High Mercury Concentrations Reflect Trophic Ecology of Three Deep-Water Chondrichthyans

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Abstract The relative contributions of proximity to mercury sources and trophic ecology to realized axial muscle mercury concentrations were explored for three deep-water chondrichthyans (*Etmopterus princeps*, *Centroscymnus coelolepis*, and *Hydrolagus affinis*), two species of which are harvested for human consumption. Samples were taken at three North Atlantic Ocean locations: the Azores, the Charlie Gibbs Fracture Zone, and the Bear Seamount. Despite the long distances between anthropogenic sources and the sampling locations, all species from all locations had muscle mercury concentrations exceeding the United States human health screening value of 0.3 mg/kg wet weight. Proximity to anthropogenic sources was not an obvious determinant of these elevated concentrations. Generally, mercury concentrations appeared to increase with increased dependence on benthic versus pelagic food sources (as indicated by interspecies differences in δ^{13}C), and with higher position in the trophic web (as indicated by differences in δ^{15}N).

Mercury is a major contaminant of concern because of its global atmospheric dispersion, its propensity to biomagnify after methylation, and its high toxicity. Numerous fish species have muscle mercury concentrations higher than

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consumption advisory limits (Kraepiel et al. 2003; Mason et al. 2006; Sunderland 2007), and health risk from fish consumption has been reported by Fujino (1994), Grandjean et al. (1997), and Crump et al. (2000). Consequently, understanding determinants of mercury concentration in fishes is important to human risk management.

Human exposure in any particular instance might involve an ill-defined mixture of mercury from natural and anthropogenic sources. Currently, natural mercury constitutes the smaller portion of total global atmospheric deposition (Ebinghaus et al. 1999; Pirrone et al. 2001), with the most prominent natural sources being volcanic activity, rock weathering, and oceanic emission. Most mercury is currently released into the environment from diverse human sources (Manohar et al. 2002). Inorganic mercury released from anthropogenic or natural sources is methylated and becomes available for biomagnification.

Although both anthropogenic and natural sources contribute to fish tissue mercury, their relative contributions vary widely among situations. In addition, the trophic position of finfish species is influential in determining mercury tissue concentrations as a consequence of methylmercury biomagnification (Cabana and Rasmussen 1994). Ruelas-Inzunza and Páez-Osuna (2005) measured high mercury concentrations in sharks from Altata-Ensensda del Pabellón lagoon (Mexico), compared them with those of the same species from Guaymas Harbor, and concluded that Altata-Ensensda del Pabellón lagoon had a higher input of anthropogenic mercury. Differences in shark trophic ecology at the two locations were not explored. Relative to accumulation from natural sources, high mercury concentrations were reported from deep-water fishes caught at the Mid-Atlantic Ridge, notionally as a consequence of hydrothermal vent mercury emissions (Martins et al. 2006). Elevated mercury concentrations

have been observed in species inhabiting still other regions remote from anthropogenic sources, e.g., polar bears in the Arctic (Eaton and Farant 1982) and deep-water sharks from Northern Australia (Lyle 1986), tropical Queensland (Denton and Breck 1981), and the Mediterranean Sea (Hornung et al. 1993; Storelli et al. 2002).

In this study, mercury concentrations and stable nitrogen and carbon isotope ratios were measured in three deep-water chondrichthyans (*Etmopterus princeps, Centroscymnus coelolepis,* and *Hydrolagus affinis*) collected from three North Atlantic Ocean locations. Stable isotope ratios that suggest trophic position (δ^{13}C and δ^{15}N) and other features of trophic ecology were compared among species and locations to better understand general patterns of mercury accumulation in deep-water chondrichthyans from remote locations, and to gauge any associated human risk from consumption of deep-water fish.

Materials and Methods

Species Descriptions

Great lanternshark (*E. princeps*) and Portuguese dogfish (*C. coelolepis*) are sharks of the order Squaliformes (subclass Elasmobranchii). The third species, small-eyed rabbitfish *(H. affinis)*, is a chimaera (subclass: Holocephali). The great lanternshark feeds on teleosts (46% of diet), cephalopods (46% of diet), and decapod crustaceans (8% of diet) (Cortés 1999) and has an estimated trophic position of 4.1 (Cortés 1999). Portuguese dogfish feed more diversely (79% cephalopods, 11% teleosts, 4% mammal, 4% decapod crustaceans, 1% other invertebrates, and 1% chondrichthyans) and has a slightly higher trophic position of 4.2 (Cortés 1999). In contrast, information from *H. mirabilis* (Mauchline and Gordon 1983) suggests that small-eyed rabbitfish consume small fishes and invertebrates nearer the ocean bottom and occupy a lower trophic position of 3.5.

Portuguese dogfish is harvested commercially for liver oil and flesh (Stevens and Correia 2003; Compagno et al. 2005; Kyne and Simpendorfer 2007). Great lanternshark is taken as by-catch (Kyne and Simpendorfer 2007) and sold for human consumption in the European market. Although rarely harvested as seafood, small-eyed rabbitfish was included in this survey because it has a contrasting trophic ecology, i.e., a lower trophic position and heavier reliance on benthic food sources than the two sharks.

Species Collection

Specimens were taken opportunistically from stations near the Azores (N42°40′, W29°00′), Charlie Gibbs Fracture
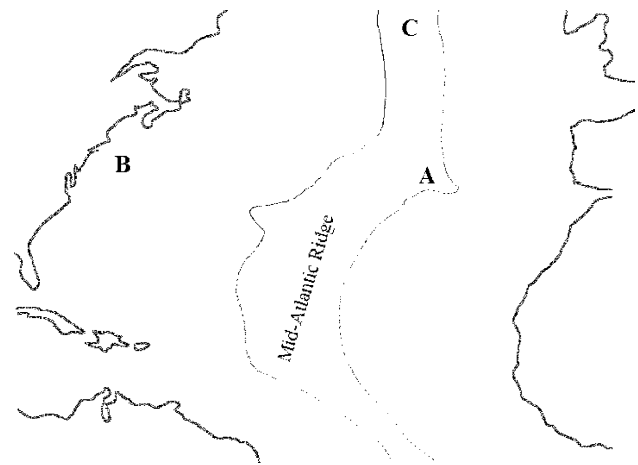
Fig. 1 Map of sampling locations in the Atlantic Ocean. (A, Azores; B, Bear Seamount; C, Charlie Gibbs Fracture Zone)

Zone (N52°40′, W32°35′), and Bear Seamount (N39°55′, W67°25′) (Fig. 1). *E. princeps* was taken at all locations and *H. affinis* was taken at the Azores and Charlie Gibbs Fracture Zone sites, but *C. coelolepis* was taken only at the Azores site. Each Azores and Charlie Gibbs Fracture Zone specimen was collected during the Mid-Atlantic Ridge Ecology (MAR-ECO) expedition (Bergstad and Godo 2003), measured and determined on board for total length and sex, and processed as described by Fossen et al. (2008). Generally, the sex ratio for individuals taken from the locations was 1:1. Slightly more female than male *C. coelolepis* (female-to-male [F:M] ratio = 7:6) were analyzed from the Azores, and *H. affinis* analyzed from the Azores (F:M ratio = 7:9) and Bear Seamount (F:M ratio = 5:4) also deviated slightly from the general 1:1 F:M ratio.

Dorsal fin spines, collected to age individuals (Cotton 2010), and attached small wedges of muscle were frozen on the vessel and remained frozen until processed. Additional specimens of *E. princeps* were taken from Bear Seamount by bottom trawl during the March 2008 Research Vessel *Delaware II* cruise, according to the methods outlined by Moore et al. (2003). Frozen whole *E. princeps* were thawed in the laboratory, measured for total length, and sexed before dissection. Bear Seamount *E. princeps* tissue samples were taken from the liver, kidney, cartilage, gill, brain, and muscle adjacent to the dorsal fin spine.

Sample Preparation

Muscle tissue was taken from the right and left sides of the first and second dorsal fin spines of the sharks and from both sides of the single dorsal fin spine of *H. affinis*. All samples were dried on a Freezone 4.5 freeze-dryer (Labconco, Kansas, MO). The dry samples were then split for mercury and stable isotope ratios (δ^{13}C and δ^{15}N) analyses.

The question about whether a single sample from any spine was sufficiently representative of axial muscle mercury concentration (wet weight [ww]) of a specimen was answered with variance components analysis (SAS PROC NESTED, version 9.2; SAS, Cary, NC) of the large MAR-ECO *E. princeps* data set ($n = 162$ individual muscle samples). Of the total variance in the data set, only minimal amounts of variance were associated with differences between two spines (2%) or between two samples taken on either side of a spine (<1%), indicating that taking one muscle sample from either spine was adequate to represent muscle concentration for an individual fish.

Total Mercury Analysis

Total mercury was measured in ground, freeze-dried samples with a Direct Mercury Analyzer-80 (Milestone, Shelton, CT), which required only a few milligrams of sample. Total mercury concentration was estimated on a dry-weight basis and then converted to ww values (mg/kg ww) using measured wet/dry quotients. Standard curves were made with the standard reference material, DORM-3 (fish protein; National Research Council of Canada). Precision and accuracy for the analytic system were checked with a second standard reference material, TORT-2 (lobster hepatopancreas; National Research Council of Canada). Analytic accuracy and precision were 101.7% recovery ($SD = 1.6\%$, $n = 80$). Tissue sampling precision, estimated with mean differences between measured concentrations for laboratory splits of randomly selected samples, was 8.9% ($SD = 6.8\%$, $n = 50$).

Stable Isotope Analysis

Nitrogen and carbon isotopes allow quantification of trophic position for omnivorous species and suggest relative contributions of different carbon sources to the diet. Nitrogen-isotope ratios are commonly applied in trophic analyses, such as those examining the general structure of the Georges Bank food web (Fry 1988), or biomagnification of mercury (Atwell et al. 1998) or persistent organic pollutants (Fisk et al. 2001; Ruus et al. 2002) in marine food webs. $\delta^{15}N$ increases 3.4 ‰ on average for every increase of one trophic level (Minagawa and Wada 1984). Although $\delta^{13}C$ increases approximately 1‰/trophic level, it is used most often to reflect the source of carbon at the base of food webs (Takai et al. 2002). As a relevant example, carbon incorporated from methane into the biomass of geothermal vent producers had a recognizable abiogenic carbon and thermally derived sediment carbon $\delta^{13}C$ signature (Van Dover and Fry 1994, Proskurowski et al. 2008). In studies such as those of the Georges Banks (Fry 1988) and Seta Inland Sea (Takai et al. 2002), $\delta^{13}C$

also tended to be more negative (i.e., more ^{13}C depleted) in individuals that obtained more carbon from pelagic than benthic sources.

To facilitate trophic analyses of these deep-water chondrichthyans with nitrogen and carbon isotopes, 1-mg portions of ground, freeze-dried samples were weighed and encapsulated in 5×9–mm tin capsules. $\delta^{13}C$ and $\delta^{15}N$ were determined at the Stable Isotope Facility of the University of California (Davis, CA) using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Results were presented as ratios relative to those of standards of Pee Dee belemnite limestone ($\delta^{13}C$) or atmospheric N_2 ($\delta^{15}N$) and expressed as per mil (‰). Quality control and accuracy for the analyses were checked with the two mixtures of ammonia and sulfate generated at the Stable Isotope Facility. The mean % recoveries (SD, n) for $\delta^{15}N$ in these mixtures were 101.3% (13.5%, 34) and 96.1% (48.5%, 16), and those for $\delta^{13}C$ were 100.0% (27%, 34) and 100.0% (0.3%, 17).

Data Analysis

Conventional statistical significance testing was not used to make inferences because that general approach has come under increasing criticism (McCloskey 1995), most notably in health sciences (Sterne and Davey Smith 2001), socioeconomics (Ioannidis 2005; Ziliak and McCloskey 2004), psychology (Cohen 1994), ecology (Fidler et al. 2004, 2006), and, recently, environmental toxicology and chemistry (Newman 2008). Confidence interval analysis, as advocated by Altman et al. (2000), Di Stefano (2004), Fidler et al. (2004), and Cummin and Finch (2005), was applied instead to avoid the problems with conventional significance testing. Confidence interval analysis has the advantage of simultaneously presenting the statistical significance, precision, and importantly, magnitude of any differences (Altman et al. 2000). *p*-values for one set of F tests were reported for descriptive purposes, not to infer statistical significance.

Results

General Mercury Concentrations

Axial muscle mercury concentrations for all species exceeded the current United States human health screening value of 0.3 mg/kg ww for fish (Table 1). The mean concentrations for *E. princeps* (1.72 mg/kg ww) and *H. affinis* (2.38 mg/kg ww) were similar to those reported by Martins et al. (2006) for specimens collected near mid–Atlantic

Table 1 Mercury, $\delta^{13}C$ and $\delta^{15}N$ (mean and 95% CI) for all locations

Species	Location	n	Tissue	Mercury (mg/kg ww)		$\delta^{15}N$ (‰)		$\delta^{13}C$ (‰)		Total length (cm)	
				Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
E. princeps	A, B, C	52	Muscle	1.72	1.51–1.94	11.82	11.70–11.94	−18.17	−18.34 to −18.00	57.88	55.62–60.15
C. coelolepis	A	26	Muscle	4.96	4.42–5.49	13.76	13.57–13.95	−16.22	−16.43 to −16.02	102.42	98.80–106.05
H. affinis	A, C	29	Muscle	2.38	1.88–2.88	15.32	15.00–15.65	−16.08	−16.43 to −15.73	105.28	101.05–109.51

A Azores, *B* Bear Seamount, *C* Charlie Gibbs Fracture Zone

Table 2 Interspecies comparisons of mercury concentrations (mg/kg ww) for Azores location

Species	N	Mercury mean (SD) (mg/kg ww)	95% CI of mean
E. princeps	11	2.36 (0.72)	1.88–2.85
C. coelolepis	25	4.96 (1.35)	4.41–5.52
H. affinis	15	1.72 (1.20)	1.04–2.38
Differences in means	n/n	Difference (mg/kg ww)	95% CI of difference
E. princeps vs. *H. affinis*	11/15	Δ 0.64	−0.20 to 1.48
C. coelolepis vs. *E. princeps*	25/11	Δ 2.60	1.72 to 3.48
C. coelolepis vs. *H. affinis*	25/15	Δ 3.24	2.38 to 4.10

Ridge hydrothermal vents. However, mercury concentrations for *C. coelolepis* (4.96 mg/kg ww) were higher than those reported for this species collected off southeast Australia by Turoczy et al. (2000) (3.08 mg/kg ww) and Pethybridge et al. (2010) (2.3 to 2.5 mg/kg ww).

Differences Among Species

Interspecies comparisons were performed with the Azores location samples (Table 2) because all three species were collected there in sufficient numbers. The $\delta^{15}N$ and $\delta^{13}C$ data indicated that the species differed relative to trophic

position, and pelagic and benthic contribution to the diet (Fig. 2). Differences were consistent with the literature (Mauchline and Gordon 1983; Marques and Porteiro 2000; Jakobsdottir 2001). The shark, *E. princeps,* is a micronekton consumer of cephalopods, teleosts (especially lanternfish), and crustaceans, and according to Mauchline and Gordon (1983) and Cortés (1999), *C. coelolepis* is more of a benthopelagic consumer of cephalopods, teleosts, decapod crustaceans, and mammal blubber. The less negative $\delta^{13}C$ of *C. coelolepis* relative to *E. princeps* reflected the slightly higher dependence of *C. coelolepis* on the benthic food web. The higher $\delta^{15}N$ for *C. coelolepis* (13.77‰; 95% confidence interval [CI] 13.57–13.97) than *E. princeps* (11.71‰; 95% CI 11.39–12.02) was also consistent with their respective trophic rankings by Cortés (1999) of 4.2 and 4.1. Chimaerids, such as *H. affinis*, are epibenthic feeders, primarily of invertebrates, although larger individuals do consume some fish (Mauchline and Gordon 1983). *H. affinis* sampled by Marques and Porteiro (2000) near hydrothermal vents consumed vent mussels. Consistent with the literature, mean $\delta^{13}C$ values for Azores *H. affinis* (−15.49‰; CI −14.93 to −16.06) suggested the higher reliance of this species on benthic food sources than either *E. princeps* (−17.88‰; CI −18.02 to −17.73) or *C. coelolepis* (−16.22‰; CI −16.43 to −16.00). The $\delta^{15}N$ values for *H. affinis* were high relative to those of the two sharks; however, this likely reflected the $\delta^{15}N$ signature of the benthos more than a higher trophic

Fig. 2 Isotopic data for three deep-water chondrichthyans collected from the Azores location. (E, *E. princeps*; C, *C. coelolepis*; H, *H. affinis*)
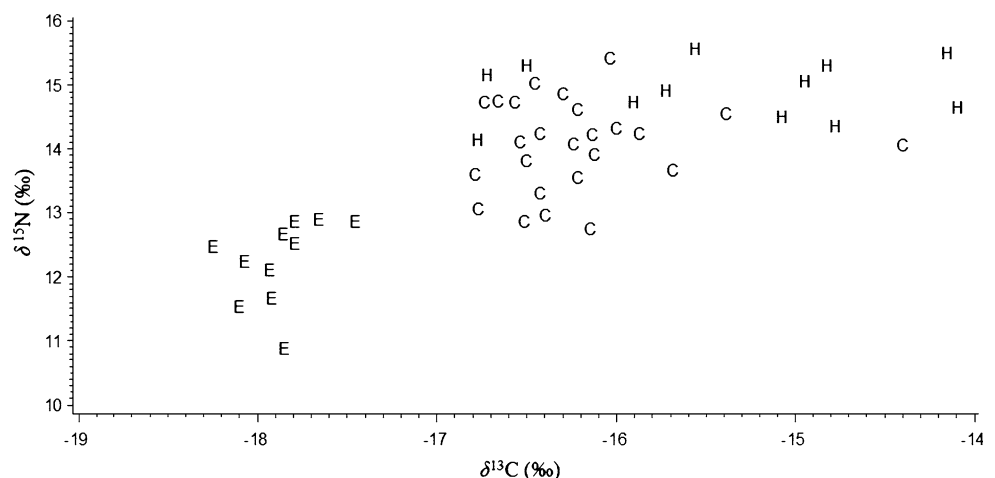
Fig. 3 Mercury concentrations in three chondrichthyans plotted against corresponding values of $\delta^{15}N$ (top panel) and $\delta^{13}C$. (E, *E. princeps*; C, *C. coelolepis*; H, *H. affinis*)
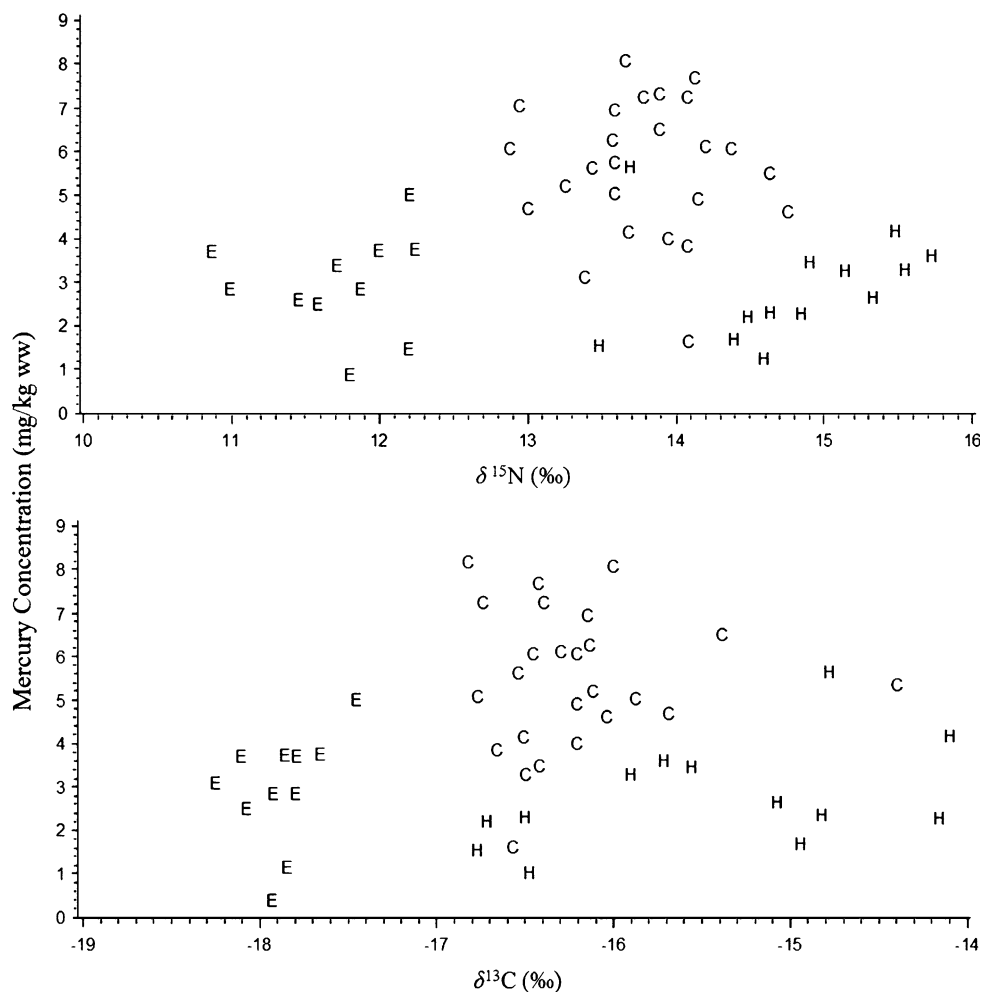
position of this species relative to the sampled shark species. Using the equation given by Cortés (1999) and diet data from a congeneric species (Mauchline and Gordon 1983), we estimated a trophic level of 3.5 for *H. affinis*, which is less than those of the two sharks (4.1 and 4.2, given in Cortés (1999)).

The mean mercury concentration in *C. coelolepis* muscle was significantly higher than those of *E. princeps* and *H. affinis* based on nonoverlapping CIs (Table 2 and Fig. 3). In contrast, the 95% CI for the difference between mean mercury concentrations for *E. princeps* and *H. affinis* included zero, suggesting the lack of statistically significant difference between these species. The highest mercury

concentrations in chondrichthyans taken from the Azores location were associated with *C. coelolepis*, the shark species with the highest reliance on the benthic food web and the chondrichthyan with the highest trophic position.

Difference Among Locations

Because only *E. princeps* was collected at all three locations, differences in mercury concentration among locations were examined for this species only (Table 3). The mean mercury concentration at the geothermally least active Bear Seamount was lower than those of the other two locations but still exceeded the United States Food and

Table 3 Mercury concentration, shark length, $\delta^{15}N$ and $\delta^{13}C$ (mean and 95% CI) for *E. princeps* at the three locations

Quality	Location		
	Azores ($n = 11$)	Charlie Gibbs Fracture Zone ($n = 19$)	Bear Seamount ($n = 12$)
Mercury (mg/kg ww)	2.36 (1.89 to 2.85)	1.71 (1.50 to 1.91)	0.92 (0.60 to 1.23)
Shark length (cm)	54 (47 to 60)	64 (62 to 66)	52 (47 to 57)
$\delta^{15}N$ (‰)	11.7 (11.4 to 12.0)	11.9 (11.7 to 12.1)	12.0 (11.7 to 12.2)
$\delta^{13}C$ (‰)	−17.9 (−18.0 to −17.7)	−18.0 (−18.2 to −17.9)	−18.8 (−19.4 to −18.2)

Drug Administration human health screening value. This location was nearest to North American anthropogenic mercury sources. The 95% CIs for mean mercury concentration in *E. princeps* taken from the Bear Seamount location did not overlap with those for the other locations, but the CIs for samples from the Azores and Charlie Gibbs Fracture Zone did overlap. Mean mercury concentration in *E. princeps* from the location most distant from terrestrial anthropogenic sources of mercury (Charlie Gibbs Fracture Zone) was not the lowest of the three locations. The non-overlapping CIs for mean $\delta^{13}C$ of the Bear Seamount location and those of the other locations indicated ^{13}C depletion at Bear Seamount relative to the other locations. However, differences in $\delta^{13}C$ among locations for *E. princeps* were much smaller in magnitude (Table 3) than the differences among species just described. ^{13}C depletion can be associated with organisms that obtain proportionally more carbon from the pelagic than the benthic trophic web (Fry 1988, Takai et al. 2002), but the small differences could also reflect interlocation differences in carbon flux into the food web from geothermal sources (see Van Dover and Fry 1994; Proskurowski et al. 2008).

General linear modeling (SAS PROC GLM; SAS, Cary, NC) of the *E. princeps* mercury data produced the following F test *p*-values: location (<0.0001), shark length (0.0009), $\delta^{15}N$ (0.0266), and $\delta^{13}C$ (0.0570). SAS PROC GLMSELECT (SAS, Cary, NC) was used to select the best multivariate model with location, shark length, $\delta^{15}N$, and $\delta^{13}C$ as candidate explanatory variables. Minimum Akaike's information criterion (AIC) estimation indicated that the best model (i.e., that with the most explanatory power per estimated parameter) included all candidate variables. Inclusion of location and $\delta^{13}C$ had the most influence on AIC, and shark length had the least influence. Several qualities appeared to be correlated with mercury concentration for this species, including those related to trophic ecology. Analysis of variance (SAS PROC NESTED; SAS, Cary, NC) with location as the only class variable indicated that most (60%) of the total variance among *E. princeps* samples could be attributed to location. The distinct $\delta^{13}C$ signature and lowest mercury concentrations from the least geothermally active location (Bear Seamount) suggested that proximity to natural, but not anthropogenic, mercury sources might influence mercury concentrations in this shark species.

Differences Among Tissues

Mercury concentrations measured in all but the cartilaginous and brain tissues of *E. princeps* exceeded current United States health-screening concentrations (Fig. 4). The liver, from which squalene oil is produced, had mercury concentrations as high as muscle tissue. Liver oil and meat of
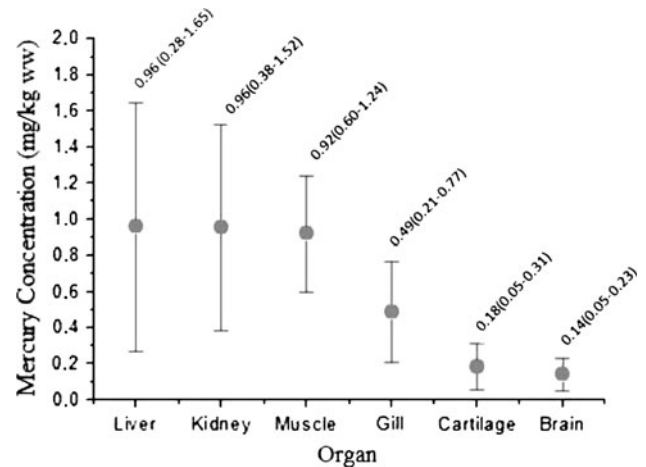
Fig. 4 Means and 95% confidence intervals of mercury concentration (mg/kg ww) in organs of *E. princeps* from the Bear Seamount location

C. coelolepis and other deep-water elasmobranch species are harvested for human consumption (Kyne and Simpendorfer 2007). Cartilagenous tissues from other shark species are used in the Asian seafood and United States health-supplements markets. Mercury in this tissue of *E. princeps* was below United States health-screening concentrations.

Discussion

Our evidence indicated that trophic ecology had a more consistent influence than proximity to anthropogenic sources on axial muscle mercury concentrations for these deep-water chondrichthyans. Our conclusion about the relative importance of trophic position was supported by the intraspecies positive relation between mercury and $\delta^{15}N$ of the rabbitfish ("H" in Fig. 3, top panel: slope = 0.66; 95% CI = 0.12–1.21), and by the higher mercury concentrations and $\delta^{15}N$ values in *C. coelolepis* relative to *E. princeps* (Fig. 3). However, the largest differences in mercury concentration among the three chondrichthyans were not associated with trophic position alone (as indicated by $\delta^{15}N$ values) (Fig. 3). Comparing *E. princeps* with *C. coelolepis*, those sharks feeding more from the benthos and higher in the food web tended to have higher muscle mercury concentrations. *H. affinis* mercury concentrations would be judged anonymously low based on $\delta^{15}N$-assigned trophic level alone; however, the distinct $\delta^{15}N$ and $\delta^{13}C$ signatures relative to those of the sharks appeared to result from the higher dependence of *H. affinis* on the benthic food web. Regardless, only by understanding trophic ecology could these trends be explained.

Although mercury concentration in *E. princeps* from the three locations increased slightly with change in $\delta^{13}C$, it

was not possible to attribute these small differences to any particular feature of the species' diet, e.g., relative dependence on pelagic versus benthic food webs. Because the geothermal activity at the three locations differed, the slight ${}^{13}C$ depletion could reflect differences in geothermal carbon movement (Van Dover and Fry 1994, Polz et al. 1998, Proskurowski et al. 2008) into the diet of *E. princeps* at these locations. The differences in *E. princeps* mercury concentrations could have been influenced by proximity to natural, geothermal sources.

Deep-water chondrichthyans at all locations had axial muscle mercury concentrations exceeding those protective of human health. Differences in species trophic ecology best explained the observed differences in mercury concentrations among species. Material influence of proximity to anthropogenic mercury sources was not evident.

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