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Quantifying maternal transfer of trace elements and stable isotopes in the endangered pelagic thresher shark (*Alopias pelagicus)*

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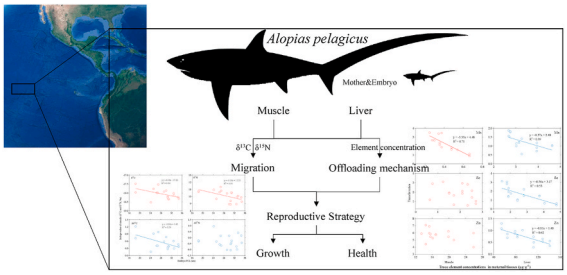
HIGHLIGHTS

- Mothers offloaded more essential elements (Cu, Cr, Mn, and Se) and less nonessential elements (As, Cd, Hg) to embryos.
- Both mothers and embryos had potentially harmful Hg concentrations.
- High Se concentrations in embryos probably inhibited the toxicity of Hg.
- Variations of embryonic isotope values reflected the diet and migration of female sharks.

GRAPHICAL ABSTRACT



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ABSTRACT

To quantify maternal provisioning of nutrients in the pelagic thresher shark (*Alopias pelagicus*) and the potential for negative impacts, the concentrations of trace elements (essential: Co, Cr, Cu, Mn, Ni, Se, and Zn; nonessential: As, Ba, Cd, Hg, and Pb) and fractionation of stable isotopes (^{13}C and ^{15}N) were analyzed in the muscle and liver of 10 pregnant females and 18 associated embryos. Essential trace elements were observed to be offloaded at higher concentrations to embryos, with the exception of Zn and Ni in liver, while nonessential trace elements were unevenly distributed between maternal-embryo tissues. Observed Hg concentrations were at levels considered toxic in *A. pelagicus*, but the Se: Hg molar ratios in all embryonic tissues were all greater than one. A negative correlation was observed between transfer ratios and concentrations of all elements in maternal tissue, indicating the existence of a regulatory mechanism in maternal ovaries of *A. pelagicus*. Compared with maternal specimens, associated embryos had higher $\delta^{13}C$ and $\delta^{15}N$ values in muscle and liver tissue. Negative correlations were observed between $\delta^{13}C$, $\delta^{15}N$, and $\Delta\delta^{13}C$ values and precaudal length in embryonic muscle tissue potentially reflecting either a dietary-habitat shift in pregnant females during the latter period of gestation or a physiological change modifying fractionation. Higher concentrations of essential elements are linked to potential benefits for embryos during early development, levels of Hg suggested a degree of anthropogenic impact with unknown consequences while the directionality of isotopic fractionation could suggest a potential reproductive migration as a protective mechanism for birthing.

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1. Introduction

Maternal transfer is the process through which pregnant females offload nutrients and if present, anthropogenic contaminants to their offspring during gestation (Lyons and Lowe, 2013; Mull et al., 2013; Olin et al., 2018). While essential trace elements, for example, Cu, Mn, and Zn are critical for the development and growth of offspring, some nonessential elements such as As, Cd, and Hg can be deleterious and toxic even at low concentrations, but are accumulated by adults and often offloaded with essential elements during the period of gestation (Wood et al., 2012a; b). Most fish transfer nutrients produced by the liver via vitellogenin, and this can include the transfer of excessive concentrations of trace elements given the role of the liver as a detoxification organ. Ultimately maternal transfer of excessive and deleterious non-essential elements has the potential to compromise the healthy development of embryos (Iida et al., 2019; Tosti et al., 2006).

Compared with teleost fish (Bang et al., 2008; Kelly et al., 2011), turtles (Guirlet et al., 2008, 2010), whales (Desforges et al., 2012) and coastal elasmobranch species (Dutton and Venuti, 2019; van Hees and Ebert, 2017), relatively little is known regarding the dynamics of maternal transfer of trace elements in pelagic species. To date, work has focused on smaller bodied coastal sharks including the smalleye smooth-hound (*Mustelus higmani*; Souza-Araujo et al., 2020) and the Atlantic sharpnose shark (*Rhizoprionodon longurio;* Frias-Espericueta et al., 2014) with only one study examining trace element trends for an individual common thresher shark (*Alopias vulpinus*; Dutton and Venuti, 2019). For rays, maternal trace element dynamics have focused on the thornback ray (*Platyrhinoidis triseriata*) and the electric ray (*Narcine brasiliensis*) (van Hees and Ebert, 2017; Lopes et al., 2019). In general, these studies reported that embryos were born with higher concentrations of most essential elements, but trends for non-essential elements were inconclusive with the need for further work identified (Dutton and Venuti, 2019).

The pelagic thresher shark, *Alopias pelagicus,* is a relatively large species considered highly migratory in oceanic regions but also occurring on coastal seamounts (Smith et al., 2008; Oliver et al., 2019). In terms of reproductive strategy, pelagic thresher shark is ovoviviparous with a gestation period of ~9 months and 1–2 embryos per litter (Romero-Caicedo et al., 2014). According to morphology and nutrition sources, embryonic development in pelagic thresher shark can be divided into five stages. During the first two stages, nutrients are provided via the embryonic yolk sac, while from the third to fifth stages, nutrients are sourced by uterine fluid and immature ova (Liu et al., 1999). Its highly k-selected life-history traits, specifically its extremely low reproductive output or fecundity combined with th1234e high interaction rates with fisheries resulted in its endangered status (Smith et al., 2008). Therefore, pelagic thresher shark has been listed by the International Union for Conservation of Nature as endangered and since 2017 in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora with an elevated risk of extinction. Understanding the maternal transfer of trace elements, especially offloading of non-essential elements that may pose threats to the health of this endangered species is therefore required.

The application of stable isotope analysis for studying food web dynamics is well-established and a proven tool for assessing various aspects of species' ecology and biology (Wada, 1980; Minagawa and Wada, 1984; Layman et al., 2012). The ratio of stable carbon isotopes ($\delta^{13}C$) can be used to indicate relative production sources that can determine carbon routing and in turn the location of foraging (Caut et al., 2009), whereas nitrogen isotope ratios ($\delta^{15}N$) are commonly used to indicate relative trophic position within a food web (Ramos and González-Solís, 2012). In the case of unborn animals, stable isotope values can be used to assess relative foraging location or feeding behavior of the mother dependent on the tissue analyzed and the associated tissue turnover rate, but must consider the reproductive mode and potential for complex fractionation dynamics tied to the stages of reproduction (Osgood et al., 2020). Several studies have evaluated $\delta^{13}C$ and $\delta^{15}N$ values in pregnant female sharks and their associated embryos and identified considerable variability in the directionality of isotope values (McMeans et al., 2009; Olin et al., 2018; Broadhurst et al., 2019; Osgood et al., 2020). For example, among ovoviviparous species, ^{13}C and ^{15}N were found to be depleted in embryonic muscle and liver tissues of bluntnose sixgill (*Hexanchus griseus*) and shortspine spurdog (*Squalus megalops*) sharks compared with those of their mothers, but the opposite trend was observed for $\delta^{13}C$ and $\delta^{15}N$ values in the embryonic tissues of small-fin gulper sharks (*Centrophorus moluccensis*) (Le Bourg et al., 2014; Osgood et al., 2020). By comparison, the embryos of a placenta-trophic species, the Atlantic sharpnose shark (*Rhizoprionodon terraenovae*), had higher $\delta^{13}C$ and $\delta^{15}N$ values than those measured in their mothers (McMeans et al., 2009). Improved knowledge regarding isotopic fractionation between maternal and embryonic tissues is consequently required to clarify the dynamics of maternal provisioning.

In the current study, we aimed to (1) quantify and compare the concentrations and distribution of 12 trace elements (essential: Co, Cr, Cu, Mn, Ni, Se, and Zn; nonessential: As, Ba, Cd, Hg, and Pb) in two maternal and embryonic tissues of pelagic thresher shark with different turnover rates to assess element transfer related to its ovoviviparous reproductive mode and the potential for negative impacts on developing young, (2) determine values for the fractionation of carbon and nitrogen between maternal and embryonic tissue considering reproductive mode and tissue turnover rate (muscle [T95 (Estimated time to reach 95% of turnover), slow, ~422 days] and liver [T95, intermediate; ~166 days]; MacNeil et al., 2006) to assist interpretation of stable isotope values, and; (3) determine trends in trace element concentrations and stable isotope values with increasing embryonic size for improved understanding of the dynamics of maternal offloading and stable isotope dynamics during the gestation phase, respectively.

2. Materials and methods

2.1. Sampling

All sampled sharks were caught as bycatch from a Chinese pelagic longline fishery targeting tuna (operating range, 1–5° S, 106–119° W, Fig. 1) in the eastern tropical Pacific Ocean (EPO) between September 2019 and January 2020. A total of 10 pregnant female *A. pelagicus* and their 18 associated embryos were measured (precaudal length, PCL, cm) and a sample of muscle tissue taken from the base of the dorsal fin and liver tissue from either the right or left lobe of all individuals. All samples were immediately stored in polyethylene bags and frozen at −20 °C prior to transport to the laboratory for trace element and stable isotope analyses.

2.2. Trace element analysis

All samples were rinsed with deionized water freeze-dried at −55 °C for 48 h, and then homogenized into a fine powder using a pestle and mortar. Approximately 0.5 g of each sample was weighed and transferred into a Teflon vessel. To digest samples, 10 mL of acid (9:1 HNO_3: HCl) was added to each sample and then placed in an automatic digestion apparatus (Auto DigiBlock S60, Lab Tech, China) at 150 °C for 4 h. Following this, samples were diluted to 25 mL with deionized water, ensuring that all sample solutions were clear and filtered through a 0.45 μm nitrocellulose membrane filter (Adel et al., 2017; Dutton and Venuti, 2019). The concentrations of 12 trace elements (dry weight, dw) (essential: Co, Cr, Cu, Ni, Mn, Se, and Zn and nonessential: As, Ba, Cd, Hg, and Pb) were determined in duplicate using inductively coupled plasma optical emission spectrometry (ICP-OES, Agilent Technologies 5110, Australia) in the Instrumental Analysis Center, Shanghai Jiao Tong University. In the meantime, a serious of the certified reference material (multi-element standard solution, GNM-M261674-2013 and DORM-4, n = 3) were detected and the detection limit values and

percentage recoveries of 12 elements in ICP-OES were shown in Table S1.

2.3. Stable isotope analysis

Homogenized muscle and liver tissue samples (~0.1 g) were extracted urea which is considered to deplete ^{15}N by adding 10 mL of deionized water, putting them at room temperature for 24 h, centrifuging for 5 min and removing the water. The water washing process was repeated twice and then samples were dried (Kim and Koch, 2012; Li et al., 2016). Thereafter, samples were lipid-extracted by the addition of 12 mL of chloroform-methanol solution (2:1, v/v) for ≥20 h, then the mixture was centrifuged for 3 min. The process was repeated twice to remove the potential effects of lipid on $\delta^{13}C$ values (Post et al., 2007). To evaporate off the remaining solvent, the solid residues were dried in a fume hood for 24 h. Approximately 1 mg of samples were weighed into tin capsules and analyzed using an IsoPrime 100 isotope ratio mass spectrometer (IsoPrime Corporation, Cheadle, UK) and a vario ISOTOPE cube elemental analyzer (Elementar Analysensysteme GmbH, Hanau, Germany) in the Laboratory of Ingestion Ecology, Shanghai Ocean University. The isotope compositions of samples are expressed as $\delta^{13}C$ and $\delta^{15}N$ notation using the following equation:

$$\delta X‰ = [\,(R_{Sample} / R_{Standard}) - 1] \times 1000$$

where ‰ is parts per thousand; R_{Sample} and $R_{Standard}$ correspond to $^{13}C/^{12}C$ and $^{15}N/^{14}N$ values of the samples and reference standard, respectively; and δ is the measure of the heavy-to-light isotope in the sample. The standard references for ^{13}C and ^{15}N were Pee Dee Belemnite carbonate and air, respectively. Standards reference materials USGS 24 (Graphite, −16.049 ± 0.04‰ VPDB) and USGS 26 (Ammonium Sulfate, 53.7 ± 0.24‰ Air) were used for quantification of $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Every tenth sample was run in triplicate with a laboratory reference standard (protein: −26.98‰ VPDB and 5.96‰ Air) to assess the within-run precision, and a blank sample was run every ten samples to clear off residual gases. The analytical errors of $\delta^{13}C$ and $\delta^{15}N$ values were approximately 0.20‰ and 0.20‰, respectively.

2.4. Data analysis

The differences between maternal trace element concentrations and those of their respective litters were determined by tissue type (muscle and liver) and assessed using a Kruskal-Wallis test. The difference of element concentrations and isotope values between siblings were estimated by paired samples *t*-test.

The Se: Hg molar ratio in both maternal and embryonic tissues was calculated by dividing the Se and Hg concentrations ($\mu g \cdot g^{-1}$, dw) by their molecular weights (Se: 78.96; Hg: 200.59) to assess the inhibitory effect of selenium on mercury toxicity. A Se: Hg value of >1 can indicate a potential protective effect of Se on reducing methylmercury toxicity (Dutton and Venuti., 2019), while a Se: Hg value of <1 indicates potential for acute toxicity of methylmercury (Peterson et al., 2009). Pearson's tests were performed to determine the relationships between Hg concentrations and Se: Hg molar ratios.

The relationships between trace element concentrations of muscle and liver tissue and transfer ratios (the ratios of element concentrations between each litter-mother pair) were examined by linear regressions. Pearson's tests were used to examine the relationships between trace element concentrations in both embryonic muscle and liver tissues and precaudal length of embryos.

Isotope fractionation of carbon and nitrogen ($\Delta\delta^{13}C$ and $\Delta\delta^{15}N$, respectively) between mother and embryo for each tissue were calculated for each litter-mother pair to facilitate among litter comparisons:

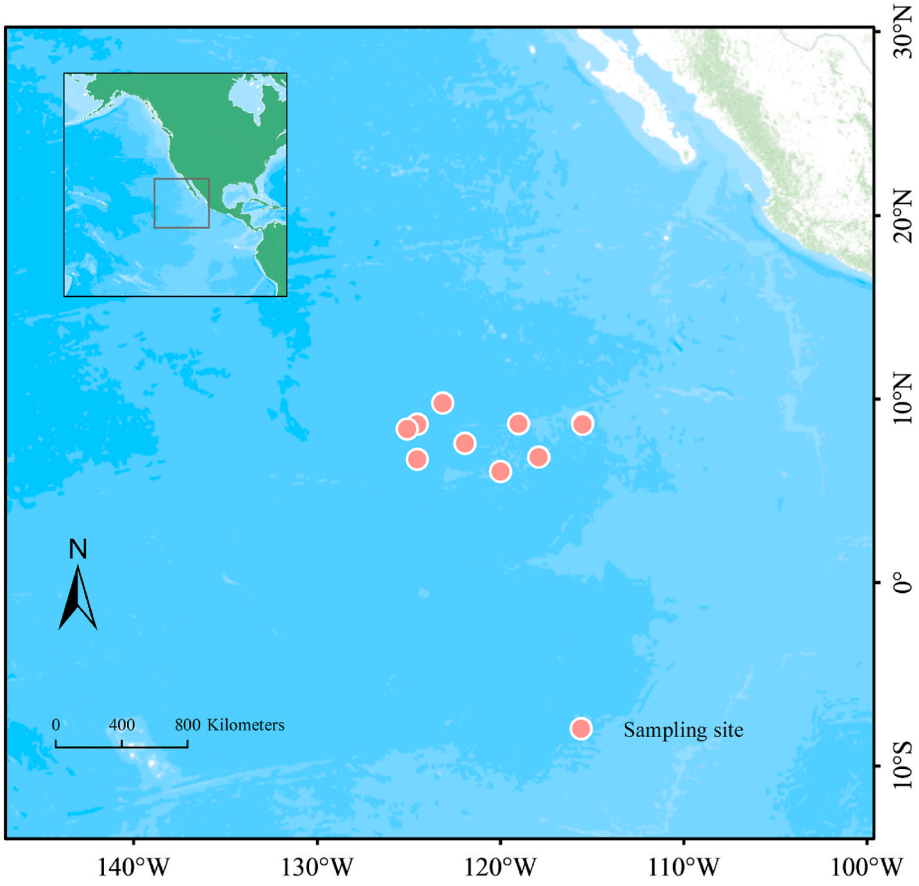

Fig. 1. Sampling locations for pelagic thresher shark (*Alopias pelagicus)* in the Eastern Pacific from bycatch in the Chinese pelagic longline fishery.

$$\Delta\delta^{13}C = \delta^{13}C_{embryo} - \delta^{13}C_{mother}$$

$$\Delta\delta^{15}N = \delta^{15}N_{embryo} - \delta^{15}N_{mother}$$

The $\delta^{13}C$ and $\delta^{15}N$ values for embryonic tissues were then compared with those of mothers using a Kruskal-Wallis test. Linear regressions between $\delta^{13}C$, $\delta^{15}N$, $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values and PCL of embryos were undertaken to examine the potential effects of maternal shifts in diet/foraging location and trends in isotope integration over the gestation period. The level of significance for all the analyses was set to $P < 0.05$.

3. Results

The mean PCL (mean ± standard deviation) of sampled pregnant female pelagic thresher and their respective embryos were 150.7 ± 6.02 cm (range: 138–158 cm) and 28.7 ± 4.88 cm (range: 18.4–34.8 cm), respectively (Table S2). Embryos were determined to be in the third and fourth stages of development (i.e., sourced by uterine fluid and immature ova) based on their PCL. There is no difference between siblings by comparing the element concentrations and δ values ($P > 0.05$).

Embryos had higher concentrations of the essential elements Cr, Cu, Mn, and Se in both tissues compared with their mothers. Concentrations of Ni and Zn in embryonic muscle were about 117% and 428% higher than those in maternal muscle, respectively, while the various trends were observed for these elements in liver ($P < 0.05$). For nonessential elements, mothers had 32% more As and 135% more Hg in muscle tissue relative to embryos (Table 1), while As and Cd were detected at lower concentrations in embryonic liver ($P < 0.05$). Although maternal liver had extremely high concentrations of Cd, it was only detected in a subset of samples; 5/10 in maternal muscle, 0/18 in embryonic muscle and 3/18 in embryonic liver. Similarly, Pb and Co were only detected in a few maternal and embryonic tissue samples (5/28 in muscle and 8/28 in liver were detected for Pb; 3/28 in muscle and 2/28 in liver were detected for Co; Table 1). For both maternal muscle and liver tissues, the Se: Hg molar ratios were mostly >1, and embryo Se: Hg molar ratios were higher than those of their mothers ($P < 0.05$, Fig. 2). The mean Se: Hg molar ratio in maternal muscle was 1.02 ± 0.24 (ranging from 0.56 to 1.45), while the mean Se: Hg molar ratio in embryo muscle was 4.65 ± 2.21 (ranging from 1.69 to 9.01). In contrast, the mean maternal and embryo Se: Hg molar ratio in liver was 7.23 ± 2.41 (ranging from 2.76 to 10.71) and 13.83 ± 9.06 (ranging from 4.70 to 46.21), respectively. The coefficients of the Se: Hg molar ratio were negatively correlated with Hg concentrations, indicated by the high Pearson's correlation coefficients (Fig. 2).

For embryos, Mn concentrations in muscle tissue were significantly positively correlated with PCL whereas Ba showed the opposite trend; no other significant relationships with body size were observed (Table 2). Most of the correlations between transfer ratios and trace element concentrations were positive and significant in both embryonic muscle and liver tissues except for Zn in both tissues and Hg in liver (Table 3). Mn concentrations in both maternal tissues and As, Ba, Cu, Se, and Zn concentrations in maternal liver showed significant negative correlations with transfer ratios (Table 3, Fig. 3).

The fractionation of carbon between mother and embryo ($\Delta\delta^{13}C$) in muscle tissue ranged from 0.16‰ to 1.22‰ and from 0.06‰ to 1.41‰ in liver. Both tissues were significantly enriched in ^{13}C in embryos compared with associated mothers (0.55‰ and 0.68‰, respectively; $P < 0.001$, Table 3, Fig. 4). When considering $\delta^{15}N$ in muscle tissue, values were comparable between mothers and embryos, but variable at the individual level, with an observed range in fractionation values ($\Delta\delta^{15}N$) of −1.04 to 1.95‰ (0.66 ± 0.44‰). In contrast, all embryonic livers were significantly enriched in ^{15}N relative to mothers (2.63‰; $P < 0.001$, Table 1), with fractionation values ranging from 1.33‰ to 3.34‰. Negative linear relationships were observed between $\delta^{13}C$, $\delta^{15}N$ and $\Delta\delta^{13}C$ values and PCL in embryonic muscle (Fig. 5).

Table 1
Concentrations of 12 trace elements (μg·g^{-1}, dry weight) and $\delta^{13}C$, $\delta^{15}N$ and fractionation values (‰) in muscle and liver tissue of pelagic thresher shark (*Alopias pelagicus)* mothers and their respective litters, sampled from the eastern tropical Pacific Ocean. Significance of the Kruskal-Wallis test results between element concentrations of maternal and embryonic tissues are indicated by *. The presented values for Co concentrations in embryonic muscle and liver tissue and the Cd concentrations in embryonic muscle were below detection limits. BDL: Below Detection Limits.

Item	Maternal muscle	Embryonic muscle	Maternal liver	Embryonic liver
Essential				
Co	0.02 ± 0.03	BDL	0.01 ± 0.03	BDL
Cr	0.79 ± 0.27	2.65 ± 2.26*	0.31 ± 0.13	0.73 ± 0.76*
Cu	0.86 ± 0.06	2.31 ± 1.07*	12.00 ± 2.35	76.17 ± 26.36*
Mn	0.40 ± 0.09	0.83 ± 0.29*	3.34 ± 0.64	3.90 ± 0.90
Ni	0.40 ± 0.22	1.07 ± 0.83*	0.38 ± 0.16	0.37 ± 0.21
Se	1.77 ± 0.68	3.48 ± 1.56*	2.70 ± 1.06	3.74 ± 1.43*
Zn	17.63 ± 3.75	96.57 ± 20.67*	96.23 ± 27.48	63.04 ± 15.74*
Nonessential				
As	5.05 ± 0.98	1.61 ± 0.69*	26.67 ± 5.74	14.12 ± 3.69*
Ba	0.47 ± 0.25	0.68 ± 0.53	0.35 ± 0.15	0.29 ± 0.15
Cd	0.16 ± 0.28	BDL	102.66 ± 45.34	0.06 ± 0.03*
Hg	5.31 ± 0.85	2.18 ± 0.52*	0.91 ± 0.24	0.81 ± 0.30
Pb	0.05 ± 0.02	0.18 ± 0.08	0.19 ± 0.10	0.15 ± 0.08
Isotope values				
$\delta^{13}C$	−18.60 ± 0.12	−18.05 ± 0.29	−18.34 ± 0.45	−17.75 ± 0.36
$\delta^{15}N$	10.34 ± 0.85	9.23 ± 0.80	10.09 ± 0.76	11.72 ± 0.91
$\Delta\delta^{13}C$		0.55 ± 0.29		0.66 ± 0.44
$\Delta\delta^{15}N$		−0.32 ± 0.33		2.49 ± 0.79

4. Discussion

Understanding maternal transfer of trace elements and stable isotopes is important for evaluating the role of maternal provisioning during gestation, especially for endangered and little-known species such as the pelagic thresher shark. The derived results for 10 pregnant female sharks and their associated embryos suggested a potential positive effect of maternal provisioning through offloading of high concentrations of essential elements to support embryonic growth and

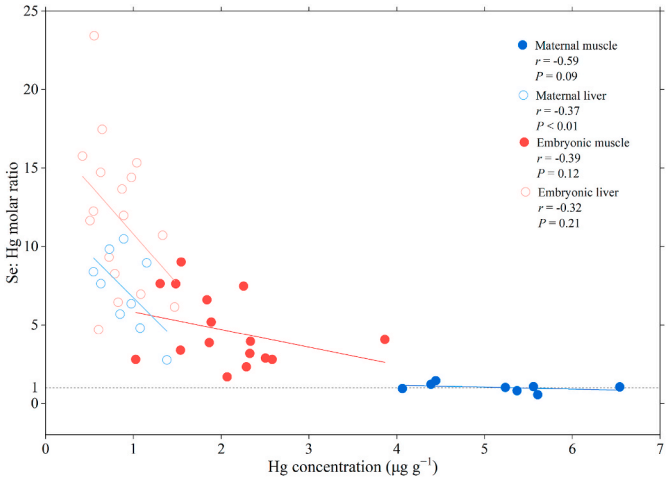

Fig. 2. The relationships of Hg concentration and Se: Hg molar ratios in maternal and embryo muscle and liver tissues. Most of the Se: Hg molar ratios are >1 and higher in embryo vs maternal tissue; Se: Hg molar ratios decrease with increasing Hg concentration.

Table 2
Correlation coefficients between embryo precaudal length and differences between mother and embryo $\delta^{13}C$ and $\delta^{15}N$ values (i.e. $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$) and trace elements measured in muscle and liver tissue of pelagic thresher sharks (*Alopias pelagicus)* sampled from the eastern tropical Pacific Ocean. * = $P < 0.05$.

Variable	Muscle		Liver	
	r	*P*	r	*P*
$\delta^{13}C$	−0.507*	0.038	−0.041	0.871
$\delta^{15}N$	−0.547*	0.019	−0.021	0.933
$\Delta\delta^{13}C$	−0.538*	0.026	−0.306	0.217
$\Delta\delta^{15}N$	−0.158	0.530	0.218	0.400
As	0.191	0.463	−0.361	0.141
Ba	−0.392*	0.002	−0.056	0.843
Hg	−0.313	0.206	−0.042	0.876
Mn	0.551*	0.018	−0.094	0.720
Ni	−0.418	0.084	0.025	0.925
Cr	0.183	0.468	−0.095	0.716
Cu	−0.303	0.222	0.049	0.849
Se	−0.188	0.456	−0.303	0.237
Zn	0.300	0.226	0.323	0.207

Table 3
Correlation coefficients between element concentrations of both embryonic and maternal tissues and transfer ratios in pelagic thresher sharks (*Alopias pelagicus)* sampled from the eastern tropical Pacific Ocean. Cd, Co and Pb were not included due to the low sample sizes. * = $P < 0.05$.

Element	Embryonic muscle	Embryonic liver	Maternal muscle	Maternal liver
As	0.72*	0.87*	−0.25	−0.63*
Ba	0.83*	0.94*	−0.42	−0.49*
Hg	0.77*	−0.43	−0.43	−0.30
Mn	0.78*	0.87*	−0.59*	−0.63*
Ni	0.69*	0.82*	−0.35	−0.35
Cr	0.98*	0.92*	−0.33	−0.41
Cu	0.85*	0.99*	−0.13	−0.64*
Se	0.73*	0.50*	−0.65	−0.61*
Zn	0.46	0.32	−0.39	−0.78*

development and protection from toxication by the offloading of low concentrations of non-essential elements. The $\delta^{13}C$ and $\delta^{15}N$ values of maternal and embryonic tissues varied through gestation potentially reflecting the diet and habitat occupied by mothers during this period. It is important, however, to consider the observed variation in transfer ratios of elements and fractionation of isotopes in terms of physiology during gestation.

4.1. Comparison of maternal and embryo trace element concentrations

4.1.1. Differences in essential trace elements between mothers and embryos

As expected, essential trace element concentrations in embryonic tissues of *A. pelagicus* were higher than those in maternal tissues (Table 1). These results are in agreement with those reported for the common thresher shark (Dutton and Venuti, 2019) and Pacific sharpnose shark (Frias-Espericueta et al., 2014). These elements are tightly regulated and incorporated into various enzymes that control metabolic reactions and physiological processes that provide protection against free-radical damage (e.g., Cu, Se, and Zn) and aid metabolism (e.g., Cr, Mn, and Zn) (Bosch et al., 2016). Pregnant sharks may provide higher concentration of essential elements to embryos to accelerate their development and growth (Endo et al., 2015). In contrast, if reduced transfer of essential elements occurs it may lead to a significant decrease in survival and a significant increase in development time (Slobodian et al., 2021).

Unlike the above essential trace elements, the concentrations of Ni and Zn were lower in embryonic liver when compared to maternal liver, likely a result of the uneven distribution of proteins that control the transfer of essential elements (Hara et al., 2017). Variable trends in element concentrations in muscle and liver were also observed in the electric ray *Narcine brasiliensis* whose Ni and Zn concentrations in embryonic livers were lower than their mothers (Lopes et al., 2019). The distribution of Zn in various tissues is managed by a complex set of Zn transporters and several cellular signal transduction pathways whose expression patterns and catalytic activities in different organs determine the magnitude of zinc absorption (Puar et al., 2020).

4.1.2. Differences in non-essential elements between mothers and embryos

Significant differences in non-essential elements were observed between mothers and embryos, but trends were variable and element specific. The liver is the most important organ in terms of storing and detoxicating non-essential elements (i.e. Cd), while it is also a key energy source for embryonic development (Iida et al., 2019; Lara et al., 2020). Due to the inhibitory effects of non-essential elements by the ovary when forming yolks, the transfer of lower concentrations of non-essential elements likely has a potential protective effect on embryonic health (Martins et al., 2022). The relatively low Pb concentrations found in muscle are similar to results reported for the common thresher shark. This could be a result of the phasing out of leaded gasoline, which has led to lowered concentrations of Pb in the atmosphere and aquatic environments (Dutton and Venuti, 2019). The strong up-welling of As-rich deep ocean water could explain the high As concentrations recorded given the pelagic deep-water environment this species occupies (Cutter and Cutter, 1995). As concentrations were measured as total As in this study, consequently whether the toxic inorganic As is maternally transferred to embryos is unknown. However, marine fish generally have high (1–10 μg g^{-1}, dw) As concentrations with 95% in the organic form as nontoxic arsenobetaine, suggesting these elevated As concentrations are likely not harmful (Schmidt et al., 2018). Cd concentrations in pelagic thresher maternal liver were notably high (102.66 μg g^{-1}, dw), but lower than those reported in the Baja California Sur (~259.59 μg g^{-1}, dw) (Lara et al., 2020). The variation in Cd recorded between these two areas was likely due to phosphorite deposits in coastal area that increase Cd concentration in coastal waters (John and Leventhal, 1995).

Mercury is a concerning heavy metal pollutant, given its influence on fish development (e.g., reduces growth rates), health (e.g., affects liver function, leads to gill deformity, and impacts the nervous system), and reproduction (e.g., reduces spawning and successive hatching rates) (Wood et al., 2012b). The Hg transfer ratios in embryonic muscle had a wide range from 0.071 to 0.635 compared with maternal muscle, which were likely due to the continued transfer of Hg through oophagy. In the common thresher, Hg transfer ratios in embryonic muscle reported from two studies were 0.066 (4.456 and 0.295 μg g^{-1} of maternal and embryonic muscle, dw) and 0.081 (1.35 and 0.11 μg g^{-1} of maternal and embryonic muscle, dw) (Lyons and Lowe, 2013; Dutton and Venuti, 2019). However, Hg concentrations previously reported in juvenile pelagic thresher muscle were similar to the results for embryonic muscle in the current study (Lara et al., 2020). In organisms, mercury exists in inorganic and organic forms with the latter toxic if present above certain levels (Wood et al., 2012a, b). In the current study, total mercury concentrations were measured, however, Pethybridge et al. (2010) reported that over 90% of total mercury in shark muscle exists in the form of methylmercury, the form that is selectively toxic to the central nervous system. The high Hg concentrations in embryonic tissues in this study could be detrimental for embryonic development given similar concentrations in blacktip sharks (*Carcharhinus limbatus*), resulted in an observed increase in melanomacrophages and lipid deposition, suggesting the negative effects on liver (Norris et al., 2021).

4.1.3. Se: Hg molar ratios and maternal transfer mechanism

High Se concentrations are considered to effectively inhibit the toxicity of methylmercury (Rafael et al., 2019). When the Se: Hg molar ratio is > 1, the toxicity of methylmercury is reduced because histidine and cysteine in selenoprotein-P (SEIP) can combine in equimolar ratios

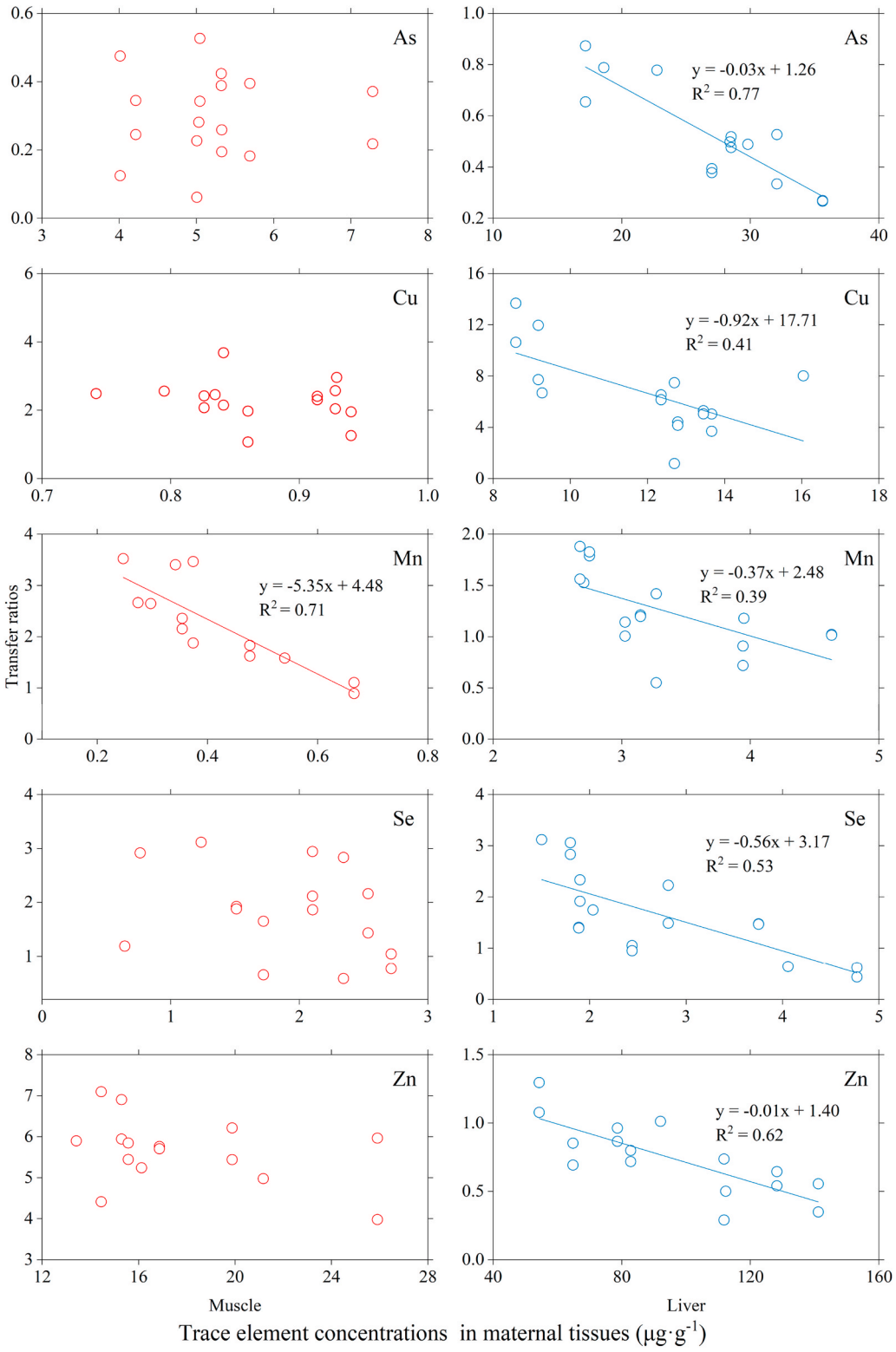

Fig. 3. Relationships between As, Cu, Mn, Se, and Zn transfer ratios and individual trace element concentrations in maternal muscle and liver tissue of pelagic thresher sharks (*Alopias pelagicus)* sampled from the eastern tropical Pacific Ocean. Linear regressions are shown only if $P < 0.05$.

with Se and Hg, forming complexes that organisms can excrete (Ralston and Raymond, 2010). In the current study, the mean Se: Hg molar ratios in embryos were above one, consistent with data reported for other shark embryo studies examining Se: Hg molar ratios (Dutton and Venuti, 2019). Recent studies have reported that methylmercury concentrations can be biologically amplified through food webs, whereas the biomagnification of Se was controversial (Kehrig et al., 2013; Okelsrud et al., 2016; Cusack et al., 2017; Acquavita and Bettoso, 2018). Therefore, when Hg concentrations increase with age of an organism and Se decrease, potentially Se: Hg molar ratios reduced to <1. If this is the case, negative effects of Hg could be observed with increasing age/size of pelagic thresher sharks.

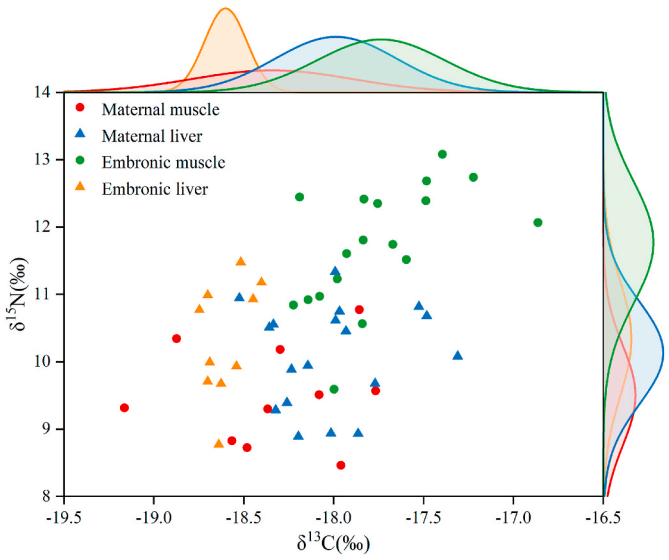

Fig. 4. $\delta^{13}C$ and $\delta^{15}N$ values in muscle and liver tissue of 10 pregnant female pelagic thresher sharks (*Alopias pelagicus)* and their associated embryos (n = 18) sampled from the eastern tropical Pacific Ocean.

Variable body size-related trends in trace element concentrations indicate that maternal transfer strategies during gestation affect the quantity of maternal offloading. Significant negative correlations were found between Al, As, Co, Cu, Mn, Ni, Pb, Se, and Zn concentrations and embryonic length of the smalleye smooth-hound (Souza-Araujo et al., 2020). Similarly, Frias-Espericueta et al. (2014) reported negative relationships between Cu, Zn, Cd, and Pb concentrations in embryonic tissues and body length of the Pacific sharpnose shark. For the pelagic thresher shark, while the muscle Mn concentration increased with embryo size, most elements showed no correlation with increasing body size, which may be related to the small range of body size (ranged from 18.4 to 34.8 cm for embryos and 138–158 cm for mothers). The increase in Mn concentration with body size may be due to the relatively higher metabolic rates of embryos requiring more Mn-dependent enzymes via maternal transfer to improve cell growth (Wood et al., 2012a). The determination of trace element concentrations in various tissues of shark species that adopt a range of reproductive strategies and span body sizes will be required to fully characterize maternal transfer.

The results of element transfer ratios in both muscle and liver tissues of pelagic thresher sharks suggest a regulatory mechanism that maintains the balance of element concentration offloading when maternal tissues contain higher concentrations of elements. Martins et al. (2022) speculated that a potential mechanism exists in sharks and rays, which decreased the concentrations of Cd in uterine content samples for Brazilian guitarfish (*Pseudobatos horkelii*), increasing offspring survival and development.

4.2. Maternal-offspring fractionation of stable isotopes

4.2.1. Difference between the $\delta^{13}C$ and $\delta^{15}N$ values of embryo and pregnant female pelagic thresher sharks

Our results indicate that the mean $\delta^{13}C$ and $\delta^{15}N$ values (−18.60 ± 0.12‰ and 10.34 ± 0.85‰, respectively) for pregnant female pelagic thresher sharks sampled in the open ocean were variable (i.e. lower) when compared with those of previous studies ($\delta^{13}C$: −16.7 ± 0.3‰, $\delta^{15}N$: 13.8 ± 1.4‰ in the Ecuadorian Pacific area, Rosas-Luis et al., 2017; $\delta^{13}C$: −16.54 ± 0.43‰, $\delta^{15}N$: 12.65 ± 1.58‰ in the Galapagos marine reserve, Paez-Rosas et al., 2018). While baseline stable isotope values were not sampled in the EPO to standardize data across studies, the isotope values for pelagic thresher sharks from these three regions indicate individuals are potentially more coastal or seamount associated (Ecuadorian Pacific and Galapagos) compared to those reported in the current study based on established coastal-pelagic isotopic relationships (Abrantes and Barnett, 2011).

Embryos were expected to have higher $\delta^{13}C$ and $\delta^{15}N$ values than their mothers as a result of isotopic discrimination of maternal resources

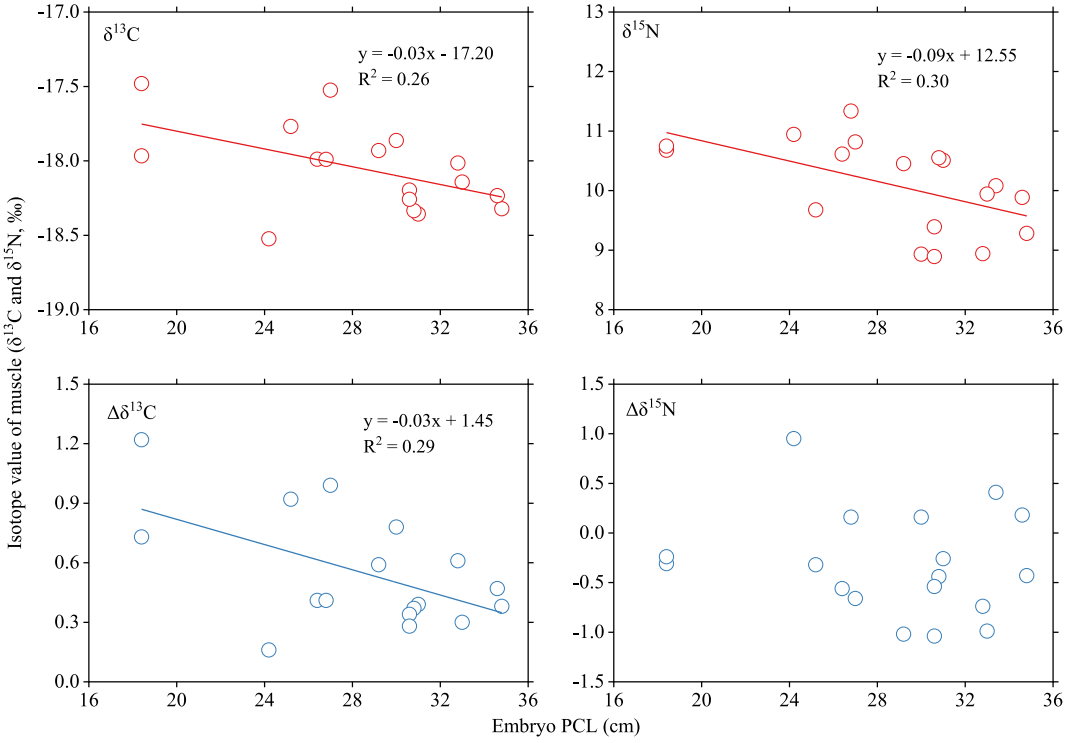

Fig. 5. Relationships between $\delta^{13}C$, $\delta^{15}N$, $\Delta\delta^{13}C$, and $\Delta\delta^{15}N$ values of embryonic muscle and embryo PCL in pelagic thresher shark (*Alopias pelagicus)* sampled from the eastern tropical Pacific Ocean. Linear regressions are shown only if $P < 0.05$; see Table 2.

throughout development (Olin et al., 2018). Since the eggs are derived from the liver, the $\delta^{13}C$ and $\delta^{15}N$ values of fertilized eggs were expected to be the same as those of maternal livers in oviparous sharks (Tosti et al., 2006; Olin et al., 2018). For ovoviviparous species, $\delta^{13}C$ and $\delta^{15}N$ values were mostly lower in embryonic muscle, whereas viviparous species were enriched in ^{13}C and ^{15}N (Table S3). The $\Delta\delta^{13}C$ values in muscle and liver of pelagic thresher shark were all positive, but lower than 1‰, contrasting negative values reported for other ovoviviparous species without oophagy (Le Bourg et al., 2014). The source of nutrition of oophagous sharks shift to unfertilized eggs after the yolk sac was depleted, just like the feeding modes of viviparous species whose $\Delta\delta^{13}C$ values are around 1‰ (Olin et al., 2018).

The $\delta^{15}N$ values in embryonic liver were markedly higher than in maternal liver ($\Delta\delta^{15}N$: 2.63 ± 0.53‰), indicating higher fractionation than muscle tissue. Similar results were observed in embryonic liver of Atlantic sharpnose and Pacific sharpnose shark, where $\delta^{15}N$ values were 1.70‰ and 4.39‰ higher than those of the mothers, respectively (McMeans et al., 2009; Baro-Camarasa et al., 2021). The $\Delta\delta^{15}N$ values for liver were reported to be 1.50 ± 0.54‰ between sharks and prey consumed in a controlled feeding experiment (Hussey et al., 2010). When considering pelagic thresher shark embryos are first fed by a yolk sac with their diet then shifting to immature yolks formed during gestation, variation in embryonic liver isotope values could reflect shifts in the foraging location of mothers. This is further supported by the fact that the nutrition of embryos is provided by the maternal liver with a faster metabolic (i.e. turnover) rate (Liu et al., 1999; Iida et al., 2019).

4.2.2. *Relationship between $\delta^{13}C$ and $\delta^{15}N$ values and embryo size*

For pelagic thresher sharks, the $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissue decreased with increasing embryo PCL (Fig. 5). Changes in isotope values of embryonic tissues could be due to a shift in the mother's diet or feeding location during pregnancy since embryonic muscle tissue provides a long-term integrated measure of their yolk sac diet which reflects maternal tissue during gestation (McMeans et al., 2009). Typically, the $\delta^{13}C$ values of a primary producer reduce from coastal to oceanic areas (Graham et al., 2010; Shipley et al., 2021) and this variation can be observed in tissues of sharks that inhabit distinct regions tied to life stage. For example, shift in isotopic values in vertebrae of blue sharks supported the idea that small juvenile and adult blue sharks (*Prionace glauca*) occur in coastal areas, versus medium-sized and large juveniles that occur in oceanic areas (Estupiñán-Montaño et al., 2019). If female sharks feed in continental shelf areas during gestation, the $\delta^{13}C$ values of yolks will likely be higher than those that feed in the pelagic realm. Whether pelagic thresher sharks migrate for reproduction is unknown but pupping has previously been observed on a coastal seamount (Oliver and Kaszo, 2015). The decrease in $\delta^{13}C$ and $\delta^{15}N$ values with increasing embryo size is speculated as indicating a maternal breeding migration, whereby copulation occurs in coastal regions followed by migration and residency in open ocean areas (Fig. 5). Alternatively, the observed trends could relate to physiological variation during gestation driving a shift in fractionation rather than a maternal diet shift (Pinnegar and Polunin, 1999).

The observed decrease in $\Delta\delta^{13}C$ values in muscle with embryo PCL (Fig. 5) could be attributed to the nutrient source of the yolk-sac during the initial two stages of embryonic development. Identical results of decreasing $\Delta\delta^{13}C$ values with body size but no observed relationship for $\Delta\delta^{15}N$ were reported for the smalleye smooth-hound shark and were suggested to occur as a result of greater depletion rates of ^{13}C in the growing embryo (Souza-Araujo et al., 2020).

5. Conclusions

In conclusion, essential trace elements were transferred between mother and embryo of pelagic thresher shark tissues at high concentrations during gestation as would be expected, whereas nonessential trace elements appeared to be inhibited by a regulatory mechanism or other physiological process within the mother. The high Se: Hg molar ratios in both tissues of embryos suggests that Se plays a protective role against Hg toxicity during the development of pelagic thresher shark embryos. Isotopic fractionation between mothers and embryos was minimal except for embryonic liver tissue, indicating either a potential shift in maternal feeding location and/or dietary shifts during gestation or regulation via physiological processes. Understanding maternal transfer of trace elements and stable isotopes provides an important tool to estimate exposure risk characteristics of young prior to parturition.

Author contributions statement

Zezheng Li: Conceptualization, Methodology, Software, Investigation, Formal analysis, Writing – original draft; Nigel E. Hussey: Visualization, Writing – review & editing; Yunkai Li: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2022.134614.

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