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^{210}Po, Cd and Pb distribution and biomagnification in the yellowfin tuna *Thunnus albacares* and skipjack tuna *Katsuwonus pelamis* from the Eastern Pacific

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ABSTRACT

We measured Cd and Pb in the muscle and stomach contents of *Thunnus albacares* and *Katsuwonus pelamis* to define the distribution of the elements in the tissues and their degrees of biomagnification. ^{210}Po was measured in the livers of both species and compared to the results of similar studies. The trophic position of the tuna species was determined by N isotope measurements. The average activity of ^{210}Po in the liver ranged from 119 to 157 (Bq kg^{-1} wet weight) in *K. pelamis* and *T. albacares*. The trophic position of *T. albacares* (4.60) was higher than that of *K. pelamis* (3.94). The Cd content of the muscle increased significantly with the trophic position of the tuna. δ^{13}C in *T. albacares* and *K. pelamis* varied, with values of 3.13 and 1.88‰, respectively. The δ^{15}N values in yellowfin tuna were higher than in skipjack tuna. The trophic position of *T. albacares* (4.60 ± 0.67) was therefore more elevated than that of *K. pelamis* (3.94 ± 1.06). Pb was biomagnified in *T. albacares* (transfer factor = 1.46).

1. Introduction

The issue of metal biomagnification in marine ecosystems remains under debate (Barwick and Maher, 2003). Prior studies have found that Hg is subjected to bioaccumulation and biomagnification (Castilhos and Bidone, 2000; Dietz et al., 2000), but that biomagnification is nonexistent for Cd and Pb (Amiard et al., 1980; Szefer, 1991). In marine ecosystems at tropical and subtropical latitudes, information on the occurrence of trace metals along food chains remains scarce. For the Eastern Pacific surrounding the west coast of Mexico, there is some evidence of Cd biomagnification (Ruelas-Inzunza and Páez-Osuna, 2008), but another study found that Cd, Cu, Pb and Zn were not positively transferred through the entire food web (Jara-Marini et al., 2009). When simulating a marine trophic chain in the Eastern Pacific, a study found that concentrations of Pb did not increase as the metal moved up through the food chain (Soto-Jiménez et al., 2011).

Classic studies on the food preferences and trophic levels of marine organisms are usually conducted using stomach contents and direct observation of the predators during feeding; however, such approaches have limitations both because these organisms feed underwater (Walker and Macko, 1999) and because their digestion process (Lesage et al., 2001) makes it difficult to identify the prey. A wider perspective of trophic relationships can be obtained using stable isotope techniques; δ^{13}C is usually employed to establish the origin of a trophic web and to recognize the carbon sources of primary producers (Hobson and Welch, 1992). δ^{15}N is usually enriched in predators with respect to the concentrations in the corresponding prey (Minegawa and Wada, 1984; Abend and Smith, 1997); it is therefore used in ecological studies to trace the trophic levels of organisms. Studies with stable isotopes of C and N are useful because they provide integrated information on the food assimilated rather than only on recent consumption (Lesage et al., 2001). Studies related to the transfer of trace metals in conjunction with stable isotopes of C and N are useful to define the degree of trophic transfer of pollutants. In the current study, measurements of ^{210}Po, Cd and Pb and of C and N isotopes were performed to assess whether biomagnification occurs in the skipjack tuna *Katsuwonus pelamis* and the yellowfin tuna *Thunnus albacares* in the Eastern Pacific Ocean.

2. Materials and methods

Tuna specimens were captured by a commercial fleet operating in the Eastern Pacific at a rate dependent on tuna availability.

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Individuals were collected in three areas: (a) adjacent to the Baja California Peninsula (NW Mexico), (b) in the Gulf of Tehuantepec (Southern Mexico), and (c) in the offshore waters of the Pacific Ocean (Fig. 1). Thirty-six skipjack (*K. pelamis*) specimens and forty-four yellowfin (*T. albacares*) individuals were collected between March and July of 2008. Fish were identified using illustrated taxonomic keys (Allen et al., 1995). The total weight, total length and fork length (from the tip of the snout to the fork of the tail) were recorded (Table 1). After each fishing trip, fish dissection was conducted in the processing plant; the liver, muscle tissue from the median dorsal part of both sides of each animal, and stomach contents were analyzed. Samples were freeze-dried (Labconco Freeze-dry System, FreeZone 6) at 80×10^{-3} mBar and −52 °C for 72 h, followed by manual grinding in an agate mortar with a pestle. Samples were processed and analyzed in a HEPA (class 1000) filtered air, trace metal clean laboratory, using high purity reagents and water. For Cd and Pb, acid digestion (J.T. Baker concentrated nitric acid, trace metal grade) of duplicate subsamples was performed using Teflon vials (Savillex™) at 120 °C for 3 h (MESL, 1997). For ^{210}Po, aliquots of 0.3 g of dried tissue spiked with ^{209}Po as a yield tracer were digested overnight in closed Teflon vials (Savillex™) on a hotplate using 10 ml of concentrated HNO_3 at 150–180 °C. The residue was converted to a chloride salt by repeated evaporation with 12 M HCl, followed by redissolution in 0.5 M HCl and 0.2 g of ascorbic acid. Po isotopes were deposited on a silver disc in contact with the acid solution overnight using an orbital shaker at room temperature. Po activity was measured by α-spectrometry using ORTEC silicon surface barrier detectors coupled with a PC running Maestro™ data acquisition software. Blanks were run in parallel to correct for any contamination. The results are expressed in Bq kg^{-1} dry weight (dw). For comparative purposes, ^{210}Po activities were converted to wet weight (ww) according to: $^{210}Po_{ww} = {}^{210}Po_{dw} \times$ (100-humidity percentage)/100 (Magalhães et al., 2007).

Before running analyses of stable isotopes of C and N in muscle samples, the lipids and carbonates were extracted from the samples using cyclohexane and HCl vapor, respectively. To accomplish this, sub-samples of ~100–200 mg of fine powder were agitated with 4 ml of cyclohexane for 1 h and then centrifuged for 10 min at 3500 rpm; the supernatant containing the lipids was discarded. Sample were dried overnight in an oven at 60 °C and then treated with HCl vapor for 4 h inside a glass desiccator and again dried overnight. Aliquots were weighed, pressed into tin capsules (Costech, Valencia, CA), and sent to the Stable Isotope Facility at the University of California in Davis for determination of the stable isotope ratios (^{13}C/^{12}C and ^{15}N/^{14}N). Analyses of the stable isotope compositions of the samples were performed using a PDZ Europa ANCA-GSL elemental analyzer interfaced with a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The results are reported as parts per thousand (‰) differences from a corresponding standard: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$. The final delta values were expressed relative to international standards V-PDB (Vienna PeeDee Belemnite) and Air for carbon and nitrogen, respectively. At least two different laboratory standards, calibrated against NIST Standard Reference Materials and compositionally similar to the samples, were analyzed with the sample batches. The long-term standard deviation was 0.2‰ for ^{13}C and 0.3‰ for ^{15}N.

Cd and Pb were measured by graphite furnace atomic absorption spectrophotometry (Varian SpectrAA220); blanks were included with every batch of samples. The quality of the analytical method was assessed by trace metal determinations of certified material consisting of fish protein (DORM-3) and shark liver (DOLT-4) in parallel with the samples. Measured concentrations in reference materials were within certified intervals (mean percentage recoveries were 103 in the case of Pb and 101 for Cd). Trace metal concentrations were expressed as μg g^{-1} dry weight. Conversions of metal concentrations from dry weight (d.w.) to wet weight (w.w.) were performed according to the water content of the tissue of interest. Significant differences in metal concentrations between species were defined using Student's t-test. Biometric variables (weight, total length and fork length) were correlated with δ^{15}N. Statistical analyses were performed using GraphPad Prism 4.0 (Graph Pad Software, San Diego, CA). Average

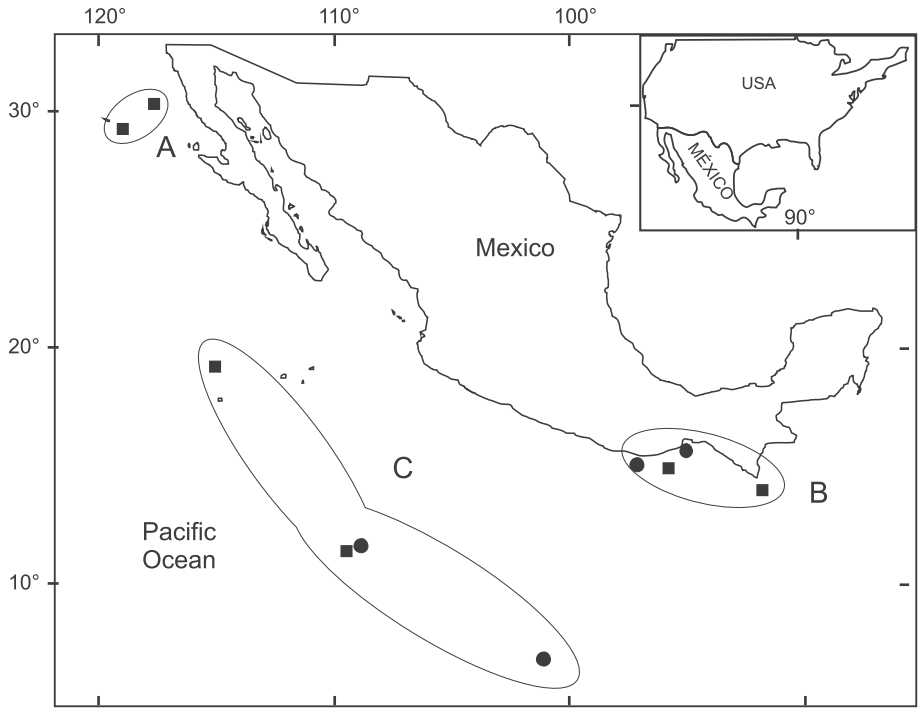

Fig. 1. Collection areas of skipjack tuna *Katsuwonus pelamis* (filled circles) and yellowfin tuna *Thunnus albacares* (filled squares) in the Eastern Pacific Ocean.

Table 1
Biological information and mean concentrations (μg g^{-1} dry weight) of Cd and Pb in muscle and stomach content and transfer factor in *K. pelamis* and *T. albacares* from the Eastern Pacific.

Species	Common name	*N*	Weight (kg) ± SD	Total length (cm) ± SD	Fork length (cm) ± SD	Element	Muscle	Stomach content[a]	Transfer factor
K. pelamis	Skipjack tuna	36	3.1 ± 1.4	55.4 ± 7.6	51.6 ± 7.1	Cd	0.23 ± 0.22	20.6 ± 9.7	0.01
						Pb	–	–	–
T. albacares	Yellowfin tuna	44	13.6 ± 17.2	86.2 ± 33.7	78.6 ± 30.2	Cd	0.18 ± 0.15	11.5 ± 9.8	0.01
						Pb	0.06 ± 0.04	0.041 ± 0.052	1.46

N, number of specimens; SD, standard deviation.
[a] Crustaceans, fish and cephalopods.

concentrations of Cd, Pb and ^{210}Po in muscle tissue of the analyzed species and the prey that were found in the stomach contents were used to estimate the transference factor (TF) according to Mackay and Fraser (2000): TF = Cc/Cp, where Cc represents the concentration of the element of interest in the predator (consumer), and Cp is the concentration of the same element in the prey (whole stomach content). TF > 1 indicates that the metal is biomagnified (Gray, 2002), and TF < 1 implies biodilution. Nitrogen isotopic ratios were used to estimate the trophic position (TP) of tuna species by the following equation (Post, 2002):

$$TP = \lambda + (\delta^{15}N_{tuna} - \delta^{15}N_{base\ of\ the\ food\ web})/\lambda^{15}N$$

where λ is the trophic level of the base of the food web, δ^{15}N tuna is the nitrogen signature of the fish of interest, and λ^{15}N is the trophic discrimination factor. In this study, we considered zooplankton as the base of the food web ($\lambda = 2$; $\delta^{15}N_{base} = 10.63 \pm 0.71$‰) (Popp et al., 2007). To effectively apply isotopic values to study the metal transfer in *K. pelamis* and *T. albacares*, we used an accurate laboratory-based value of λ^{15}N of 1.9‰ measured in white muscle of *T. orientalis* (Madigan et al., 2012). Regression analyses were performed to establish the relationships between metal concentrations (Cd, Pb and ^{210}Po) and the TP estimated for each specimen. A reciprocal or square root transformation of datasets was first performed to obtain normal distributions and homogeneous variances.

3. Results and discussion

From the biometric data of the collected tuna (Table 1), it can be observed that the *K. pelamis* were adults; it has been documented that when this species reaches sexual maturity, the fork length is approximately 45 cm (Collette, 1995). For *T. albacares*, weights of the specimens indicate (Schaefer, 1998) a mix of mature and juvenile specimens.

Concentrations of Cd and Pb in muscle tissue and stomach contents and the corresponding transfer factors (TF) of the analyzed tuna are presented in Table 1. Stomach contents were composed of crustaceans, fish and cephalopods. Pb was not measured in *K. pelamis*. The mean concentrations of Cd in muscle from both tuna species were not significantly different ($p > 0.05$). Similarly, the Cd levels in the stomach contents of *K. pelamis* and *T. albacares* did not differ significantly ($p > 0.05$). Regarding the TF of the analyzed elements, the Pb values in *T. albacares* were above unity. This value indicates that this element is biomagnified during transformation of the stomach contents into muscle in the yellowfin tuna. In some studies (Amiard et al., 1980; Szefer, 1991), it has been concluded that Cd and Pb are not biomagnified. In another study (Barwick and Maher, 2003) in a temperate estuarine ecosystem, the authors reported a positive transference of Cd and Pb, but only in some of the analyzed trophic interactions. In an experimental four-level food chain (*Tetraselmis suecica*-phytoplankton, *Artemia franciscana*-brineshrimp, *Litopenaeus vannamei*-white shrimp, *Haemulon scudderi*-fish), it was found that Pb in phytoplankton increased with respect to the solution but decreased during successive trophic transfers (Soto-Jiménez et al., 2011). In a study of the trophic distribution of Cd and Pb from a subtropical lagoon in the Gulf of California, Ruelas-Inzunza and Páez-Osuna (2008) found that the TFs of Cd were >1.0 in 64.5% of the examined trophic links; in the case of Pb, the TFs were >1 in only 45.2% of the trophic interactions. The concentrations of elements in prey and the approach used to estimate TF (homogenized stomach contents versus separate food items) can lead to contrasting conclusions about whether biomagnification is occurring. In addition, elemental bioavailability can be influenced by environmental parameters, such as metal speciation, mineralogy, pH, redox potential, temperature, and total organic content (Luoma, 1989).

The ^{210}Po activity in the livers of *T. albacares* and *K. pelamis* analyzed in our study and published information about the muscles and livers of multiple tuna species are presented in Table 2. The average activity of ^{210}Po was higher in the liver than in muscle. ^{210}Po activity in liver ranged from 119 Bq kg^{-1} in *K. pelamis* from the Eastern Pacific (this study) to 777 Bq kg^{-1} in *T. albacares* from the Eastern Pacific (15°N; 100°W). In muscle, values ranged from 0.45 Bq kg^{-1} in *T. albacares* from the Eastern Pacific (from 16 to 21°N) to 137 Bq kg^{-1} in *T. thynnus* from the Mediterranean Sea (35°N). Clearly, the ^{210}Po activity in muscle varied by several orders of magnitude; inherent differences between the species of tuna or in their latitudes may explain this variance. In this context, Carvalho (2011) mentioned that ^{210}Po activity in marine biota varies by several orders of magnitude and such variations are not related to water depth or geographical location.

With respect to the feeding behavior of the analyzed tuna, jumbo squid *Dosidicus gigas* and pelagic red crab *Pleuroncodes planipes* are the main prey reported for *T. albacares* from the Eastern tropical Pacific (Ordiano-Flores et al., 2012). Feeding habits of the smallest individuals of tropical tuna are poorly known (Graham et al., 2006). A study of small *T. albacares* found that specimens with less than a 40 cm fork length (FL) fed on crustaceans, while larger specimens (>50 cm FL) used fish as food (Maldeniya, 1996). δ^{15}N and δ^{13}C in the muscle of *T. albacares* and *K. pelamis* and the variation of δ^{15}N with tuna dimensions are presented in Fig. 2. δ^{13}C values in *T. albacares* varied from −18.82 to −15.69‰; in *K. pelamis* they ranged from −18.2 to −16.32‰. The wide variations in δ^{13}C indicate that organic matter is supplied from diverse sources. For δ^{15}N, values ranged from 17.93 to 12.24‰ in *T. albacares* and from 16.41 to 10.45‰ in *K. pelamis*. The variation of ^{15}N values in marine predators can be due to differences in diet composition, the physiology of the individuals and the nutrient dynamics at the base of the food web (Popp et al., 2007). A change in prey composition with age for tuna, from mainly crustaceans to a more varied diet composed of both fish and crustaceans, implies that an increase and a higher variability in δ^{15}N values is expected with age, especially in individuals with a larger than 45 cm FL (Bearhop et al., 2004). Trophic positions estimated for *T. albacares* averaged 4.60 ± 0.67 (2.85–5.84) and 3.94 ± 1.06 (1.91–5.04) for *K. pelamis*. TP increased with FL for both species ($r \geqslant 0.89$, $p < 0.05$). Based on diet data, Olson and Watters (2003) reported values of 4.66 and 4.57 for large yellowfin and skipjack from the Eastern tropical Pacific.

Table 2
Comparison of ^{210}Po activity (Bq kg^{-1} wet weight) in *K. pelamis* and *T. albacares* from the Eastern Pacific with other studies.

Species	Common name	Tissue	^{210}Po	Site	Reference
T. albacares, T. obessus, T. alalunga, K. pelamis[a]	Yellowfin tuna, bigeye tuna, albacore, skipjack tuna	Muscle	5	Portugal coasts	Carvalho (1988)
T. albacares, T. obessus, T. alalunga, K. pelamis[a]	Yellowfin tuna, bigeye tuna, albacore, skipjack tuna	Liver	288	Portugal coasts	Carvalho (1988)
T. albacares	Yellowfin tuna	Muscle	0.45	Eastern Pacific	Ruelas-Inzunza et al. (2012)
K. pelamis	Skipjack tuna	Muscle	1.76	Eastern Pacific	Ruelas-Inzunza et al. (2012)
K. pelamis	Skipjack tuna	Muscle	18.5	Gulf of Panama	Hoffman et al. (1974)
K. pelamis	Skipjack tuna	Liver	407	Gulf of Panama	Hoffman et al. (1974)
T. albacares	Yellowfin tuna	Liver	777	15°N;100°W	Hoffman et al. (1974)
T. albacares	Yellowfin tuna	Muscle	14.8	15°N;100°W	Hoffman et al. (1974)
T. thynnus	Bigeye tuna	Muscle	137	Mediterranean Sea	Heyraud and Cherry (1979)
T. thynnus	Bigeye tuna	Liver	629	Mediterranean Sea	Heyraud and Cherry (1979)
T. obesus	Bigeye tuna	Muscle	3	Madeira Island (Portugal)	Carvalho (2011)
T. obesus	Bigeye tuna	Liver	268	Madeira Island (Portugal)	Carvalho (2011)
T. albacares	Yellowfin tuna	Liver	157	Eastern Pacific	This study
K. pelamis	Skipjack tuna	Liver	119	Eastern Pacific	This study

[a] Composite samples.

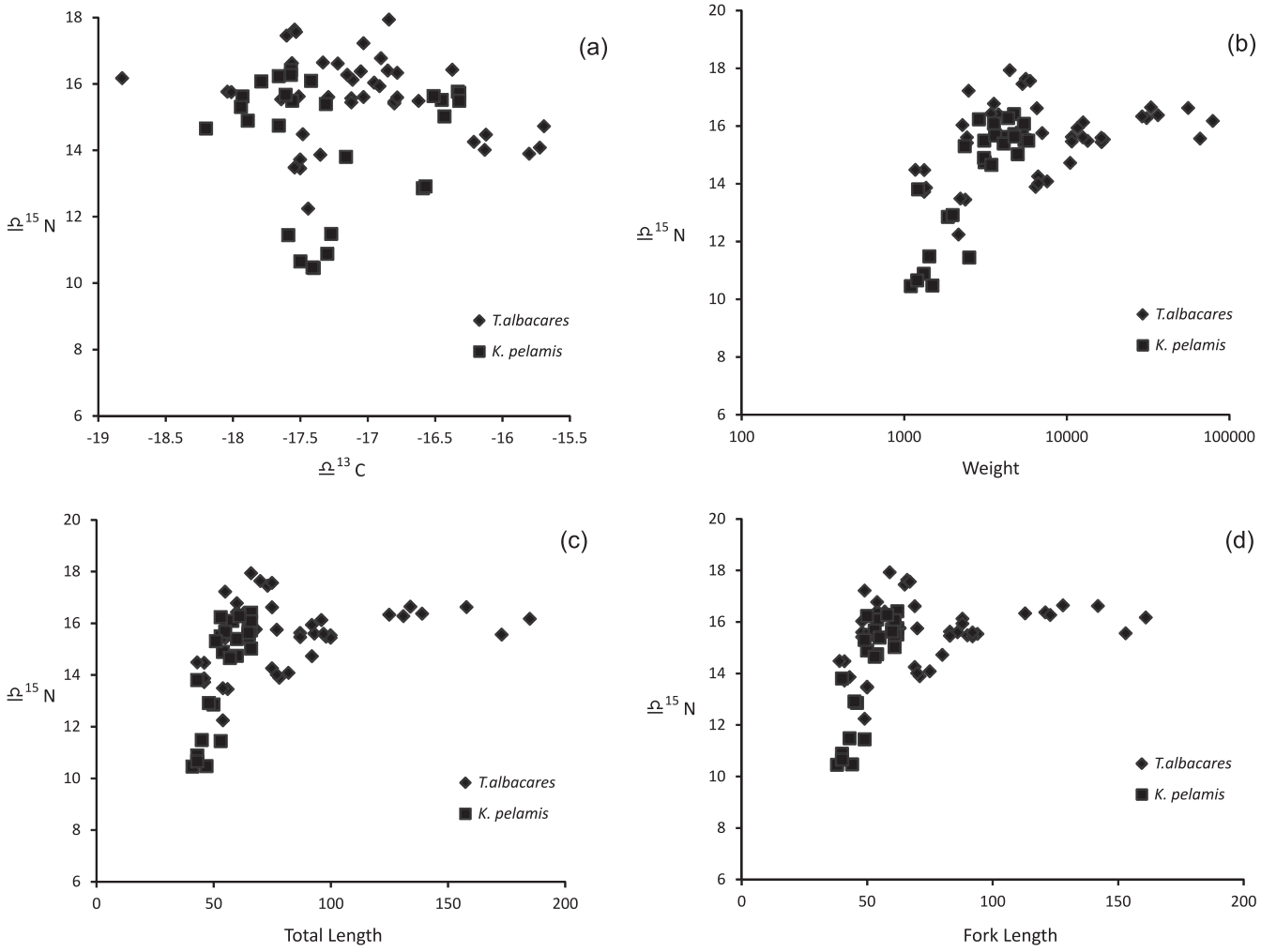

Fig. 2. Relation of δ^{15}N with δ^{13}C in muscle of *Thunnus albacares* and *Katsuwonus pelamis* (a); variation of δ^{15}N as a function of tuna dimensions (b–d).

Variations of Cd and Pb (μg g^{-1} dry weight) and ^{210}Po activity (Bq kg^{-1} dry weight) with TP of analyzed tuna species are included in Fig. 3. The results revealed that Cd in muscle increased significantly with TP for *K. pelamis* ($R^2 = 0.24$, $F = 12.366$, $p = 0.0011$) and for *T. albacares* ($R^2 = 0.19$, $F = 6.164$, $p = 0.0198$), while Pb decreased in *T. albacares* ($R^2 = 0.27$, $F = 11.79$, $p = 0.0017$). Similarly, there was a significant trend of a decrease in ^{210}Po in the liver of *T. albacares* ($R^2 = 0.29$, $F = 5.01$, $p = 0.0449$), excluding two outlier ^{210}Po values (Bq kg^{-1} dry weight) of 3145 and 3535.

The transfer of Pb from stomach contents to the muscle tissue of *T. albacares* was positive (higher than one); this result implies that this element was biomagnified in the interaction between the whole stomach content and the muscle tissue. As for ^{210}Po activity, values in the liver of analyzed tuna were higher than in muscle of

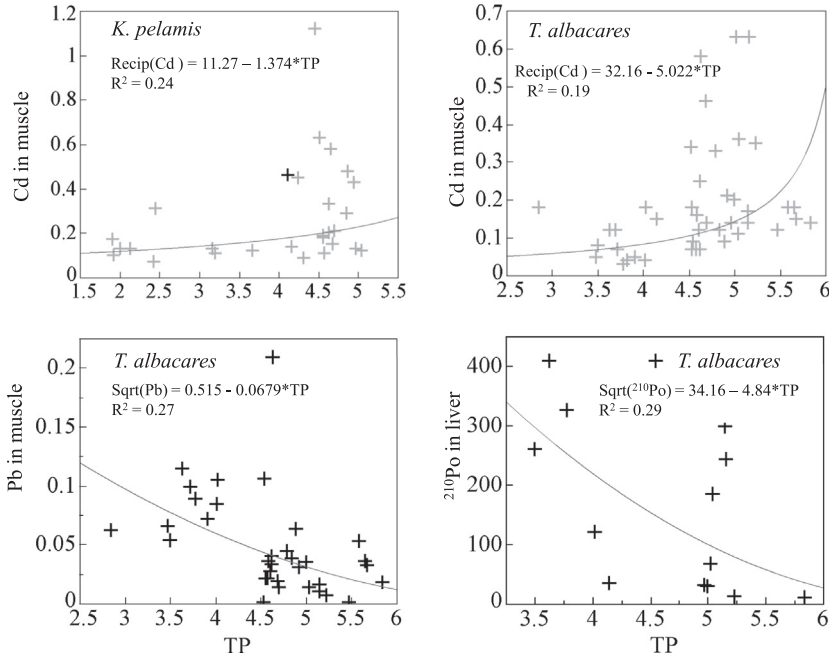

Fig. 3. Variation of Cd and Pb concentrations (μg g^{-1} dry weight) and ^{210}Po activity (Bq kg^{-1} dry weight) with trophic position of *Katsuwonus pelamis* and *Thunnus albacares*.

the same species from different regions of the Eastern Pacific. δ^{13}C in *T. albacares* and *K. pelamis* varied by 3.13 and 1.88‰, respectively, which indicates that organic matter sources are more varied in the yellowfin tuna *T. albacares*. δ^{15}N values in yellowfin tuna were higher than in skipjack tuna. Therefore, the trophic position of *T. albacares* (4.60 ± 0.67) was more elevated than in *K. pelamis* (3.94 ± 1.06). In some cases, the analyzed elements increased in concentration as a function of the trophic position; such behavior was observed for Cd in the muscle of both *T. albacares* and *K. pelamis*.

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