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Ontogenetic niche expansion influences mercury exposure in the Antarctic silverfish *Pleuragramma antarcticum*

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ABSTRACT: The effects of body size and age class on mercury concentrations were examined in the Antarctic silverfish *Pleuragramma antarcticum*, an ecologically important prey species in the Antarctic marine food web. Stable isotope analysis was used to investigate variation in mercury concentrations related to ontogenetic changes in diet and/or foraging habitat. Specimens of *P. antarcticum* were collected along the Ross Sea shelf in February 2008 and mercury concentrations in homogenized whole fish and muscle tissue were analyzed relative to standard length, age class (juvenile vs. adult) and isotopic measures of diet ($\delta^{15}N$) and foraging habitat ($\delta^{13}C$). While mercury concentration in muscle tended to be higher than whole-fish values, concentrations in these 2 matrices were highly correlated. A positive relationship was found between standard length and mercury concentration; further, adult *P. antarcticum* had significantly higher mercury concentrations than juveniles. Adult mercury concentrations were also more variable: the coefficient of variation in adult muscle (58.3%) was more than twice that found in juveniles (25.0%). Though no linear relationships were detected between standard length and $\delta^{15}N$ or $\delta^{13}C$ values when all individuals were combined, juvenile and adult size classes of fish differed in their isotopic niche position and width. In addition, $\delta^{13}C$ values explained the greatest amount of variation in whole-fish mercury across all age classes and for adult fish alone. By expanding both the horizontal and vertical components of their foraging habitat, adult *P. antarcticum* may have a wider range of exposure to mercury compared with juvenile fish.

KEY WORDS: Mercury · Ontogenetic shift · Trophic position · Stable isotope analysis · Foraging niche · Antarctic silverfish

INTRODUCTION

Many marine fish undergo indeterminate growth, increasing body mass by 5 or more orders of magnitude throughout their lives (Mommsen 2001), and as they grow, many expand their dietary niche (Karpouzi & Stergiou 2003). In fact, body size is a well-documented determinant of niche dynamics during ontogeny, with size-related dietary shifts in trophic position prevalent in many marine and freshwater fishes (Scharf et al. 2000, Eagles-Smith et al. 2008, Hammerschlag-Peyer et al. 2011, Szczebak & Taylor 2011). In addition, niche shifts in terms of changes in foraging habitat with growth occur in which consumers may exploit prey in new habitats with different basal nutrient sources or forage at greater spatial scales (Karpouzi & Stergiou 2003, Layman et al. 2005). Stable isotope analysis has become a common

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tool in studies documenting ontogenetic niche shifts, as the composition of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes in animal tissues can be used to infer a marine consumer's diet and habitat use (Szczebak & Taylor 2011, Kim et al. 2012, Polito et al. 2013). For example, Hammerschlag-Peyer et al. (2011) used an isotopic approach to identify a distinct ontogenetic habitat shift in gray snapper *Lutjanus grieus* in the southeastern Atlantic Ocean in which juveniles forage exclusively within oyster reefs while sub-adults expand their foraging habitat into nearby mangrove habitats due to decreased predation pressure and/or increased mobility associated with a larger body size. This finding highlights how different ontogenetic stages of an organism may form functionally distinct groups which could vary in their risk of exposure to mercury due to behavioral ontogenetic changes.

Accounting for nearly 90% of the icthyoplankton and pelagic fish biomass in the Antarctic marine food web (Pinkerton et al. 2013), *Pleuragramma antarcticum* (Family: Nototheniidae) may serve as a major biovector of mercury into Antarctic predators such as flying seabirds, penguins, and marine mammals. *P. antarcticum* functions as a critical trophic link between the lower trophic positions (invertebrates such as krill, *Euphausia* spp.) and top predators including 11 species of fish, the south polar skua *Stercorarius maccormicki*, Adélie penguin *Pygoscelis adeliae*, emperor penguin *Aptenodytes forsteri*, and Weddell seal *Leptonychotes weddellii* (La Mesa et al. 2004). *P. antarcticum* has a variable diet driven by shifting prey availability, seasonality, geographic location, and body size (Pinkerton et al. 2013). In fact, distinct dietary and trophic differences have been detected among post-larval, juvenile, and adult *P. antarcticum* in the Ross Sea and East Antarctic marine food webs (Hodum & Hobson 2000, Giraldo et al. 2011, Pinkerton et al. 2013). As mercury biomagnifies (Atwell et al. 1998), it is possible that mercury exposure to predators foraging on *P. antarcticum* may vary depending upon the ontogenetic stage of the prey item consumed. However, to our knowledge, mercury concentrations in this ecologically important prey fish have not previously been assessed.

In *P. antarcticum*, stomach contents and stable isotope data suggest that trophic shifts and dietary expansions can occur with ontogeny. Larvae feed on phytoplankton and small zooplankton, post-larval stages consume primarily copepods, juveniles consume equal parts copepods and euphausids, and adults feed primarily on krill and small fishes (Giraldo et al. 2011, La Mesa & Eastman 2012, Pinkerton et al. 2013). The magnitude of these dietary shifts varies among ontogenetic stages and can differ by as much as 0.4 to 1.0 trophic level (Giraldo et al. 2011, Pinkerton et al. 2013). In addition to dietary shifts, there is ontogenetic variation in the vertical and horizontal foraging habitats used by *P. antarcticum*. Larval stages are found at shallow depths (0–100 m), but with ontogeny, juveniles and adults are transported to progressively deeper (0–400 m and 400–700 m, respectively) and more offshore waters of the continental shelf (La Mesa & Eastman 2012). Adults also make seasonal migrations to nearshore waters adjacent to continental ice shelves to spawn (Kellermann 1986). While yet unexamined, these ontogenetic changes in diet and foraging habitat have the potential to lead to significant differences in tissue mercury concentrations among *P. antarcticum* life stages. For example, dietary niche expansion via the consumption of higher trophic zooplankton and fish prey is likely to lead to higher mercury concentrations in adult stages through biomagnification, while differences in mercury availability across foraging habitats may lead to differential mercury exposure. These concerns are warranted as similar trends have been found in other fish species, advocating the importance of subtle ontogenetic changes in dietary composition and foraging habitat in terms of assessing risk of exposure to mercury in marine food webs (Szczebak & Taylor 2011).

Predators such as penguins, whales, seals, and flying seabirds in the Antarctic marine food web preferentially forage on specific size classes of *P. antarcticum* (Eastman 1985, La Mesa et al. 2004), which creates the potential for varied risk of exposure to mercury with ontogeny in this species. For example, the rate at which mercury is accumulated has been shown to vary with ontogeny in bluefish: mercury concentrations in juvenile fish were relatively high owing to rapid accumulation rates in age 0 fish relative to older fish (Szczebak & Taylor 2011). Understanding mercury at lower trophic positions is essential to understanding the process of biomagnification at higher trophic levels and predicting risk of exposure to predators. Thus, the purpose of the present study was to determine whether mercury concentrations change with ontogeny in *P. antarcticum*. Specifically, we aimed to: (1) document mercury concentrations in this ecologically important prey fish, (2) determine whether mercury concentrations in *P. antarcticum* varied with body size or age class, and (3) investigate the relationship between mercury accumula-

tion and ontogenetic variation in diet and foraging habitat in this species.

MATERIALS AND METHODS

Specimens of *Pleuragramma antarcticum* were collected along the Ross Sea shelf (74° 31.27′ S, 177° 34.02′ E) via a single trawl from the R/V 'Tangaroa' during the International Polar Year cruise in February 2008. Trawling occurred after the spawning period for *P. antarcticum* (early austral spring with larvae appearing in October–December; Radtke et al. 1993), allowing for the collection of post-larval and adult fish. The trawl was conducted using a National Institute of Water and Atmospheric Research fine-mesh mid-water trawl with a circular mouth opening (~12 m) and a cod-end mesh of 10 mm at a depth of 272–276 m. A random sub-set of individuals (n = 52) was made available for mercury analysis from this single haul of 220 kg of *P. antarcticum*. Whole fish were frozen at –20°C until analysis.

Sample preparation

Fifty-two fish of unknown sex were thawed and measured for standard length (SL; rostrum to the base of the tail; mm), and body mass was recorded to the nearest 0.01 g. To aid comparison with other work, we divided fish into 2 age classes based on SL: juveniles (90–151 mm SL, 3–6 yr of age) and adults (>151 mm SL, >7 yr of age; La Mesa & Eastman 2012). To investigate the relationship between muscle and whole-fish mercury concentrations, a muscle biopsy representing 1% of the body mass was collected from the right dorsolateral region of each fish, posterior to the second dorsal fin. Smaller specimens having a body weight <5 g had muscle biopsies >1% of their body weight to ensure a large enough sample for mercury analysis. Each fish was then homogenized using a razor and/or food processor (larger fish required a food processor for homogenization) and a sub-sample (~0.20 g) was taken for mercury analysis. Wet weight of muscle samples and homogenate subsamples were recorded and samples were subsequently freeze-dried to a constant weight (–62°C for ~36 h). Once dry, samples were reweighed in order to determine moisture loss prior to mercury analysis (mean % moisture loss ± SD, muscle: 22.6 ± 5.6%, whole fish: 22.0 ± 4.5%). The whole-fish homogenate will be referred to as 'whole fish' and the muscle biopsies as 'muscle' throughout the remainder of the manuscript.

Mercury analysis

Samples of whole fish and muscle were analyzed separately for total mercury via atomic absorption spectrophotometry on a Tri-Cell Direct Mercury Analyzer (DMA-80) at the University of North Carolina Wilmington. As ≥95% of the mercury in fish tissue is methylmercury, total mercury was used as a proxy for this highly bioavailable form (Bloom 1992, Piraino & Taylor 2009, Payne & Taylor 2010). Each set of 20 samples analyzed was preceded and followed by 2 method blanks, a sample blank, and 2 samples each of standard reference material (DORM-3 and DOLT-4; fish protein and dogfish liver certified reference materials, respectively, provided by National Research Council Canada). Total mercury concentrations were reported as parts per million (ppm) wet weight (ww). Mean (± SD) percent recoveries for standard reference materials were 102.0 ± 1.4% (DORM-3) and 99.8 ± 2.0% (DOLT-4). The relative percent difference between 40 pairs of duplicate standards was less than 2.0%. Detection limit of the assay was 0.0015 ng mercury. A subset of samples of whole-fish homogenate (n = 19) was analyzed in duplicate to examine the homogenization of the tissue sample; the mean (± SD) percent difference between duplicates was 6.97 ± 4.66%.

Stable isotope analysis

Approximately 0.5 mg of individual whole fish was loaded into separate tin cups, flash-combusted (Thermo-Finnigan elemental analyzer), and analyzed for $\delta^{13}C$ and $\delta^{15}N$ values via a Con-Flo II interfaced with a Thermo-Fisher Delta Plus XL continuous flow stable isotope ratio mass spectrometer (CFIRMS). Raw δ values were normalized on a 2-point scale using glutamic acid reference materials USGS-40 and USGS-41. Normalized $\delta^{13}C$ values were also corrected for lipid content based on their carbon to nitrogen ratio following the methods of Post et al. (2007). Sample precision was 0.1‰ and 0.2‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively. Stable isotope abundances are expressed in δ notation in per mil units (‰), according to the following equation:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The $R_{standard}$ values were based on

the Vienna PeeDee Belemnite (VPDB) for $\delta^{13}C$ and atmospheric N_2 (air) for $\delta^{15}N$. While no studies have estimated isotopic turnover rates in *P. antarcticum*, studies of other fishes suggest a temporal integration of dietary and habitat information into whole fish on the scale of weeks to months (e.g. Herzka 2005).

Statistical analysis

Fish muscle and whole-fish mercury concentrations were log-transformed prior to analyses in order to generate data sets that did not differ from a normal distribution (Shapiro-Wilk, $p > 0.05$ in all cases). We used paired *t*-tests and linear regression (ordinary least square regression) to examine relationships between fish muscle and whole-fish mercury concentrations. Similar linear regression analyses were used to investigate the influence of SL (using body size as a continuous variable) on mercury concentration, trophic position ($\delta^{15}N$), and foraging habitat ($\delta^{13}C$). We then used Bartlett's test of homogeneity of variance and *t*-tests adjusted for equal or unequal variance data (Zar 1999) for bivariate comparisons to assess differences in the degree of variation and mean mercury concentrations between juvenile and adult size classes of *P. antarcticum.* Coefficients of variation were calculated as (standard deviation × 100)/mean. In addition, we explicitly tested for ontogenetic shifts in the trophic ($\delta^{15}N$) and habitat ($\delta^{13}C$) niches of *P. antarcticum* following the methods of Hammerschlag-Peyer et al. (2011). First, we tested for differences in isotopic niche position by computing the Euclidean distance (ED) between group centroids ($\delta^{13}C$ and $\delta^{15}N$ bivariate means) of juvenile and adult fish (Turner et al. 2010). Isotopic niche positions were considered to be different if the ED between these 2 size classes was significantly greater than zero after comparison with null distributions generated by a residual permutation procedure. We then used *t*-tests for equal or unequal variance data to determine which niche axis ($\delta^{13}C$ and/or $\delta^{15}N$) drove the observed shifts in niche position. Trophic shifts were further quantified by estimating the trophic level (TL) of each *P. antarcticum* size-class using a baseline (TL = 1) mean $\delta^{15}N$ value from concurrently collected phytoplankton (0.3‰; Pinkerton et al. 2013) while assuming a +3.2‰ change in $\delta^{15}N$ per successive trophic transfer (Sweeting et al. 2007).

To test for differences in niche width between size classes, we computed the mean distance to centroid (MDC; a measure of dispersion) for each size class (Turner et al. 2010). Using an analysis of nested linear models and residual permutation procedures, the absolute value of MDC differences was evaluated among groups with absolute values greater than zero indicating a difference in niche width among size classes (see Turner et al. 2010 for more details). When pair-wise comparisons indicated significant differences in MDC and thus niche width, Bartlett's test of homogeneity of variance was used to examine the homogeneity of variance of $\delta^{13}C$ and $\delta^{15}N$ values among size classes to determine which isotopic niche axis drove the observed difference. We then measured pair-wise niche overlap between juvenile and adults by quantifying the percentage of individuals in each age class that was encompassed by a comparison age class's convex hull, the area of the smallest convex polygon that contains all individuals of a single size class in a $\delta^{13}C$ and $\delta^{15}N$ bi-plot (Layman et al. 2007).

Finally, we investigated the relative influence of age class (juveniles and adults), body size (SL), trophic position ($\delta^{15}N$), and foraging habitat ($\delta^{13}C$) on mercury concentration in *P. antarcticum* using a generalized linear modeling (GLM) approach. We parameterized a global model using whole-fish mercury concentration as the dependent variable and age class, SL, $\delta^{15}N$ and $\delta^{13}C$ values as possible covariates. Models with all possible combinations of these 3 covariates were compared using Akaike's information criteria (AIC; Akaike 1973). While AIC methods are generally robust to collinearity, some biases can arise when predictor covariates are highly correlated ($r > 0.60$; Freckleton 2011). As correlations between the 3 continuous covariates used in our GLM model were weak ($r = 0.19$ to 0.38), it is likely that any such effects of collinearity were negligible. The model with the lowest AIC score was selected as the model most strongly supported by the data. Models with ΔAIC scores (difference in AIC between a given model and the model with the lowest AIC) ≤2.0 were considered competitive with the most strongly supported model and any model with a $\Delta AIC \leq 10.0$ was considered well supported. Model fits were further assessed by R^2 and AIC weight (ω_i), which is a measure of the relative likelihood that a given model is the best among a set of models fitted (Burnham & Anderson 2002). As the above analysis indicated the importance of age classes as a predictor variable, we supplemented this approach by conducting GLM analyses separately for both juveniles and adults. This allowed us to assess the relative influences of SL, $\delta^{15}N$, and $\delta^{13}C$ on mercury values across individuals within each age class. Significance was

assumed at $\alpha = 0.05$ and all means are presented ±SD. Statistical comparisons were conducted using SAS (version 9.1), SPSS (version 18.0) and Program R (version 2.14.1).

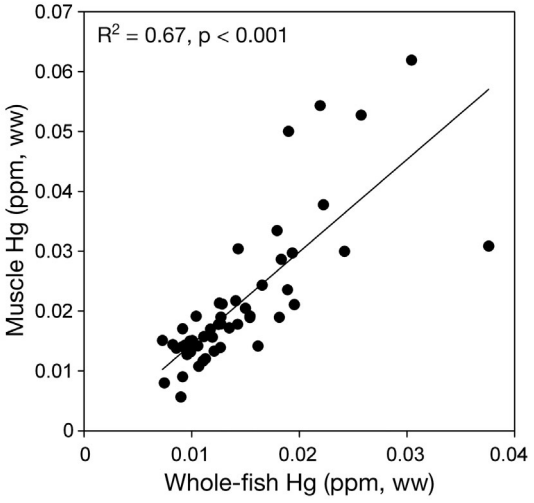

Fig. 1. Relationship between muscle and whole-fish mercury concentration (wet weight, ww) in Antarctic silverfish *Pleuragramma antarcticum*

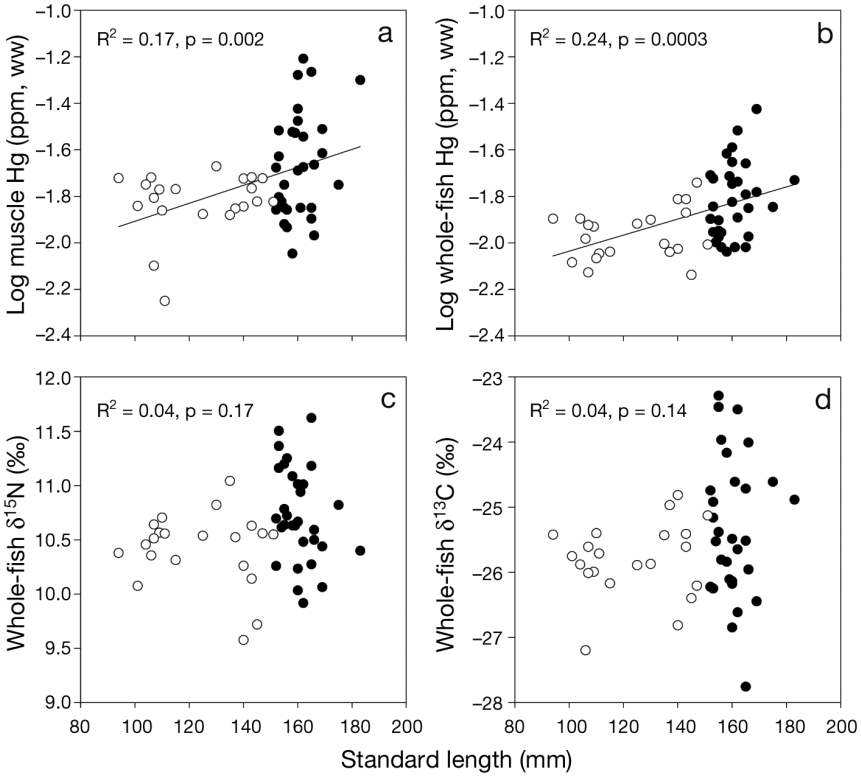

Fig. 2. Relationships between standard length and (a) muscle and (b) whole-fish mercury concentration (wet weight, ww) and (c) $\delta^{15}N$ and (d) $\delta^{13}C$ stable isotope values in adult (●) and juvenile (○) *Pleuragramma antarcticum*

RESULTS

There was a significant, positive relationship between mercury concentration in muscle and whole fish ($R^2 = 0.67$, $p < 0.001$; Fig. 1); however, the mercury concentration in muscle (0.021 ± 0.012 ppm) tended to be higher than in whole fish from the same individual (0.014 ± 0.006 ppm; $t = 9.01$, df = 54, $p < 0.001$). Relatively weak, positive relationships were detected between body length and muscle ($R^2 = 0.17$, $p = 0.002$) and whole-fish mercury concentrations ($R^2 = 0.24$, $p < 0.001$; Fig. 2). When examined by age class, adults had significantly higher muscle and whole-body mercury concentrations compared with juveniles (Table 1). Further, the coefficient of variation of mercury concentration in adults was approximately twice that of juveniles in both whole fish and muscle, though this difference was only significant for muscle tissue (Table 1).

Overall, no significant relationships were found between body length and $\delta^{13}C$ or $\delta^{15}N$ ($R^2 = 0.04$, $p = 0.140$ and $R^2 = 0.04$, $p = 0.170$, respectively; Fig. 2). However, upon examination by age class, mean isotopic niche positions (ED) of juveniles and adults were found to differ by 0.5‰ ($p = 0.042$; Fig. 3). Univariate tests found that $\delta^{13}C$ values did not differ between age classes and that differences in ED between age classes were due to significantly higher $\delta^{15}N$ values in adults relative to juveniles (Table 1). This difference in $\delta^{15}N$ values (0.3‰) between age classes translated into a small, but statistically significant shift in trophic level between juvenile (4.1 ± 0.1) and adult (4.2 ± 0.1) *P. antarcticum* ($t = 2.89$, $p = 0.006$). Furthermore, the width of isotopic niches also differed among age classes, with significantly higher MDC (+0.5‰; $p = 0.001$) in adults relative to juveniles (Fig. 3). Broader niches in adults were driven by the significantly higher variance in $\delta^{13}C$, but not $\delta^{15}N$ values, with coefficients of variation approximately twice that of juveniles for $\delta^{13}C$ (Table 1). The isotopic niches of juveniles overlapped substantially with those of adults (85.7%), while only 38.7% of adults were encompassed by the convex hull (i.e. isotopic niche area) of juveniles (Fig. 3).

Table 1. Mean (±SD), range and coefficient of variation (CV) of mercury concentrations (muscle and whole fish, ppm, wet weight) and stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) in juvenile and adult *Pleuragramma antarcticum* collected from the Ross Sea shelf in February 2008. Test statistics for homogeneity of variance (Bartlett's test) and mean (*t*-tests for equal or unequal variance data) comparisons between age groups are provided

Metric, age class	n	Mean ± SD	Range	CV	Bartlett's test	*t*-test
Muscle Hg (ppm)						
Juvenile	21	0.016 ± 0.004	0.006 to 0.021	25.0	$K^2 = 5.71$, p = 0.017	$t = 49.33$, p = 0.003
Adult	31	0.024 ± 0.014	0.009 to 0.062	58.3		
Whole-fish Hg (ppm)						
Juvenile	21	0.011 ± 0.003	0.007 to 0.018	27.3	$K^2 = 3.51$, p = 0.061	$t = 50.00$, p < 0.001
Adult	31	0.016 ± 0.007	0.009 to 0.038	43.8		
Whole-fish $\delta^{13}C$ (‰)						
Juvenile	21	−25.8 ± 0.6	−27.2 to −24.8	2.3	$K^2 = 8.01$, p = 0.005	$t = 1.92$, p = 0.061
Adult	31	−25.4 ± 1.1	−27.8 to −23.3	4.3		
Whole-fish $\delta^{15}N$ (‰)						
Juvenile	21	10.4 ± 0.3	9.6 to 11.0	2.9	$K^2 = 1.83$, p = 0.176	$t = 2.89$, p = 0.006
Adult	31	10.7 ± 0.4	9.9 to 11.6	3.7		

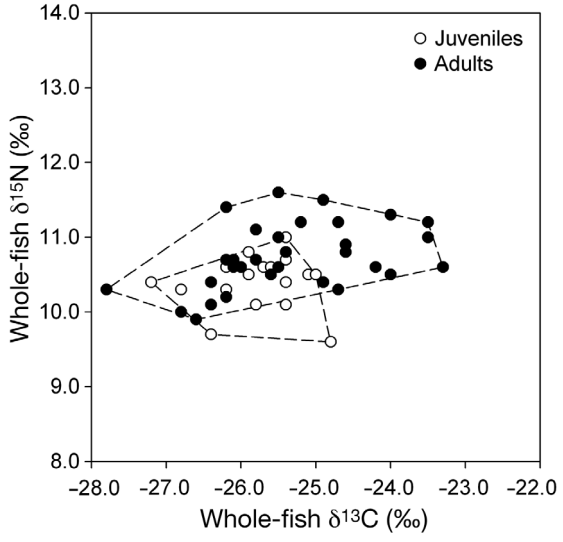

Fig. 3. Relationships between mean isotopic niche positions (Euclidian distance between group centroids of $\delta^{15}N$ and $\delta^{13}C$ bivariate means) and isotopic niche width (mean distance of centroids) in adult (●) and juvenile (○) *Pleuragramma antarcticum*

When examining mercury concentration across all individuals, the model that included age (adult vs. juvenile) and $\delta^{13}C$ as covariates was the most parsimonious model selected by AIC analysis, with no other competitive models (i.e. $\Delta AIC \leq 2.0$; Table 2). Whole-fish mercury concentration was higher in adults relative to juveniles ($F_{1,52} = 25.18$, $p < 0.001$) and was negatively correlated with habitat niche ($\delta^{13}C$: $\beta = -0.077$, $R^2 = 0.10$, $F_{1,52} = 16.21$, $p < 0.001$). When the analysis included only juvenile fish, a model that included a positive ($\beta = 0.070$) but non-significant ($F_{1,21} = 0.96$, $p = 0.340$) relationship between mercury concentration and $\delta^{15}N$ was the most strongly supported by the data (Table 2). A model that included an effect of $\delta^{13}C$ on juvenile mercury concentration was also competitive, but was similarly non-significant ($F_{1,21} = 0.96$, $p = 0.344$). Finally, the most parsimonious model explaining variation in adult mercury concentration included a significant negative relationship with habitat niche ($\delta^{13}C$: $\beta = -0.084$, $R^2 = 0.32$, $F_{1,31} = 13.78$, $p < 0.001$). Two other competitive models included either both $\delta^{13}C$ and $\delta^{15}N$ or solely $\delta^{15}N$ when explaining adult mercury concentration (Table 2). Whole-fish $\delta^{15}N$ values had a negative relationship ($\beta = -0.180$ to -0.110, $R^2 = 0.07$ to 0.24; Fig. 4) with adult mercury concentration, but this was significant only when the model did not also contain an effect of $\delta^{13}C$ ($p = 0.005$ to 0.071). AIC weights illustrated that the adult model using $\delta^{13}C$ had twice as much support as the model with $\delta^{15}N$ as the sole covariate (Table 2).

DISCUSSION

While an increasing number of studies have begun to address mercury concentrations in marine predators in the Antarctic marine food web (Aubail et al. 2011, Brasso et al. 2012a,b), few have investigated mercury availability at lower trophic levels. To our knowledge, the present study is the first to report mercury concentrations in *Pleuragramma antarcticum*, a major prey resource in the Antarctic marine food web. On average, mercury concentrations in muscle and whole-fish samples of *P. antarcticum*

Table 2. Summary of model selection statistics used to investigate the influence of age class (juvenile and adult), body size (SL), diet ($\delta^{15}N$), and foraging habitat ($\delta^{13}C$) on mercury concentration in *Pleuragramma antarcticum* collected from the Ross Sea shelf in February 2008. ΔAIC is the difference in Akaike's information criterion (AIC) score between a given model and the model with the lowest AIC score. AIC weight (ω_i) indicates the relative percent support for each the model. Only models with a ΔAIC ≤ 10.0 are reported

Model structure	R^2	AIC	ΔAIC	ω_i
All ages				
Age, $\delta^{13}C$	0.41	−53.40	0.00	0.75
Age, $\delta^{13}C$, $\delta^{15}N$	0.43	−50.50	2.90	0.18
SL, $\delta^{13}C$	0.42	−46.90	6.50	0.03
Age, $\delta^{15}N$	0.29	−46.00	7.40	0.02
Age, SL, $\delta^{13}C$	0.45	−45.40	8.00	0.01
Age	0.21	−45.20	8.20	0.01
Juveniles				
$\delta^{15}N$	0.05	−25.00	0.00	0.56
$\delta^{13}C$	0.05	−23.90	1.10	0.32
$\delta^{13}C$, $\delta^{15}N$	0.10	−22.70	2.30	0.10
SL	0.06	−21.50	3.50	0.01
SL, $\delta^{15}N$	0.13	−17.30	7.70	0.00
Adults				
$\delta^{13}C$	0.31	−25.20	0.00	0.43
$\delta^{13}C$, $\delta^{15}N$	0.40	−24.80	0.40	0.35
$\delta^{15}N$	0.24	−23.80	1.40	0.21
SL, $\delta^{13}C$	0.37	−17.80	7.40	0.01
SL, $\delta^{13}C$, $\delta^{15}N$	0.42	−16.30	8.90	0.00

were relatively low (0.021 ± 0.012 ppm and 0.014 ± 0.006 ppm, respectively), as was to be expected at a low trophic position in this remote food web. Mercury concentrations in *P. antarcticum* detected in the present study are below threshold concentrations in prey fish known to cause adverse effects in piscivorous birds (>0.16 ppm, WW; Evers et al. 2008). Further, mercury concentrations reported for the Antarctic toothfish *Dissostichus mawsoni* (mean: 0.16 ppm, range: 0.02–0.70 ppm, n = 253 individuals; Hanchet et al. 2012), for which adult *P. antarcticum* are a major prey item, were an order of magnitude higher than concentrations in *P. antarcticum*, indicating significant biomagnification of mercury in the Antarctic marine food web.

Similar to studies of other fishes, we found a significant relationship between mercury concentrations in muscle tissue and whole fish (Goldstein et al. 1996, Szczebak & Taylor 2011). In all cases, muscle mercury concentrations were significantly higher than whole-body concentrations. Mercury concentration in muscle is likely higher than whole-fish mercury as mercury bioaccumulates at a faster rate in muscle compared with the whole body in fish (Szczebak & Taylor 2011). A significant, positive relationship was detected between whole-fish mercury and standard length when all fish were combined; however, when separated into age classes, we found adult fish to have significantly higher mercury concentrations than juveniles. Interestingly, adult mercury concentrations were significantly more variable than those of juveniles, with the coefficient of variation in muscle being twice as high in adults compared with juveniles (58.3% and 25.0%, respectively). Stable isotope analysis revealed significant differences in isotopic niche width between age classes, in which 61.3% of adults were foraging outside of the isotopic niche area of juveniles (Fig. 3), providing explanatory power to the observation of variation in mercury concentrations in adult, relative to juvenile fish.

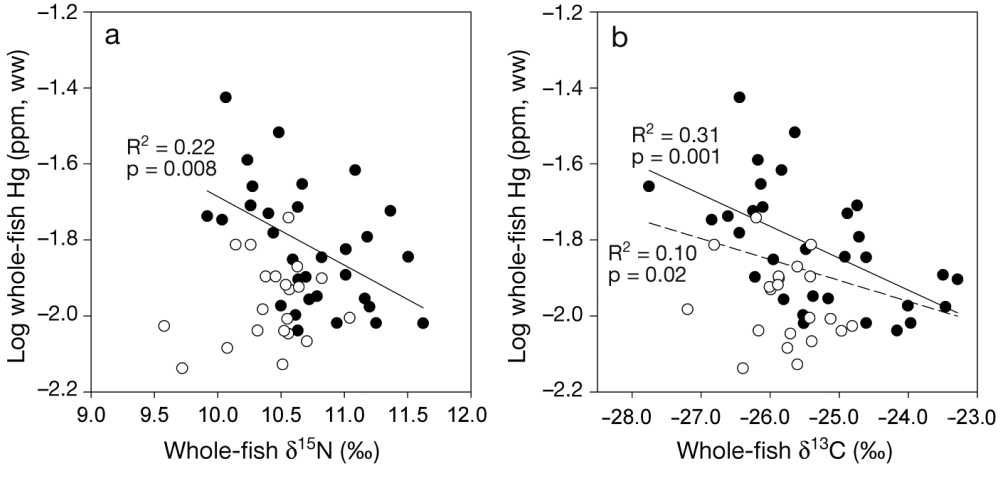

Fig. 4. Relationships between whole-fish mercury concentration and whole-fish (a) $\delta^{15}N$ and (b) $\delta^{13}C$ values in *Pleuragramma antarcticum*. ●, solid lines: adults; ○: juveniles. For comparison, the model fit for adults and juveniles combined (dashed line) is also shown in (b)

Influence of size, age class and trophic dynamics on mercury concentrations

Mercury concentrations in *P. antarcticum* did vary with both size and age class. We found an overall positive relationship between mercury and standard length in *P. antarcticum* in which mercury increased with body length; however, this trend was not driven by a uniform increase in mercury with body length across the entire size range of individuals sampled. The overall relationship between body length and mercury across all fish, as well as the higher mean mercury concentration for adult fish, was driven by significantly higher variance in mercury among individuals in the adult age class compared with the juvenile age class (Table 1). As in other studies, we first predicted that a trophic shift or dietary expansion might have led to the higher and more variable mercury concentrations in adults relative to juveniles. When we tested for relationships between $\delta^{15}N$ and mercury across all individuals, $\delta^{15}N$ was not considered significant in explaining variation in whole-fish mercury (Table 2, Fig. 4). However, when age classes were analyzed separately, adult mercury concentrations were found to decrease with increasing $\delta^{15}N$ (Fig. 4). While we did find, in support of this hypothesis, that mean $\delta^{15}N$ values were higher in adults compared with juveniles (+0.3‰; Table 1), the magnitude of the increase in trophic level (+0.1 TL), though statistically significant, was unlikely to be ecologically relevant in terms of driving differences between age classes. This suggests that the slight increase in trophic level and expansion of dietary resources in adults relative to juvenile *P. antarcticum* is likely not the sole or primary determinant of the observed increase in mercury concentration and variability with ontogeny.

Evidence for a similar trophic shift (+0.1 TL) in *P. antarcticum* in the Ross Sea was also reported by Pinkerton et al. (2013), with adult $\delta^{15}N$ values 0.5‰ higher than juveniles, with significant differences in prey composition detected between age groups. Juvenile *P. antarcticum* in Pinkerton et al.'s (2013) study fed primarily on copepods (30.3% by mass) and euphausids (45.8% by mass), while small adult fish fed on a mix of fish (36.1% by mass), euphausids (37.3% by mass), and copepods (15.0% by mass). It should be noted that Pinkerton et al. (2013) identified a larger trophic shift (+1.6‰ $\delta^{15}N$ and +0.5 TL) between post-larval individuals (50–89 mm) and large adults (>190 mm), with evidence of increased piscivory in the largest adult *P. antarcticum* (54.8% of diet by mass). Unfortunately, in our study we were not able to assess mercury for the full range of *P. antarcticum* size classes, and it remains likely that larger trophic shifts may still play a role in mediating mercury exposure across size classes in *P. antarcticum*.

Ontogenetic changes in foraging behavior reflected in mercury concentrations

The lack of a clear relationship between ontogenetic changes in trophic level and mercury exposure is not without precedent in fishes. A comparable trend was found in bluefish, in which individuals changed their diets, but occupied similar trophic levels across multiple life stages while mean mercury concentration increased with age (Szczebak & Taylor 2011). In marine fish, the direction and degree of change in dietary composition with ontogeny is correlated with ontogenetic changes in gape morphology, morphological specializations, and foraging tactics (Scharf et al. 2000, Karpouzi & Stergiou 2003, Bacha et al. 2010), which can lead to expansion or contraction of their foraging niche. For example, the anchovy *Engraulis encrasicolus* increases its dietary diversity index and niche width between age 0 and age 2, but then becomes more specialized, consuming a greater proportion of larger prey items after age 3+ (Bacha et al. 2010). However, other marine fishes (including *P. antarcticum*) continue to include small, lower trophic level prey items in their diet throughout their lifetime, leading to ontogenetic increases in niche breadth rather than niche position (Scharf et al. 2000, Karpouzi & Stergiou 2003). Scharf et al. (2000) found the absolute size range of prey items consumed by 18 species of predatory marine fish to increase with predator body size; while all species increased the maximum size prey taken, 67% of species also increased the minimum prey size consumed.

Ultimately, the trophic position of a given individual may increase due to the inclusion of higher trophic prey with age, but a concurrent increase in dietary diversity may limit the exposure to bioavailable mercury, uncoupling trophic position and the risk of exposure to mercury. The continued incorporation of lower trophic level prey into the diet throughout the life history of the fish can reduce the rate of dietary mercury uptake, leading to a decelerating risk of mercury exposure with age in some marine fish (Szczebak & Taylor 2011). Ontogenetic changes in foraging behavior can also manifest in the form of shifts in foraging habitat. Variation in

foraging habitat (as measured by $\delta^{13}C$) has been found to correlate with variation in mercury concentrations among individuals in several species of fish (Nisbet et al. 2002, Eagles-Smith et al. 2008, Szczebak & Taylor 2011). Szczebak & Taylor (2011) found a significant proportion of the variability in $\delta^{13}C$ to be explained by length in bluefish; $\delta^{13}C$ values in age +1 fish were significantly lower than in age 0 fish, reflecting an ontogenetic shift from foraging on a mix of benthic and pelagic prey in estuarine and coastal habitats to solely pelagic prey as adults. An ontogenetic niche shift between juvenile and sub-adult gray snapper was found to occur primarily along the $\delta^{13}C$ axis as well (Hammerschlag-Peyer et al. 2011), though the effect of this shift on mercury concentrations were not assessed in their study.

In the present study, a significant relationship was detected between mercury concentration and $\delta^{13}C$, especially in adult fish, in which mercury concentrations decreased as $\delta^{13}C$ values increased (Fig. 4). Ultimately, $\delta^{13}C$ explained the greatest amount of variation in whole-fish mercury, when age classes were combined as well as for adults alone (Fig. 4). Adult fish had a significantly wider foraging niche than juvenile fish, with adult fish exploiting a larger and/or more variable habitat range than juveniles (Fig. 3). Compared with the relatively shallow waters inhabited by post-larval and juvenile fish, adult *P. antarcticum* have been found offshore at mesopelagic depths of 400–700 m (La Mesa & Eastman 2012), and recent studies have found enhanced bioaccumulation of mercury by fishes in the mesopelagic realm (Monteiro et al. 1996, Choy et al. 2009). The expansion of the foraging niche of adult *P. antarcticum* into deeper waters further offshore (supported by lower $\delta^{13}C$ values in this group) appears to be a stronger determinant of mercury concentrations relative to the slight increase in trophic position ($\delta^{15}N$) (Table 1). These findings suggest that a more integrated set of factors are influencing dietary mercury uptake than proposed by the traditional trophic-level-focused model of biomagnification, which may be relevant to future efforts in biomonitoring and ecological risk assessment.

While not assessed in the present study, it is important to consider how changes in energy allocation with ontogeny could also play a role in the variation detected in mercury concentrations between age classes of *P. antarcticum*. It should be noted that there was a wider range of possible ages for adult fish relative to juvenile fish in the present study (>7 yr vs. 3–6 yr, respectively), and changes in bioenergetics as fish age have been shown to play a significant role in the rate of mercury uptake, assimilation, and elimination. It is well documented that the rate of mercury elimination is negatively correlated with body size in fish (Trudel & Rasmussen 1997). As fish age, metabolic costs of activity and growth efficiency change; energy is reallocated towards behaviors such as mating and decreased growth efficiency can lead to an increase in prey consumption (Trudel & Rasmussen 2006). Subsequently, an increase in prey consumption rate to maintain a larger body size can lead to increased tissue mercury concentrations regardless of trophic position (Trudel & Rasmussen 2006, Sackett et al. 2013). In addition, mercury elimination rates in fish have also been shown to be positively correlated with water temperature (Sackett et al. 2013). Foraging in colder, deeper waters, it is possible that adult *P. antarcticum* eliminate mercury at a slower rate than juveniles; however, this has not been tested in this species. With the limited difference in trophic position and documented difference in foraging habitat between age classes in the present study, future research into differences in bioenergetics between age groups is certainly warranted.

Implications for individual-level variation in mercury

Documenting mercury concentrations as well as establishing sources of variation in mercury both between age classes and across all individuals in *P. antarcticum* is essential for gauging the risk of exposure to dietary mercury for top predators in the Antarctic marine food web, as many species rely almost exclusively on this low trophic level prey during the austral summer. The results of the present study suggest that predators foraging on adult *P. antarcticum* are not necessarily at a greater risk of exposure to elevated mercury due to the high degree of inter-individual variation resulting from the wide foraging niche of adult fish. In fact, the increased niche width in adult *P. antarcticum* creates a more heterogeneous prey base, in terms of mercury availability, than would result from a complete niche shift in which all individuals would increase (or decrease) in mercury concentration with age. To this end, when estimating a predator's risk of exposure to mercury, our results along with those of the other studies cited herein provide sound support for expanding beyond the common population-level trophic comparison.

Conclusions

Recent studies utilizing stable isotope analysis have cautioned against assuming diet or habitat use is a 'species-level trait' (Hammerschlag-Peyer et al. 2011, Kim et al. 2012). Therefore, studies assessing the risk of mercury exposure in marine food webs should assess the degree of ontogenetic and individual-level variation in the foraging ecology of marine consumers (Szczebak & Taylor 2011). Stable isotope analysis allows for the assessment of not only trophic disparities ($\delta^{15}N$), but also differences in foraging habitat ($\delta^{13}C$), a factor long known to affect the risk of exposure to mercury uptake among populations (Evers et al. 1998, 2005, Scheuhammer et al. 2007), but rarely examined at the individual level (but see Nisbet et al. 2002). We found that by expanding both the horizontal and vertical components of their foraging habitat, adult *P. antarcticum* have a wider range of exposure to mercury compared with juvenile fish. Further analysis is needed to determine mercury concentrations in the smallest (post-larval, 50–89 mm) and largest (>190 mm) size classes of *P. antarcticum* to gain a more complete understanding of mercury uptake in this important prey species. Ultimately, our findings suggest that mercury exposure in marine ecosystems is based on an integrated set of factors relating to foraging ecology and life history; while shifts in trophic position may occur, more subtle changes in the form of niche expansions may better explain inter-individual and among-age-class variation in mercury concentrations.

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