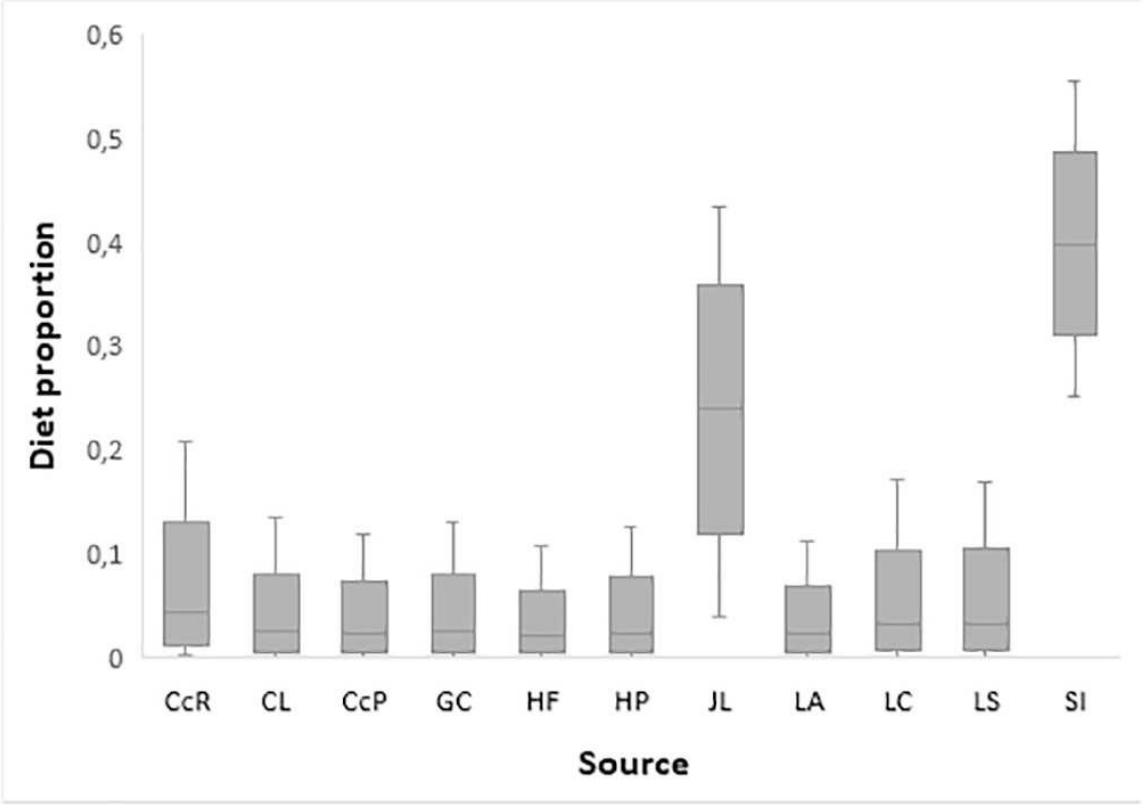

Fig. 4. Posterior estimates of bottlenose dolphins (*Tursiops truncatus*) diet in Bocas del Toro, Panama according to MixSIAR Bayesian stable isotope mixing model. Median diet proportions (%) of each source are: blue runner *Caranx crysos* (CcR, 4.4%), horse-eye jack *Caranx latus* (CL, 2.6%), foureye butterflyfish *Chaetodon capistratus* (CcP, 2.4%), yellow fin mojarra *Gerres cinereus* (GC, 2.6%), French grunt *Haemulon flavolineatum* (HF, 2.2%), white grunt *Haemulon plumieri* (HP, 2.4%), dwarf round herring *Jenkinsia lamprotaenia* (JL, 23.9%), mutton snapper *Lutjanus analis* (LA 2.4%), northern red snapper *Lutjanus campechanus* (LC, 3.3%), lane snapper *Lutjanus synagris* (LS, 3.3%), and striped parrotfish *Scarus iseri* (SI, 39.7%).

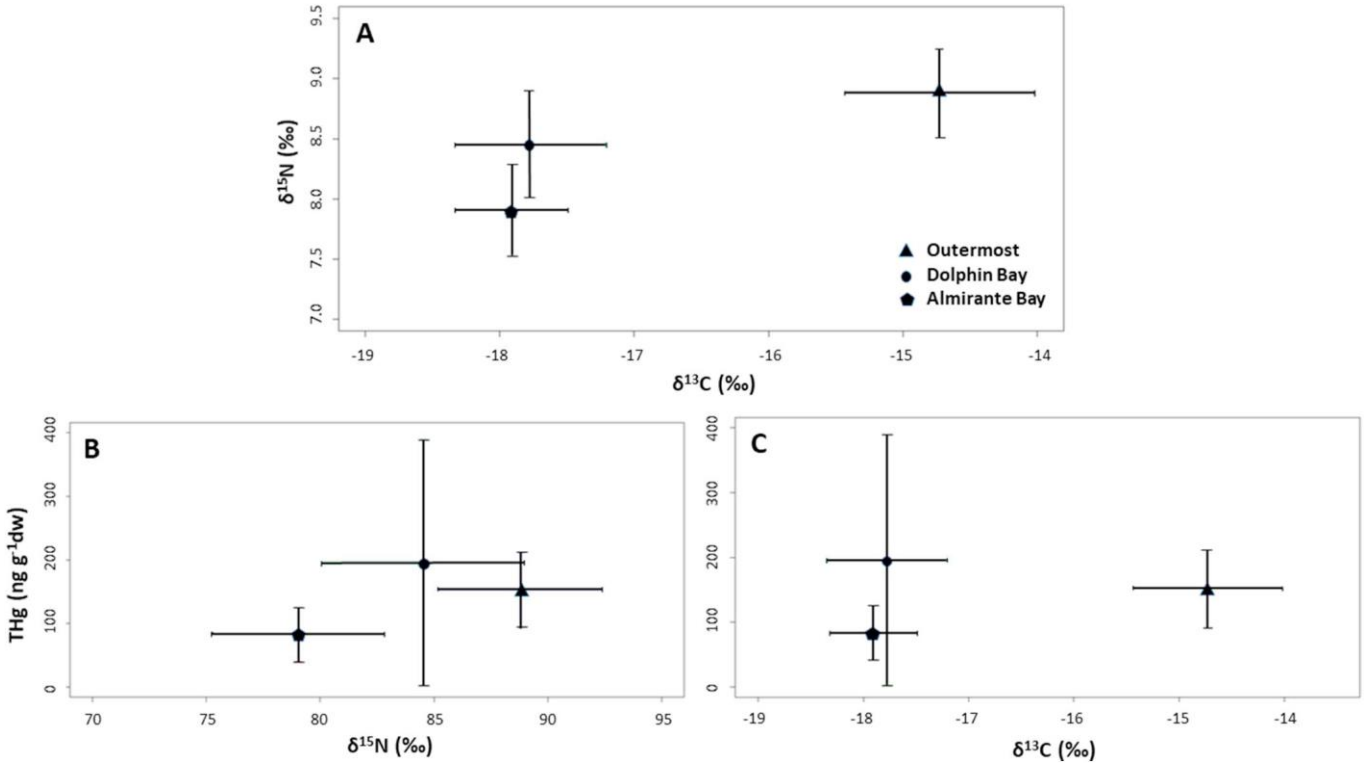

Fig. 5. Mean ± SD carbon and nitrogen isotopic signatures (‰) and THg concentrations (ng g^{-1} dw) of dwarf round herring from three main locations within the Bocas del Toro Archipelago, Panama (triangle: Almirante Bay, circle: Dolphin Bay, square: 'Outermost' area). In (A) is represented relationship between carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values; in (B) and (C) is represented relationship between THg with $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

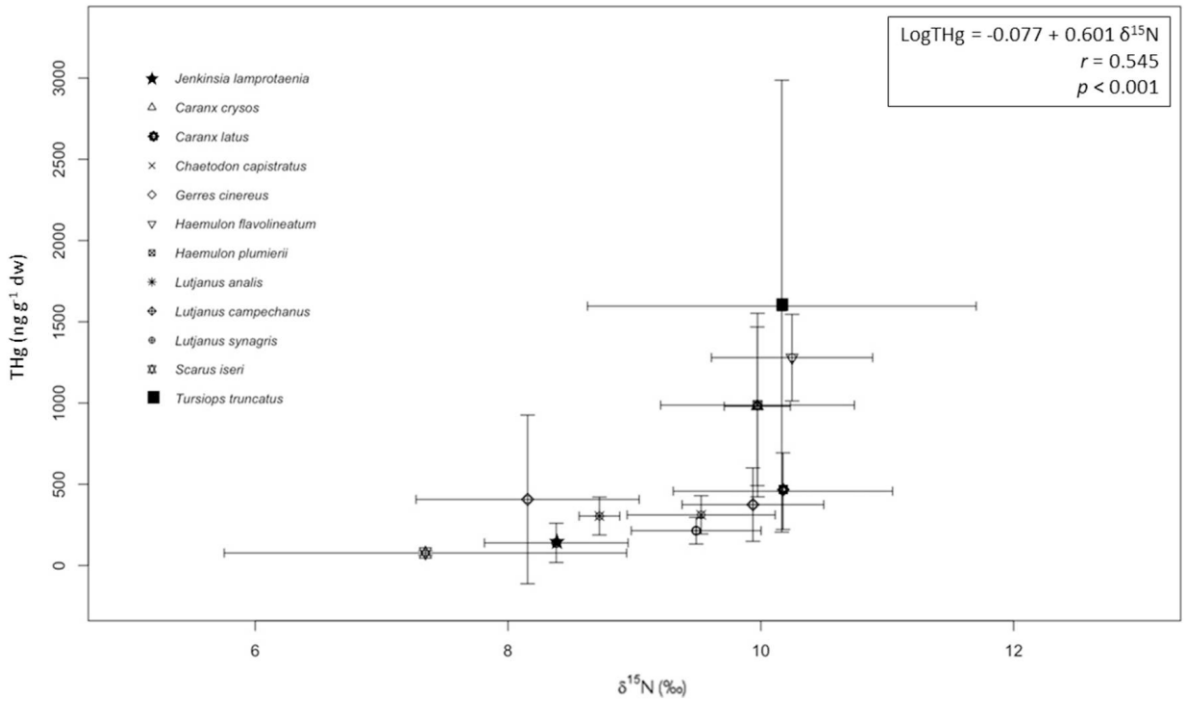

Fig. 6. Relationship between nitrogen isotopic ratio ($\delta^{15}N$ in ‰), and THg concentrations (ng g^{-1} dw) in bottlenose dolphins (*Tursiops truncatus*) and 11 fish species that compose a trophic chain in Bocas del Toro, Panama.

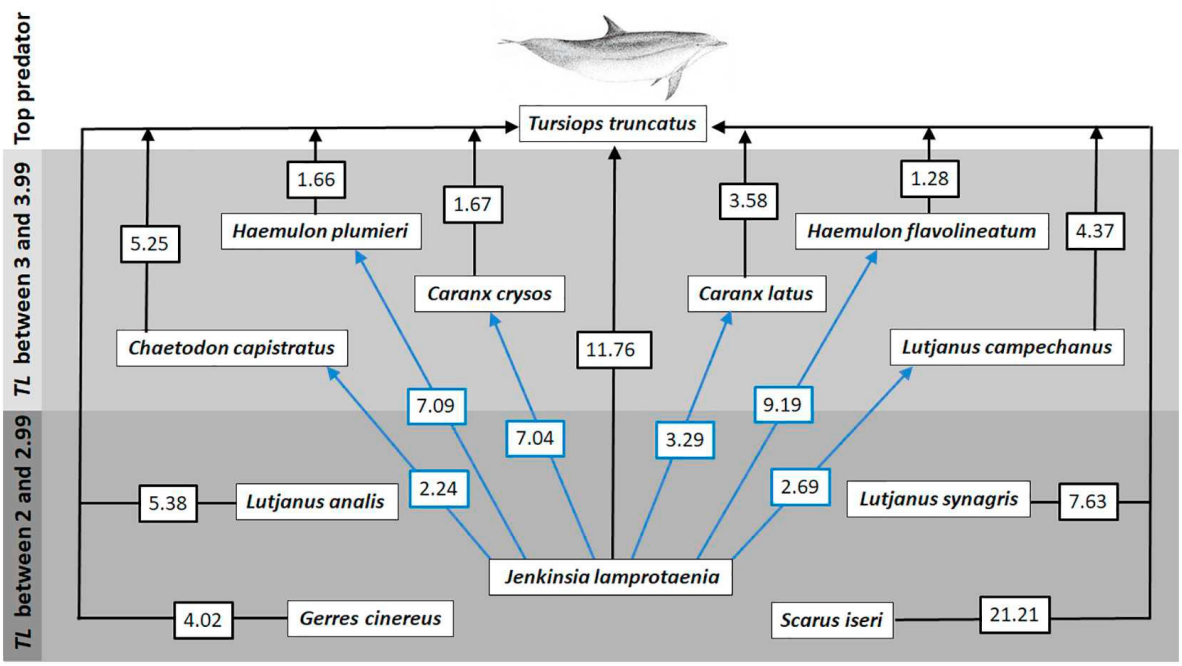

Fig. 7. Bioaccumulation factor (*BAF*) and biotransference factor (*BTF*) of THg through a trophic chain representation in Bocas del Toro, Panama with bottlenose dolphins (*Tursiops truncatus*) as top predator. Indicated numbers on black arrows refer to *BAT* among all fish and bottlenose dolphins. Indicated number on blue arrows refer to *BFT* between the dwarf round herring (*Jenkinsia lamprotaenia*) as prey in second trophic level (*TL* = 2), fish from third trophic level (*TL* = 3) and dolphins as last trophic level.

Table 1. Stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$ in ‰), total mercury (THg in ng g^{-1} dw) concentration and trophic level (*TL*) of bottlenose dolphins, *Tursiops truncatus*, and potential prey fish species collected in Bocas del Toro, Panama.

Species	Common name	*N*	$\delta^{13}C$ Mean ± SD	$\delta^{15}N$ Mean ± SD	THg Min–max	THg Mean ± SD	*TL*
Caranx crysos	Blue runner	10	−16.21 ± 0.27	9.97 ± 0.26	253 – 1813	980 ± 488	3.33
Caranx latus	Horse-eye jack	48	−14.64 ± 1.06	10.18 ± 0.87	141 – 1438	458 ± 236	3.14
Chaetodon capistratus	Foureye butterflyfish	14	−13.27 ± 0.76	9.53 ± 0.59	138 – 527	312 ± 118	3.20
Gerres cinereus	Yellow fin mojarra	4	−11.85 ± 0.84	8.16 ± 0.88	64 – 1162	407 ± 519	2.79
Haemulon flavolineatum	French grunt	3	−13.57 ± 0.47	10.24 ± 0.64	977 – 1480	1279 ± 267	3.41
Haemulon plumieri	White grunt	18	−14.15 ± 1.54	9.97 ± 0.77	343 – 2557	987 ± 565	3.33
Jenkinsia lamprotaenia	Dwarf round herring	50	−16.85 ± 1.58	8.38 ± 0.57	45 – 830	139 ± 121	2.86
Lutjanus analis	Mutton snapper	3	−11.84 ± 0.98	8.72 ± 0.17	180 – 411	304 ± 117	2.96
Lutjanus campechanus	Northern red snapper	8	−14.94 ± 2.22	9.94 ± 0.56	164 – 865	375 ± 226	3.32
Lutjanus synagris	Lane snapper	14	−14.77 ± 1.02	9.49 ± 0.51	118 – 364	214 ± 83	2.99
Scarus iseri	Striped parrotfish	3	−12.70 ± 0.14	7.35 ± 1.59	61 – 89	77 ± 14	2.56
Tursiops truncatus	Bottlenose dolphin	37	−13.05 ± 1.89	10.25 ± 1.48	113 – 4627	1637 ± 1387	3.41

Table 2. Summarized values used in the risk assessment of bottlenose dolphins (*Tursiops truncatus*) in Bocas del Toro, Panama based on Hung et al. calculations (2004; 2006).

Variable	Bottlenose dolphin values
Hg concentration in all prey (CF)	5587 ng g^{-1} ww
Ingestion rate (*IR*)	5.48 kg day^{-1} (Kastelein et al., 2002)
Exposure frequency (*EF*)	365 days $year^{-1}$
Exposure duration (*ED*)	45 years – assuming life-time exposure (Jefferson et al., 2002)
Body weight (*BW*)	260 kg (Kastelein et al., 2002)
Average time (*AT*)	16425 (45 × 365 days)
Reference dose (*RfD*)	118 mg kg^{-1} ww day^{-1}
Maximum Allowable Concentration (MAC_{Rfd})	5587 ng g^{-1} ww

Table 3. Total mercury (THg) skin concentrations (ng g^{-1} dw) of free ranging bottlenose dolphins (*Tursiops truncatus*), Commerson's dolphin (*Cephalorhynchus commersonii*), common dolphin (*Delphinus delphis*), harbour porpoise (*Phocoena phocoena*), and striped dolphin (*Stenella coeruleoalba*) reported in the literature. Data are shown as sample location, sample year, sample number (n), THg mean ± standard deviation, and the study reference.

Location	Year	n	THg Min–max	THg Mean ± SD	Reference
Bottlenose dolphin (*Tursiops truncatus*)					
Bocas del Toro (Panama)	2012–2016	37	113 – 4627	1637 ± 1387	This study
La Guajira (Colombia)	2016	7	2720 – 10590	5526 ± 3209	This study
Lower Florida Keys (FL, USA)	2008	9	294 – 5713	2936 ± 2083	Damseaux et al., 2017
Everglades (FL, USA)	2013	9	Female: 4509 – 29M125	12313 ± 8735	Damseaux et al., 2017
Everglades (FL, USA)	2013	13	Male: 2221 – 28761	10048 ± 6637	Damseaux et al., 2017
Sarasota Bay (FL, USA)	2003–2005	54	322 – 7685	2152 ± 1680	Woshner et al., 2008
Charleston (SC, USA)	2003–2005	74	650 – 4900	1700 ± 920	Stavros et al., 2007
Indian River Lagoon (FL, USA)	2003–2005	75	330 – 31000	7000 ± 5900	Stavros et al., 2007
Bay of Biscay, Atlantic Ocean	2001–2008	16	2200 – 14400	5700 ± 2900	Aubail et al., 2013
Commerson's dolphin (*Cephalorhynchus commersonii*)					
Tierra del Fuego (Argentina)	2010–2012	9	680 – 3110	1380 ± 850	Cáceres-Saez et al., 2015
Common dolphin (*Delphinus delphis*)					
Bay of Biscay, Atlantic Ocean	2001–2008	79	200 – 3500	1700 ± 700	Aubail et al., 2013
Harbour porpoise (*Phocoena phocoena*)					
Bay of Biscay, Atlantic Ocean	2001–2008	17	800 – 3300	1600 ± 700	Aubail et al., 2013
Striped dolphin (*Stenella coeruleoalba*)					
Bay of Biscay, Atlantic Ocean	2001–2008	19	1030 – 6800	3000 ± 1700	Aubail et al., 2013