

# Mercury in Forage Fish from Mexico and Central America: Implications for Fish-Eating Birds

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**Abstract** Mercury (Hg) is a global contaminant of aquatic food chains. Aquatic birds, such as the osprey (*Pandion haliaetus*), with migratory populations breeding in Canada and the northern United States and wintering in the Central and South America, can be exposed to mercury on both the breeding and wintering ranges. We examined Hg levels in 14 fish taxa from 24 osprey wintering sites identified from satellite telemetry. Our main goal was to determine whether fish species that feature in the diet of overwintering and resident fish-eating birds reached toxicity thresholds for Hg. Mean Hg levels in fish whole carcasses ranged from a high of 0.18  $\mu\text{g g}^{-1}$  (wet weight) in

*Scomberomorus sierra* to a low of 0.009  $\mu\text{g g}^{-1}$  in Catostomidae. Average Hg levels were within published toxicity threshold values in forage fish for only two sites in Mexico (Puerto Vallarta and San Blas Estuary), and all were marine species, such as mackerel (*Scomberomorus sierra*), sea catfish (*Ariopus* spp.), and sardinas species (*Centropomus* spp.). Except for one sample from Nicaragua, sea catfish from Puerto Morazan, none of the fish from sites in Central America had Hg levels which exceeded the thresholds. Nonmetric multidimensional scaling revealed geographical differences in Hg levels with significant pairwise differences between sites along the Pacific Ocean (Mexico) versus the Bay of Campeche, partly due to differences in species composition of sampled fish (and species distributions). Hg increased with trophic level, as assessed by nitrogen stable isotope ratios ( $\delta^{15}\text{N}$  but not  $\delta^{13}\text{C}$ ), in freshwater and marine, but not estuarine, environments. Hg concentrations in forage fish do not account for the elevated Hg reported for many osprey populations on the breeding grounds, thus primary sources of contamination appear to be in the north.

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Migratory behaviour can be an important factor in determining contaminant exposure in a bird population. Among the species of most concern are piscivores, such as the osprey (*Pandion haliaetus*), which are at the apex of aquatic food chains and represent important and charismatic indicators for toxic chemicals (Elliott et al. 2007; Grove et al. 2009). The aquatic ecosystems where these species forage are sinks for persistent contaminants from both local and distant sources (Wang et al. 2004). One of these is the global contaminant mercury (Hg) and its highly toxic biological form, methylmercury (MeHg), which has both natural and anthropogenic

sources and biomagnifies in aquatic systems (Eagles-Smith et al. 2009). MeHg can have significant effects on a variety of biological functions and potentially impact both reproduction and survival of wildlife (Wiener et al. 2003; Scheuhammer et al. 2007, 2008, 2012; Dietz et al. 2013).

Major industrial point sources of Hg have been curtailed largely. Currently, Hg is released and distributed primarily from low-level atmospheric combustion activities and can be transported to remote environments, including Arctic and alpine systems (Schuster et al. 2002; Wang et al. 2004; Scheuhammer et al. 2007; Guigueno et al. 2012). For example, mercury levels in arctic species, including toothed whales, polar bears (*Ursus maritimus*), and various bird and fish species were reported often to exceed putative toxicity thresholds (Dietz et al. 2013). Given that Hg emissions are predicted to continue to increase until at least 2050 (Streets et al. 2009), although this may be attenuated by the global impact of the Minamata Convention on Mercury, it is important to continue to identify sources of Hg in the food chain and monitor indicator species.

Compared with northern breeding sites, relatively little is known about contaminant exposure in the overwintering areas of many migratory species, such as the Osprey *Pandion halieetus* (Elliott et al. 1998, 2000, 2007, 2012). Similarly, compared with the extensive data base on Hg in fish, largely collected as part of game fish monitoring in the United States and Canada (Kamman et al. 2005; Davis et al. 2008; Wyn et al. 2010), there is much less from wintering areas in Mexico and Central America, with some exceptions (Mol et al. 2001; Ruelas-Inzunza and Páez-Osuna 2005). We targeted sampling sites based on our knowledge of where satellite tagged osprey were wintering and some information collected by local biologists on forage fish species preyed upon by ospreys (Elliott et al. 2007). We examined interspecific differences in Hg concentrations and the toxicological implications of those findings for apex piscivores. We also compared Hg levels among different geographic regions to test for spatial patterns. Finally, we modelled relationships between Hg levels and two stable isotopes of carbon ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). Those stable isotopes have been used to relate the activities of piscivorous species and Hg contamination, as well as trace pathways and sources of Hg in aquatic ecosystems (Choy et al. 2010; Braune et al. 2013). Generally, sources of carbon ( $\delta^{13}\text{C}$ ) can be used to examine the relationship between contaminant concentrations and foraging area, because marine sources are more enriched than freshwater ones; sources of nitrogen ( $\delta^{15}\text{N}$ ) are used to indicate trophic level (Post 2002).

## Materials and Methods

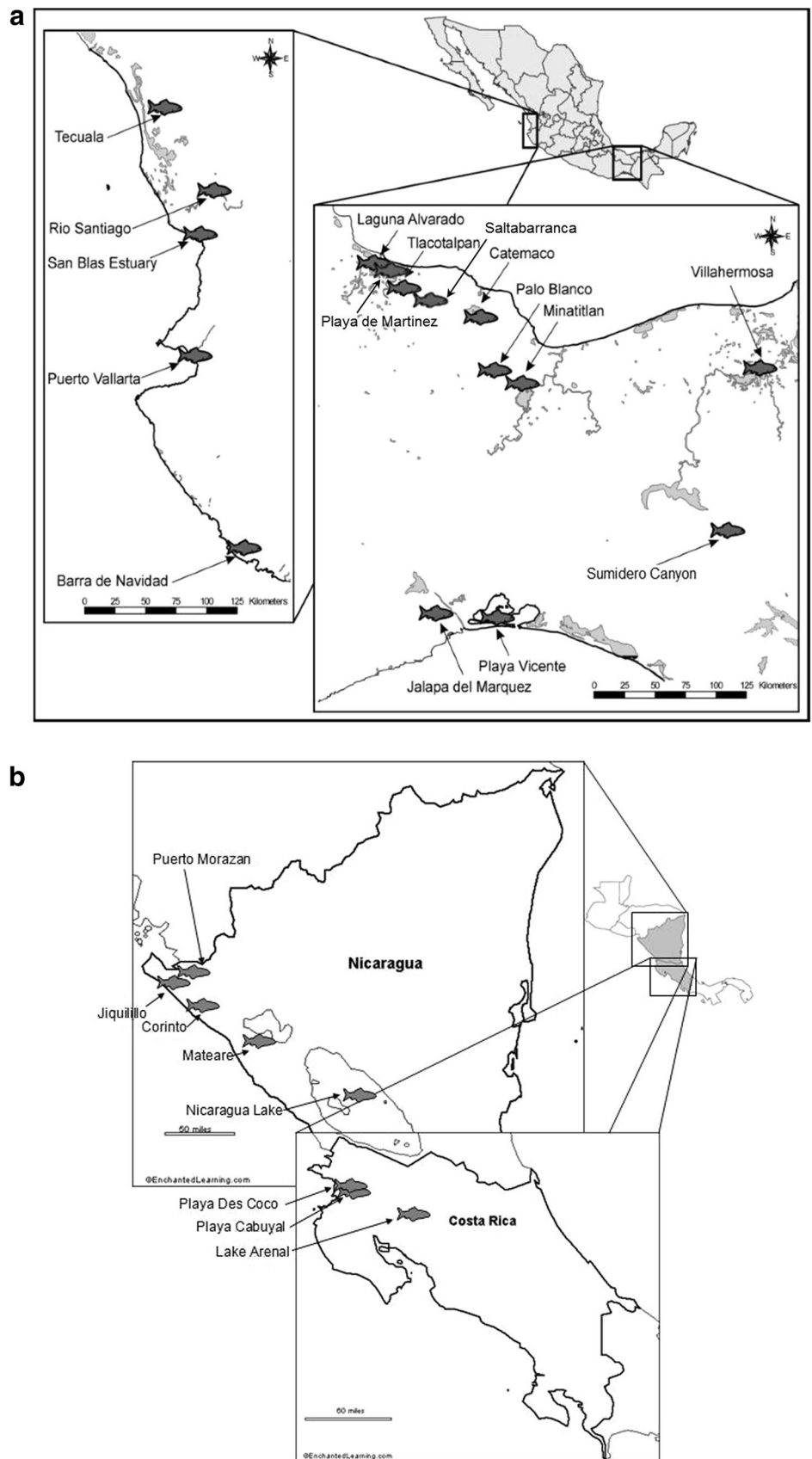
### Study Areas

Between 2000 and 2003, fish were sampled from 18 sites in Mexico, including 2 from the state of Jalisco, 3 from Nayarit, 2 from Oaxaca, 2 from Tabasco, and 8 from Veracruz (Fig. 1a, b). In 2007, samples were obtained from five locations in Nicaragua and three in Costa Rica, also as close as possible to known osprey wintering sites (Elliott et al. 2007; Martell et al. 2001, 2014). We worked with local biologists, subsistence fisherman, small commercial fishing operations, and occasionally anglers to collect several fish species that are potential prey of Osprey, including mojarra (*Gerridae* spp.), catfish (*Ariusspp.*, *Rhamdia* spp.), guavina (*Gobiomorus dormitor*), mackerel (*Scomberomorus sierra*), mullet (*Mugil* spp.), snook (*Centropomidae* spp.), surgeonfish (*Acanthuridae* spp.), and tilapia (*Cichlasoma urophthalmus*, *Oreochromis niloticus*). We attempted to collect fish from areas where satellite-tagged Ospreys were wintering or from known Osprey wintering sites in Mexico, including the Laguna de Alvarado in Veracruz State, Villahermosa in Tabasco State, Barra de Navidad in Jalisco State, and the San Blas estuary in Nayarit State (Elliott et al. 2007). To sample a larger geographic area, we collected several samples from small commercial fisherman at dockside or in local markets in other Mexican states and some locations in Central America. A minimum of three to five individual fish of approximately the same length for the same species at each site was used to make up a single composite pool. All samples were labeled and stored in polyethylene bags on ice until frozen locally. Frozen samples were transported by air in coolers on ice to Canada in the accompanying baggage of two of the authors (JEE and/or KEE). All samples were stored at  $-20\text{ }^{\circ}\text{C}$  at the Pacific Wildlife Research Centre, Delta, BC. Each fish sample was partially thawed, weighed, measured, and dissected at the Pacific Environmental Science Centre (PESC) in North Vancouver, British Columbia, Canada to separate the muscle fillets from the remaining carcass. Muscle fillets and composite samples of the remaining carcasses were weighed and analyzed separately according to protocols previously established to assess risk to human consumers of only muscle tissue as well as wildlife that consume whole carcasses.

### Mercury Analysis

Frozen fish samples were shipped on dry ice to the Environment Canada laboratory at the National Wildlife Research Centre (NWRC) in Ottawa, Canada. All muscle tissue analyses were conducted by staff at the NWRC

**Fig. 1** Locations where forage fish samples were collected in: **a** Mexico, 2000–2003 and **b** Nicaragua and Costa Rica, 2007



(Neuberger et al. 2000). For samples collected before 2001, approximately 0.5 g of homogenate was placed in a pre-weighed acid-washed test tube and then freeze-dried until reaching a constant mass. In order to express concentrations in terms of dry or wet weight, dry weights of samples were recorded and moisture content calculated. These dry samples were then placed in plastic, acid-washed tubes, and 0.25 mL of deionized water and 0.5 mL of HNO<sub>3</sub> (70 %) was added. Samples were left overnight and then on the following day, loosely capped and placed in dry heating blocks at 100 °C for 4 h. Following this, samples were diluted to 2.0 mL in deionized water and analyzed by continuous-flow cold vapour atomic absorption spectrophotometry (CVT-AAS, Perkin-Elmer 3030B with VGA-76; see Scheuhammer and Bond 1991).

For fish samples collected from 2001 onward, total Hg concentrations were determined directly using an automated mercury analyzer (AWA-254, Analytical, Burlington, Ontario, Canada). Using a combustion/catalyst tube to macerate the sample, this process removes interfering elements in an oxygen-rich environment. A gold amalgamator then traps any Hg that occurs in the expelled gases; Hg content is determined by a dual-path length cuvette/spectrophotometer. For the average 0.020-g dry mass sample, the effective detection limit was 0.12 ng Hg or 0.006 µg g<sup>-1</sup>. Two methods were deployed to achieve quality control: (1) repeated concurrent analyses of procedural blanks, and (2) certified/in-house standard reference materials. The latter included: National Research Council of Canada (NRCC) dogfish liver (DOLT2), dogfish muscle (DORW-2), mussel tissue (ERW<sup>®</sup>-CE278), and tuna fish (BCR<sup>®</sup>-463). To assess analytical variability, duplicate sampling was performed. Furthermore, method accuracy was assessed through analysis of the concentration of certified reference materials (DOLT-3, TORT-2 from NRRC, and Oyster Tissue 1566b from NIST). To verify the comparability of results following method changes (e.g., before and after 2001), in-house standard reference materials from NWRC (pooled Herring Gull *Larus argentatus* egg samples) that have been repeatedly analyzed for Hg over multiple years were used. All results are reported as µg g<sup>-1</sup> dry weight, except for the wet weight conversions to determine toxicity thresholds. For the latter, we used weight toxicity thresholds for Osprey from Heinz et al. (2009).

### Stable Isotope Analysis

Stable isotope analysis was performed using the same freeze-dried muscle homogenates used for mercury analyses. Detailed methods are described elsewhere (Elliott et al. 2014). Briefly, 1-mg subsamples were freeze-dried, loaded into tin cups and analyzed using a PDZ Europa ANCA-

GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS; Sercon Ltd., Cheshire, UK) at the Stable Isotope Facility, University of California, Davis (<http://www.stableisotopefacility.ucdavis.edu>). Samples were analyzed for <sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N isotopes. During analysis, samples were interspersed with several replicates of at least two different laboratory standards. The final delta values were presented in parts per thousand (‰) relative to international standards Vienna PeeDee Belemnite and Vienna Cañon Diablo Troilite (δ<sup>13</sup>C) and air (δ<sup>15</sup>N), respectively. We accounted for variation in lipid content algebraically following Logan et al. (2008):

$$\delta^{13}C' = \delta^{13}C + \frac{7.415C : N - 22.73}{C : N + 0.746}$$

### Statistical Analysis

Concentrations of contaminants in whole body fish composites were calculated as [(muscle mass x muscle concentration) + (composite carcass mass x composite carcass concentration)]/(sum of fish masses). For samples collected in 2002 ( $n = 8$  pools), individual muscle fillets were not analyzed with the remaining carcasses. Therefore, we used linear regression equations for mercury and methyl-mercury to predict the whole body composite concentrations given only data from the carcasses (Elliott et al. 2007).

We conducted four types of data summary and analysis. First, we averaged total Hg for each fish species to determine whether there were interspecific differences in levels; we also correlated length of individual fish with Hg levels, because generally Hg levels are positively correlated with size in fish (Bank et al. 2007). Second, we graphed these average Hg values and compared them with conservative critical thresholds in forage fish associated with behavioral effects (0.1 µg g<sup>-1</sup> wet weight) and reproductive effects (0.18 µg g<sup>-1</sup> wet weight) in Common Loons (*Gavia immer*) (Depew et al. 2012) to see which species or sample locations exceeded the thresholds recommended. See below for more discussion of threshold values. For these analyses, we converted dry weights to wet weight using the formula Wet Wt = Dry Wt x [1 - (% moisture/100)]. Third, to see if we could detect any geographical patterns in Hg levels in fish, we averaged the Hg levels for each species by sample location and then conducted a nonmetric multidimensional scaling (nMDS) ordination (Bray-Curtis; Clarke and Gorley 2006). We took averages for Hg for each species, because there were multiple specimens from each location and statistically these were not independent (the data matrix was too sparse to calculate similarity matrices based on individual fish). Following nMDS ordination, we tested pairwise differences between fish samples

in different regions using Analysis of Similarity (ANO-SIM). We also conducted a cluster analysis (group linkage) to see whether sites were grouped by geographic location based on their fish species composition and Hg levels in fish.

We used linear mixed models in SAS (Proc. GLIMMIX in SAS Inst. 2000) to model the relationship between total Hg as response variable and various predictors. We used an Information-theoretic (IT) approach (Akaike's Information Criterion, AIC<sub>c</sub> corrected for small sample size; Burnham and Anderson 2002), developing a series of candidate models with different combinations of species, site location,  $\delta^{13}\text{C}$ , and  $\delta^{15}\text{N}$  (Table S1). We included site location and species in models, because Hg and stable isotope values vary according to those parameters.

## Results and Discussion

Mean total Hg levels in whole body composite samples ranged from a high level of  $0.18 \mu\text{g g}^{-1}$  (wet weight) in *Scomberomorus sierra*, a marine fish from Puerto Vallarta, Jalisco State, Mexico to a low of  $0.005 \mu\text{g g}^{-1}$  in *Tilapia Oreochromis niloticus* from freshwater sites in Veracruz State, Mexico (Table 1). Even the greater concentrations reported are not particularly elevated compared with mean Hg in fish collected at many northern locations. For example, in a study of lakes across northeastern North America, Hg concentrations in whole carcass samples of brook trout (*Salvelinus fontinalis*) were  $0.294 \mu\text{g g}^{-1}$  and in yellow perch (*Perca flavescens*) were  $0.290 \mu\text{g g}^{-1}$  (Kamman et al. 2005). Mercury in fish samples from some individual lakes in the northeast of North America were more than an order of magnitude greater than those overall means. Similar or higher mercury levels in fish have been measured from hotspot sites across North America, such as former mercury or gold mines and other industrial activities, or lakes receiving particularly high deposition rates (Weech et al. 2004; Davis et al. 2008; Wyn et al. 2010). In contrast, the limited reports on mercury in fish from Mexico are consistent with our findings reported, with relatively low,  $<0.1 \mu\text{g g}^{-1}$  w. w. concentrations reported in forage fish from sites in Mexico (Ruelas-Inzunza and Páez-Osuna 2005). Other data on mercury in fish from the region has been from mainly coastal and estuarine sites (ibid; García-Hernández et al. 2007). Mercury contamination may be greater around areas of Mexico where gold and silver mining was greatest, given the large quantities of mercury used in such mining in Mexico and elsewhere in Latin America (Nriagu 1994; Malm 1998; Mol et al. 2001). However, those mining regions in Mexico tend to be more arid, and so the biogeochemistry may not only be different, but also there are fewer water bodies that would

**Table 1** Total mercury levels (mean  $\pm$  SD, range for analyses of individual samples) in muscle filets and whole-body samples, and stable isotope ratios in muscle filets for fish collected in Mexico 2000–2003, and Central America, 2007

Country	Region	Species	Diet	Environment	Type	N <sup>a</sup>	% Lipid	% Moisture	Hg $\mu\text{g g}^{-1}$ (wet wt)	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Mexico	Chiapas	Tilapia Sumidero Canyon ( <i>Oreochromis sp.</i> )	Omnivorous; plants, small invertebrates	Freshwater	Muscle	(12)	NA <sup>b</sup>	76.0	0.05	-25.16	10.96
					Whole body	(12)	NA	63.2	0.03	NA	NA
Mexico	Jalisco	Pacific Flagfin ('Mojarra') ( <i>Cichlasoma sp.</i> )	Omnivorous; plants, small invertebrates, fish	Marine	Muscle	4	NA	$76.3 \pm 1.15$	$0.05 \pm 0.005$	-16.07	15.32
					Whole body	(4)	5.95	70.2	0.03	NA	NA
					Mullet ( <i>Mugil sp.</i> )	5	NA	$76.5 \pm 1.08$	$0.02 \pm 0.01$	$-17.18 \pm 0.91$	$15.67 \pm 0.71$
Puerto Vallarta	Pacific Sierra ( <i>Scomberomorus sierra</i> )	Small fish, zoobenthos	Marine; pelagic-neritic	Muscle	3	NA	$75.3 \pm 0.74$	$0.24 \pm 0.13$	-16.05 $\pm$ 0.53	17.80 $\pm$ 0.36	
				Whole body	(2)	6.69	71.2	0.18	NA	NA	

Table 1 continued

Country	Region	Species	Diet	Environment	Type	N <sup>a</sup>	% Lipid	% Moisture	Hg $\mu\text{g g}^{-1}$ (wet wt)	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	
<i>Nayarit</i>												
Rio	Mullet	Omnivorous;	Marine,	Muscle	5	NA	77.5 ± 1.38	0.04 ± 0.02	-19.75 ± 2.15	10.52 ± 1.42		
Santiago, St. Ixcuintla	( <i>Mugil</i> sp.)	plants, small invertebrates, fish	freshwater, brackish	Whole body	(4)	2.28	68.8	0.05	NA	NA		
San Blas Estuary	Sea Catfish species ( <i>Arius</i> sp.)	Omnivorous; plants, small invertebrates, fish	Marine	Muscle	5	NA	77.7 ± 1.19	0.20 ± 0.06	-16.32 ± 0.20	15.8 ± 0.17		
				Carcass	(5)	9.12	63.8	0.18				
	Blue Tang Surgeonfish ('Tang')	Zooplankton, algae	Marine	Muscle	3	11.81	77.4 ± 0.58	0.12 ± 0.06	-17.42 ± 0.40	15.22 ± 0.24		
	( <i>Acantharus coeruleus</i> )			Whole body	(3)		63.1	0.10	NA	NA		
<i>Tecuala Market</i>	Mullet ( <i>Mugil</i> sp.)	Omnivorous; plants, small invertebrates, fish	Marine, brackish	Muscle	5	NA	79.4 ± 3.31	0.03 ± 0.006	-19.65 ± 0.93	12.34 ± 1.35		
				Whole body	(5)	3.33	72.4	0.03	NA	NA		
	Snook ( <i>Centropomus</i> sp.)	Benthic crustaceans, small fish	Marine, brackish	Muscle	5	NA	77.4 ± 0.82	0.30 ± 0.14	-18.03 ± 1.42	15.69 ± 0.84		
<i>Oaxaca</i>				Whole body	(5)	4.49	67.3	0.15	NA	NA		
Playa Vicente	Catfish ( <i>Rhamdia</i> sp.)	Omnivorous; plants, small invertebrates, fish	Freshwater, brackish	Muscle	5	2.00 ± 0.45	76.5 ± 0.42	0.16 ± 0.01	-20.04	14.70		
				Whole body	(5)	11.43	NA	<b>0.08 ± 0.005</b>	± 0.67	± 0.46		
									NA	NA		
Region of Jalapa del Marquez	Nile Tilapia ( <i>Oreochromis niloticus</i> )	Omnivorous; plants, small invertebrates	Freshwater	Muscle	4	0.54 ± 0.22	76.08 ± 1.63	0.02 ± 0.007	-27.45 ± 1.38	8.68 ± 1.50		
<i>Tabasco</i>				Whole body	(4)	NA	NA	<b>0.01 ± 0.004</b>	NA	NA		
Villahermosa	Hardhead Sea Catfish ( <i>Arius felis</i> )	Omnivorous; plants, small invertebrates, fish	Freshwater	Muscle	3	0.29 ± 0.19	77.4 ± 2.30	0.15 ± 0.14	-27.66 ± 0.58	12.35 ± 0.26		
				Whole body	(5)	8.17	76.1 ± 2.40	0.14 ± 0.09	NA	NA		
	Catfish ( <i>Rhamdia</i> sp.)	Omnivorous; plants, small invertebrates, fish	Freshwater	Muscle	2	NA	74.3 ± 1.01	0.09 ± 0.01	-31.20 ± 1.00	12.66 ± 0.14		
				Whole body	(2)	NA	NA	<b>0.04 ± 0.007</b>	NA	NA		
	Bigmouth Sleeper ( <i>Gobiomorus dormitor</i> )	Benthos, small fishes	Freshwater	Muscle	5	NA	78.4 ± 0.79	0.03 ± 0.006	-30.08 ± 0.29	9.68 ± 0.45		
				Whole body	(5)	7.95	NA	<b>0.01 ± 0.003</b>	NA	NA		

Table 1 continued

Country	Region	Species	Diet	Environment	Type	N <sup>a</sup>	% Lipid	% Moisture	Hg $\mu\text{g g}^{-1}$ (wet wt)	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Veracruz	Catemaco	Mayan Cichlid (Mexican Mojarra) ( <i>Cichlasoma</i> <i>urophthalmus</i> )	Benthos, small fishes	Freshwater	Muscle	5	0.97 ± 0.45	77.4 ± 0.88	0.04 ± 0.026	-30.31 ± 0.91	9.06 ± 0.92
					Whole body	(5)	NA	na	<b>0.02 ± 0.014</b>	NA	NA
Laguna Alvarado	Silver Mullet ( <i>Mugil curema</i> )	Omnivorous; plants, small invertebrates, fish	Freshwater, brackish	Muscle	2	0.66 ± 0.23	77.7 ± 0.43	0.02 ± 0.01	-22.30 ± 0.72	9.97 ± 0.96	
				Whole body	(2)	NA	NA	<b>0.01 ± 0.005</b>	NA	NA	
Laguna Alvarado	Nile Tilapia ( <i>Oreochromis</i> <i>niloticus</i> )	Omnivorous; plants, small invertebrates	Freshwater, brackish	Muscle	2	0.35 ± 0.29	79.3 ± 2.10	0.01 ± 0.007	-25.62 ± 1.48	8.13 ± 3.02	
				Whole body	(2)	7.1	NA	<b>0.005 ± 0.004</b>	NA	NA	
Laguna Alvarado	Hardhead Sea Catfish ( <i>Arius felis</i> )	Omnivorous; plants, small invertebrates, fish	Marine, brackish	Muscle	(4)	2.57	77.2	0.27	-22.59	12.20	
				Whole body	(4)	8.58	NA	<b>0.15</b>	NA	NA	
Minatitlan	Silver Mullet ( <i>Mugil curema</i> )	Omnivorous; plants, small invertebrates, fish	Marine, freshwater, brackish	Muscle	(4)	1.20	74.3	0.03	-22.42	11.56	
				Whole body	(4)	6.79	66.64	<b>0.02</b>	NA	NA	
Minatitlan	Fat Snook ( <i>Centropomus</i> <i>parallelus</i> )	Benthic crustaceans, small fish	Marine, freshwater, brackish	Muscle	(4)	2.02	74.0	0.18	-25.34	14.56	
				Whole body	(4)	12.9	62.75	<b>0.09</b>	NA	NA	
Minatitlan	Nile Tilapia ( <i>Oreochromis</i> <i>niloticus</i> )	Omnivorous; plants, small invertebrates	Freshwater	Muscle	(4)	0.35	65.5	0.02	-30.94	7.21	
				Whole body	(4)	8.91	68.66	<b>0.005</b>	NA	NA	
Minatitlan	White Mullet ( <i>Mugil curema</i> )	Omnivorous; plants, small invertebrates, fish	Freshwater, brackish	Muscle	7	0.22 (0.08 –	77.66 ± 0.51	0.14 ± 0.08	-25.34 ± 3.65	8.74 ± 0.82	
				Whole body	(5)	0.45) 12.58	65.63	<b>0.07 ± 0.04</b>	NA	NA	
Palo Blanco	Fat Snook ( <i>Centropomus</i> <i>parallellus</i> )	Fish and crustacean	Freshwater, brackish	Muscle	5	NA	76.0 ± 0.52	0.29 ± 0.11	-24.24 ± 1.30	11.59 ± 0.31	
				Whole body	(5)	8.52	65.39	<b>0.15 ± 0.06</b>	NA	NA	
Palo Blanco	Mexican Mojarra ( <i>Cichlasoma</i> <i>urophthalmus</i> )	Benthos, small fishes	Freshwater, brackish	Muscle	5	0.26 ± 0.11	78.15 ± 1.21	0.10 ± 0.07	-25.71 ± 0.79	7.38 ± 0.27	
				Whole body	(5)	8.47	66.52	<b>0.05 ± 0.04</b>	NA	NA	
Palo Blanco	Bigmouth Sleeper ( <i>Gobiomorus dormitor</i> )	Benthos, small fishes	Freshwater, brackish	Muscle	3	NA	78.2 ± 0.28	0.11 ± 0.02	-25.17 ± 0.43	8.43 ± 0.11	
				Whole body	(3)	6.86	NA	<b>0.06 ± 0.007</b>	NA	NA	

Table 1 continued

Country	Region	Species	Diet	Environment	Type	N <sup>a</sup>	% Lipid	% Moisture	Hg $\mu\text{g g}^{-1}$ (wet wt)	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Costa Rica	Paso de Salta Barranca	Hardhead Sea Catfish ( <i>Arius felis</i> )	Omnivorous; plants, small invertebrates, fish	Marine, brackish	Muscle	4	3.31 ± 1.98	75.17 ± 3.48	0.14 ± 0.08	-25.85	11.43 ± 0.15
					Whole body	(4)	13.74	NA	<b>0.07 ± 0.04</b>	± 0.78	NA
	Playa Martinez	Mexican Mojarra ( <i>Cichlasoma urophthalmus</i> )	Benthos, small fishes	Freshwater, brackish	Muscle	5	1.26 ± 1.06	78.0 ± 0.02	0.11 ± 0.06	-25.22 ± 0.65	10.49 ± 0.98
					Whole body	(5)	14.25	77.7 ± 1.45	0.14 ± 0.05	NA	NA
	Playa Martinez	Bigmouth Sleeper ( <i>Gobionorus dormitor</i> )	Benthos, small fishes	Freshwater, brackish	Muscle	2	0.15 ± 0.07	77.5 ± 0.00	0.12 ± 0.02	-25.01 ± 0.17	8.32 ± 0.05
					Whole body	(2)			<b>0.06 ± 0.009</b>		
	Tlacotalpan	Fat Snook ( <i>Centropomus parallelus</i> )	Fish and crustacean	Freshwater, brackish	Muscle	2	5.68 ± 1.86	72.4 ± 1.33	0.16 ± 0.04	-26.33 ± 1.74	10.43 ± 0.56
					Whole body	(2)			<b>0.08 ± 0.02</b>		
	Playa Cabuyal	Bigmouth Sleeper ( <i>Gobionorus dormitor</i> )	Benthos, small fishes	Freshwater, brackish	Muscle	2	0.20 ± 0.00	77.25 ± 0.37	0.12 ± 0.001	-25.25 ± 0.17	8.19 ± 0.27
					Whole body	(2)			<b>0.06 ± 0.006</b>		
	Arenal Lake	Fat Snook ( <i>Centropomus parallelus</i> )	Fish and crustacean	Freshwater, brackish	Muscle	2	1.45 ± 1.97	77.42 ± 2.56	0.13 ± 0.02	-23.39 ± 0.69	14.07 ± 3.76
					Whole body	(2)			<b>0.07 ± 0.01</b>		
Mojarra Bonita	Machacha ( <i>Brycon guatemalensis</i> )	Benthos, small fishes	Freshwater	Muscle	1	NA	73.7	0.20	-23.42	8.41	
				Whole body	1	NA	58.2	0.12	NA	NA	
Playa Del Coco (Processi)	Mayan Cichlid ( <i>Cichlasoma urophthalmus</i> )	Benthos, small fishes	Freshwater	Muscle	(4)	NA	80.4	0.16	-25.21	10.10	
				Whole body	(4)	NA	61.0	0.08	NA	NA	
Playa Cabuyal	Sucker sp. F. Catostomidae	Omnivorous, invertebrates, algae, detritus	Marine, brackish	Muscle	(5)	NA	74.5	0.06	-16.63	15.46	
				Whole body	(5)	NA	70.3	0.05	NA	NA	
Playa Del Coco (Processi)	Red Snapper ( <i>Lutjanus campechanus</i> )	Benthos, small fishes	Marine	Muscle	1	NA	73.4	0.01	-13.63	13.81	
				Whole body	1	NA	67.9	0.01	NA	NA	
Playa Del Coco (Processi)	Red Snapper ( <i>Lutjanus campechanus</i> )	Benthos, small fishes	Marine	Muscle	(3)	NA	80.1	0.03	-15.96	15.26	
				Whole body	(3)	NA	74.6	0.02	NA	NA	



Table 1 continued

Country	Region	Species	Diet	Environment	Type	N <sup>a</sup>	% Lipid	% Moisture	Hg $\mu\text{g g}^{-1}$ (wet wt)	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Nicaragua	<i>Chinandega</i>										
	Coronito	Sea Catfish ( <i>Arius</i> sp.)	Omnivorous; plants, small invertebrates, fish	Marine, brackish	Muscle	(5)	NA	78.9	0.11	-15.26	15.88
					Whole body	(5)	NA	75.5	0.08	NA	NA
	Jiquillo— El Viejo Mercado	Sea Catfish ( <i>Arius</i> sp.)	Omnivorous; plants, small invertebrates, fish	Marine, brackish	Muscle	(5)	NA	78.1	0.12	-16.85	15.41
					Whole body	(5)	NA	72.7	0.08	NA	NA
	Puerto Morazan Estuary	Sea Catfish ( <i>Arius</i> sp.)	Omnivorous; plants, small invertebrates, fish	Marine, brackish	Muscle	(5)	NA	76.5	0.17	-22.25	10.57
					Whole body	(5)	NA	72.3	0.12	NA	NA
		Fat Snook ( <i>Centropomus parallelus</i> )	Benthos, small fishes	Marine, brackish	Muscle	(5)	NA	78.0	0.12	-20.85	8.74
					Whole body	(5)	NA	73.5	0.09	NA	NA
		Tilapia ( <i>Oreochromis</i> sp.)	Omnivorous; plants, small invertebrates, fish	Marine, brackish	Muscle	(5)	NA	78.4	0.03	-24.42	5.05
					Whole body	(5)	NA	70.5	0.03	NA	NA
	<i>Managua</i>										
	Mateare, Managua Lake	Tilapia ( <i>Oreochromis</i> sp.)	Omnivorous; plants, small invertebrates, fish	Freshwater	Muscle	(5)	NA	78.8	0.06	-23.20	11.28
					Whole body	(5)	NA	69.2	0.04	NA	NA
	<i>Rio San Juan</i>										
	Lake Nicaragua	Mexican Mojarra ( <i>Cichlasoma urophthalmus</i> )	Benthos, small fishes	Freshwater	Muscle	(7)	NA	80.5	0.04	-24.25	11.61
					Whole body	(7)	NA	69.7	0.03	NA	NA

Sample size (N) within brackets represents data from muscle or carcass composite (pooled) samples. Whole-body Hg levels in bold represent values calculated from a regression

<sup>a</sup> Sample size

<sup>b</sup> Not analyzed

attract wintering osprey and other water birds compared to the coastal wetlands. Thus, fish-eating birds may be present in smaller numbers.

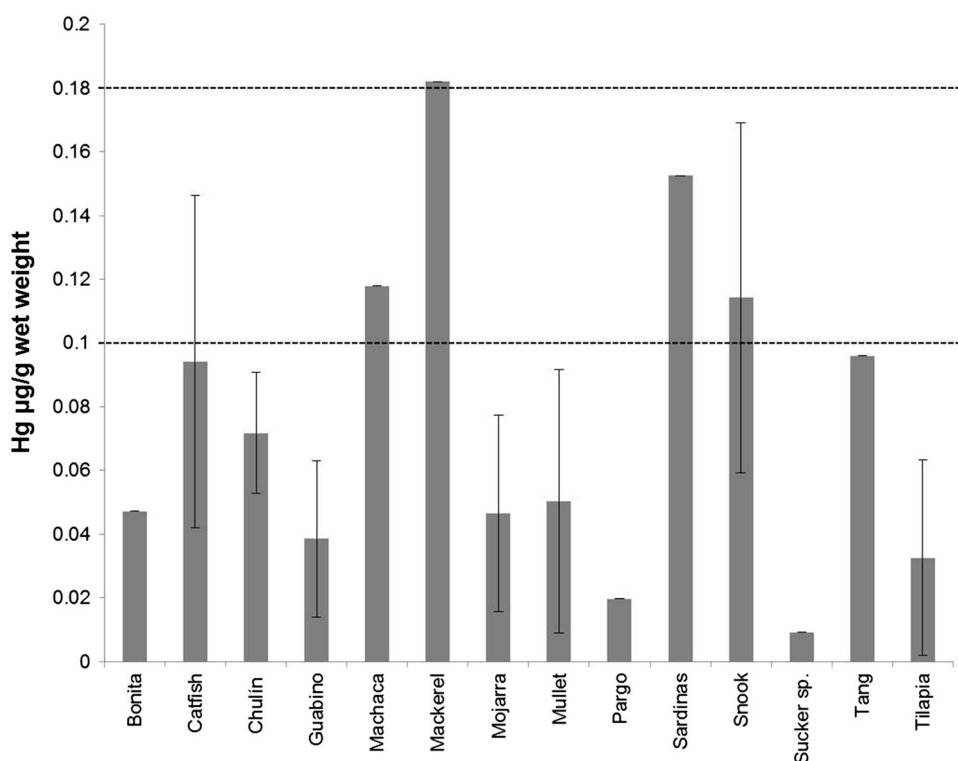
Mean Hg levels (converted to wet weight) exceeded the published conservative toxicity threshold range in forage fish ( $0.10\text{--}0.18\ \mu\text{g g}^{-1}$ ) for four (28.6 %) taxa (*Scomberomorus sierra*, *Centropomus* spp., *Brycon guatemalensis*, *Centropomus paralellus*), whereas the threshold was within one standard deviation for some of these taxa, as well as *Ariopsis felis* (Fig. 2). Geographically, none of the fish from Central American sites had Hg levels that exceeded the toxicity threshold, whereas average Hg levels were above the toxicity threshold at two sites in Mexico (Puerto Vallarta and San Blas Estuary) or were within one standard deviation for five (21 %) sites (Arenal Lake, Laguna Alvarado, Minatitlan, Puerto Morazan Estuary, and Tecuala Market; Fig. 3). The mercury threshold value of  $0.1\ \mu\text{g g}^{-1}$  w.w. in forage fish is a screening value for risk assessment and is based on field studies of common loons. It is the midpoint ( $0.05\text{--}0.15\ \mu\text{g g}^{-1}$  w.w.) of fish Hg values associated with altered behaviours. Although behavioural changes have been found to be sensitive endpoints for other contaminants (Harris and Elliott 2011), they also are difficult to evaluate and control for rigorously, particularly in the field. The reproductive impairment threshold of  $0.18\ \mu\text{g g}^{-1}$  w.w. also is a screening value and is lower than the  $0.25\ \mu\text{g g}^{-1}$

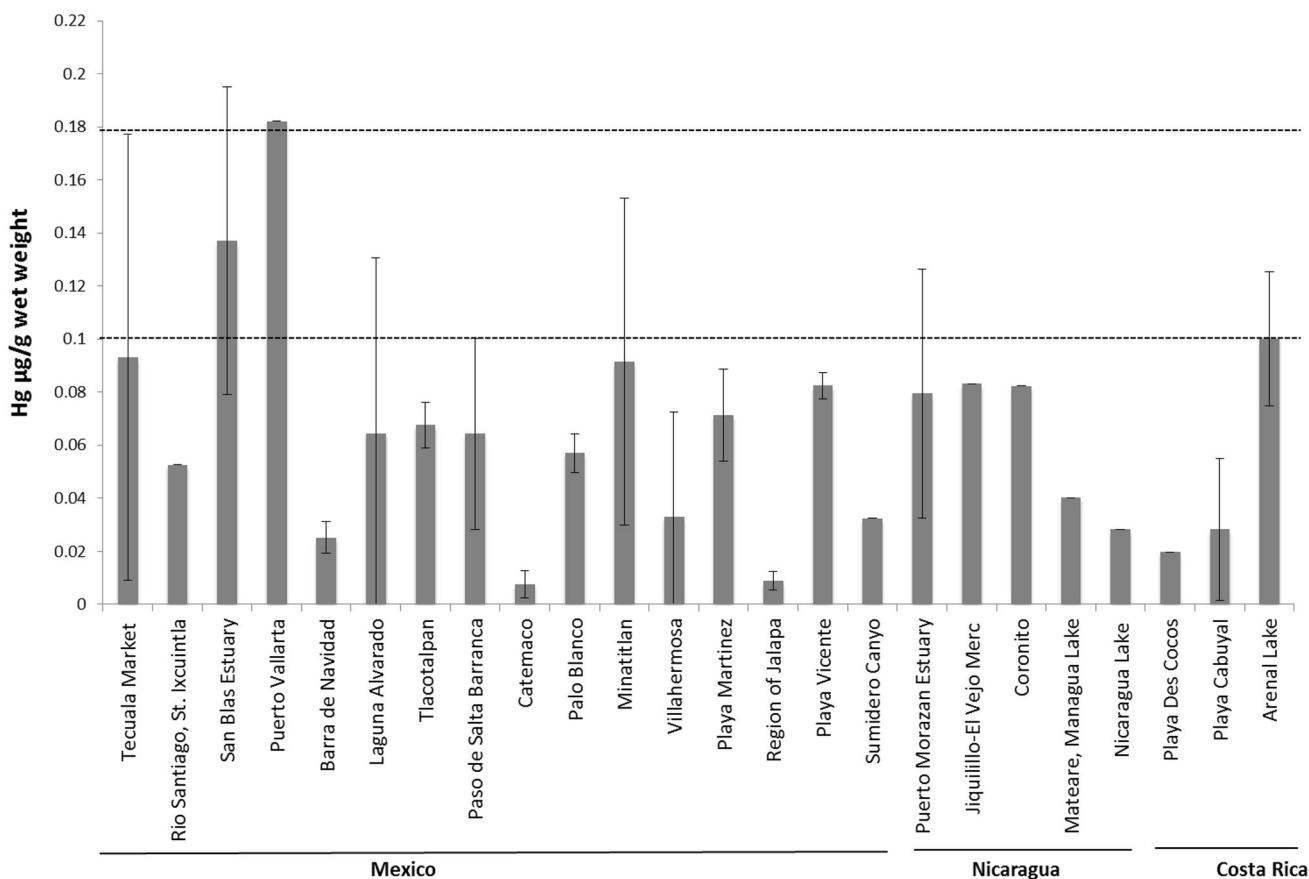
w.w. conservative screening value in an avian diet proposed by Shore et al. (2011). There is little in the way of comparative toxicity data for mercury in fish eating birds. Our original study species, the osprey, was found to be among the more mercury sensitive species based on egg injection experiments (Heinz et al. 2009). However, nest success of osprey populations was within the normal range at a number of sites with greater mercury concentrations in forage fish than the mean values that we report in the present study (Desgranges et al. 1998; Anderson et al. 2008).

Nitrogen stable isotopes ( $\delta^{15}\text{N}$ ) were positively correlated with carbon stable isotopes ( $\delta^{13}\text{C}$ , lipid-corrected) across habitats ( $t_{106} = 9.83$ ;  $P < 0.00001$ ;  $R^2 = 0.48$ , Fig. 4a), as marine habitats (enriched in  $^{13}\text{C}$ ) usually have longer food chains and therefore are more enriched in  $\delta^{15}\text{N}$ . They were similarly correlated within the estuarine habitat ( $t_{22} = 3.48$ ,  $P = 0.002$ ;  $R^2 = 0.33$ ), but not in either the freshwater or marine habitat (all  $P > 0.05$ ). Carbon stable isotope ratios were good signatures for habitat, with  $\delta^{13}\text{C}$  usually  $< -22$  in freshwater and  $> -17$  in marine habitats, with estuarine fish largely in between.

Hg was not correlated with  $\delta^{13}\text{C}$  across all habitats combined or within any of the three habitats (all  $P > 0.05$ ). Hg was correlated with  $\delta^{15}\text{N}$  in freshwater ( $t_{68} = 3.21$ ,  $P = 0.002$ , Fig. 4b) and marine ( $t_{13} = 3.10$ ,  $P = 0.009$ , Fig. 4b), but not estuarine ( $P > 0.05$ ) habitats. Thus, fish

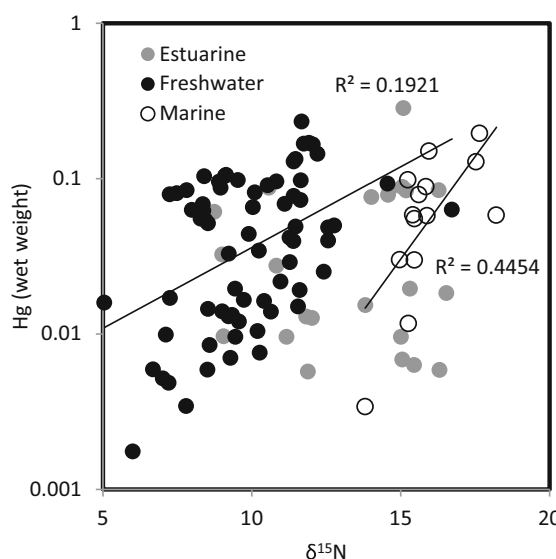
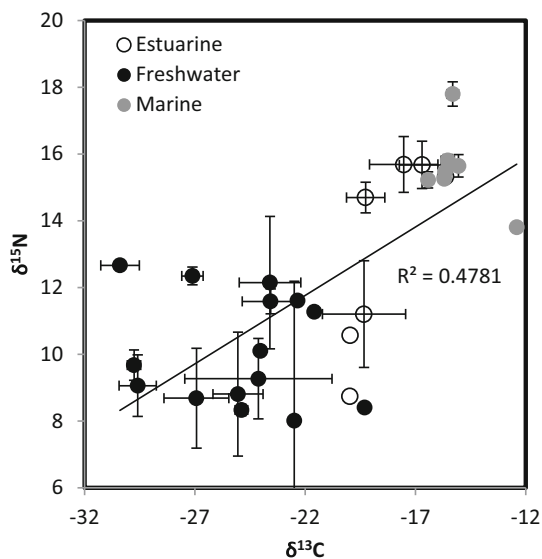
**Fig. 2** Average (SD as error bar where available) Hg levels ( $\mu\text{g g}^{-1}$ /wet weight) grouped by overall species means for forage fish samples collected 2000–2003. Samples are a mix of composites and calculated composite values from a mix of single composite values and means from individual muscle values. Dotted horizontal lines show putative toxicity thresholds for avian consumers: the first ( $0.1\ \mu\text{g g}^{-1}$ ) is the threshold for adverse behavioural impacts in adult Common Loons (*Gavia immer*). The  $0.18\ \mu\text{g g}^{-1}$  correspond to MeHg levels in prey fish associated with reproductive impairment in wild adult loons (Depew et al. 2012)





**Fig. 3** Average (SD as error bar where available) Hg levels ( $\mu\text{g g}^{-1}$ /wet weight) for forage fish collected from Mexico (2000–2003) and Central American fish (2007), by location. Samples are a mix of composites and calculated composite values from a mix of single composite values and means from individual muscle values. Dotted

lines show toxicity thresholds: the first ( $0.1 \mu\text{g g}^{-1}$ ) is the threshold for adverse behavioural impacts in adult Common Loons (*Gavia immer*). The  $0.18 \mu\text{g g}^{-1}$  correspond to MeHg levels in prey fish associated with a reproductive impairment in wild adult loons (Depew et al. 2012)



**Fig. 4 a** Values for  $\delta^{15}\text{N}$  plotted against  $\delta^{13}\text{C}$  in forage fish from Mexico, 2000–2003 and Central America, 2007; **b** concentrations of mercury plotted against  $\delta^{15}\text{N}$  in forage fish from Mexico, 2000–2003

and Central America, 2007. Regression lines are fitted for freshwater and marine fish, and were not significant for estuarine species

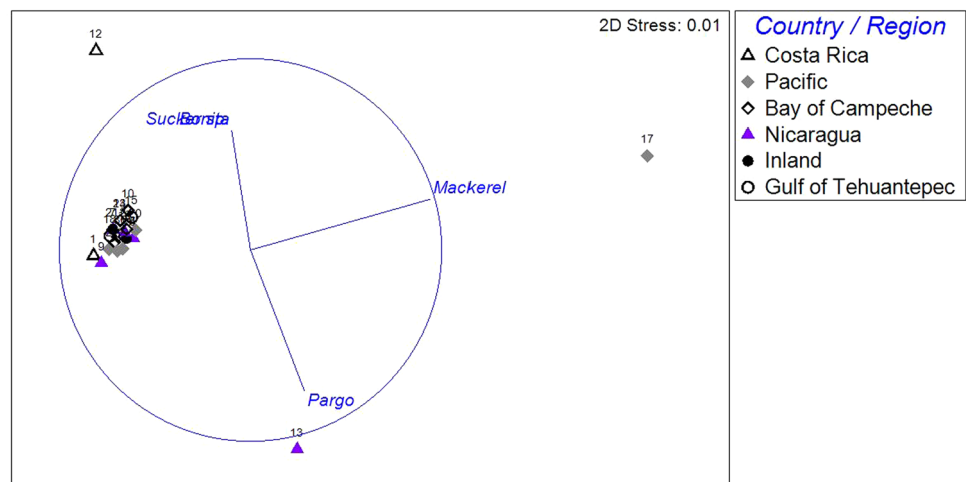
feeding at higher trophic levels had higher Hg. In the estuarine environment, anthropogenic inputs, movement between fresh and saltwater, and habitat differences may have altered the  $\delta^{15}\text{N}$  levels at the base of the food web, making  $\delta^{15}\text{N}$  a poor indicator of trophic level. Indeed, similar processes among different lakes and river systems may explain why the  $R^2$  value was also lower for freshwater than marine systems. In the marine environment, larger predatory fish, such as mackerel (*Scomberomorus sierra*) had both high  $\delta^{15}\text{N}$  ( $\sim 18$ ) and Hg ( $\sim 0.07 \mu\text{g g}^{-1}$ ), whereas other fish, such as northern red snapper (*Lutjanus* sp.) had both lower  $\delta^{15}\text{N}$  ( $\sim 15$ ) and Hg ( $\sim 0.01 \mu\text{g g}^{-1}$ ). When averaged across species in the marine environment, variation in  $\delta^{15}\text{N}$  explained 60 % of the variation in Hg. In contrast, Hg levels were highly variable in brackish, estuarine water. Some apparently more marine, higher trophic level species, such as mullet, mojarra, and sardines, varied between 0.01 and 0.18  $\mu\text{g g}^{-1}$  Hg, whereas more freshwater, lower trophic level species, such as catfish, snook, *Mugil* sp., and *Rhamdia* sp., likewise varied between 0.02 and 0.09  $\mu\text{g g}^{-1}$ . Within freshwater systems, the highest levels of Hg (0.08  $\mu\text{g g}^{-1}$ ) were from catfish (*Arius felles*) with relatively high trophic level ( $\delta^{15}\text{N} \sim 12.4$ ), whereas the lowest levels were in tilapia (*Oreochromis niloticus*) in Oaxaca and Chiapas, tilapia (*Chichlasoma urophthalmus*) in Tabasco, and guabino (*Gobiomorus dormitor*) in Tabasco with Hg  $\sim 0.01\text{--}0.02 \mu\text{g/g}$  and  $\delta^{15}\text{N} \sim 8\text{--}9$ . However, levels were quite variable. For instance, the guabino in Veracruz had Hg  $\sim 0.06$ , although they had  $\delta^{15}\text{N}$  of 8.3.

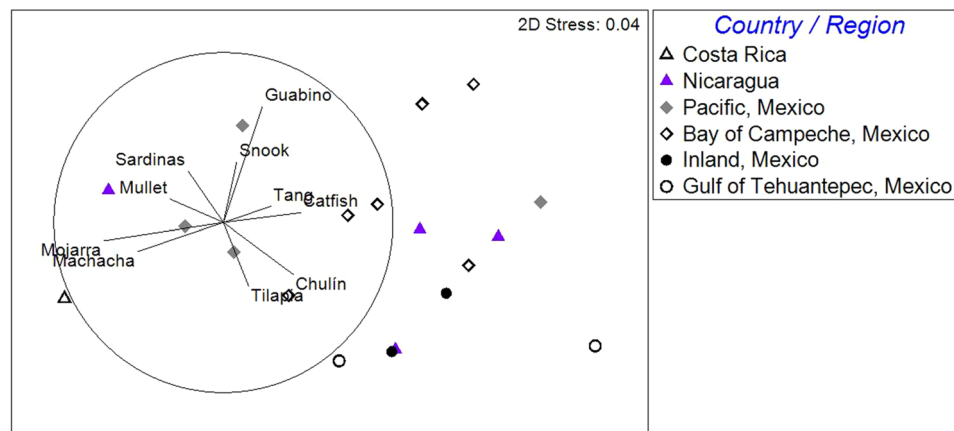
According to nMDS ordination there were some geographic patterns in fish species and Hg concentrations (Figs. 5, 6). However, the initial nMDS (Fig. 5) had three strong outliers—Playa Cabuyal (Costa Rica), Playa del Coco (Processi, Nicaragua) and Puerto Vallarta (Mexico). We removed these outliers in a second ordination (Fig. 6) to enable us to better determine patterns in species

composition and Hg levels. On axis 1 of the ordination, sites from inland, the Gulf of Tehautepec, the Bay of Campeche, and most sites from Nicaragua were on the right side of the ordination. Most sites from the Pacific were on the centre of the ordination, and the one site from Costa Rica on the left. Overall the ANOSIM demonstrated significant differences among areas based on fish species distributions and mercury levels (global  $R = 0.294$ ,  $P = 0.002$ ). Significant pairwise differences occurred between fish species composition and total Hg between the Pacific Coast of Mexico and Bay of Campeche ( $R = 0.36$ ,  $P = 0.024$ ), and marginally significant differences between the Bay of Campeche and the Gulf of Tehautepec ( $R = 0.429$ ,  $P = 0.056$ ), between the Bay of Campeche and Nicaragua ( $R = 0.23$ ,  $P = 0.067$ ), between the Pacific and Inland ( $R = 0.482$ ,  $P = 0.067$ ) and between the Pacific and Nicaragua ( $R = 0.2$ ,  $P = 0.095$ ). We identified seven groups at 35 % similarity from the cluster analysis; there was considerable mixing of geographic locations (Figs. S1 and S2). The linear mixed model indicated that species and site location had important influences on total Hg; however, the best model included site and  $\delta^{15}\text{N}$  (Table 2).

There is potential variability around the precise locations of fish samples from small local markets. However, those retailers invariably stated that the small forage species were very local in origin. Thus, we can assume with some confidence that the geographical comparison we conducted does reflect real spatial differences. There was, however, some confounding of the ordination analyses by differences in fish species distributions. Substantial variation in Hg concentrations can occur in marine fish which can be influenced by factors such as point source pollution, various abiotic and biotic factors that influence MeHg production, as well as diet and local prey dynamics (Bank et al. 2007).

**Fig. 5** Nonmetric multidimensional scaling ordination of mercury concentrations in forage fish for all 24 sites in Mexico, 2000–2003, and Central America, 2007





**Fig. 6** Nonmetric multidimensional scaling ordination of mercury concentrations in forage fish from Mexico and Central America, with outliers removed. Site codes are (all sites in Mexico, unless stated otherwise): 1 = Arenal Lake (Costa Rica), 2 = Barra de Navidad, 3 = Catemaco, 4 = Coronito (Nicaragua), 5 = Jiquilillo-El Vejo Merc (Nicaragua), 6 = Laguna Alvarado, 7 = Mateare, Managua Lake (Nicaragua), 8 = Minatitlan, 9 = Nicaragua Lake (Nicaragua), 10 = Palo Blanco, 11 = Paso de Salta Barranca, 12 = Playa Cabuyal, 13 = Playa del Coco, 14 = Playa Martinez, 15 = Playa

Vicente, 16 = Puerto Morazan Estuary, Nicaragua, 17 = Puerto Vallarta, 18 = Region of Jalapa del Marquez, 19 = Rio Santiago, St. Ixcuintla, 20 = San Blas Estuary, 21 = Sumidero Canyon, 22 = Tecuala Market, 23 = Tlacotalpan, 24 = Villahermosa. Species acronyms are: Guabino = *Gobiomorus dormitor*, Snook = *Centropomus parallelus*, Tang = *Acantharus coeruleus*, Catfish = *Ariopsis felis*, Chulin = *Rhamdia* sp., Tilapia = *Oreochromis niloticus*, Mojarra = *Cichlasoma urophthalmus*, Machacha = *Brycon guatemalensis*, Mullet = *Mugil* sp., Sardinas = *Centropomus* sp.

**Table 2** Modelling results for linear mixed models with Hg as dependent variable and various candidate models for site, species, and stable isotopes ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ). Only models with  $\Delta\text{AIC} \leq 2.0$  are included

Model parameters	$\Delta\text{AIC}$	$\chi^2$	df	$P$
Site	0.00	38.18	9	<0.0001
$\delta^{15}\text{N}$		22.47	1	<0.0001

The mercury concentrations in forage fish measured in this study do not account for the high mercury concentrations reported in many osprey populations on the breeding grounds (Hughes et al. 1997; Desgranges et al. 1998; Elliott et al. 2000; Hopkins et al. 2007; Anderson et al. 2008; Henny et al. 2009; Guigueno et al. 2012). Consistent with our previous study of persistent organic pollutants in western migratory populations of osprey, primary sources of contamination appear to be in the north. That is consistent with the much greater industrial development of the landscapes, particularly in the United States and the continuing legacy of contaminated sites, such as former mercury and gold mines in both the United States and Canada (Henny et al. 2002; Weech et al. 2004, 2006; Anderson et al. 2008). Our results do not preclude the possibility of other Hg contaminated hotspots in Mexico and Central America. The situation also may be different for ospreys breeding in eastern North America, many of which winter in South America, including the Amazon basin (Martell et al. 2014).

Sources of Hg contamination in Mexico and Central America have not been well researched, and this paper provides further baseline information for the region. Of interest, we found that Hg levels were above the conservative screening level toxicity thresholds for some fish species some sampling locations, but the values are not really cause for concern. The limited published data highlights the need for further surveys of diet of apex predators in their overwintering areas.

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