



Evidence for Interrupted Biomagnification of Cadmium in Billfish Food Chain Based on Stable Carbon and Nitrogen Isotopes from Southwestern of Gulf of California

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Abstract

We report cadmium (Cd) concentrations in muscle, liver, and blood of striped marlin (*Kajikia audax*) and blue marlin (*Makaira nigricans*), caught in the southwest of the Gulf of California. The average Cd concentration was higher in the liver followed by blood and muscle in descending order. This pattern of Cd concentration may be related to the differences in the physiological function of these tissues. In *K. audax*, the Cd concentration in muscle and liver increased proportionally with body size, but only in animals that have reached the body size corresponding to first sexual maturity (animals exceeding 155 cm of postorbital length). Interspecific differences in dietary composition and Cd content evidenced that food preferences have a significant effect on the bioaccumulation of Cd. No evidence of Cd biomagnification (progressive bioaccumulation of an element along the food web) was found, as the correlation between logarithmic Cd concentrations and $\delta^{15}\text{N}$ values was not significant when both billfish and their prey items were included in the calculations. Furthermore, the calculated biotransference factor (transfer of an element from food to consumer) suggested that Cd transference is interrupted from prey to marlins.

Keywords Cadmium · Interrupted biomagnification · Billfish · Stable isotope analysis · Mexican Pacific

Introduction

The Gulf of California lies in a semi-closed basin surrounded by desertic areas and semi-arid areas and constitutes an ecosystem nearly pristine with high biodiversity [1], at the north of Mexico. However, environmental pollution by trace metals, nutrients, and diverse organic compounds, caused by natural or anthropogenic sources, has been reported in recent studies [2]. Notably, a study conducted in sailfish (*Istiophorus platypterus*) and striped marlin (*Tetrapturus audax*) from the southern Gulf of California revealed that arsenic (As) and

cadmium (Cd) in the edible portion of muscle exceeded by 20% from the guideline levels of the World Health Organization (WHO), the U.S. Food and Drug Administration, and the European Union [3].

Billfish play a very important role in maintaining the equilibrium of marine ecosystems. These fish became of great economic importance for both sport and commercial fisheries [4]. Striped marlin (*Kajikia audax*) and blue marlin (*Makaira nigricans*) are major recreational fishing resources in the southern Gulf of California, and most of them are obtained within their area of distribution in Cabo San Lucas (about 500 nautical miles from the coast) [5]. Although many marlins are caught and released, most of them are retained and consumed locally. However, eating large predatory fish may place the people at risk of serious illness, due to the ingestion of metal-contaminated edible portions [3].

Previous studies have documented that the accumulation of elements depends on the type and amount of food consumed [6]. Elements, such as mercury, show a phenomenon of biomagnification (i.e., the progressive bioaccumulation along food web) when they are transferred from a low trophic level to a high trophic level [7]. The existence of Cd biomagnification in marine ecosystems remains under debate [8], and some studies have found that Cd is not biomagnified

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[9, 10]. There is evidence of biomagnification of Cd around the west coast of Mexico, [11], although other studies have found that Cd, Cu, Pb, and Zn were not positively transferred through the entire food web [12] (i.e., there was no increase of chemicals from lower to higher trophic levels). In the case of Cd, what is not clear yet is what process of Cd accumulation takes places in organisms, whether the accumulation of Cd progresses to higher trophic levels, or to what extent accumulation occurs. In any case, it is worth noting that all trace elements exert toxic effects on organisms as concentrations increase.

Ratios of stable carbon ($^{13}\text{C}:^{12}\text{C}$, $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}:^{14}\text{N}$, $\delta^{15}\text{N}$) isotopes are related to exposure to contaminants and are tools currently used in studies of feeding ecology. The $\delta^{13}\text{C}$ signature (i.e., the proportion of heavy isotopes over light isotopes) is used to establish the origin of a trophic web and to identify the carbon sources of primary producers [13]. The $\delta^{15}\text{N}$ is usually enriched in predators, relative to concentration in the corresponding prey [14]. Because stable isotope ratios of consumers are related to those of its prey [7], $\delta^{15}\text{N}$ is used to evaluate the biomagnification of trace elements in distinct trophic links along the food web. Different studies have shown a direct relationship between the type of diet and the isotopic composition of an organism, which is dependent on the turnover rate of the tissue analyzed [15]. For example, along the life of animals, the muscle shows the longest time of bioaccumulation through dietary intake, depending on age. On the other hand, blood samples reflect recent (from previous days to weeks) dietary intake of consumers and exposure to contaminants in their food areas [16].

To assess the biomagnification of Cd and to compare the bioaccumulation of Cd in different tissues, we collected muscle tissue, liver, and blood samples from the billfish species striped marlin (*Kajikia audax*) and blue marlin (*Makaira nigricans*), during summer and autumn of 2015. Here, we report the relationship between Cd concentrations and stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in the muscle of billfish and their prey items. We also discuss the relationships between Cd concentrations and some biological factors (i.e., body length, sex, and species).

Materials and Methods

Sampling

Billfish samples were collected monthly from the sport-fishing fleet that operated from autumn to winter of 2015 in Cabo San Lucas, Baja California Sur (22°53'N, 109°54'W), in the southern Gulf of California. Once a specimen was identified, the post-orbital length (POL), sex, and species were recorded. Samples of dorsal white muscle tissue and liver (~100 g) were collected from billfish. With help of a syringe and

needle, blood samples (~5 mL) were collected from the caudal vein and deposited in heparin tubes to prevent coagulation. Also, from each billfish, the stomach was removed to obtain information on recently consumed food. Samples were kept in coolers during transport to the laboratory. All biological samples were kept frozen (−20 °C) until further analysis.

In the laboratory, stomach of each fish was thawed, and the contents were then separated into the lowest determinable taxonomic groups. The prey items in the stomach contents were identified on descriptions given elsewhere [5], and the number of individuals was counted and weighed. To estimate feeding habits, the number of individuals of each prey type, the weight of each prey type, and the frequency of prey occurrence, expressed as percentage of the total number (%N), weight (%W), or occurrence (%OF) of all prey items in the stomach contents, were calculated [5]. Undigested individual prey were extracted from the gut contents of marlins and thoroughly washed in double-distilled deionized water to remove contaminants. We took samples of muscle tissue of prey items from stomach contents and prepared total body homogenates for small species. The tissue samples and the prey from the stomach contents were separately homogenized and freeze-dried for 72 h (−45 °C and 27×10^{-3} mbar) and then pulverized in a semi-automatic agate mortar and by hand.

Chemical Analysis

A detailed description of the method used for Cd determination is given by Páez-Osuna and Tron-Mayén [17]. Aliquots of the sample (0.250 ± 0.003 g of dry tissue) were digested in Teflon vials with caps (Savillex) with 5 mL of concentrated HNO_3 (Ultrapure $\geq 65\%$). The livers were digested using 2 mL of H_2O_2 (30%) and 3 mL of concentrated HNO_3 at 120 °C for 3 h. The samples were diluted with purified Milli-Q water to a final volume of 20 mL. All samples were analyzed in duplicate. Blank samples and the standard reference material, DORM-4 (fish protein; NRC-CNRC) and DOLT-5 (dogfish liver; NRC-CNRC), were digested (one in each batch of 20 samples alternately) using the same procedure to control for accuracy and precision. Quantitative determination of Cd was performed by Graphite Furnace Atomic Absorption Spectrometry (GFAAS) (Varian SpectrAA220).

The GFAAS system was initially calibrated with a carrier blank and 0.5, 1.0, and 2.0 ng mL^{-1} aqueous solution of cadmium. The solution was prepared from a 1000 $\mu\text{g mL}^{-1}$ stock standard solution (Merck, Germany). The limit of detection was 0.2 $\mu\text{g L}^{-1}$, and the limit of quantification was 0.03 $\mu\text{g g}^{-1}$. Replicate analyses of DORM-4 (0.303 ± 0.020 $\mu\text{g g}^{-1}$ dry weight) and DOLT-5 (14.0 ± 1.8 $\mu\text{g g}^{-1}$ dry weight) were within the range of the certified reference material for Cd (DORM-4, 0.299 ± 0.018 $\mu\text{g g}^{-1}$; DOLT-5, 14.5 ± 0.6 $\mu\text{g g}^{-1}$). Concentrations of trace metals were expressed as $\mu\text{g g}^{-1}$ dry weight. Conversion of metal

concentrations from dry weight to wet weight was performed according to the water content of the tissue of interest.

Stable isotope ratios were measured using an elemental analyzer (Costech) coupled with an isotope ratio mass spectrometer (Thermo Scientific Delta V Plus-IRMS) and expressed as $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ relative to the international standard (Pee Dee Belemnite for carbon and atmospheric N_2 for nitrogen), according to the following equation:

$$\delta X(\text{‰}) = \left(\left[\frac{R_{\text{sample}}}{R_{\text{standard}}} \right] - 1 \right) \times 10^3,$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$.

At least two different laboratory standards, calibrated against reference materials of the National Institute of Standards Technology (NIST) with a composition similar to the samples, were analyzed along with the samples. Long-term standard deviation was 0.2‰ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{15}\text{N}$.

Data Analysis

Cadmium concentrations and stable isotope values are presented as mean \pm standard deviation (SD) based on dry weight. In order to define significant differences in Cd concentrations and stable isotopes of carbon and nitrogen between animals, an analysis of variance was conducted using the Kruskal–Wallis test. A Mann–Whitney U test was used to compare Cd concentrations across sex and species in each tissue. Additionally, we performed Mann–Whitney U test to compare stable isotope values between marlin species and between sex. Single regression and Spearman's rank correlation analyses were conducted to test whether body size had significant effects on metal concentrations and stable isotope values. A p value equal or less than 0.05 indicated statistical significance [18]. The statistical analysis was performed using Statistica 7.0 for Windows (StatSoft, Inc.).

In this study, we evaluated biomagnification across the food chain of billfish by plotting the linear relationships between trace elements and $\delta^{15}\text{N}$ or trophic position and using the regression slope (β) as a measure of biomagnification power, i.e., the magnitude of the increment when the chemical passes up through two or more trophic levels [19], as follows:

$$[\text{trace element}] = \beta * (\delta^{15}\text{N}) + a;$$

where a is the y -intercept.

Biotransference factor (BTF), the transference of trace elements from a food source (prey) to a consumer (predator), was calculated using the following equation:

$$\text{BTF} : \left(\frac{\text{Metal}_{\text{predator}}}{\text{Metal}_{\text{prey}}} \right) / \left(\frac{\delta^{15}\text{N}_{\text{predator}}}{\delta^{15}\text{N}_{\text{prey}}} \right)$$

where $\text{Metal}_{\text{predator}}$ and $\text{Metal}_{\text{prey}}$ are the concentrations of the selected metals in micrograms per gram of dry weight of the

predator and prey [20]. BTF was corrected for differences in trophic position (based on $\delta^{15}\text{N}$) between predator and prey. Positive biotransference of a trace element from source to consumer occurs when $\text{BTF} > 1$. BTF was calculated for muscle in marlin fish and for its prey items. Metal concentrations of total body homogenates were used for calculations for invertebrates and small fish.

Results

Cd Concentration in Marlins

Table 1 shows the body size (as POL) and Cd concentration in males and females of *K. audax* and *M. nigricans*. Fifty-seven specimens of *K. audax* (length range 140–183 cm) and 21 specimens of *M. nigricans* (length range 134–261 cm) were collected. Although the average body length of *M. nigricans* (mean \pm SD 182 \pm 38 cm) was longer than that of *K. audax* (162 \pm 15 cm), the difference was not significant (Mann–Whitney U test, $p > 0.05$). No differences in body length were observed between males and females of *K. audax* (Mann–Whitney U test, $p > 0.05$; Table 1) or *M. nigricans* (Mann–Whitney U test, $p > 0.05$; Table 1).

No significant sex-related differences of Cd concentration were observed in the muscle, liver, and blood samples (Mann–Whitney U test, $p < 0.05$; Table 1), except for the liver where the average Cd concentration was higher in females than in males of *M. nigricans*. In *K. audax*, the average Cd concentration was higher in the liver followed by blood and muscle, in a descending order. There were only significant differences in Cd concentration between liver and muscle and between liver and blood (Kruskal–Wallis one-way ANOVA, pair-wise multiple comparison, $p < 0.05$; Table 1). The Cd concentration in the blood of *K. audax* increased slightly and proportionally as body length increased, but the relationship was not significant ($r = 0.24$, $p > 0.05$; Fig. 1). In contrast, Cd concentration in the liver and muscle increased significantly with body size (in muscle $r = 0.42$, $p < 0.05$; in liver $r = 0.41$, $p < 0.05$; Fig. 1).

In *M. nigricans*, the pattern of average Cd concentrations in different tissues was similar to that of *K. audax* (liver $>$ blood \geq muscle); all pair-wise multiple comparisons showed statistically significant differences between liver and muscle and liver and blood (Kruskal–Wallis one-way ANOVA, pair-wise multiple comparison, $p < 0.05$; Table 1). Figure 1 shows that in *M. nigricans*, there was no correlation between Cd concentration in tissues and body length (in muscle $r = 0.28$, $p > 0.05$; in liver $r = 0.27$, $p > 0.05$; in blood $r = -0.11$, $p > 0.05$). The average Cd concentration in the liver (435.4 \pm 505.2 $\mu\text{g g}^{-1}$ dry weight) and muscle (0.63 \pm 0.70 $\mu\text{g g}^{-1}$ dry weight) of *K. audax* was higher than that in the liver (196.3 \pm 117.9 $\mu\text{g g}^{-1}$ dry weight) and muscle (0.29 \pm

Table 1 Body length and metal concentrations (mean \pm SD and range, on dry weight) in muscle, liver, and blood and values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (mean \pm SD and range, on dry weight) in muscle of *Kajikia audax* and *Makaira nigricans* by sex

Species	Length (POL, cm)	Muscle			Liver	Blood
		Cd ($\mu\text{g g}^{-1}$)	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	Cd ($\mu\text{g g}^{-1}$)	Cd ($\mu\text{g g}^{-1}$)
<i>K. audax</i>	162 \pm 15 ¹	0.63 \pm 0.70 ^{a1}	-16.7 \pm 0.5 ¹	17.4 \pm 1.3 ¹	435.4 \pm 505.2 ^{b1}	1.0 \pm 1.5 ^{a1}
Range	140–183	0.03–4.80	-17.6 to -15.3	13.8–19.7	2.0–2295.7	0.1–6.4
Male	157 \pm 13	0.70 \pm 0.89	-16.7 \pm 0.4	17.3 \pm 1.1	559.5 \pm 639.2	0.8 \pm 1.1
Female	161 \pm 12	0.52 \pm 0.47	-16.6 \pm 0.6	17.5 \pm 1.5	361.4 \pm 335.9	1.2 \pm 1.7
<i>M. nigricans</i>	182 \pm 38 ¹	0.29 \pm 0.18 ^{a2}	-16.5 \pm 0.4 ¹	16.6 \pm 1.5 ¹	196.3 \pm 117.9 ^{b2}	1.3 \pm 1.2 ^{a1}
Range	134–261	0.08–0.80	-17.1 to -15.7	13.5–19.6	20.8–499.4	0.1–4.2
Male	176 \pm 33	0.25 \pm 0.18	-16.5 \pm 0.6	16.7 \pm 1.8	115.3 \pm 76.0	1.2 \pm 1.1
Female	197 \pm 37	0.31 \pm 0.21	-16.8 \pm 0.3	16.5 \pm 1.2	249.5 \pm 120.0	1.3 \pm 1.3

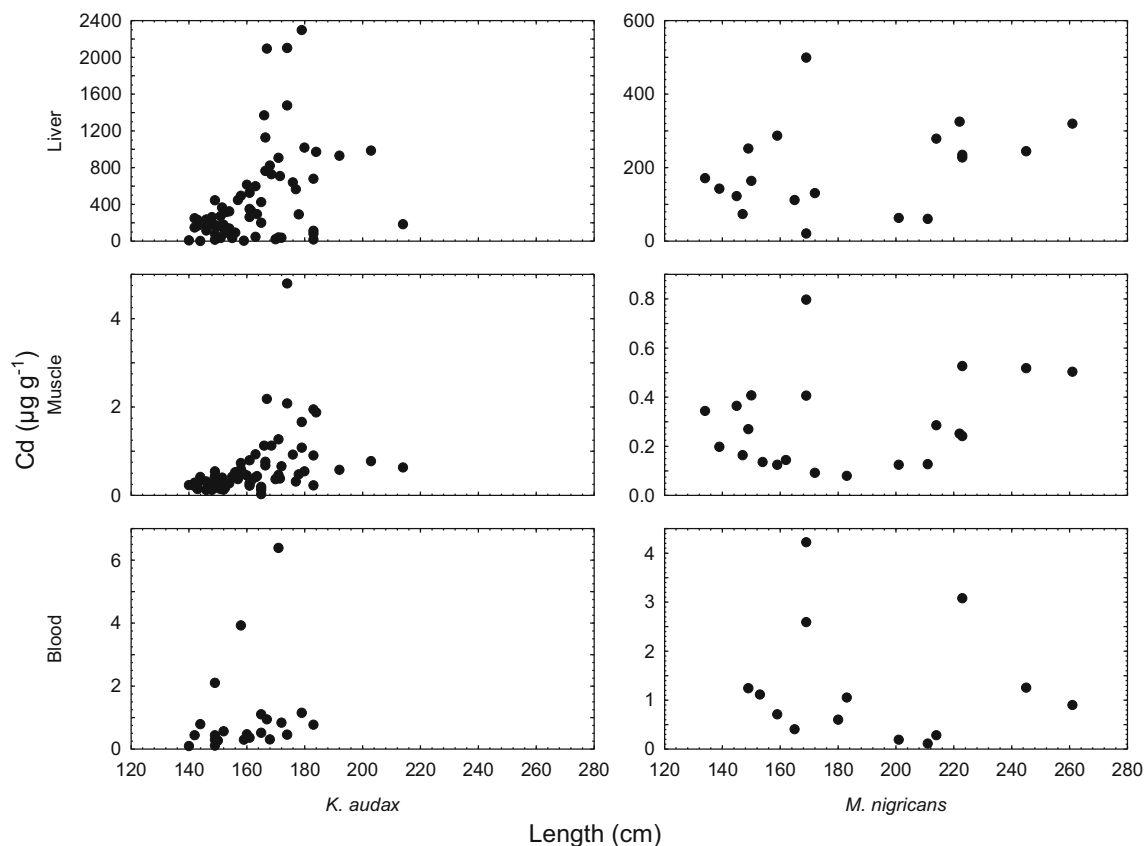
Different superscript letter indicates significantly different ($p < 0.05$) mean concentrations between tissues in a same species; different superscript number indicates significantly different ($p < 0.05$) body length, mean concentrations, and stable isotopes between species in a same tissue

POL, post-orbital length

0.18 $\mu\text{g g}^{-1}$ dry weight) of *M. nigricans* (Mann–Whitney U test, $p < 0.05$; Table 1). In contrast, the average Cd concentration in blood (1.0 \pm 1.5 $\mu\text{g g}^{-1}$ dry weight) of *K. audax* was comparable to the Cd concentration in blood (1.3 \pm 1.2 $\mu\text{g g}^{-1}$ dry weight) of *M. nigricans* (Mann–Whitney U test, $p > 0.05$; Table 1).

Stable Isotopes in Marlins

In the muscle of *K. audax*, the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were -16.7 \pm 0.5 and 17.4 \pm 1.3‰, respectively. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of *M. nigricans* were -16.5 \pm 0.4 and 16.6 \pm 1.5‰, respectively. There were no significant differences in $\delta^{13}\text{C}$

**Fig. 1** Relationship between body length (cm) and Cd concentration ($\mu\text{g g}^{-1}$ dry weight) in liver, muscle, and blood of *Kajikia audax* and *Makaira nigricans*

and $\delta^{15}\text{N}$ between *K. audax* and *M. nigricans* (Mann–Whitney U test, $p > 0.05$; Table 1), and there were no differences in the isotopic values between males and females (Mann–Whitney U test, $p > 0.05$; Table 1).

The correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and body length was not significant in *K. audax* (for $\delta^{13}\text{C}$ $r = 0.19$, $p > 0.05$; for $\delta^{15}\text{N}$ $r = 0.14$, $p > 0.05$; Fig. 2) or in *M. nigricans* (for $\delta^{13}\text{C}$ $r = 0.19$, $p > 0.05$; for $\delta^{15}\text{N}$ $r = -0.13$, $p > 0.05$; Fig. 2). Figure 3 shows Cd concentration in muscle plotted against $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. Cadmium concentrations did not increase with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ($p > 0.05$) in either *K. audax* or *M. nigricans*.

Diet and Trophic Transference of Cd

Stomach content analysis (Table 2) indicates that the diet in %N of *K. audax* was composed mainly of cephalopods (23.53% squid *Dosidicus gigas* and 20.00% *Argonauta* spp.) and fish to a lower extent (9.41% *Selar crumenophthalmus* and 8.24% *Lagocephalus lagocephalus*). On the other hand, *M. nigricans* mainly consumed fish (e.g., 14.89% *Auxis* spp., 6.38% *Balistes polylepis*), crustaceans (19.15% *Euphyllax dovii*), and cephalopods (14.89% *Sthenoteuthis oulaniensis*, 8.5% *D. gigas*). The Kruskal–Wallis test revealed significant differences ($p < 0.05$) in Cd concentrations between prey items.

The prey species in the initial state of digestion were considered in the quantification of Cd from their muscle tissue. Among 12 prey analyzed, the highest concentration of Cd was

shown in cephalopods followed by crustaceans and fish in descending order (Table 3). In the muscle of *K. audax*, positive biotransference (BTFs > 1) of Cd from prey to predator was observed in 1 out of 11 trophic interactions. In contrast, in *M. nigricans*, all the calculated BTFs were < 1 (Table 3). However, when the global stomach content (all prey items) was used for analyses, the BTF did not show a positive trophic transfer from all the prey items (considered as a single source) to the predator, either in *K. audax* or *M. nigricans*.

Integrating Stable Isotopes and Cd Data

The food chain of marlins was structured according to $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of prey items (Fig. 4). The $\delta^{13}\text{C}$ values ranged from -18.3‰ in *Mastigoteuthis dentata* to -16.7‰ in *K. audax* and from -19.2‰ in *Sphoeroides* spp. to -16.5‰ in *M. nigricans*. Values of $\delta^{15}\text{N}$ ranged from 10.1‰ in *Fistularia* spp. to 17.4 in *K. audax* and from 11.9‰ in *Euphyllax dovii* to 16.6 in *M. nigricans*. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the food chain of *K. audax* and *M. nigricans* were significantly different (Kruskal–Wallis, $p < 0.05$).

K. audax and *M. nigricans* had the highest $\delta^{15}\text{N}$ values and thus represented the highest trophic level of all animals analyzed (Fig. 4). In the food chain of both marlin species, there was no significant correlation between logarithmic Cd concentrations and $\delta^{15}\text{N}$ ($r = -0.09$, $p > 0.05$; Fig. 5). The slope of the regression curve between logarithmic Cd concentrations and $\delta^{15}\text{N}$ was negative (slope = -0.032).

Fig. 2 Relationship between body length (cm) and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) in the muscle of *Kajikia audax* and *Makaira nigricans*

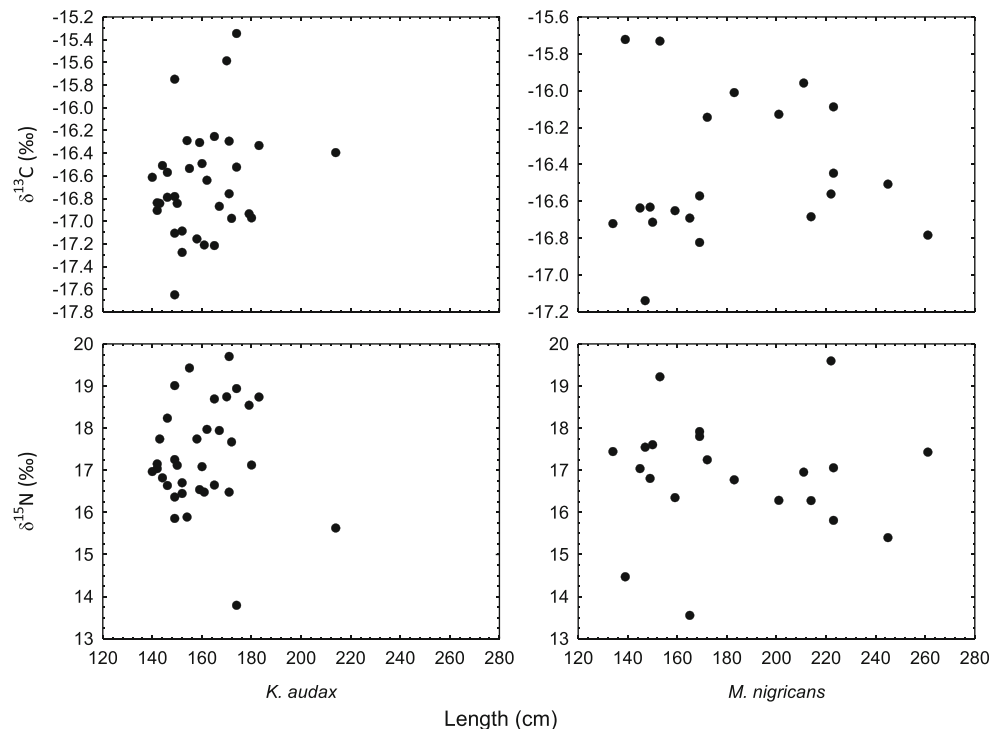
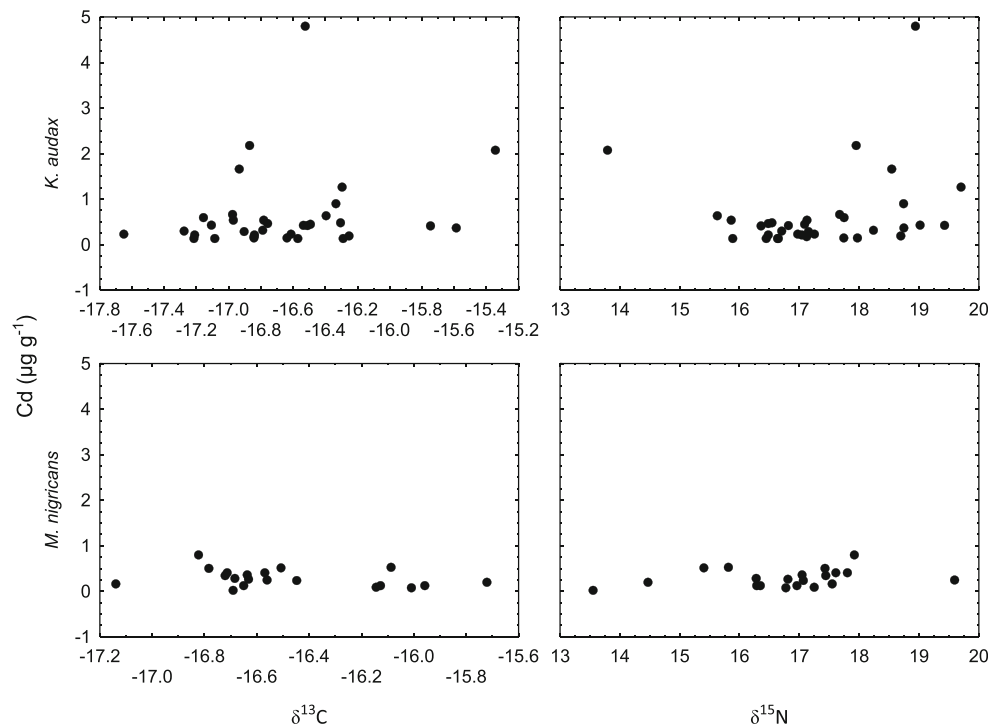


Fig. 3 Relationship between Cd concentration ($\mu\text{g g}^{-1}$ dry weight) and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (‰) in the muscle of *Kajikia audax* and *Makaira nigricans*



Discussion

We have found that, both in *K. audax* and *M. nigricans*, the average Cd concentration was higher in the liver, followed by blood and muscle. This pattern of Cd concentration may be related to the differences in physiological function of these tissues, as has been suggested [21, 6]. For example, the elemental turnover rates are, as a general rule, higher in liver than in muscle [22]. One of the most common detoxification strategies in the liver of marine animals is the binding of metals to metallothioneins [23], which provides protection against the toxic effects of certain metals (particularly against Cd) by sequestering and reducing the amount of free metal ions and by restoring the function of structures that are altered by inappropriate metal binding [21]. Metallothioneins have been found in a large number of animals, including fish, in the liver, kidney, gill, and muscle [6]. Hence, although metallothioneins were not investigated in the present study, the high concentration of Cd found in the liver of marlin species may provide clues to whether metallothioneins play a detoxifying in these fish.

Because chemicals absorbed by the gut are transported by the blood [22], the Cd detected in blood samples can be associated with direct uptake from prey that were consumed in the study area, which suggests that Cd is bioavailable for *K. audax* and *M. nigricans* in the southwestern coasts of the Gulf of California. In the Gulf of California region (coastal lagoons), Cd has been detected at concentrations ranging from 9.0 to 39.3 ng L^{-1} [24]. Blood can represent recent dietary intake on consumers and the exposure to contamination in their food areas (from previous days to weeks) [16], while muscle may reflect intake on a larger

time dietary intake along the life of animals. Unlike *M. nigricans*, *K. audax* concentrated large amounts of Cd in the muscle and liver; therefore, attention must be paid to the capture of *K. audax* in the southwestern coasts of the Gulf of California. In terms of food safety, the muscle tissue of *M. nigricans* may be suitable for human consumption, as the Cd concentrations in wet weight (*K. audax*, $0.15 \pm 0.16 \mu\text{g g}^{-1}$; *M. nigricans*, $0.06 \pm 0.04 \mu\text{g g}^{-1}$) were below the $1.0 \mu\text{g g}^{-1}$ limit (in wet weight) adopted by the Mexican Secretariat of Health [25]. Nevertheless, the Cd levels in the muscle exceeded the limit set by the European Union [26] ($0.1\text{--}0.3 \mu\text{g g}^{-1}$); this limit was exceeded in 36 and 14% in *K. audax* and *M. nigricans*, respectively. For food safety, it is important to take into consideration the quantity and frequency consumption of marlins, because consuming small amounts of contaminated fish over long periods of time may lead to Cd intoxication.

The mean levels of Cd in muscle tissues of *K. audax* in our study are lower to those reported by Soto-Jiménez et al. [3] in specimens of striped marlin (*Tetrapturus audax*) and sailfish (*Istiophorus platypterus*) caught in the southeast Gulf of California. These differences may be derived from differences in body size, because specimens of a larger size (from 159 to 254 cm lower jaw fork length) from southeast Gulf of California exhibited much higher values than specimens of *K. audax* (140–183 cm) caught in the southwestern coasts of the Gulf of California. In keeping with this idea, we found that Cd concentration in the muscle of *K. audax* increased with body size. Nonetheless, Cd concentration in the liver and muscle increased with body size only in marlins over 155 cm POL (Fig. 1). Marlins over 155 cm POL may represent mature fish, as reported

Table 2 Stomach contents of *Kajikia audax* and *Makaira nigricans* sampled from southwest Gulf of California, Mexico. Values in bold represent the most important prey items in billfish diet expressed as

percentage in number of all prey items (%N); wet weight of the total weight or all prey items (%W); and percentage frequency of occurrence (%FO); *, not present in the diet

Presa	<i>Kajikia audax</i>			<i>Makaira nigricans</i>		
	%N	%W	%FO	%N	%W	%FO
Crustacean						
<i>Euphylax dovii</i>	*	*	*	19.15	0.80	14.28
Cephalopoda						
<i>Dosidicus gigas</i>	23.53	0.44	10.00	8.51	0.44	7.14
<i>Sthenoteuthis oulaniensis</i>	*	*	*	14.89	1.71	7.14
<i>Mastigoteuthis dentata</i>	3.53	2.65	4.00	*	*	*
<i>Argonauta</i> spp.	20.00	0.00	12.00	2.12	0.00	3.57
<i>Vitreledonella richardi</i>	1.18	0.08	2.00	*	*	*
<i>Japetella diaphana</i>	6.38	0.00	3.57	*	*	*
<i>Onychoteuthis borealijaponica</i>	1.18	0.09	2.00	*	*	*
<i>Pholidoteuthis boschmaii</i>	*	*	*	6.38	0.00	3.57
Fish						
<i>Acanthurus</i> spp.	3.53	0.78	6.00	*	*	*
<i>Auxis</i> spp.	1.18	13.28	4.00	14.89	39.59	17.86
<i>Balistes polylepis</i>	3.53	0.52	6.00	6.38	0.14	7.14
<i>Coryphaena hippurus</i>	*	*	*	2.12	54.33	3.57
<i>Balistes</i> spp.	1.18	0.19	2.00	*	*	*
<i>Strongylura</i> spp.	1.18	0.20	2.00	*	*	*
<i>Caranx caballus</i>	2.35	10.12	4.00	*	*	*
<i>Fistularia</i> spp.	1.18	0.08	2.00	*	*	*
<i>Johnrandallia nigrirostris</i>	1.18	1.75	2.00	4.25	0.24	7.14
<i>Lagocephalus lagocephalus</i>	8.24	28.75	6.00	4.25	0.40	7.14
<i>Mulloidichthys dentatus</i>	1.18	1.58	2.00	*	*	*
<i>Selar crumenophthalmus</i>	9.41	38.65	16.00	2.13	2.12	3.57
<i>Porichthys</i> spp.	*	*	*	2.13	0.02	3.57
Unidentifiable	8.23	0.55	12.00	6.38	0.18	10.71

by Eldridge and Wares [27], who analyzed striped marlin from Buenavista (Baja California Sur), Mazatlán (Sinaloa), and San Diego (California) and reported that fish size at first sexual maturity was 155 cm for males and 165 cm for females. The increased hepatic Cd concentrations after maturation may be explained by the continuous intake of Cd through the diet, reduction in growth at maturation, and formation of metallothioneins–Cd complexes [6, 21]. On the other hand, the lack of correlation between body length and Cd concentrations in *M. nigricans* may be attributed to low Cd uptake along the fish life and/or to passive or active excretion of Cd that may lead to the stabilization of Cd accumulation.

Interspecific differences in dietary composition and Cd content evidenced that food preferences have a significant effect on the bioaccumulation of Cd. Our analysis of stomach content indicated that *M. nigricans* fed on shoals of epipelagic fish, mainly on *Auxis* spp. while the diet of *K. audax* included more mesopelagic prey (e.g., squids of the family Ommastrephidae,

particularly *Dosidicus gigas*). These results are consistent with previous studies showing that marlin fish are top predators that can also consume cephalopods and invertebrates, in addition to fish [5]. Moreover, studies on the stomach contents of marlin fish from the Gulf of California [5, 28, 29] indicated that *K. audax* is a generalist predator while *M. nigricans* is unique in its reliance on pelagic fish. In this study, prey fish show low Cd concentrations, and their consumption by *M. nigricans* can drive a lower Cd uptake in this species.

On the other hand, it has been reported that inclusion of a large percentage of cephalopods in the diet results in accumulation of higher Cd concentrations [30]. This report is in agreement with our finding that cephalopods had the highest Cd levels among all prey analyzed (Table 3). The higher Cd concentrations in the liver and muscle of *K. audax*, relative to *M. nigricans*, can thus be explained by the higher abundance of cephalopods in the diet of *K. audax*. The similarity of Cd levels between marlin fish males and females that we report here reveals dietary similarities.

Table 3 Scientific name, taxon, number of specimens (n), and Cd concentration (mean \pm SD, on a dry weight basis) for prey items, and biotransference factor (BTF) for striped marlin *Kajikia audax* and blue marlin *Makaira nigricans*

Species	Taxon	<i>K. audax</i>			<i>M. nigricans</i>		
		n	Cd ($\mu\text{g g}^{-1}$)	BTF	n	Cd ($\mu\text{g g}^{-1}$)	BTF
<i>Acanthurus</i> spp.	Fish	4	3.93 \pm 2.97	0.13	0	–	–
<i>Auxis</i> spp.	Fish	2	1.40 \pm 1.37	0.70	5	5.98 \pm 4.59	0.08
<i>Balistes polylepis</i>	Fish	2	2.57 \pm 1.03	0.16	2	2.94 \pm 0.41	0.06
<i>Caranx caballus</i>	Fish	2	1.45 \pm 0.45	0.33	0	–	–
<i>Fistularia</i> spp.	Fish	1	0.72	0.22	0	–	–
<i>Johnrandallia nigrirostris</i>	Fish	3	1.72 \pm 0.14	0.20	2	2.67 \pm 0.30	0.06
<i>Lagocephalus lagocephalus</i>	Fish	11	3.18 \pm 2.44	0.21	2	1.65 \pm 0.39	0.12
<i>Selar crumenophthalmus</i>	Fish	9	0.69 \pm 0.34	1.01	1	0.51	0.42
<i>Dosidicus gigas</i>	Cephalopod	1	19.81	0.02	0	–	–
<i>Mastigoteuthis dentata</i>	Cephalopod	3	64.29 \pm 55.22	0.21	0	–	–
<i>Sthenoteuthis oulaniensis</i>	Cephalopod	1	51.11	0.01	2	2.74 \pm 0.95	0.08
<i>Euphylax dovii</i>	Crustacean	0	–	–	4	8.13 \pm 4.44	0.02
Global		39	8.65 \pm 22.29	0.41	18	4.61 \pm 3.89	0.08

Global means all prey items are considered as a single source

This is consistent with previous reports on male and female marlin fish [5, 28]. The similar dietary composition of male and female marlins can be related to the high abundance of available food resources in the Gulf of California during autumn and winter [31, 32]. For instance, here, we show that male and female marlins share the same prey items during autumn and winter, resulting in similar uptake of Cd from the diet and similar isotopic values (Table 1).

Average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were slightly higher in *K. audax* than in *M. nigricans* (Table 1), which may indicate a greater trophic enrichment in *K. audax*. However, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were not significantly different, which suggests that the carbon sources of *K. audax* and *M. nigricans* are similar because both species are located at the same feeding grounds and trophic level. In *K. audax* and *M. nigricans*, Cd concentration in muscle was not correlated with $\delta^{13}\text{C}$ and

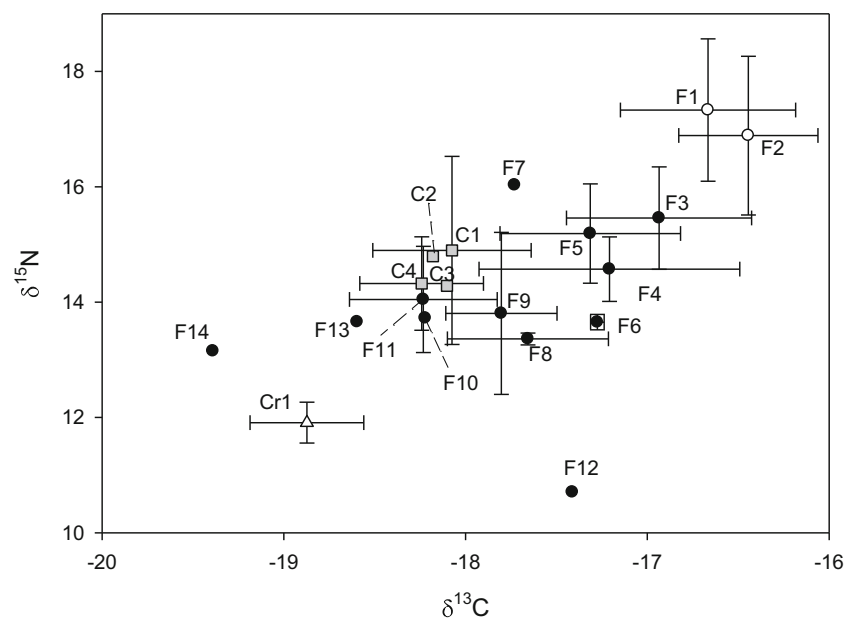
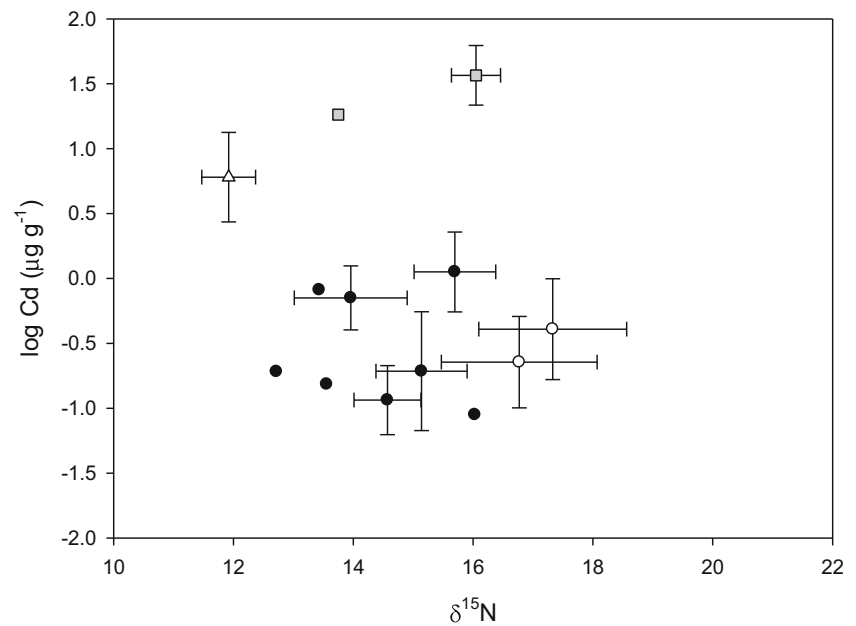


Fig. 4 Diagram of stable isotopes of the food chain of striped marlin and blue marlin in the Gulf of California, $\delta^{15}\text{N}$ ‰ (mean \pm SD) versus $\delta^{13}\text{C}$ ‰ (mean \pm SD). Open circles, top-predator marlin fish; closed circles, fish; gray squares, cephalopods; open triangles, crustaceans. F1, *Kajikia audax*; F2, *Makaira nigricans*; F3, *Auxis* spp.; F4, *Caranx caballus*; F5, *Selar crumenophthalmus*; F6, *Coryphaena hippurus*; F7, *Mulloidichthys*

dentatus; F8, *Johnrandallia nigrirostris*; F9, *Acanthurus* spp.; F10, *Balistes polylepis*; F11, *Lagocephalus lagocephalus*; F12, *Fistularia* spp.; F13, *Porichthys* spp.; F14, *Sphoeroides* spp.; C1, *Sthenoteuthis oulaniensis*; C2, *Dosidicus gigas*; C3, *Vitreledonella richardi*; C4, *Mastigoteuthis dentata*; Cr1, *Euphylax dovii*

Fig. 5 Relationship between $\delta^{15}\text{N}$ and Cd concentration (log-transformed) in *Kajikia audax* and *Makaira nigricans* and their prey items. Open circles, top-predator marlin fish; closed circles, fish; gray squares; cephalopods; open triangles, crustaceans



$\delta^{15}\text{N}$ values (Fig. 3). This lack of correlation may be related to the lack of correlation between body size and isotopic values (Fig. 2). It is worth noting that our sample size could not represent the full range of body lengths of marlins, which makes it difficult to establish an accurate correlation between Cd concentrations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ levels, and body length. It is also possible that, in the Gulf of California, billfish belonging to different size groups have similar diets [28, 29], which implies that $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are constant (i.e., there are similar food sources and trophic level of prey items) and a continued Cd uptake is expected to increase with body length. Nonetheless, there were wide variations of $\delta^{13}\text{C}$ levels with respect to body size in *K. audax* (Fig. 2) indicating organic matter is supplied from diverse sources. There were also wide variations in the levels of $\delta^{15}\text{N}$ in *K. audax*, which can be due to differences in diet composition, physiology of the individuals, and nutrient dynamics at the base of the food web [13].

The BTFs that we report here indicate negative trophic transfer between billfish and each prey item (Table 3). Furthermore, considering all prey items as single source of Cd intake (depredator/diet), the BTF < 1 indicated negative trophic transfer. In keeping with our findings, previous studies on the marine biota for a coastal lagoon of Mexico reported a negative trophic transference of Cd through the entire food web [12]. Similar results were found in the yellowfin tuna (*Thunnus albacares*) and skipjack tuna (*Katsuwonus pelamis*), from the Eastern Pacific [8]. The authors of these studies reported that BTFs of Cd were < 1.0, according to the analysis of the content of the whole stomach. Because BTF values over 1 are a sign of positive transfer trophic, the BTF values in our study indicate that Cd is sequestered in hard tissues of fish and is diluted during the transformation of food into the muscle of marlins.

On the other hand, by analyzing $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, it was possible to structure the food chain of marlins (Fig. 4). In particular, $\delta^{15}\text{N}$ is considered to be a suitable indicator of the trophic level of an animal; this idea is supported by several field studies that show an increment of $\delta^{15}\text{N}$ along the food chain [19, 7]. Taking these studies into account, we expected that the positive correlation between the concentration of a given element and $\delta^{15}\text{N}$ indicated that the element accumulated along the food chain. However, when we plotted the linear relationships between trace elements and $\delta^{15}\text{N}$, Cd concentrations were negatively correlated with $\delta^{15}\text{N}$ values in the food chain of marlins (Fig. 5). This suggests that, according to our expectation, Cd is reduced in *K. audax* and *M. nigricans* animals along their food chains. Similar results have been found for other toxic elements, for example, Yoshinaga et al. [33] found that lead is not biomagnified in animals consumed by the Gidra people of Papua New Guinea. Some of these relationships can be interpreted as a pattern of interrupted biomagnification. In fact, Cd was biomagnified only in lower and intermediate trophic levels up to the top-predator marlin, because Cd concentrations in *K. audax* and *M. nigricans* were lower than those in cephalopods and crustaceans.

Conclusion

The differences in Cd levels show that there are different Cd accumulation patterns between species, which is a consequence of food preferences, trophic position, and particular physiology of each species. The variability of the correlation between body size and Cd concentration (positive correlation or no correlation) may be attributed to Cd bioaccumulation that depends on environmental conditions, but also on the

physiological needs of fish at a given stage of their life [6]. No evidence of Cd biomagnification was found, as the relation between logarithmic Cd concentrations and $\delta^{15}\text{N}$ values was not significant in the food chain of *K. audax* and *M. nigricans*. Furthermore, the calculated biotransference factors (BTF < 1) suggested that Cd transference is interrupted from preys to marlins.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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