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Ecologically-framed Mercury Database, Exposure Modeling and Risk/Benefit Communication to Lower Chesapeake Bay Fish Consumers

Xiaoyu Xu
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Ecologically - framed Mercury Database, Exposure Modeling and Risk/Benefit Communication to Lower Chesapeake Bay Fish Consumers

A Dissertation
Presented to
The Faculty of the School of Marine Science
The College of William and Mary in Virginia

In Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy

by

Xiaoyu Xu

Dec 2012
APPROVAL SHEET

This dissertation is submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

Xiaoyu Xu

Approved, by the Committee, December 2012

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Robert Hale, Ph.D.

Jian Shen, Ph.D.

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ABSTRACT

Mercury concentrations and determinants of mercury accumulation were examined for ten finfish species from the lower Chesapeake Bay and its tributaries. None of the sampled fish had total mercury concentrations approaching the U.S. EPA human health screening value. Mercury concentrations in different fish species generally increased with increasing δ^{15}N, but not δ^{13}C, suggesting that trophic position, but not dietary carbon source was a dominant determinant. A methylmercury biomagnification model was built to estimate a food web magnification factor of approximately 10-fold increase per trophic level in Chesapeake Bay. Based on otolith strontium-calcium ratios, Atlantic croaker inhabiting less saline waters might accumulate more mercury than those inhabiting more saline waters. Positive intraspecies relationships between methylmercury concentration and δ^{13}C were identified for summer flounder, weakfish, American eel, Atlantic croaker, and spot.

Fish consumption and associated mercury exposure were explored for two ethnic (Chinese and Vietnamese) church communities along coastal Virginia, as well as two general population (non-Asian) churches in this region. Individual seafood consumption rates for the ethnic communities were higher than the general U.S. fish consumption rate of 12.8 g/person/day. People from the general population churches and Chinese church took in most of their mercury from market fish (distributed and sold nationally) whereas people from the Vietnamese church took in mercury from both the market and local fish as they tended to eat a large amount of diverse local species.

Hair mercury concentrations in the Chinese and the Vietnamese church were higher than the overall level for U.S. women (0.20 µg/g), but lower than the published WHO exposure threshold of 14 µg/g. Regression between seafood consumption rates and hair mercury concentrations suggested that dietary mercury ingestion through seafood was positively related to mercury exposure. Mercury exposure of the Vietnamese community was higher compared to the Chinese community, which itself was higher than the general church communities. Regardless, the daily methylmercury intake rates for all studied communities were lower than the U.S. EPA Reference Dose of 0.1 µg/kg BW-day.

Keywords:
Mercury, methylmercury, trophic ecology, biomagnification, fish, Chesapeake Bay, exposure assessment, seafood consumption, Chinese, Vietnamese
Ecologically-framed Mercury Database, Exposure Modeling and Risk/Benefit Communication to Lower Chesapeake Bay Fish Consumers
INTRODUCTION
Concern about mercury as a major environmental contaminant has increased in recent years due to its increasing deposition from anthropogenic sources, global atmospheric dispersion, propensity to magnify after methylation, and high toxicity to wildlife and humans.

**Mercury as a Global Concern**

Mercury, as an element existing in the earth's crust, can be emitted from a range of natural sources including volcanoes, deep sea vents, fossil fuels such as coal and petroleum, mercury-rich geological zones, as well as soils, freshwaters and oceans, plants, forest fires, sea salt spray and meteoric dust (Lindqvist and Rodlhe 1985, Schierow 2006). With the Industrial Revolution, anthropogenic mercury gradually increased to account for the larger portion of mercury deposition. Roughly three to five times more mercury is mobilized today relative to 200 years ago (Lamborg et al. 2002, Manohar et al. 2002). One recent study even argued that the current atmospheric mercury deposition in the biosphere is at least ten times higher than it was before global industrialization (Bindler 2003). Among the major sources are a variety of industrial and combustion processes, including metal mining and smelting, incineration of municipal and medical wastes, coal-fired power plants and certain industrial processes such as cement manufacturing, chlorine production, paper industry, mineral ores processing, steel manufacturing, petroleum refining, and fossil fuel combustion (Schierow 2006, Manohar et al. 2002). In addition, agricultural and urban activities contribute to mercury releases. Pesticides and fungicides with high mercury concentrations were widely used in agriculture for a long period (Smart 1968, Wang et al. 2004).
The capacity for long distance atmospheric transport makes mercury a global concern. Mercury falls to the earth in dust, rain, and snow, meanwhile, mercury recycles from the oceans, leaves of plants, and other surfaces back into the air (Mason et al. 1994). Approximately half the mercury released into the air is then deposited locally (Mason et al. 1994, Jackson 1997). The rest is entrained by general atmospheric circulation and transported miles away before being returned to terrestrial or aquatic systems. The spatial distribution of atmospheric mercury depends largely on wind direction (Davies and Notcutt 1996; Pirrone et al. 1996), but is also a function of other factors that could affect the speciation, partitioning, deposition, and reemission of the mercury (Jackson 1997).

**In situ Methylation and Bioaccumulation**

Most methylmercury in coastal marine systems results from bacterial methylation in sediments (Hammerschmidt and Fitzgerald 2006). Deposited inorganic mercury in the aquatic system is converted to methylmercury by the active communities of sulfate reducing bacteria in anoxic sediments (Compeau and Bartha 1985). The methylation capacity of an aquatic system is influenced by concentrations of sulfate (and/or sulfide) and organic content in sediment (Benoit et al. 1999). Although elevated sulfate concentrations in water will enhance sulfate reduction during the process of *in situ* methylation, buildup of sulfide can limit the process (Benoit et al. 1999). In addition, high organic content is likely to inhibit mercury accumulation from media. In Mason and Sheu's study (2002), methylmercury availability to accumulate in food webs appeared to be a function of organic content, with higher organic content in sediment or water resulting in relatively lower mercury accumulation to the trophic web. This is why highly
contaminated environments may not have elevated methylmercury concentrations in fish, that is, highly contaminated and generally eutrophic systems have higher concentrations of sulfide and organic content (Mason and Sheu 2002).

Once methylmercury enters the food web, it will be efficiently accumulated and then magnified to organisms at higher trophic levels (Mason and Sheu 2002). In aquatic systems, methylmercury and inorganic mercury are both concentrated by unicellular organisms first (Mason et al. 1996), then enter the aquatic food web via phytoplankton, benthic algae, or bacteria, which can be consumed by primary consumers, forage fish and piscivorous animals. To successfully accumulate and increase in concentration with trophic position, mercury must be taken up efficiently and retained by the organisms at the bottom of the food web, as well as passed on to consumers (Morel et al. 1998). Although methylmercury and inorganic mercury (Hg(II)) are both reactive with cellular components and efficiently retained by microorganisms, the transfer efficiency between a marine diatom and a microorganism is about four times greater for methylmercury than for Hg(II) (Mason et al.1996). Mercury(II) becomes bound chiefly to particulate membranes of the diatoms which are eliminated rather than absorbed. In contrast, methylmercury is associated with the soluble fraction of the diatom cell and is efficiently assimilated by unicellular microorganisms and their consumers (Watras and Bloom 1992). In a study of mercury dynamics in Atlantic cod (Amlund et al. 2007), methylmercury constituted 90-95% of the mercury in muscle tissue, suggesting that methylmercury is the main chemical form accumulated in fish muscle, where it is incorporated into larger peptides or proteins.

Methylmercury can similarly be accumulated in terrestrial trophic webs where mercury may come from direct atmospheric deposition, periodic flooding, or trophic flux from aquatic
systems (Brasso and Cristol 2009, Cristol 2008, Tom et al. 2010). Terrestrial animals are exposed to mercury primarily through diet. For example, female tree swallows on the contaminated stretch of South River had significantly elevated blood and feather total mercury concentrations (blood: 3.56 ± 2.41 ppm wet weight vs. 0.17 ± 0.15 ppm wet weight of reference site; feather: 13.55 ± 6.94 ppm wet weight vs. 2.34 ± 0.87 ppm wet weight of reference site), and the insects collected by adults for nestlings also had higher total mercury concentrations (0.97 ± 1.11 ppm dry weight) compared to reference sites (Brasso and Cristol 2009). In a recent study on the methylmercury magnification in the floodplain adjacent to contaminated South River in Virginia (Tom et al. 2010), a consistent progression was described from low mercury concentrations in land plants through the herbivory-based food web (including plants, insects, and birds) to realized high mercury concentrations in apex avian predators (e.g., eastern screech owl).

**Mercury in Fish**

The slow depuration of mercury relative to its high uptake rate contributes to mercury accumulation and magnification in fish (Amlund et al. 2007). For the past thirty years, elevated mercury concentrations have been observed in diverse fish species (Bodaly et al. 1984, Burger et al. 2001), some even containing mercury surpassing human toxicological thresholds (U.S. EPA 2004). Consequently, fish consumption becomes a major pathway of human exposure to mercury. In order to generate accurate fish consumption advisories and make appropriate human risk judgments, it is crucial to understand determinants of mercury concentrations in fish, which
depends on many variables such as trophic ecology, age and migratory behavior of fish, as well as proximity to sources of mercury, and water chemistry.

Risks from Fish Consumption

The accumulation of mercury in fish, provides an exposure pathway to humans and creating concerns about public health. Neurotoxicity of methylmercury manifests primarily as central nervous system damage, including sensory and motor deficits and behavioral impairment (Wren 1986, 1988). Methylmercury is also readily transferred across the placenta and concentrates selectively in the developing fetal brain, which is more sensitive to methylmercury than the developed adult brain (Wolfe et al. 1998). It was reported that methylmercury concentration in the fetal brain are roughly five to seven times the concentration in maternal blood (Cernichiari et al. 1995). Methylmercury exposure of a fetus can seriously affect brain development, as evidenced by the physical or behavioral deficits after birth, or even cause fetal death (Amin-Zaki et al. 1978, Chang and Annau 1984, Eccles and Annau 1987, U.S. EPA 1997, Schierow 2006).

Methylmercury has also been linked to adverse effects on the cardiovascular system. As a risk factor for cardiovascular disease (e.g., coronary heart disease, carotid atherosclerosis and myocardial infarction), methylmercury causes heart disease through a variety of mechanisms potentially involving pro-oxidant effects via the generation of radical species and the inactivation of cellular antioxidant systems such as glutathione peroxidase and catalase (Guallar et al. 2002). Methylmercury can exert toxic effects on the vascular endothelium by depletion of sulphydryls, increased oxidative stress, and activation of phospholipases (Hagele et al. 2007; Mazerik et al.
Evidence suggests that oxidation of low-density lipoprotein (LDL) in the arterial intima has an important role in atherogenesis (Steinberg 1991). Several studies provide evidence of increased risk of coronary heart disease (CHD) in relation to mercury exposure indicated by hair or toenail mercury concentrations in men (Guallar et al. 2002; Salonen et al. 1995). In a prospective study with measurements of the common carotid intima-media thickness (IMT) from 1,014 Finnish men, those with hair methylmercury concentration higher than 2.81 ppm had an accelerated thickening of the carotid artery, which suggests carotid atherosclerosis (Salonen et al. 2000). Methylmercury exposure was also associated with a higher risk of myocardial infarction (MI) in an European multicenter study (Guallar et al. 2002), and a similar but statistically insignificant association was found in nondentist health professionals in the United States (Yoshizawa et al. 2002).

Benefits of Fish Consumption

A primary benefit of fish consumption is the intake of omega-3 fatty acids that belong to a family of long-chained polyunsaturated fatty acids (PUFA). Nutritionally important omega-3 PUFAs include plant-derived α-linolenic acid (C18:3n-3), marine-derived eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3). Omega-3 PUFAs, in particular DHA, have been associated with a number of beneficial effects on neurodevelopment, both in early life and in adult (Ginsberg and Toal 2009). These associations include improved early brain development and visual acuity (Helland et al. 2003, Jørgensen et al. 2001), better scores on neurodevelopmental test batteries (Oken et al. 2005, 2008). Furthermore, imbalances in fatty acid status are linked to behavioral and learning disorders such as attention deficit hyperactivity
disorder (ADHD), dyslexia, dyspraxia and autism (Richardson and Ross 2000). Limited evidence from supplementation trials suggests beneficial effects on prevention of a number of neurologic and psychological disorders in adults, such as attention deficit disorder, Alzheimer's disease, schizophrenia, and depression (Calon and Cole 2007; Young and Conquer 2005).

Cardiac societies including the American Heart Association, American College of Cardiology and the European Society for Cardiology recommend the intake of 1 g/day of the omega-3 PUFAs (EPA and DHA) for prevention of cardiovascular disease, treatment after a myocardial infarction, and prevention of sudden death (De Backer et al. 2003, Priori et al. 2003, Smith et al. 2006). This is because omega-3 PUFAs have benefits on a variety of cardiovascular diseases, including MI, ischemic stroke, atrial fibrillation, atherosclerosis, and congestive heart failure (Mozaffarian and Rimm 2006, Von Schacky 2007). Epidemiological data reported by Hu et al. in 2002, who followed 84,688 women enrolled into the Nurses Health Study for 16 years, suggested that deaths related to cardiovascular disease were 50% lower in women who consumed fish five times weekly, and 20% lower for those consuming fish one to three times monthly. The same authors also investigated a subgroup of diabetic women in 2003, and discovered an even stronger inverse relationship between fish intake and cardiovascular disease. A more than 60% risk reduction was discovered for the highest fish intake group, and an approximately 30% reduction for those consuming one to three fish meals each month.

Conclusion

Mercury accumulation in members of food webs, especially finfish and shellfish, creates the major exposure pathway to most humans. Understandably, mercury in fish is a central public
health concern for people who are not occupationally exposed to inorganic mercury, prompting much effort to ensure seafood safety. Given the above context, it was necessary to examine mercury in the lower Chesapeake Bay (LCB) fish that were consumed by ethnic groups who live in the LCB region (coastal Virginia) with notionally distinct dietary customs.

In this study, a mercury database of ten finfish species was established for the lower Chesapeake Bay. Interpretation of mercury (total and methylmercury) concentrations were based on ecological features, including trophic position ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), fish size (total length), and salinity influences (otolith Sr:Ca). The ability to interpret data using a trophic framework is essential to understanding the concentration variations among species, locations, and years. Secondly, fish consumption and associated mercury exposure (hair mercury) were explored for two ethnic (Chinese and Vietnamese) church communities along the coastal Virginia, as well as two general population churches in this area. Distributions of mercury exposure for consumers were generated and compared to the Reference Dose of the U.S. EPA.
Literature Cited


CHAPTER I

Ecologically-framed Mercury Database of Lower Chesapeake Bay Finfish
Abstract

Total and methyl-mercury concentrations, and determinants of mercury accumulation were examined for 10 finfish species from the lower Chesapeake Bay (LCB) and its tributaries. There was no suggestion of potential human harm from mercury due to LCB fish consumption: none of the sampled fish had total mercury concentrations approaching the U.S. EPA human health screening value. Mercury concentrations in different fish species generally increased with increasing δ¹⁵N, but not δ¹³C, suggesting that trophic position but not dietary carbon source was a dominant determinant. A methylmercury biomagnification model was built that estimated a food web magnification factor of approximately 10-fold increase per trophic level. Based on otolith strontium-calcium ratios, Atlantic croaker inhabiting less saline waters might accumulate more mercury than those inhabiting more saline waters. The Statistical Analysis System (SAS) mixed procedure identified significant positive intraspecies relationships between methylmercury concentration and δ¹³C for summer flounder, weakfish, American eel, Atlantic croaker, and spot.

Keywords:

Mercury, methylmercury, trophic ecology, biomagnification, fish, Chesapeake Bay
1. Introduction

Concern about mercury as a major environmental contaminant has grown in recent years due to its increasing anthropogenic emission (Lamborg et al. 2002, Manohar et al. 2002), global atmospheric dispersion (Mason et al. 1994), propensity to magnify after methylation (Compeau and Bartha 1985, Newman et al. 2011 a), and high toxicity to wildlife and humans (Ginsberg and Toal 2009, Oken et al. 2005). Mercury accumulation in fish is a major exposure pathway for humans, creating a justifiable public concern about seafood safety. Elevated mercury concentrations have been observed in diverse fish species over the past fifty years (Bodaly et al. 1984, Burger et al. 2001, Newman et al. 2011 b), some within the range of human toxicological thresholds (U.S. FDA 1990-2010, U.S. EPA 2004). Perception of many in the public is that harmful levels of mercury are pervasive in fish and constitute a serious health risk. This perception can lead to less consumption of fish that are rich in omega-3 fatty acids. A clear understanding by the public of the hazard and benefits of consuming local fish is critical to making sound dietary decisions. In addition, understanding the determinants of mercury concentrations in fish, such as trophic ecology, age, and residence time in different salinity regimes (Cizdziel et al. 2002, Mason 2006) is critical to generating accurate fish consumption advisories and making appropriate risk judgments.

Identifying which features play a prominent role in determining tissue concentrations of different edible species is a common goal in efforts to manage human exposure via seafood consumption. Trophic ecology can be a prominent determinant, and isotopic ratios of nitrogen and carbon are often employed to define trophic dynamics of mercury. Stable nitrogen isotopic ratios ($^{15}N$) provide a reliable indicator of relative trophic position of a species in the food web
(Cabana and Rasmussen 1994, Füredier 2003), and carbon isotope ratios ($\delta^{13}C$) are used to infer carbon (food) sources (Füredier 2003). For example, methylmercury (MHg) concentrations in members of a floodplain food web increased from plants, to herbivorous insects and invertebrate detritivores, to birds, and apex avian predators (Newman et al. 2011 a). In the adjacent river, aquatic organisms occupying different trophic positions also had MHg tissue concentrations dictated by their trophic ecology (Tom et al. 2010).

For a given species, age can be an important determinant of mercury bioaccumulation; for fish, length or weight is sometimes used as a surrogate for age, although these morphometrics are imprecise indicators of age for most fishes. Total mercury concentrations of striped bass, channel catfish, largemouth bass, bluegill, and blue tilapia from Lake Mead (AZ and NV, USA) increased with fish size and trophic level (Cizdziel et al. 2002). In another study, mercury concentrations were correlated positively with age, weight and length of yellow perch from lakes in Michigan’s upper peninsula (Grieb et al. 1990).

Individual fish movement among estuarine and marine habitats differing in salinity could also lead to variations in mercury accumulation. Although not relevant to all species, such movements might potentially explain some differences in mercury concentrations among and within species that move among coastal habitats, where they are subject to different sources of mercury and different water chemistries that influence methylation and the movement of mercury through trophic webs (Sveinsdottir and Mason 2005). Strontium (Sr) to calcium (Ca) molar ratios of fish sagittal otoliths have been related to the salinity of waters in which the fish live. The Sr:Ca molar ratios of ocean fishes range from 8.5 to 9, in contrast to those of freshwater fishes that have ratios below 5 (Kraus and Secor 2004). In a study of MHg
accumulation in three upper Chesapeake Bay fishes (Mason et al. 2006), MHg concentrations of similarly-sized largemouth bass captured in tidal tributaries were approximately three to five times lower than those residing in freshwater reservoirs, supporting the notion that estuarine fish accumulate less MHg than fish from freshwater systems such as reservoirs and lakes. In contrast, striped bass from these different habitats did not have a similar trend for some undetermined reason.

Limited studies have explored mercury accumulation in Chesapeake Bay edible fish, but none of the studies intended to examine the influence of trophic ecology directly. Mason et al. (2006) explored mercury concentrations and the influence of fish size and migratory behavior (Sr:Ca ratio) in striped bass, largemouth bass and white perch in the upper Chesapeake Bay and its tributaries. The Virginia DEQ Fish Tissue and Sediment Contaminants Monitoring Program monitored concentrations of diverse contaminants, including mercury in biota of Virginia rivers and coastal waters. Information about MHg concentrations and fish trophic ecology (δ¹⁵N and δ¹³C) were not collected in either of these studies, limiting interpretation of within and among species variation from a trophic vantage, or prediction of concentrations for unsampled species or locations in the Chesapeake Bay.

In this study, a mercury database was established for ten commonly consumed finfish species from the lower Chesapeake Bay (LCB) and its tributaries by subsampling fish captured by the VIMS (Virginia Institute of Marine Science) Juvenile Fish Trawl Survey. Interpretations of mercury (total and methyl-mercury) concentrations within and among species were attempted based on fish trophic ecology (δ¹³C and δ¹⁵N), size (total length), and residence time in different salinity regimes (otolith Sr:Ca molar ratio). General linear mixed models were used to
explore the effects of trophic ecology, size, and salinity on mercury concentration within each species.

2. Materials and Methods

2.1. Field Sampling

Ten species of the LCB and its tributaries were selected for this study: American eel (*Anguilla rostrata*), Atlantic croaker (*Micropogonias undulatus*), blue catfish (*Ictalurus furcatus*), channel catfish (*Ictalurus punctatus*), spot (*Leiostomus xanthurus*), striped bass (*Morone saxatilis*), summer flounder (*Paralichthys dentatus*), weakfish (*Cynoscion regalis*), white catfish (*Ictalurus catus*), and white perch (*Morone americanus*). These species exhibit a range of life histories and trophic ecologies, and inhabit different portions of the LCB for different durations, which allowed the exploration of influences of these factors on inter- and intraspecies variation in mercury concentration. These species are also consumed by recreational anglers that fish these waters. Fish were collected by personnel from the VIMS Juvenile Fish Trawl Survey which operates monthly in the LCB, and the James, York, and Rappahannock rivers (Figure 1, Tuckey and Fabrizio 2011). The present mercury study involved advantageous sampling to obtain edible finfish samples representative of what is consumed locally. All catfishes were from the upper reaches of the rivers (Figure 1, regions R4, R3, Y4, Y3, J4, J3, J2); whereas, striped bass and white perch were taken throughout the rivers. American eel and weakfish were caught primarily from the rivers with a few individuals taken from the Bay. Atlantic croaker, spot, and summer flounder were caught throughout the entire sampling area (Figure 2 (1)-(10)). Fish of harvestable
size were taken, that is, fish larger than the minimum legal size permitted by the recreational fishery in Virginia; in the absence of minimum size regulations, the smallest size sampled was the size that anglers are likely to retain. A total of 348 individual fish was collected between 2009 and 2010 (Table 1). Because of the selectivity of the bottom trawl, a relatively narrow range of sizes was realized by our sampling. Most importantly, we lacked samples of large (> 69 cm total length) striped bass even though these sizes are periodically taken by local anglers.

2.2. Sample Preparation

Individual fish were euthanized in an ice slurry onboard the research vessel according to accepted IACUC (Institutional Animal Care and Use Committees) protocols, placed in plastic bags, and stored frozen until processed. Axial muscle tissue taken from each fish was weighed, freeze dried with a Freezone 4.5 freeze-dryer (Labconco Company, Kansas, MO) for 48 hours, and the dry weight of each sample was measured after freeze drying. Wet to dry weight quotients were calculated. Each dried muscle sample was ground before analysis. Sagittal otolith pairs were removed, rinsed with deionized water, and freeze dried for 48 hours prior to storage and processing for metal analysis.

2.3. Mercury Analysis

Total mercury (THg, mg/kg dry weight) in freeze-dried tissue was measured by atomic absorption spectrophotometry with a Direct Mercury Analyzer-80 (Milestone Company, Shelton, CT) and expressed as a dry weight concentration (mg/kg dry weight). Standard curves were established with a certified standard reference material DORM-3 (fish protein, National Research
Council of Canada) for each analytical session. Precision and accuracy for the analytical system were quantified with blanks, a second certified standard reference material TORT-2 (Lobster Hepatopancreas, National Research Council of Canada), and 10% replicated samples. Method precision expressed as relative percent difference for duplicate samples averaged 1.4% (SD = 0.9, n = 44). The mean percent recovery of TORT-2 samples was 102.8% (SD = 2.1, n = 93).

Methylmercury (MeHg) was analyzed by modified U.S. EPA Method 1630, aqueous phase ethylation, Tenax trap collection, GC separation, and CVAFS detection (U.S. EPA 1998; Liang et al. 1994 a). Fish tissue samples were alkaline digested at 75°C for 3 hours in closed vials, and appropriate aliquots were directly placed into bubblers for ethylation (Liang, et al, 1994 b). Methylmercury standard solutions prepared from CH₃HgCl (Cebam Analytical, Bothell, WA) were used for calibration of results. A mercury analyzer, BRIII (Seattle, WA) was employed as the detector. A certified reference material, DORM-3, was used for monitoring the analytical accuracy. Duplicate samples (1 per 10 samples) were also prepared and analyzed for monitoring the precision of analyses. The analytical precision expressed as relative percent difference (RPD) of duplicate analyses averaged 5.7% (SD = 4.8, n = 55). The mean recovery of DORM-3 was 96.2% (SD = 6.2, n = 115).

2.4. Nitrogen and Carbon Isotopes

Stable nitrogen and carbon isotopic signatures (δ¹⁵N and δ¹³C) in fish muscle tissue were determined at the Stable Isotope Facility of the University of California (Davis, CA). One milligram portions of freeze dried and ground muscle tissue were weighed and compressed into 5x9 mm tin capsules. The δ¹³C and δ¹⁵N were analyzed using a PDZ Europa ANCA-GSL
elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer. Results were expressed as ratios (per mil (%)) normalized to isotopic composition of Pee Dee Belemnite Limestone ($\delta^{13}C$) or atmospheric $N_2 (\delta^{15}N)$ standards. Analytical accuracy and precision were assessed using recoveries and associated standard deviations for replicate analyses of five standard reference materials G-9 (glutamic acid), G-11 (nylon), G-12 (glutamic acid-enriched), G-13 (bovine liver), and G-17 (USGS-41 glutamic acid). The $\delta^{13}C$ mean percent recovery of G-9, G-11, G-12, G-13 and G-17 were 100.1% (SD = 0.5%, n = 26), 100.0% (SD = 0.3%, n = 89), 100.0% (SD = 0.4%, n = 13), 100.4% (SD = 0.7%, n = 8), and 100.0% (SD = 0.5%, n = 4), respectively. The $\delta^{15}N$ mean percent recovery of G-9, G-11, G-12, G-13 and G-17 were 99.7% (SD = 3.2%, n = 28), 100.0% (SD = 1.1%, n = 98), 100.1% (SD = 0.5%, n = 13), 99.8% (SD = 1.9%, n = 8), and 100.00% (SD = 0.1%, n = 4), respectively.

2.5. Methylmercury Biomagnification Model in Chesapeake Bay

A conventional biomagnification model with MHg concentrations and estimated relative trophic levels (TLs) for sampled species was built using the PROC REG procedure in SAS® (SAS Institute, Inc., Cary, North Carolina). Trophic levels for sampled species were estimated from $\delta^{15}N$ values assuming a phytoplankton base line value of $\delta^{15}N$ (TL = 1) in the trophic web of Chesapeake Bay (Baird and Ulanowicz 1989), and that $\delta^{15}N$ changed 3.4‰ per trophic level (Minagawa and Wada 1984). The $\delta^{15}N$ of 13.0‰ (estimated as the average $\delta^{15}N$ of Acartia tonsa and Mnemiopsis leidyi results in Figure 4 and 7 of Montoya's study, 1990) and trophic level of 2.16 (Baird and Ulanowicz 1989) in zooplankton of Chesapeake Bay were used to calculate relative trophic levels for sampled species with the following equation:
The length of the food web containing these ten fish species was calculated as the difference between the highest and lowest trophic level for the species considered. A linear regression model was fit to the trophic level data to predict Log$_{10}$-transformed MHg concentration (wet weight basis) for sampled species:

$$\text{Log}_{10}[\text{MHg}] = a \cdot TL + b.$$  \hfill (2)

Food web magnification factor (FWMF) was estimated as the following,

$$FWMF = 10^a.$$  \hfill (3)

where $a$ is the slope from the linear regression in (2) (Newman et al. 2011). Because the species we sampled occupy relatively high trophic positions in the Chesapeake Bay food web (Baird and Ulanowicz 1989), the resulting narrow range of trophic level constrained the biomagnification modeling and associated FWMF estimation. A literature search was performed to augment the species data with published values for Chesapeake Bay species in other trophic levels, but limited trophic position data were available for species for which MHg was measured (Mason 2006, Mason et al. 1999). Most mercury monitoring programs report THg concentrations in commonly eaten finfish and shellfish, but MHg concentrations in lower trophic level species and nonseafood species are not available. Two studies that analyzed MHg accumulation in relatively low trophic level species from Chesapeake Bay (Mason 2006, Mason et al. 1999) were appropriate for inclusion in the mercury biomagnification model. Ranges of MHg concentrations (wet weight basis) in anchovy, blue crab, clams, copepods, and amphipods were obtained from
the average of log_{10}-transformed maximum and minimum reported MHg concentrations for each species. Neither of Mason’s studies collected δ^{15}N information or examined trophic position, so trophic levels of these species were determined according to the average annual trophic level in Chesapeake Bay as reported by Baird and Ulanowicz (1989). A biomagnification model was built using the SAS® 9.2 software procedure, PROC REG for species collected in this study and for those reported in the literature. Equation (2) was used to estimate FWMF from the regression results.

2.6. Otolith Strontium and Calcium Analysis

Strontium and Ca concentrations in otoliths were measured with a Perkin-Elmer AAnalyst 800 atomic absorption spectrophotometer (Norwalk, CT). Sagittal otolith pairs were cleaned with 3% (v/v) H_2O_2 and 1% (v/v) hydrochloride acid (HCl), dried, and stored until digested. Cleaned otoliths were digested in 1 to 3 ml of Teflon-distilled concentrated HCl for four to six hours, and diluted appropriately with deionized water for analysis. Calcium was determined using air-acetylene flame atomic absorption spectrophotometry with deuterium background correction at 422.7 nm; strontium was determined using air-acetylene flame emission spectrophotometry at 460.7 nm. Hydrochloric acid was used for digestions instead of nitric acid to avoid signal suppression during Sr analyses. Lanthanum (LaCl_3, 50g/L) was also added to both Ca (10% v/v) and Sr (1% v/v) digests to minimize ionization and chemical signal suppression. Standard curves were established with commercial standards (Ricca Chemical, Pocomoke, MD) for each analytical session. Analyte concentrations for a test sample of each fish species were estimated by comparison to an aqueous standard curve and also by standard
additions to generate signal suppression factors (SSFs). Concentrations of Sr and Ca were adjusted with the SSFs for any signal suppression, and converted to molar values to generate final Sr:Ca molar ratios.

2.7. Statistical Analysis

The use of conventional null hypothesis significance tests was minimized herein due to the emerging discussion about their general validity (Altman 2004, Anderson et al. 2000, Fidler 2006, Gigerenzer 2004, Sterne and Davey Smith 2001), and when possible, inferences depended more on confidence interval interpretation as advocated by Altman et al. (2000), Cumming and Finch (2005), and Cumming (2012). Mercury concentrations, $\delta^{15}$N and $\delta^{13}$C, and Sr:Ca molar ratios in this study were expressed as geometric means with 95% confidence intervals (CIs). All mercury concentrations were Log$_{10}$-transformed, and the calculated mean and 95% CIs were back-transformed. Comparison of mercury concentrations and isotopic ratios among species followed Cumming's general rules-of-thumb: non-overlap of 95% CIs indicated $P$-value of less than 0.01, and a proportion of overlap of 95% CIs less than 0.5 indicated $P$-value of less than 0.05 (Cumming and Finch 2005, Cumming 2012). Log$_{10}$-transformed mercury concentrations were used to explore relationships between mercury accumulation and $\delta^{15}$N, $\delta^{13}$C, and Sr:Ca molar ratios (Figure 4, 5 and 6).

The SAS MIXED procedure was used (SAS® 9.2 package, SAS Institute, Inc., Cary, North Carolina) to explore variations in MHg concentrations within each species relative to fish total length, trophic indicators ($\delta^{15}$N and $\delta^{13}$C), salinity exposure as indicated by Sr:Ca molar ratios, and sampling locale. In this model, "river" was designated as a fixed effect and fish total
length, δ¹⁵N and δ¹³C values, and Sr:Ca molar ratios were treated as random effects. An interaction term "river*total length" was used to test for significant differences in trends in MHg with length among locales. Methylmercury concentrations were log-transformed in this procedure to meet the assumptions of normality. The transformation also resulted in homogeneous variances across the levels of the factors by examining the residuals from this model.

3. Results

3.1. General Mercury Concentrations

No sampled fish had total mercury concentrations (wet weight basis) approaching the human health screening value of 300 μg/kg wet weight (U.S. EPA 2009) (Table 1). The percentage of THg present as MHg (%MHg) increased with increasing mercury concentrations (Figure 3). Spot had the lowest THg and MHg concentrations among the sampled finfish and its 95% CIs did not overlap with those of other species (Table 1 and Figure 3). White catfish, striped bass, and white perch had higher THg and MHg concentrations (dry weight basis) compared with the other seven species, but there were no statistically significant differences among white catfish, striped bass, and white perch as suggested by their 95% CI (Table 1 and Figure 3). White and blue catfish are omnivorous bottom feeders that reside in fresh and brackish waters. Concentrations of THg and MHg, and %MHg in white catfish were significantly greater than those observed for blue catfish based on the non-overlapping 95% CIs (Table 1 and Figure 3). Striped bass and white perch are both members of the family Moronidae and use upriver
habitats for spawning; however, most Chesapeake Bay striped bass participate in coastal feeding migrations during late spring, summer, and fall, whereas white perch remain in riverine habitats year round (Murdy 1997). For the size ranges examined here, these species appear to occupy similar trophic positions as determined by δ¹⁵N, feed on similar food sources (δ¹³C), and have similar mercury concentrations and %MHg ratios (Table 1 and Figure 3).

3.2. Trophic Influence on Mercury Accumulation among Species

Methylmercury increased with increasing values of δ¹⁵N (Figure 4) among studied species, but there was no obvious relationship between MHg and δ¹³C. Similarly, %MHg increased with increasing values of δ¹⁵N, but not δ¹³C (Figure 4). Channel catfish were excluded from Figure 4 because of the uncertainty in estimating MHg, δ¹⁵N, and δ¹³C resulting from the associated small sample size (n = 7). The range of δ¹⁵N values indicated 2.2 trophic levels between fish species occupying the lowest (Atlantic croaker) and highest (white catfish) trophic positions (ΔTL = TL_white catfish − TL_Atlantic croaker = (Mean δ¹⁵N_white catfish − Mean δ¹⁵N_Atlantic croaker) / 3.4 = 2.2). Inconsistent with the general pattern of methylmercury accumulation with increasing trophic positions (Figure 4), Atlantic croaker had relatively low values of δ¹⁵N but high concentrations of MHg.

3.3. Methylmercury Biomagnification Model

The MHg biomagnification model for Log₁₀-transformed MHg concentrations (wet weight basis) and trophic level of the sampled species was: Log₁₀ [MHg] = 0.56 [TL] − 0.10 (regression r² = 0.27). The model was not informative because the 95% CI of the slope (-0.20 to
1.31) included zero. The second MHg biomagnification model was fitted to data from the sampled species and data from species occupying lower trophic positions in the Chesapeake Bay as reported by others: Log$_{10}$[MHg] = 1.03 [TL] - 1.78. The solid line, the area of shadow, and the area of dashed line in Figure 5 indicated the fit of these data, 95% confidence limits, and 95% prediction limits, respectively. The regression $r^2$ was 0.62 and the 95% CI of the slope (0.54-1.51) did not include zero. The food web magnification factor was estimated as FWMF = $10^a$ = $10^{1.03}$ = 10.60 (95% CI: 3.46-32.42). Methylmercury concentration increased approximately 10-fold for each trophic level of the Chesapeake Bay food web. The length of the trophic web encompassed by the modeled species was estimated as the difference between species in the lowest and highest TL (Baird and Ulanowicz 1989): $\Delta_{TL} = TL_{white \ catfish} - TL_{clam} = 1.3$.

3.4. Salinity Regime Influence on Mercury Accumulation within Species

Wide confidence intervals of THg and MHg in white perch, striped bass, white catfish and channel catfish (Table 1) suggested the possible influence of factors other than trophic ecology on mercury accumulation within these species, such as different rivers, diversities of individual life histories, fish size, and residence time in different salinity regimes. (As mentioned previously, the small sample size for channel catfish (n = 7) likely contributed to the observed high variation in mercury concentrations.) Otoliths from white perch, striped bass and white catfish were analyzed for Sr:Ca molar ratios to explore the association between accumulated mercury and relative time spent in fresh and mesohaline waters. Atlantic croaker otoliths were also analyzed because their low $\delta^{15}$N values and high MHg concentrations (Table 1 and Figure 4)
were inconsistent with the general assumptions of mercury biomagnification through the trophic web.

The Sr:Ca molar ratios of Atlantic croaker and striped bass ranged from 2.2 to 7.6 and 2.4 to 9.6, respectively, and as expected, Sr:Ca ratios of white perch and white catfish were generally lower, ranging from 1.4 to 3.9 and 1.2 to 4.1, respectively. Spatial distribution of fish samples were not associated with their Sr:Ca molar ratios within each river, indicating that sampling locations were not fully indicative of the habitats used by these fish over their lifetime. The mean Sr:Ca molar ratios of Atlantic croaker and striped bass from the Rappahannock River were higher than those from the York and James rivers suggested by the non-overlapping 95% CIs. No obvious relationship was identified between MHg concentrations and Sr:Ca molar ratios among these four species. There was a weak negative relationship between MHg and Sr:Ca ratios (slope = -0.078, 95% CI: -0.13 to -0.02, r² = 0.32) for Atlantic croaker (Figure 6). No such relationship was observed for white perch, striped bass, or white catfish in the sampled size range.

3.5. Mixed Model for Mercury Accumulation

We found significant positive relationships between MHg concentrations (dry weight basis) and stable isotope δ¹³C for American eel (p < 0.01, t = 3.22, df = 23), Atlantic croaker (p < 0.05, t = 2.13, df = 21), spot (p < 0.05, t = 2.27, df = 41), summer flounder (p < 0.01, t = 2.96, df = 36), and weakfish (p < 0.0001, t = 5.63, df = 15). Of the ten species sampled, only blue catfish showed a significant relationship between δ¹⁵N and MHg accumulation (p < 0.0001, t = 5.51, df = 27). Fish total length had a significant positive influence on MHg concentrations for summer flounder (p < 0.05, t = 2.21, df = 36) and white catfish (p < 0.001, t = 4.14, df = 17). The
interaction term "river*total length" was not significant for any species and therefore did not help explain the variation in MHg accumulation in sampled fish. Relationships between fish total length and MHg concentrations in white catfish and summer flounder were invariant to river and thus, a general relationship could be described for these two species in lower Chesapeake Bay.

4. Discussion

4.1. General Mercury Concentrations

Based on U.S. EPA and FDA criteria there is no evidence suggesting possible human harm from mercury consumption associated with selected LCB finfish species sampled from 2009 to 2010 (Table 1). All sampled finfish had THg concentrations lower than the human health screening value of 300 µg/kg wet weight (EPA 2009). More specific statements of LCB human subpopulation risk require detailed exposure assessment of fish consumption. In one such study involving a LCB African-American community (Holloman and Newman 2010, 2012), dietary contribution to mercury exposure from locally caught fishes did not stand out as substantial relative to the contributions from nonlocal seafood. The large amount of total seafood eaten daily by this LCB African-American community was most important in determining the risk from mercury in finfish. A similar study is underway now of a local Vietnamese and Chinese community based on these mercury data and specific seafood consumption information.

4.2. Trophic Influence on Mercury Accumulation among Species
Trophic position was a major factor influencing axial muscle mercury concentrations and $\delta^{15}N$ was a general predictor of relative mercury accumulation in Chesapeake Bay biota. For the sampled LCB finfish species, MHg concentration and %MHg generally increased with increasing $\delta^{15}N$ (Figure 4). A MHg biomagnification model based on data from our study and data published previously indicated a FWMF of approximately 10-fold for the LCB food web. The FWMF was only roughly estimated because of the absence of information for species occupying other trophic positions. There were limited studies exploring mercury biomagnification in Chesapeake Bay, but a similar study completed in the South and Holston Rivers (VA, USA) found that the methylmercury FWMF was 4.6 (95% CI: 3.6-5.7, Newman et al. 2011 a). The Chesapeake Bay FWMF was higher than that estimated from the South and Holston Rivers. However, with the exception of blue catfish, we were unable to identify a significant intraspecific influence of $\delta^{15}N$ on MHg for the sampled species. Though many studies found a positive influence of intraspecies trophic position on mercury accumulation (Hammerschmidt and Fitzgerald 2006, Tom 2010), the narrow size range sampled in this study (Table 1) may have limited the ability to detect such a trend. Alternatively, the fish species considered here may exhibit high intraspecific variability in diets, leading to high variability in MHg concentrations.

The overall influence of dietary carbon source was judged to be less important than trophic position ($\delta^{15}N$) on determining mercury concentration due to the lack of a linear relationship between MHg and $\delta^{13}C$ across species. However, $\delta^{13}C$ did appear to have some influence on intraspecies differences in mercury concentrations in a few species. For example, distinct differences in mercury concentrations between white and blue catfish may be explained by their stable isotope signatures ($\delta^{15}N$ and $\delta^{13}C$). White catfish (21.2-22.7 cm), appear to
occupy a higher trophic position than blue catfish (38.6-42.8 cm) as indicated by δ^{15}N (table 1), and also had higher mercury concentrations (figure 3). This is somewhat surprising because blue catfish greater than 30 cm begin incorporating fish into their diet (Schloesser et al. 2011) and would therefore be expected to occupy a fairly high trophic level. In addition, white catfish with less negative δ^{13}C values relative to blue catfish suggested a greater dependence on benthic food items (Fry 1998). This is consistent with the speculation that mercury concentrations appeared to increase with increased dependence on benthic versus pelagic food sources (Newman et al. 2011 b). The SAS mixed procedure identified significant positive intraspecies relationships between MHg concentrations (dry weight basis) and δ^{13}C for American eel, Atlantic croaker, spot, summer flounder, and weakfish, suggesting that different food sources might also influence mercury accumulation within these species.

4.3. Salinity Regime Influence on Mercury Accumulation within Species

Time spent in different salinity regimes resulting from fish movement (Sr:Ca ratio) suggested weak intraspecies influence on mercury accumulation in Atlantic croaker only (Figure 6). Although the results of otolith Sr:Ca ratio were not relevant to all species, these ratios may explain some differences in mercury concentrations. For instance, low values of δ^{15}N and high concentrations of MHg in Atlantic croaker were inconsistent with the general pattern of increasing MHg concentration with increasing trophic status (Figure 4), indicating the presence of some confounding influence(s) on mercury accumulation, like movement between habitats with prey items that differ in MHg concentrations as studied for other fish species (Farmer et al. 2010). Otolith Sr:Ca ratios suggested that Atlantic croaker inhabiting less saline environments
tended to accumulate more mercury than those inhabiting more saline waters, which might be due to consumption of prey exposed to point sources from the terrestrial environment. This could help explain the inconsistency observed in the relation between δ^{15}N and MHg concentrations for Atlantic croaker (Figure 4). Yet there was no such relationship for white perch, white catfish and striped bass, three species that also make use of upriver and freshwater habitats. Additionally, no interspecies relationship between MHg accumulation and Sr:Ca molar ratios were found. The narrow range of fish size sampled (Table 1) might be one reason for the failure to establish a clear relationship between mercury concentration and Sr:Ca molar ratio within and among species. White perch and white catfish generally reside in the upper reaches of the tidal rivers (Sr:Ca: 1.4-3.9, and 1.2-4.1, respectively), so their modest movements would not be expected to result in a strong relationship between X and Y. Striped bass is an anadromous fish that undertakes coastal migrations (Sr:Ca: 2.4-9.6), but the narrow range of sampled fish size, pooled sexes, and differences in individual life histories (e.g., Secor 1999, Secor et al. 2001) might confound the relationship. Larger samples sizes may be required to elucidate these relationships for striped bass. Furthermore, water temperature, salinity, point sources of Sr, and fish biological states contribute to the observed variance in otolith Sr (Kalish 1989, Secor et al. 1995). The higher Sr:Ca molar ratios of Atlantic croaker and striped bass from the Rappahannock River relative to the York and James rivers may also be due to the combined influence of these factors.

4.4. **Other Influences of Mercury Accumulation in Fish**

Mercury accumulation in fish is also influenced by fish age, sex, lipid content, sampling season and location, water chemistry, and possibly other factors. A large portion of variance in
MHg was apportioned into the random error term of the mixed model, implying the existence of explanatory factors other than those used to construct the model. In this study, the significant influence of fish size was identified only for white catfish and summer flounder. Although many studies report a positive influence of intraspecies fish size and trophic position on mercury accumulation (Hammerschmidt and Fitzgerald 2006, Tom 2010), the narrow size range sampled in this study (Table 1) limited our ability to detect a relationship. Furthermore, mercury concentrations within species sampled from different tidal rivers were not statistically different, which indicated that either there is no difference of species-specific mercury accumulation in different rivers, or that the relatively small sample size from each river made it difficult to discern relationships. Additionally, Atlantic croaker is believed to have higher lipid content compared with species such as summer flounder and striped bass (R. Schloesser and M. Fabrizio, pers. obs.). The accumulation of the lipophilic MHg is closely related to tissue lipid content, so Atlantic croaker might take up more MHg compared to the species with lower lipid contents. Such a relationship may also contribute to the inconsistent pattern observed in low values of δ¹⁵N and high MHg concentrations of (Figure 4) in Atlantic croaker.

5. Conclusion

There is no evidence suggesting possible human harm from mercury in the selected LCB finfish species. Trophic position emerged as the most important determinant of interspecies mercury concentration differences. Effects of dietary food sources, residence time in different salinities, and other factors on mercury accumulation in the ten species examined in this study
were less obvious. The ability to interpret data using a trophic framework is useful for predicting mercury concentrations of unsampled species or locations particularly for freshwater and marine species in estuarine environments, and can help understand variation in mercury concentrations within and among species, locations, and years. This study adopted such a context for mercury in commonly eaten finfish from LCB, and also established a much needed mercury database. It will be used as a fisheries tool for understanding and communicating mercury risks associated with LCB fish consumption, informing seafood consumers (market, recreational or tourism-related consumption), and potentially dispelling misperceptions about fish safety.

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TABLE 1  Means and 95% confidence intervals of mercury concentrations, δ 15N and δ 13C values, and total length for sampled fish species.
<table>
<thead>
<tr>
<th>N</th>
<th>Total length (cm)</th>
<th>THg (mg/kg, wet)</th>
<th>THg (mg/kg, dry)</th>
<th>MHg (mg/kg, wet)</th>
<th>MHg (mg/kg, dry)</th>
<th>%MHg</th>
<th>δ^{15}N (%)</th>
<th>δ^{13}C (%)</th>
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<td>13.92</td>
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FIGURE 1  Sampling areas in the lower Chesapeake Bay and tidal tributaries.

The dashed line is the boundary between Virginia and Maryland. Solid lines separate the geographic regions sampled by the random stratified design (B1/B2/B3: Chesapeake Bay regions 1, 2, and 3; J1/J2/J3/J4: four regions in the James River; Y1/Y2/Y3/Y4: four regions in the York River; R1/R2/R3/R4: four regions in the Rappahannock River; and MB: Mobjack Bay).
FIGURE 2  Distributions of fish samples in the lower Chesapeake Bay.
FIGURE 2 A. Distributions of American eel samples in the lower Chesapeake Bay.
FIGURE 2 B Distributions of Atlantic croaker samples in the lower Chesapeake Bay.
FIGURE 2 C Distributions of blue catfish samples in the lower Chesapeake Bay.
FIGURE 2 D Distributions of channel catfish samples in the lower Chesapeake Bay.
FIGURE 2 E Distributions of spot samples in the lower Chesapeake Bay.
FIGURE 2 F Distributions of striped bass samples in the lower Chesapeake Bay.
FIGURE 2 G Distributions of summer flounder samples in the lower Chesapeake Bay.
FIGURE 2 H Distributions of weakfish samples in the lower Chesapeake Bay.
FIGURE 2 I Distributions of white catfish samples in the lower Chesapeake Bay.
FIGURE 2 J Distributions of white perch samples in the lower Chesapeake Bay.
FIGURE 3  Total and methyl-mercury concentrations, and %methylmercury for ten finfish species from the lower Chesapeake Bay. Columns and filled box indicate means, and error bars indicate 95% confidence intervals.
FIGURE 4  Influence of trophic position ($\delta^{15}$N) on methylmercury accumulation in 10 finfish species from the lower Chesapeake Bay. Filled box and error bars reflect means ± 95% confidence intervals.
FIGURE 5  Methylmercury biomagnification model for Chesapeake Bay.

A, white catfish; B, blue crab; C, white perch; D, striped bass; E, weakfish; F, summer flounder; G, American eel; H, blue catfish; I, spot; J, Atlantic croaker; K, channel catfish; L, anchovy; M, copepod; N, amphipod; O, clam.
FIGURE 6  Relationship between methylmercury accumulation and otolith Sr: Ca molar ratios in Atlantic croaker.
CHAPTER II.

Mercury Exposure through Fish Consumption of Two Ethnic Communities of Coastal Virginia
Abstract

Fish consumption and associated mercury exposure were explored by food frequency questionnaires for two ethnically Asian dominated church communities along coastal Virginia, as well as two general population churches in this area treated as the reference group. Hair samples were also collected for mercury analysis. Associated mercury exposure was modeled by Monte Carlo simulation with consumption data obtained by the questionnaires. Individual seafood consumption rates for the ethnic communities (geometric mean: 36.9 g/person/day and 52.7 g/person/day for the Chinese and Vietnamese church communities) were higher than the general U.S. fish consumption rate of 12.8 g/person/day. People from the general population churches and Chinese church took in most of their mercury from market fish (distributed and sold nationally) whereas people from the Vietnamese church took in mercury from both the market and local fish as they tended to eat a large amount of diverse local species. Hair total mercury concentrations in the Chinese and the Vietnamese church were higher than the overall level for U.S. women (0.20 µg/g), but lower than the published WHO exposure threshold of 14 µg/g. Regression between seafood consumption rates and hair mercury concentrations suggested that dietary mercury ingestion through seafood was positively related to mercury exposure. The annual-average daily methylmercury intake rate calculated by Monte Carlo simulation indicated a higher mercury exposure of the Vietnamese community compared to the Chinese community, and a higher exposure of the Chinese community compared to the general churches communities. Regardless, the daily methylmercury intake rates for all studied communities were lower than the U.S. EPA RfD of 0.1 µg/kg BW-day. In conclusion, fish consumption patterns differed among communities, which resulted in different levels of mercury exposure. The higher seafood and
mercury ingestion rates of ethnic groups compared to the general populations suggests the need for specific seafood consumption advice for ethnic groups within the lower Chesapeake Bay region of Virginia.

Keywords:

Mercury, exposure assessment, seafood consumption, Chinese, Vietnamese
1. Introduction

Concern about environmental mercury continues to grow due to its increasing anthropogenic emissions (Lamborg et al. 2002, Manohar et al. 2002), global atmospheric dispersion (Mason et al. 1994), propensity to biomagnify after methylation (Compeau and Bartha 1985, Newman et al. 2011a), and relatively high toxicity (Ginsberg and Toal 2009, Oken et al. 2005). Methylmercury, the form most readily transferred in food webs, is a neurotoxicant (Guallar et al. 2002) and a risk factor for cardiovascular disease (Oken et al. 2005). Elevated mercury concentrations, including some that exceeded human toxicological thresholds, have been observed in diverse fish species over the past fifty years (U.S. FDA 1990-2010, U.S. EPA 2004). Understandably, mercury in fish and shellfish has become a central public health theme, prompting much effort to ensuring seafood safety.

Although there is general agreement about methylmercury toxicity, key features of present day fish consumption advisories remain incompletely defined (Ginsberg and Toal 2009). The nutrients in fish, including high-quality protein, vitamins and omega-3 polyunsaturated fatty acids (ω-3 PUFA), are often correlated with an array of health benefits (Helland et al. 2003, Oken et al. 2005, 2008, De Backer et al. 2003, Priori et al. 2003, Smith et al. 2006). Regardless, a common misconception in many public sectors is that harmful levels of mercury are pervasive in fish and constitute a serious health risk to be avoided. This perception can lead to low consumption of fish that are rich in ω-3 PUFA. Sound dietary decisions require a clear understanding by the public of both the hazard and benefits of consuming local fish.

Mercury concentrations in commonly eaten fish from the lower Chesapeake Bay (LCB) were lower than the human health screening value of 0.3 μg/kg (U.S. EPA 2009, Xu et al. 2012,
unpublished); however, this information alone is insufficient to fully inform all LCB residents about risks from seafood consumption. Consumption advisories are based on general public seafood consumption rates, but consumption rates can be quite high for specific LCB subpopulations such as a recently surveyed African-American community in Newport News (Virginia, USA). Due to their distinct seafood consumption habits, the mean consumption rate (147.8 g/day) for women aged 16 to 49 in this community was substantially higher than that reported for U.S. women (1.8 g/day) (Holloman and Newman 2010). People from other LCB ethnic groups could also have distinctive seafood consumption patterns as a result of differences in choice of consumed species, meal size, consumption frequency, and parts of finfish consumed (Sechena et al. 2003). Such cultural differences will change mercury exposure so that the generic consumption advisory would be misleading for a member of a LCB ethnic group. So far, there has been limited exploration of seafood consumption and mercury exposure for ethnic groups in the LCB region; therefore, insufficient consumption advisory information is available for important LCB communities. The purpose of this study is to generate a better understanding of mercury exposure through seafood consumption for two ethnic communities of the LCB coastal area, provide specific seafood consumption advice, and enhance understanding of seafood safety.

2. Materials and Methods

2.1. The Studied Communities
Preliminary data of hair mercury concentrations in Chinese and Vietnamese communities along coastal Virginia were collected in 2008 to 2011. The geometric mean hair mercury concentrations in these two communities (0.30 μg/g for the Chinese community, and 0.26 μg/g for the Vietnamese community) were both higher than 0.2 μg/g, which is the geometric mean concentration of U.S. women aged from 16 to 49 as reported by National Health and Nutrition Examination Study (Margaret et al. 2004). These preliminary results suggested mercury exposure levels in the two ethnic communities were elevated relative to the general U.S. population. Based on these preliminary findings, we conducted follow-up surveys of two typical Chinese and Vietnamese communities along coastal Virginia.

Church-based sampling was adopted because engagement through church leaders provided access to large numbers of individuals of a common ethnic background who came together on specific occasions. This approach is particularly appropriate for sampling ethnic groups in the study area where families might live in several distant neighborhoods. The largest Chinese and Vietnamese churches along the James and York rivers were selected (Peninsula Chinese Baptist Church, Yorktown, Virginia, USA (GPS: 37.1386, -76.4562); Our Lady of Vietnam Chapel, Hampton, Virginia, USA (GPS: 37.0304, -76.3634)), as well as two reference churches that served the general community in the study area (Unity Fellowship Church, Newport News, Virginia, USA (GPS: 37.0921, -76.4736); Gloucester Point Baptist Church, Gloucester Point, Virginia, USA (GPS: 37.2561, -76.4923)).

2.2. Survey Design
The Lower Chesapeake Bay Seafood Consumption Survey included a food frequency questionnaire to characterize seafood consuming patterns, and hair sample collection for direct mercury exposure assessment. The questionnaire was drafted by modification of the Southeast Seafood Consumption Survey (Holloman and Newman 2010) and the Asian and Pacific Islander Seafood Consumption Study (Sechena et al. 2003). Information about seafood consumption patterns (such as: species, consumption frequency, meal size and consumed finfish parts), hair treatments, and demographic background (age, gender, and ethnic group) were solicited using both English, and as appropriate, Mandarin or Vietnamese languages. A quality control procedure was designed such that some questions about seafood consumption patterns (consumed items, consumption frequency and meal size) were asked in two ways within the survey and the precision of answers were checked. The relative percent difference was 25% for questions on meal size, and 17% for questions on consumption frequency. Study participants needed to be 18 years or older and have lived in the study area for more than a year. The survey was done as a community-based partnership between the church community and the College of William and Mary. The survey design, instruments and implementation plan were approved by the College of William & Mary Human Subjects Committee and the sampling method and mercury measurement process for hair samples protocols were approved by the college’s Institutional Biosafety Committee.

2.3. Survey Administration

Essential to the success of such a survey is the involvement of a community opinion leader, that is, the church ministers. One week before the survey, the church minister
communicated the study goal to church members, encouraged participation, and explained the value of knowing one’s mercury exposure level regardless of whether it was low or high. At least two volunteers from each church who speak both English and their ethnic language were also recruited, trained, and engaged during the entire survey.

During the spring and summer of 2011 to 2012, bilingual written surveys were administered after Sunday services in each community. At their convenience, participants were sampled during a four week period. Every respondent answered a written questionnaire and gave a hair clipping. Approximately 10 to 100 mg of scalp hair was collected from at least two areas of the head, placed in an envelope, and marked with the respondent’s name or initials. Hair mercury results were sent to respondents through mail along with general information about mercury exposure, and the typical hair mercury levels of the U.S. population.

2.4. Survey Instrument

We chose written questionnaires instead of face-to-face verbal interviews due to the church-based sampling strategy. Trained College of William & Mary staff and church volunteers administered the survey, communicated with respondents, and took samples. Two types of visual aids were used to maximize the recall survey reliability. First, a seafood brochure including the names and pictures of commonly consumed species was provided to each respondent. The English names were also translated into the appropriate language, and more than one name provided for some species because people from different parts of the country apply different common names. The second visual aid was a series of weighted seafood meals (1oz, 2oz, 4oz,
8oz, 16oz; 25g, 50g, 100g, 200g, 400g) with different serving sizes on a plate. Weights of uncanned seafood items were estimated according to the provided weights of the meals, but weights of canned seafood items were estimated according to the weights given on the can (Holloman and Newman 2010). In addition, bilingual posters were also displayed to explain the project and encourage participation.

2.5. Hair Mercury Analysis

The proximal 2 cm of each respondent's hair was analyzed for mercury by atomic absorption spectrophotometry with a Direct Mercury Analyzer-80 (Milestone Company, Shelton, CT) and expressed as microgram per gram (µg/g). Batches of 15 to 50 samples were analyzed during each analytical session. Calibration curves were established with a certified standard reference material DORM-3 (fish protein, National Research Council of Canada) for every batch of samples, and linearity with an $r^2$ of greater than 0.99 was required for each calibration curve. Precision and accuracy for the analytical system were quantified with a second certified standard reference material TORT-2 (lobster hepatopancreas, National Research Council of Canada), and 10% replicated samples. The mean percent recovery of TORT-2 was 104.5% (standard deviation = 1.2%, n = 32). Method precision expressed as relative percent difference for duplicate samples averaged 0.9% (standard deviation = 0.7%, n = 16).

2.6. Data Collection and Analysis

2.6.1. Seafood items, consumption frequency, meal size, and body weight
A maximum of 10 commonly eaten seafood items was reported by respondents in the questionnaire, as well as the consumption frequency (number of meals/month) and meal size (g/meal) estimates for each. All reported items were grouped into seafood categories for a church-based community and the number of items in each category applied as the consumption frequency of that item. Each seafood category was defined as a seafood group. The relative proportion of an item was calculated by dividing its consumption frequency by the number of all items reported by the community, which was treated in later Monte Carlo simulations as the probability of selecting a seafood item by a person. Given that certain local fish, such as striped bass, oyster, and flounder, were only available seasonally, the number of meals within a month (number of meals/month) for a certain item in spring, summer, fall, and winter were estimated separately for each respondent. The "number of meals within a month" for each season was multiplied by 3 and summed to get an annual consumption frequency (number of meals/year) of an item, which was divided by 365 to yield a daily consumption frequency (number of meals/day). When meal size was asked in the questionnaire, respondents were asked to exclude weights of inedible parts and side dishes. Different units of meal size (gram, kilogram, ounce, pound) and body weight (kilogram, pound) reported by respondents were converted to grams for meal size and kilograms for body weight.

2.6.2. Missing and suspicious data

One respondent in the general population church was Asian, and one respondent in the Chinese church was a non-Asian. The answers of these two questionnaires were deemed unrepresentative and excluded from further analysis.
Two methods were applied for missing data of consumption frequency and meal size: if the item with a missing value was reported by other respondents, the missing value was replaced by randomly selecting one from the answers of other respondents; if the item with a missing value was not reported by any other respondent, the value was replaced by the answer of that respondent’s general consumption frequency or meal size. Two questions about a person’s general (not species-specific) seafood consumption frequency and meal size were also included in the questionnaire as part of the quality control procedure.

Arithmetic means and standard deviations (SD) of consumption frequency and meal size for each seafood group were calculated after preliminary examination of the associated distribution of observations. A few respondents reported suspiciously high consumption data that suggested unreliability. If an observation was greater than three standard deviations above the arithmetic mean, the observation was treated as a suspicious value (Sechena et al. 2003). Only two suspicious values for meal size (e.g., 2 kg of shrimp per meal) in this study were replaced by the largest value lower than the arithmetic mean plus three standard deviations in that seafood group (Sechena et al. 2003).

An item was omitted from the data set if an item name was missing or vaguely expressed. Among the 390 seafood items reported, 18 (4.6%) values of consumption frequency and 18 (4.6%) values of meal size were missing, 2 (0.5%) values of meal size were suspicious, and 11 (2.8%) values were dropped because of the missing or vague seafood names.

2.6.3. Seafood consumption rate
Seafood consumption rate (g/day) of each reported item was calculated by multiplying its consumption frequency (number of meals/day) and meal size (g/meal). All reported items were placed into several seafood groups, and the group seafood consumption rate (g/person/day) calculated by summing all seafood consumption rates in that group and dividing the result by the number of respondents in that community.

Seafood consumption rates of different items reported by the same respondent were summed to get an individual seafood consumption rate (g/person/day). Geometric means, standard deviations, and distributions of the individual seafood consumption rates for each community were determined and compared.

2.6.4. Mercury concentrations in seafood

Reported species were classified as local (LCB) fish (Kirkley 1997) which included the commonly eaten species caught from the LCB, or market fish which were distributed and sold nationally. Mercury concentrations of the local fish were derived from a LCB finfish mercury database (Xu et al. 2012, unpublished) and another recent mercury exposure study in a nearby LCB community (Holloman and Newman 2012). Mercury concentrations of the market fish were derived from the studies of mercury exposure in U.S. seafood markets and reports from the U.S. EPA and FDA (Table 1). General mercury concentrations of fresh tuna were used instead of those for canned albacore and light tuna because most respondents could not recollect which kind of tuna was consumed.

2.6.5. Mercury intake rate
Mercury intake rate (μg/day) of each reported item was calculated by multiplying its seafood consumption rate (g/day) and the mean total mercury concentrations (μg/g) from Table 1. All reported items were placed into several seafood groups, and the group mercury intake rate (μg/person/day) was calculated by summing all mercury intake rates in that group and dividing by the number of eligible respondents in that community.

2.7. Statistical Analysis

The SAS 9.2® software (SAS Institute, Inc., Cary, North Carolina) was used for data analysis. A simple linear model between hair total mercury concentrations and mean individual seafood consumption rates of the studied communities was built using the PROC REG procedure in SAS. To avoid the current debate about the interpretation of P-vales from conventional statistical tests (Altman et al. 2000), general discussion of significant differences for data from the communities conformed to Cumming’s general rules-of-thumb: non-overlap of 95% confidence interval (CI) indicated P-value of less than 0.01, and a proportion of overlap of 95% confidence intervals less than 0.5 indicated P-value of less than 0.05 (Cumming 2012, Cumming and Finch 2005).

2.8. Exposure Modeling

2.8.1. Calculation of daily methylmercury intake rate

The daily methylmercury intake rate was estimated using the following equation (U.S. EPA 2011):
\[ DMIR = \frac{CF \times MS \times THg \times a \times b}{BW} \]  \hspace{1cm} (1)

Where,

- **DMIR** = daily methylmercury intake rate (µg/kg BW-day)
- **CF** = consumption frequency (number of meals/day)
- **MS** = meal size (g/meal)
- **THg** = total mercury concentration (µg/g)
- **BW** = body weight (kg)
- **a** = methylmercury conversion factor (unitless)
- **b** = food preparation/cooking adjustment factor (g/g)

The consumption frequency, meal size for each item, and body weight for each respondent were derived from questionnaire answers.

Current mercury risk assessment is based on methylmercury exposure (U.S. EPA 2011), so total mercury concentrations applied in this equation needed to be converted to methylmercury concentrations. Methylmercury concentrations of catfish, croaker, eel, flounder, perch, seatrout, spot, and striped bass were taken from results in Chapter I. For the species lacking methylmercury information, a methylmercury conversion factor (a) was adopted for converting total mercury concentrations to methylmercury concentrations. The percentage of methylmercury in total mercury for LCB finfish ranged from 55% in spot to 80% in white perch (Xu et al. 2012, unpublished). In contrast, Bloom (1992) reported higher percentages ranging
from 86% in striped seaperch to 112% in stablefish. Some of the variation in percent methylmercury can be explained by the analytical variability of mercury determinations (Bloom 1992), and the different trophic positions of the studied species (Chapter I). The Mercury Study Report to Congress (U.S. EPA 1997) came to the general conclusion that, for purposes of assessing exposure, more than 90% total mercury in fish can be assumed to be methylmercury. Based on this conclusion, the value of 95% was adopted in the national-scale assessment of mercury risk by U.S. EPA (2011), which represents a middle ground between an assumption that all mercury in a fish is methylmercury and the lower bound value of 90% in the Mercury Study Report to Congress. Due to the different percentage of methylmercury in total mercury reported by different studies, the methylmercury conversion factor (a) of 0.95 adopted by U.S. EPA (2011) was used here for the species with no methylmercury information.

Food preparation and cooking will primarily remove moisture and the non-muscle tissue (Burger et al. 2003), potentially increasing mercury concentration per unit of fish mass. Consequently, mercury concentrations of raw muscle obtained from the literature should be adjusted with a food preparation/cooking adjustment factor (mercury concentrations in raw item/mercury concentrations in cooked item, Morgan et al. 1997, Burger et al. 2003) to reflect concentrations after food preparation. In the Holloman and Newman study (2012), the adjustment factor measured for seafood items from grocery stores and markets in the coastal Virginia ranged from 1.1 (perch) to 1.6 (croaker) for finfish, and from 1.2 (snow crab legs) to 1.5 (crab cake) for shellfish. In Burger et al. study (2003), the factor of 1.5 to 1.8 had been reported for largemouth bass, so they suggested a conservative adjustment factor of approximately 2. In addition, the U.S. EPA adopted an adjustment factor of 1.5 in their national-scale assessment of
mercury risk (U.S. EPA 2011), based on estimated factors between 1.1 and 1.5 for walleye and 1.5 and 2.0 for lake trout (Morgan et al. 1997). As the target area of the current study was close to the sampling locations of the Holloman and Newman study (2012), food preparation/cooking adjustment factors (b) of 1.6 for finfish and 1.5 for shellfish were adopted here. This represents the highest adjustment factors in Holloman and Newman (2012) and avoids potentially underestimating the actual human exposure (Burger et al. 2003).

2.8.2. Monte Carlo simulation of daily methylmercury intake rate

Monte Carlo simulation with the Crystal Ball® 11.1.1.1.00 package (Oracle, Redwood Shores, CA, USA) was applied to generate a cumulative probability distribution of daily methylmercury intake rate. Estimated distributions of parameters (consumption frequency, meal size, body weight, and mercury concentrations) in Equation (1) need to be defined before running the model. Customized distributions of consumption frequency and meal size of a seafood group were built directly with the data due to the small sample size that excluded the fitting any conventional distribution to the data. Data on body weight for each community were fit to beta distributions. Mercury (total and methylmercury) concentrations were fit to two-parameter lognormal distributions if the original data were available. It was necessary to assume a normal distribution for concentration data for which only arithmetic means and standard deviations were reported in the literature. Customized distributions were produced for the total mercury distributions of cobia and goby because only the means were provided in the literature. In addition, the individual seafood consumption rates were defined by fitting to two-parameter lognormal distributions. To avoid unrealistic values being generated during simulations, such as negative body weights or extremely high mercury concentrations, a minimum of zero and a
maximum of three standard deviations above the mean were set as limits for each distribution (Wang and Newman 2012).

A dataset of 100 possible seafood items was produced and items were ranked by the relative proportions. Distributions of consumption frequency (number of meals/day), meal size (g/meal), mercury concentrations (µg/g), and the calculated seafood consumption rate (consumption frequency * meal size, g/day) and mercury intake rate (seafood consumption rate * mercury concentrations, µg/day) for each item were also tabulated on the same row in Crystal Ball®. In each model trial the following occurred:

1. The simulation began by randomly selecting an item from the dataset and then picking observation values from the corresponding distributions of seafood consumption rate and mercury intake rate;
2. The simulation continued to randomly select seafood items, and calculate the corresponding seafood consumption and mercury intake as a total amount of seafood and mercury ingested daily;
3. The simulation then randomly picked one value of individual seafood consumption rate (g/person/day) from the lognormal distribution;
4. The simulation stopped when the total amount of seafood consumed reached the selected individual seafood consumption rate, and the adjusted amount of mercury ingested daily was calculated;
5. At this step, one value of body weight was randomly selected from its beta distribution;
6. Finally, the adjusted amount of mercury ingested daily (µg/day) was divided by the selected body weight to yield a daily methylmercury intake rate (µg/kg BW-day).

The procedure of each trial simulated a hypothetical community member’s daily seafood consumption. The model included 10,000 trials that produced a distribution of 10,000 estimates of daily methylmercury intake rates for the community.
2.8.3. Monte Carlo simulation of annual-average daily methylmercury intake rate

The U.S. EPA Oral Reference Dose (RfD) for methylmercury was 0.1 μg/kg BW-day, the estimate of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (U.S. EPA, 2001a). Mercury concentrations in maternal hair and cord blood monitored over the long term (months to years) from three epidemiological studies in the Seychelles Islands (Myers et al. 1995a-c, 1997, Davidson et al. 1995, 1998), the Faroe Islands (Grandjean et al. 1997), and New Zealand (Kjellstrom et al. 1986, 1989) were used to predict daily dietary intake levels of methylmercury and to establish the U.S. EPA RfD value.

The 10,000 estimates of daily methylmercury intake rates generated in the model described above simulated 10,000 hypothetical daily mercury intake values. In order to compare the Monte Carlo results to the U.S. EPA RfD, an annual-average daily methylmercury intake rate must also be modeled by Monte Carlo simulation.

(1) The 10,000 simulated values of daily methylmercury intake were fit to a lognormal distribution;

(2) In each trial of the model, 365 (based on 365 days of a year) values were selected randomly from the lognormal distribution of daily methylmercury intake rates, and the arithmetic means of the 365 values were calculated as the annual-average daily mercury intake rate.

The procedure simulated the distribution of the community’s annual-average daily methylmercury intake. The model generated 10,000 values of annual-average daily methylmercury intake rate and a cumulative probability distribution of these intake rates was
produced. The cumulative probability distribution was plotted, and the arithmetic mean, standard deviation, and median of the 10,000 values were determined. These plotted distributions were compared to the U.S. EPA oral reference dose (RfD) for methylmercury of 0.1 μg/kg BW-day (U.S. EPA 2001a) and methylmercury dose value that resulted in adverse health effects.

3. Results

3.1. Participation Rates

A total of 140 people in the four communities responded to the survey, and the average participation rate was 41% (Table 2). The number of church members was counted on four consecutive Sundays and estimated as an mean. The survey participation rates differed among communities, ranging from 37% in the Vietnamese church to 45% in the Chinese church.

3.2. Seafood Consumption Patterns

The relative proportion for a specific seafood item was calculated by dividing its consumption frequency by the reported number of all items, which was then treated in Monte Carlo simulations as the probability of that seafood item being consumed. Data of the two general churches were pooled for presentation here, and the relative proportions of commonly consumed seafood items were presented in Table 3. Shrimp purchased from grocery stores or seafood markets was the most frequently consumed items for all three communities, and blue
crab was the most frequently consumed local species (Table 3). Two out of the top five commonly consumed items were local species in the general and the Chinese church communities, but four out of the top five items were local species in the Vietnamese church community (Table 3). The summed relative proportions of consumed market fish species in the general population, Chinese, and Vietnamese church were 52%, 67%, and 51%, and the summed relative proportions of consumed local species were 48%, 33%, and 49%. Market fish were consumed more frequently compared to the local species especially for the Chinese church with a higher difference between relative proportions of market and local species. Although four out of the top five commonly eaten species were from local waters, the studied Vietnamese communities still consumed market fish more frequently than local species.

3.3. Individual Seafood Consumption Rates

Means and 95% confidence intervals of individual seafood consumption rates in the general population church-1 (Unity Fellowship Church), general population church-2 (Gloucester Point Baptist Church), Chinese Church, and Vietnamese Church were 33.0 g/person/day (95% CI: 5.3-60.8, n = 15), 28.3 g/person/day (95% CI: 15.2-41.3, n = 47), 36.9 g/person/day (95% CI: 25.1-48.7, n = 45), and 52.7 g/person/day (95% CI: 31.1-74.3, n = 33) g/day, respectively. For the U.S. population (male and female from 14 years old to 45 and older), the daily consumption rate of prepared fish (finfish and shellfish from freshwater, estuarine and marine) was estimated to be 12.8 g/person/day (90% CI: 12.05-13.61) (U.S. EPA 2002). Except the general population church-1, individual seafood consumption rates of the other church
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communities were significantly higher than 12.8 g/day as gauged by their non-overlapping confidence intervals.

3.4. Consumption Rates of Seafood Groups

The seafood consumption rates of each seafood group (category) were materially different for the studied communities (Figure 1, Table 4). Data from the two general population church groups were combined as the reference group in this figure. For the market fish, all studied communities favored shrimp, especially the Vietnamese community which had a shrimp consumption rate of approximately 1.3g/day. The communities from the general population churches and the Chinese church both consumed large amounts of salmon. Tuna was only consumed at a high rate in the general population church communities. For the local fish, people from the Vietnamese church tended to eat a large amount of diverse local species. In contrast, people attending the general population churches favored blue crab and flounder, and people from Chinese church ate only modest amounts of local species.

3.4. Mercury Intake Rates of Seafood Groups

Tuna, salmon, and crabs (snow crab and king crab) were the primary species that contributed to mercury intake by the communities of the general population church and the Chinese church; however, tuna, mackerel, and crabs contributed the major amount of mercury to the Vietnamese church (Figure 2, Table 4). People from the general population churches and Chinese church took in most of their mercury from market fish. However, people from the
Vietnamese church took in mercury from both the market and locally harvested species such as croaker, striped bass, and catfish. In addition, snapper and crayfish were species only consumed by Chinese church communities that also contributed materially to mercury intake.

3.5. Hair Mercury Concentrations

The overall geometric mean of hair total mercury in U.S. female was 0.20 μg/g (Margaret et al. 2004, Table 5). No statistical difference of mean hair mercury concentrations was identified between communities of the general population church and the NHANES value of 0.20 μg/g (Margaret et al. 2004). However, the geometric mean of hair mercury concentrations in the Chinese church and Vietnamese church were both higher than 0.20 μg/g (P<0.01). Frequencies with which hair mercury concentrations were higher than or equal to the value of 0.2 μg/g were 46%, 90% and 100% in the general population churches, the Chinese church, and the Vietnamese church, respectively (Figure 3).

3.6. Seafood Consumption and Hair Mercury Level

Simple linear regression was developed in SAS for mean individual seafood consumption rates and mean hair mercury concentrations of the four studied communities (Figure 4). A strong positive relationship was identified: [Hair mercury] = 0.05 [seafood consumption rates] - 1.29. The regression $r^2$ was 0.98 and the 95% CI of the slope was 0.03 to 0.07. Consequently, hair mercury concentration will increase 0.05 (μg/g) with an increase in seafood consumption rate of 1 gram per day for the studied communities. Dietary mercury ingestion through seafood was
concluded to be positively related to mercury exposures in the studied church communities of coastal Virginia.

3.7. **Daily Methylmercury Intake Rate**

Data from the two general population churches were pooled to generate a reference group. Arithmetic means and 95% confidence intervals of daily methylmercury intake rate generated by the Monte Carlo simulation in the general population church, the Chinese church, and the Vietnamese church populations were 0.046 µg/kg BW-day (95% CI: 0.045-0.048), 0.092 µg Hg/kg BW-day (95% CI: 0.088-0.095), 0.119 µg Hg/kg BW-day (95% CI: 0.116-0.122), respectively. The mean daily methylmercury intake rate in the pooled general church community was statistically lower than the value of the Chinese church communities (P<0.01), which was itself statistically lower than the value of the Vietnamese church communities (P<0.01).

3.8. **Annual-average Daily Methylmercury Intake Rate**

Arithmetic means and 95% confidence intervals of the annual-average daily methylmercury intake rate generated by the Monte Carlo simulation in the general population church, the Chinese church, and the Vietnamese church were 0.04014 µg Hg/kg BW-day (95% CI: 0.04008-0.04021), 0.06825 µg Hg/kg BW-day (95% CI: 0.06817-0.06833), 0.07737 µg Hg/kg BW-day (95% CI: 0.07724-0.07750), respectively. The mean daily methylmercury intake rate of Vietnamese church communities was statistically higher than the value of the Chinese church communities (P<0.01), which was itself statistically higher than the value of the general
church populations (P<0.01). The means and upper 95% confidence limits for all studied communities were below the U.S. EPA RfD of 0.1 μg/kg BW-day. Annually, there was no chance that members of the studied communities would take in more mercury than the U.S. EPA RfD (Figure 5).

4. Discussion

4.1. Community Seafood Consumption

Seafood consumption rates varied among the studied communities. Except for the general population church-1 (Unity Fellowship Church), individual seafood consumption rates (g/person/day) of the general population church-2 (Gloucester Baptist Church), the Chinese and the Vietnamese communities in coastal Virginia were statistically higher than the daily fish consumption rate of 12.8 g/person/day (90% CI: 12.0-13.6) reported for the U.S. population for both sexes and all ages (U.S. EPA 2002). Difference of consumption rate between the two general churches could be influenced by their different sample sizes. The small sample size (n = 15) from general population church-1 resulted in a wide 95% confidence intervals that included the value of 12.8 g/person/day.

Seafood consumption patterns were distinct for these communities (Figure 1, Table 4), and they were also different from those reported for the nearby African-American community (Holloman and Newman 2010). High consumption rate by African-American women was
estimated as 147.8 g/person/day, which was much higher than the individual consumption rates for the LCB communities of this study.

Compared to the general communities, the Chinese community reported a larger seafood meal size (arithmetic mean: 248.6 g/meal; 95% CI: 165.8-331.4) than the general population churches (arithmetic mean: 156.2 g/meal; 95% CI: 127.4-175.0) (P<0.05), and the Vietnamese community reported an even higher seafood consumption frequency (arithmetic mean: 11.3 meals/month; 95% CI: 8.5-14.2) than the general population church (arithmetic mean: 4.2 meals/month; 95% CI: 3.3-5.1) (P<0.01). The study of seafood consumption on 260 Vietnamese refugees in northern Florida also reported a high consumption frequency of 32 meals/month (Crane and Green 1980). However, their results are not directly comparable to ours because the Vietnamese sampled in the current study are not recent refugees.

Relative proportions of those surveyed who reported consuming local fish were 48%, 33%, and 49% in the polled general, Chinese, and Vietnamese church communities, respectively. The general and Vietnamese communities tended to select their seafood items from both the local water and the market, but the Chinese community showed more of a preference for market fish (Table 3 and 4, Figure 1). Reasons for this difference might be related to the fishing activities in a community. Only 6% of the respondents in the Chinese community reported that they often fished in the summer, but 29% and 43% of the respondents in the general and the Vietnamese communities reported fishing in the spring, summer and fall. High proportion of recreational fishers in the Vietnamese community was one important contributor to their high consumption rates of diverse local species (Figure 1, Table 4).
People with different ethnic backgrounds do not eat fillets only, but also consume other parts of finfish (Sechena et al. 2003). Fish head, roe and stomach were consumed by people from the studied ethnic groups; skin, tail, bones and eyes were also consumed by people from the Chinese community. In contrast, no respondents in the general communities reported consumption of the finfish parts other than the fillet. Because mercury concentration varies in different parts of finfish (Newman et al. 2011 b), such differences could further contribute to differences in seafood consumption risks between some ethnic communities and the general population.

4.2. Community Mercury Intake

Seafood consumption patterns contribute importantly to dietary mercury intake of these communities. The studied Chinese and general communities both received most of their mercury exposure from commercial market fish (Figure 1 and 2, Table 4). In contrast, the Vietnamese community was exposed to mercury through the consumption of both, market and local fish (Figure 1 and 2, Table 4). The species contributing the most ingested mercury for the general, Chinese, and Vietnamese church communities were tuna, salmon and mackerel (Figure 2).

The geometric mean and cumulative frequencies of daily methylmercury intake rate and the annual-average daily methylmercury intake rate of the Vietnamese community were statistically higher than those for the Chinese church community (P<0.01), which in turn, were also statistically higher than the values of the general church communities (P<0.01). Though
mercury exposures were lower than the U.S. EPA RfD, the Vietnamese exposure was the highest of the studied communities due to their seafood consumption habits.

4.3. Mercury Exposure

Total mercury concentrations in blood and hair are common indicators of methylmercury exposure through fish consumption for people who are not occupationally exposed to inorganic mercury (Carrington and Bolger 2002, Iwasaki et al. 2003). Hair total mercury in this study was used to assess the validity of estimates derived from food frequency questionnaire and provide a more accurate estimate of actual mercury exposure through fish consumption (Mina et al. 2007).

According to the World Health Organization (WHO), health effects were not apparent in adults with hair total mercury as high as 50 μg/g (Tsubaki 1968), and apparent fetal effects would be unlikely if maternal hair total mercury was less than 10 μg/g for pregnant women (Grandjean et al. 1997). The published exposure threshold of the WHO is 14 μg/g of mercury concentration in the hair. All observed hair total mercury concentrations in this study were below the WHO threshold although hair total mercury from the Chinese and Vietnamese were statistically higher than the U.S. general concentration of 0.2 μg/g (McDowell et al. 2004). Correspondingly, all calculated methylmercury intake rates from Monte Carlo simulation were lower than the EPA oral RfD of 1 μg/kg BW-day (U.S. EPA, 2001 a, 2001 b). Hair mercury concentrations for the women in the nearby African American community can be predicted by the linear regression between hair mercury and consumption rates (Figure 4). With the reported seafood consumption rate of 147.8 g/day (95% CI: 117.6-185.8) (Holloman and Newman 2010),
their hair mercury concentrations would be 6.1 μg/g (95% CI: 4.6-8.0), which was still lower than the WHO threshold.

Hair analysis is a reliable and convenient way to determine personal mercury exposure. The clear advantages are that mercury in the hair is not remobilized once deposited and sampling is simple. The Lower Chesapeake Bay Seafood Consumption Survey was designed on an annual-average basis. Assuming the hair growth rate of 1 cm per month, a segment of 12 cm hair measured from the scalp would correspond to that deposited in one year. Only 40% of the respondents’ hair samples were of sufficient length to obtain 12 cm. Consequently, the proximal 2 cm of each respondent’s hair from the scalp was analyzed and was assumed to reflect mercury exposure during the year. Since hair samples were taken during the season of greatest fish consumption (springs and summers), the estimated annual-average exposure reflected by hair results would likely be higher than the actual annual exposure. Also the influence of hair treatment on hair mercury levels was not included in this study. The NHANES study (McDowell et al. 2004) indicated there was no difference in hair total mercury concentrations between the treated hair and untreated hair groups, but Dakeishi (2005) found out that the process of artificial hair-waving could reduce hair total mercury concentrations by approximately 30%.

4.4 Survey biases

(1) Recall bias is a characteristic of surveys asking about a long term consumption habits (Sechena et al. 2003).

(2) Participation rates of the four churches were lower than 50%. Small sample size could have biased the results to an undetermined degree; and for this reason, the relationship between
demographic questions and mercury exposure were not explored in more depth. In addition, participation rates differed among communities.

(3) It was difficult to randomly sample the targeted populations and select completely unbiased samples. Willingness to engage in the survey might have been lower for people who do not usually eat fish, or people who eat large amounts of fish and would be unwilling to change their diets. Only one respondent in the Gloucester Point Baptist Church reported not being a consumer of seafood.

(4) The survey method of written questionnaires might create some misunderstandings of the questions, particularly after translation to the primary community language.

(5) Imprecision of answers were checked with a quality control procedure. Questions about important information (e.g., seafood items, consumption frequency, and meal size) were asked in two ways and the answers were checked for the relative percent difference. The average relative percent difference of meal size and consumption frequency was 22%. The memory imprecision would either decrease or increase the calculated mercury exposure. However, the author believes that the inclusion of specific validations of hair total mercury concentration provide a reasonable certainty as to the accuracy of the estimated consumption data produced in this study.

5. Conclusion
Fish consumption patterns differed among communities, which resulted in different levels of mercury ingestion. People from the general population churches and Chinese church took in most of their mercury from market fish whereas people from the Vietnamese church took in mercury from both the market and local fish as they tended to eat a large amount of diverse local species. Individual seafood consumption rates for the Chinese and Vietnamese communities were higher than the general U.S. fish consumption rate of 12.8 g/person/day.

Dietary mercury ingestion through seafood was positively related to hair mercury concentrations. Hair total mercury concentrations in the Chinese and the Vietnamese church were higher than the overall level for U.S. women (0.20 μg/g), but lower than the published WHO exposure threshold of 14 μg/g.

The annual-average daily methylmercury intake rate calculated by Monte Carlo simulation indicated a higher mercury exposure of the Vietnamese community compared to the Chinese community, and a higher exposure of the Chinese community compared to the general churches communities. Regardless, the daily methylmercury intake rates for all studied communities were lower than the U.S. EPA RfD of 0.1 μg/kg BW-day.

Although mercury exposure levels in the studied ethnic communities were explored to be lower than the level of the general U.S. populations and the EPA threshold, the relatively higher seafood and mercury ingestion rates of ethnic communities compared to the general populations suggests the need for specific seafood consumption advice related to contaminants for ethnic groups within the lower Chesapeake Bay region of Virginia.
Acknowledgements

The minister Muliang Gong of the Peninsula Chinese Baptist Church, Joseph Phien Nguyen of Our Lady of Vietnam Chapel, Ginny Roll of the Unity Fellowship Church, and Bud Goude of the Gloucester Point Baptist Church communicated the goal of this study to their church communities and encouraged people to take the survey. Solomon Chak, Yuan Dong, Vi Nguyen, Harry Wang, and Jude Eastman assisted by communicating with people, sending out and taking back questionnaires, and taking hair samples.
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fish diet on developmental milestones in the Seychelles Child Development Study. Neurotoxicology 18, 819-830.


United States Environmental Protection Agency. 2009. Fish contamination in U.S. lakes and reservoirs, EPA-823-F-09-008


TABLE 1  Arithmetic mean and standard deviations of mercury concentrations for uncooked seafood items.

Notes:
*: Only means and standard deviations were reported for the species. Primary data were available for species without asterisk.
:- No values were reported.

Latin names: blue crab (Callinectes sapidus), catfish (a mixture of Ictalurus furcatus, Ictalurus punctatus, and Ictalurus catus), crayfish (Astacoidea), croaker (Micropogonias undulatus), eel (Anguilla rostrata), summer flounder (Paralichthys dentatus), perch (Morone americanus), seatrout (Cynoscion regalis), spot (Leiostomus xanthurus), striped bass (Morone saxatilis), cod (Gadus morhua), crabs (a mixture of Callinectes sapidus, Cancer irroratus, Cancer magister, Chinoecetes spp., Menippe mercenaria, Paralithodes camtschatica), goby (Acanthogobius flavimanus), mussels (Mytilus edulis), snapper (Lutjanus campechanus), squid (not mentioned in the reference), tuna (A mixture of Euthynnus alletteratus, Katsuwonus pelamis, Thunnus alalunga, Thunnus albacares, Thunnus atlanticus, Thunnus thynnus).

Scientific names of clams, oyster, scallop, scup, butterfish, dolphin, kingfish, lobster, mackerel, salmon, sardine, shrimp, tilapia, and whiting were not reported.

LCB: lower Chesapeake Bay
<table>
<thead>
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<th>Species</th>
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<th>Methylmercury (μg/g)</th>
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<th>Sampling location</th>
<th>Reference</th>
</tr>
</thead>
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<td>SD</td>
<td>Mean</td>
<td>SD</td>
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<td>LOCAL FISH</td>
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<td></td>
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<td>Snapper*</td>
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<td>Squid</td>
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<td>Tilapia</td>
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<td>Tuna (fresh)*</td>
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<td>Whiting</td>
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</table>
TABLE 2  Survey participation rates by communities.

<table>
<thead>
<tr>
<th>Community</th>
<th>Number of respondents</th>
<th>Number of church members</th>
<th>Participation rates</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>General population church-1</td>
<td>15</td>
<td>35</td>
<td>42.9%</td>
<td>30.1%-56.7%</td>
</tr>
<tr>
<td>(Unity Fellowship Church)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General population church-2</td>
<td>47</td>
<td>120</td>
<td>39.2%</td>
<td>32.1%-46.7%</td>
</tr>
<tr>
<td>(Gloucester Point Baptist Church)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chinese church</td>
<td>45</td>
<td>100</td>
<td>45.0%</td>
<td>37%-53.2%</td>
</tr>
<tr>
<td>Vietnamese church</td>
<td>33</td>
<td>90</td>
<td>36.7%</td>
<td>28.8%-45.3%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>140</td>
<td>345</td>
<td>40.6%</td>
<td>36.3%-45.0%</td>
</tr>
</tbody>
</table>
TABLE 3  Relative proportions of consumption frequency for commonly consumed seafood items in different communities.

Note:
*: local fish. Species without asterisks were market fish.
<table>
<thead>
<tr>
<th>Species</th>
<th>General population church</th>
<th>Chinese church</th>
<th>Vietnamese church</th>
</tr>
</thead>
<tbody>
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<td></td>
<td>Relative proportion</td>
<td>95% CI</td>
<td>Relative proportion</td>
</tr>
<tr>
<td>Shrimp</td>
<td>14.6%</td>
<td>10.9%-19.3%</td>
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<td>Salmon</td>
<td>11.5%</td>
<td>8.2%-15.8%</td>
<td>Salmon</td>
</tr>
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<td>Blue crab*</td>
<td>11.5%</td>
<td>8.2%-15.8%</td>
<td>Blue crab*</td>
</tr>
<tr>
<td>Flounder*</td>
<td>10.9%</td>
<td>7.8%-15.2%</td>
<td>Tuna</td>
</tr>
<tr>
<td>Tuna</td>
<td>9.9%</td>
<td>6.9%-14%</td>
<td>Tilapia</td>
</tr>
<tr>
<td>Oyster*</td>
<td>5.3%</td>
<td>2.4%-11.4%</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 4  Community-based seafood consumption rates (g/person/day) and total mercury ingestion rates of major reported items (μg/person/day) for studied communities.

Note:
- : seafood consumption rates and mercury ingestion rates were not reported by the community.
<table>
<thead>
<tr>
<th>Species</th>
<th>Seafood Consumption Rate (g/person/day)</th>
<th>Mercury Intake Rate (μg/person/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>General</td>
<td>Chinese</td>
</tr>
<tr>
<td>shrimp</td>
<td>3.07</td>
<td>4.22</td>
</tr>
<tr>
<td>salmon</td>
<td>3.34</td>
<td>5.72</td>
</tr>
<tr>
<td>tuna</td>
<td>3.59</td>
<td>1.17</td>
</tr>
<tr>
<td>crabs</td>
<td>1.32</td>
<td>1.22</td>
</tr>
<tr>
<td>mackerel</td>
<td>-</td>
<td>0.08</td>
</tr>
<tr>
<td>tilapia</td>
<td>0.78</td>
<td>0.90</td>
</tr>
<tr>
<td>snapper</td>
<td>0.00</td>
<td>1.33</td>
</tr>
<tr>
<td>cod</td>
<td>0.27</td>
<td>1.02</td>
</tr>
<tr>
<td>whiting</td>
<td>0.21</td>
<td>-</td>
</tr>
<tr>
<td>squid</td>
<td>0.15</td>
<td>0.13</td>
</tr>
<tr>
<td>mussels</td>
<td>0.08</td>
<td>0.23</td>
</tr>
<tr>
<td>lobster</td>
<td>0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>other</td>
<td>0.02</td>
<td>0.11</td>
</tr>
<tr>
<td>BLUE CRAB</td>
<td>1.59</td>
<td>0.76</td>
</tr>
<tr>
<td>FLOUNDER</td>
<td>2.70</td>
<td>0.17</td>
</tr>
<tr>
<td>CROAKER</td>
<td>0.51</td>
<td>0.24</td>
</tr>
<tr>
<td>SPOT</td>
<td>0.34</td>
<td>-</td>
</tr>
<tr>
<td>CLAM</td>
<td>0.45</td>
<td>0.05</td>
</tr>
<tr>
<td>CATFISH</td>
<td>0.17</td>
<td>0.31</td>
</tr>
<tr>
<td>STRIPED BASS</td>
<td>0.14</td>
<td>0.00</td>
</tr>
<tr>
<td>SCALLOP</td>
<td>1.21</td>
<td>0.34</td>
</tr>
<tr>
<td>SCUP</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PERCH</td>
<td>0.14</td>
<td>0.68</td>
</tr>
<tr>
<td>CRAYFISH</td>
<td>-</td>
<td>0.45</td>
</tr>
<tr>
<td>OYSTER</td>
<td>1.33</td>
<td>0.28</td>
</tr>
<tr>
<td>OTHER</td>
<td>0.12</td>
<td>0.08</td>
</tr>
</tbody>
</table>
TABLE 5  Hair totals mercury concentrations (μg/g) from the current study and the NHANES study (McDowell et al. 2004).

<table>
<thead>
<tr>
<th></th>
<th>NHANES</th>
<th>General population church-1</th>
<th>General population church-2</th>
<th>Chinese church</th>
<th>Vietnamese church</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>1726</td>
<td>15</td>
<td>47</td>
<td>45</td>
<td>33</td>
</tr>
<tr>
<td>Geometric mean</td>
<td>0.20</td>
<td>0.28</td>
<td>0.21</td>
<td>0.52</td>
<td>1.46</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.16-0.24)</td>
<td>(0.14-0.56)</td>
<td>(0.16-0.28)</td>
<td>(0.41-0.65)</td>
<td>(1.21-1.76)</td>
</tr>
<tr>
<td>Arithmetical mean</td>
<td>0.47</td>
<td>0.43</td>
<td>0.32</td>
<td>0.69</td>
<td>1.68</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(0.35-0.58)</td>
<td>(0.05-0.81)</td>
<td>(0.22-0.42)</td>
<td>(0.51-0.86)</td>
<td>(1.31-2.04)</td>
</tr>
<tr>
<td>10th</td>
<td>0.04</td>
<td>0.11</td>
<td>0.08</td>
<td>0.22</td>
<td>0.69</td>
</tr>
<tr>
<td>25th</td>
<td>0.09</td>
<td>0.16</td>
<td>0.11</td>
<td>0.30</td>
<td>1.15</td>
</tr>
<tr>
<td>50th</td>
<td>0.19</td>
<td>0.18</td>
<td>0.20</td>
<td>0.57</td>
<td>1.49</td>
</tr>
<tr>
<td>75th</td>
<td>0.42</td>
<td>0.65</td>
<td>0.40</td>
<td>0.82</td>
<td>1.85</td>
</tr>
<tr>
<td>90th</td>
<td>1.11</td>
<td>1.58</td>
<td>0.87</td>
<td>1.44</td>
<td>2.75</td>
</tr>
<tr>
<td>95th</td>
<td>1.73</td>
<td>1.58</td>
<td>1.00</td>
<td>1.75</td>
<td>3.76</td>
</tr>
</tbody>
</table>
FIGURE 1  Community-based consumption rates of major reported items (g/person/day).

The distance between each concentric ring represents 2 g/person/day. Names in capital letters identify local fish and those in lowercase indicate market fish.
FIGURE 2  Mercury ingestion rates of major reported items (μg/person/day).

The distance between each concentric ring of market fish represents 0.4 μg/person/day, and the distance between each concentric ring of local fish represents 0.1 μg/person/day. Capital letters represent names of local fish and lowercase letters represent names of market fish.
FIGURE 3  Cumulative frequencies of hair total mercury concentrations of respondents in the general population churches, the Chinese church, the Vietnamese church, and the NHANES studies.
FIGURE 4  Relationship between individual seafood consumption rates and hair total mercury concentrations of the studied communities. A: general population church-1, B: general population church-2, C: Chinese church, D: Vietnamese church. The solid line, the area of shadow, and the area of dashed line in indicated the fit of these data, 95% confidence limits, and 95% prediction limits.
FIGURE 5  Cumulative frequency of annual-average daily methylmercury intake rates (µg/kg BW-day) among different communities.
SUMMARY
1. Mercury in local fish

There is no evidence suggesting possible human harm from mercury in the selected lower Chesapeake Bay finfish species. All mercury concentrations were lower than the human health screening value of 0.3 μg/g. Trophic position emerged as the most important determinant of interspecies mercury concentration differences. Effects of dietary food sources, residence time in different salinities, and other factors on mercury accumulation in the ten species examined in this study were less obvious.

The ability to interpret data using a trophic framework is useful for predicting mercury concentrations of unsampled species or locations particularly for freshwater and marine species in estuarine environments, and can help understand variation in mercury concentrations within and among species, locations, and years. This study adopted such a context for mercury in commonly eaten finfish from lower Chesapeake Bay, and also established a much needed mercury database. It will be used as a fisheries tool for understanding and communicating mercury risks associated with local fish consumption, informing seafood consumers (market, recreational or tourism-related consumption), and potentially dispelling misperceptions about fish safety.

2. Mercury ingestion through fish consumption

Seafood consumption patterns contribute importantly to dietary mercury intake of these communities. Individual seafood consumption rates for the Chinese and Vietnamese communities were higher than the general U.S. fish consumption rate of 12.8 g/person/day. People from the general population churches and Chinese church took in most of their mercury
from market fish (distributed and sold nationally) whereas people from the Vietnamese church took in mercury from both the market and local fish as they tended to eat a large amount of diverse local species. The species contributing the most ingested mercury for the general, the Chinese, and the Vietnamese church communities are tuna, salmon and mackerel.

3. Mercury exposure assessment

Dietary mercury ingestion through seafood was positively related to hair mercury concentrations. Hair total mercury concentrations in the Chinese and the Vietnamese church were higher than the overall level for U.S. women (0.20 μg/g), but lower than the published WHO exposure threshold of 14 μg/g. The annual-average daily methylmercury intake rate calculated by Monte Carlo simulation indicated a higher mercury exposure of the Vietnamese community compared to the Chinese community, and a higher exposure of the Chinese community compared to the general churches communities. Regardless, the daily methylmercury intake rates for all studied communities were lower than the U.S. EPA RfD of 0.1 μg/kg BW-day.

In summary, fish consumption patterns differed among communities, which resulted in different levels of mercury exposure. Though examined fish mercury concentrations were lower than the human health screening value, the higher seafood and mercury ingestion rates of ethnic groups compared to the general populations suggest the need for specific seafood consumption advice for ethnic groups within the lower Chesapeake Bay region of Virginia.
APPENDIX

APPENDIX 1. Lower Chesapeake Bay Seafood Consumption Surveys
Name: ______________________(print)  Home address: ______________________________________(print)

SEAFOOD means FISH or SHELLFISH, including FROZEN, FRESH, and PROCESSED products. Please consider seafood eating during BREAKFAST, LUNCH, DINNER, or

1. Age____  Gender____  Body weight ____ (lbs) OR ____ (kg)  Ethnic group ______________________

2. Do you eat seafood?  □ YES (Please continue)  □ NO (Please go to part-2 directly)

3. HOW OFTEN do you usually have a seafood meal? Please choose only one answer.

<table>
<thead>
<tr>
<th></th>
<th>&lt;1 Per month</th>
<th>1 Per month</th>
<th>2 Per month</th>
<th>3 Per month</th>
</tr>
</thead>
<tbody>
<tr>
<td>everyday</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3 everyday</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. How much weight of seafood do you usually eat for one meal? ______

A 3-OUNCE portion of fish = a deck of cards, or a checkbook.

- You can use ANY WEIGHT UNIT (ounce (oz), pound (lbs), gram (g)...).
- Please DON'T INCLUDE weights of SIDE DISHES or INEDIBLE PARTS (lobster or clam shells).

5. Please check the seafood you USUALLY eat. The seafood pictures can help you identify fish names.

FISH: □ Bass (margemouth bass _smallmouth bass _striped bass/rockfish) □ Bluefish □ Butterfish □ Carp □ Catfish □ Cod □ Crappie □ Croaker □ Dogfish □ Drum □ Eel □ Flounder/sole/fluke □ Halibut □ Herring □ Kingfish □ Mackerel □ Monk fish/goosefish □ Perch □ Porgie/sea bream □ Salmon □ Sardines □ Sea trout/weakfish □ Shads □ Smelt □ Snapper □ Snow fish/Chilean sea bass □ Spot □ Sturgeon □ Suckers □ Tuna (light white) □ Tilapia □ Whiting/hake

SHELLFISH: □ Abalone □ Ark shell □ Blue crab □ Butter clam □ Dungeness crab □ Geoduck clam □ Hard clam/quahog □ Horse clam □ King crab □ Lobster □ Macoma clam □ Moonsnail □ Mussels □ Oyster □ Razor clam □ Rock crab □ Scallop □ Sea cucumber □ Sea urchin □ Shrimp □ Snow crab □ Soft clam/longneck □ Squid □ Surf clam/ocean clam □ Whelks

Please indicate OTHER fish you usually eat but not listed above __________________________
6. Please list the seafood items you USUALLY eat and answer related questions:

<table>
<thead>
<tr>
<th>Seafood name (Please indicate the SPECIFIC NAME, and avoid using vague names as &quot;crab or fish&quot;)</th>
<th>How much do you eat this item PER MEAL? (You can use any weight unit, and don't include inedible parts)</th>
<th>How many times do you eat this item PER MONTH?</th>
<th>Where do you usually get this item? (Multiple choices)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXAMPLE: blue crab</td>
<td>4oz (or 110g)</td>
<td>1 4 3 1</td>
<td>Self-caught ✓</td>
</tr>
</tbody>
</table>
7. Have you dyed, colored, highlighted, permed or bleached your hair in the last 6 months?
   - YES  NO
Did you put gel, oil, creams, hair spray or any other hair product on your hair today?
   - YES  NO

How to collect hair sample?

a) Choose two areas on your scalp.
b) Cut hair from the scalp.
c) The total weight should be about 0.1 to 0.25 grams.

8. Are you born in the US?
   - YES  NO (Please indicate how many YEARS you have been stayed in the US__________)

9. Do you live in Virginia currently?
   - YES  NO (Please indicate the time you lived in Virginia: from YEAR ____ to YEAR ____)

10. Please choose the closest percentage of western style cuisine in your usual diet:
    - 0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

11. Please check the seasons that you usually go fishing fish
    - Never  Spring  Summer  Fall  Winter

12. Did you make a major change in your diet in the last 5 years? (For example, you became a vegetarian, or you stopped eating a certain food like pork, crayfish, kiwi, nuts...)
    - NO
    - YES (Please indicate the details________________________________________________________)

13. Do you eat other parts of the fish except the fish fillet?
    - NO  YES (Please indicate: which parts__________, and which fish ______________________)

    Thank you for your cooperation! We’ll give you the results of your hair mercury in a month!
姓名或缩写：____________________

家庭住址：______________________________（只需要回答街道和城市名称）

年龄 ______ 性别 ______ 体重 ___________ 种族 ___________

海鲜是鱼、虾、蟹、螺、贝类等的统称，包括冷冻的、新鲜的和加工过的（例如腌制、晒干、瓶装、塑料包装、罐装）产品。请把早餐、中餐、晚餐以及零食（例如鱿鱼丝、蟹黄蛋糕、生鱼片、海鲜汤和海鲜沙拉）中食用的海鲜都考虑在你的回答范围之内。

1. 你来美国居住多久了？ ________________

2. 来美国之前，你在哪个国家的哪个城市居住时间最长？ ____________国 ____________市

3. 你吃海鲜么？ □ 吃（请继续回答下面的问题） □ 不吃（请直接跳到第二页的“第二部分”）

4. 请回答西式饮食在你的整体饮食中的比例。请选择一个最接近的答案：
   ○ 0% ○ 10% ○ 20% ○ 30% ○ 40% ○ 50% ○ 60% ○ 70% ○ 80% ○ 90% ○ 100%

5. 过去5年中，你的饮食习惯是否发生过巨大改变？（例如：你成为了素食主义者；或者你停止了对某些食物的食用，如猪肉、贝类、猕猴桃、坚果……）
   □ 否
   □ 是（请具体说明__________________________________________）

6. 你大概多久吃一次海鲜？请选择一个最接近的答案：
   ○ 一个月少于1次（请说明多久吃一次 ___________ ） ○ 一个月1次 ○ 一个月2次 ○ 一个月3次
   ○ 一周1次 ○ 一周2次 ○ 一周3次 ○ 一周4次 ○ 一周5次 ○ 一周6次
   ○ 每天1次 ○ 每天2次 ○ 每天多于2次(请说明每天吃几次______________)

7. 请估计一下，平时你一餐吃多少重量的海鲜？ ________________
   你可以选择任何熟悉的重量单位来回答这道题，例如“盎司，磅，克，斤，两等”。
   请排除配菜和不可食用部分的重量，例如龙虾、贝类或者螃蟹的壳。
   桌子上已经称重的海鲜样品可以帮助你回答这个问题。

8. 除了鱼的肉质部分，你还食用鱼的其它部分么？
   □ 否
   □ 是（请说明你还食用哪一种鱼的哪个部分__________________________________________）
9. 请标出你经常食用的海鲜种类（桌子上的图片能够帮你回想各种海鲜的名称）。
鱼：
- Bass 鲈鱼（如知道请指出具体种类：□ black/largemouth bass 大口黑鲈/加州鲈
  □ brown/smallmouth bass 小口黑鲈 □ striped bass/rockfish 花条鲈/海鲈）
- Bluefish 蓝鱼 □ Butterfish 鳅鱼 □ Carp 鲤鱼 □ Catfish 鲶鱼 □ Cod 啮鱼
- Crappie 刺盖太阳鱼/小翻车鱼 □ Croaker 黄花鱼 □ Dogfish 弓鳍鱼/狗鱼/泥鱼 □ Drum 敲鱼
- Eel 鳗鱼 □ Flounder/sole/fluke 比目鱼 □ Halibut 大比目鱼 □ Herring 青鱼 □ Kingfish 石首鱼
- Mackeral 鳀鱼 □ Monk fish/goosefish 安康鱼/鹅嘴鱼 □ Perch 河鲈鱼 □ Porgie/sea bream 灯鱼
- Salmon 三文鱼 □ Sardines 沙丁鱼 □ Sea trout/weakfish 海鳟鱼 □ Shads 鮰鱼 □ shark 鲨鱼
- Smelt 香鱼 □ Snapper 鲷鱼 □ Snow fish/Chilean sea bass 智利海鲈鱼 □ Spot 斑鱼 □ Sturgeon鲟
- Suckers 腮脂鱼 □ swordfish 剑鱼 □ Tuna 吞拿鱼/金枪鱼（□ light □ white)
- Tilapia 罗非鱼/吴郭鱼/非洲鲫鱼 □ Whiting/hake 白鱼
贝：
- Abalone 鲍鱼 □ Ark shell 赤贝 □ Blue crab 蓝蟹 □ Butter clam 奶油蛤蜊 □ Dungeness crab 珍宝蟹
- Geoduck clam 象拔蚌 □ Hard clam/quahog 文蛤 □ Horse clam 马蛤 □ King crab 帝王蟹
- Lobster 龙虾 □ Macoma clam 海白樱蛤 □ Moonsnail 蛤蜊 □ Mussels 贻贝 □ Oyster 牡蛎
- Razor clam 箭子 □ Rock crab 蟹类 □ Scallop 扇贝 □ Sea cucumber 海参 □ Sea urchin 海胆
- Shrimp 虾 □ Snow crab 雪蟹 □ Soft clam/longneck 软蛤 □ Squid 鱿鱼/墨鱼
- Surf clam/ocean clam 浪蛤 □ Whelks 海螺

请列举出你常食用但是上面没有列出的海鲜：________________________________________

10. 请列出几个你常去买海鲜的超市或市场，以及常去吃海鲜的餐馆名称：
超市或市场：________________________________________
餐馆：________________________________________

11. 请指出你常去钓鱼的季节：
- □ 从不 □ 春季 □ 夏季 □ 秋季 □ 冬季

1. 在近6个月中，你是否染发、烫发或漂白了头发？ □ 是 □ 否
2. 今天早晨你是否使用了发乳、着哩、摩斯、喷雾或者其它产品？ □ 是 □ 否

谢谢您的合作！我们会在一个月内把您的发言结果反馈给您。
2. 对于全年食用频率相同的海鲜，请列举出你经常食用的几种，并回答相关问题：

<table>
<thead>
<tr>
<th>海鲜名称 (请尽量回答海鲜的具体名称，避免使用模糊的名称，如“鱼、蛋等”)</th>
<th>每餐吃多少？ (可使用任何重量单位，请排除配菜和不可食用部分的重量)</th>
<th>你每个月吃多少次这种海鲜？</th>
<th>你经常在哪里买/吃这种海鲜？ (多项选择)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EXAMPLE: shrimp</td>
<td>2oz (or 60g)</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>自己钓鱼</td>
<td>从渔民处购买</td>
<td>商店超市</td>
<td>商店超市名称</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Wal-Mart, Food lion, E-mart, Trader Joe’s</td>
</tr>
</tbody>
</table>
Tên học: Tên tật của bạn: __________ Địa chỉ nhà: __________

Chú ý: Chúng tôi chỉ cần tên đường và thành phố.

---

Tôi sẽ hỏi bạn một số câu hỏi quan trọng cho bảng câu hỏi này. Hãy cố gắng trả lời tất cả các câu hỏi. Xin Cảm ơn!

1. Xin cho biết TUỔI _______; Giới tính _______; Trọng lượng _________; Dân tộc ________________.

2. Bạn đã nuốt, lên mâu, làm nội bất, chảy nước ấm và đá tay trắng trong sau mấy tháng qua? □ YES □ NO

   Bạn có giữ gel/dầu/kem/keo xịt tóc hoặc dùng bất kỳ sản phẩm khác trên tóc ngày hôm nay? □ YES □ NO

3. Xin vui lòng UỐC LƯỢNG tỷ lệ phần trăm của các món ăn phong cách phương Tây trong bữa ăn bình thường của bạn. Xin vui lòng chọn câu trả lời gần nhất:

   ○ 0% ○ 10% ○ 20% ○ 30% ○ 40% ○ 50% ○ 60% ○ 70% ○ 80% ○ 90% ○ 100%

4. Quy vị có ăn hải sản? □ YES (tiếp tục trả lời câu hỏi)
   □ NO (trả lời "PHẦN 3- MÃU TÓC" trên trang tiếp theo)

5. Bạn thực hiện một thay đổi lớn trong cách ăn uống của bạn trong năm qua? (Ví dụ, bạn dùng một loại thực phẩm ăn nhiều nhất định, như thịt lợn, kiwi, tôm càng...)
   □ NO □ YES (Xin cho biết các chi tiết) ____________________________________________

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Có một số thông tin có thể giúp bạn trả lời câu hỏi này:

- HẢI SẢN có nghĩa là cá hoặc động vật có vỏ, bao gồm cả động vật, tưới, và chế biến (ví dụ, uốt muối/kho/thuy tính/nhua/dòng họ/đồng họ/c) sán phâm
- Xin hãy xét hải sản ăn trong thời gian ăn trưa, ăn sáng, ăn tối, hoặc ĂN NHE (ví dụ, fish stick, bún cua, sushi, súp hải sản và salad).
- Khi bạn UỐC LƯỢNG trong lượng của bữa ăn hải sản, làm ơn dùng BAO GOM trong lượng của món ăn phụ hoặc các bộ phận không ăn được (ví dụ, vỏ tôm hùm hoặc vỏ sò). Bạn có thể sử dụng HẢI SẢN trong chung tôi chuẩn bị như là một trợ giúp thi giải.
- Bạn có thể sử dụng hình ảnh trong sách để giúp bạn xác định những loại cá bạn ăn.
- Dán đầu "X" nếu bạn không biết hoặc không chắc chắn về câu trả lời.
6. Hãy chọn tất cả các mặt hàng thủy sản đã từng ăn từ danh sách dưới đây:

**Finfish:** Cá có vây
- Bass: cá vuốt (O largemouth bass O smallmouth bass O striped bass)
- Bluefish
- Butterfish
- Carp: cá chép
- Catfish: cá tre ở nước mặn
- Cod: cá thu
- Croaker
- Dogfish: cá mập
- Drum
- Eel: con luồn
- Flounder/sole/fluke: cá bơn
- Halibut: cá chim lợn
- Herring: cá trích/cá mồi
- King fish
- Mackerel: cá thu
- Monk fish/goosefish
- Perch: cá rò
- Porgie/sea bream: cá mẻ
- Salmon: cá hồi
- Sardines: cá mẻ
- Sea trout/weakfish: cá hồi biển
- Shads
- Smelt: cá dơi
- Snapper: cá họng
- Snow fish/Chilean sea bass
- Spot
- Sturgeon: cá tầm
- Suckers
- Tilapia: cá rô phi
- Tuna: cá ngừ (O light O white)
- Whiting/hake

**Shellfish:** Động vật có vò

- Abalon: bảo ngư
- Ark shell
- Blue crab: cua xanh
- Butter clam: sò mỏ
- Dungeness crab: Dungeness nghêu
- Geoduck clam: geoduck nghêu
- Hard clam/quahog
- Horse clam
- King crab
- Lobster: tôm hùm
- Macoma clam
- Mussels: con sò
- Oyster: con hàu
- Razor clam
- Rock crab
- Scallop: con ngao
- Sea cucumber
- Sea urchin: con hùi
- Shrimp: Tôm
- Snow crab: cua đá
- Soft clam/longneck Squid
- Surf clam/ocean clam
- Whelks: ốc biển lông

7. Bao nhiêu hải sản bạn thường ăn cho một bữa ăn?

________ (oz)

Chú ý: Bạn có thể sử dụng HẢI SAN trong lượng chúng tôi chuẩn bị như là một trợ giúp thi giác.

8. Mức độ thường xuyên bạn thường ăn có hài sành?

Xin vui lòng chọn câu trả lời ở gần nhất.

- ít hơn 1 lần mỗi tháng (cho biết mức độ thường xuyên chính xác __________) 1 lần mỗi tháng
- 2 lần mỗi tháng 3 lần mỗi tháng 1 lần mỗi tuần 2 lần mỗi tuần 3 lần mỗi tuần
- 4 lần mỗi tuần 5 lần mỗi tuần 6 lần mỗi tuần Hàng ngày 2 lần 1 ngày
- Hôm 2 lần 1 ngày (Xin vui lòng cho biết mức độ thường xuyên chính xác __________) 8. Mức độ thường xuyên bạn thường ăn có hài sành?

Xin vui lòng chọn câu trả lời ở gần nhất.

- ít hơn 1 lần mỗi tháng (cho biết mức độ thường xuyên chính xác __________) 1 lần mỗi tháng
- 2 lần mỗi tháng 3 lần mỗi tháng 1 lần mỗi tuần 2 lần mỗi tuần 3 lần mỗi tuần
- 4 lần mỗi tuần 5 lần mỗi tuần 6 lần mỗi tuần Hàng ngày 2 lần 1 ngày
- Hôm 2 lần 1 ngày (Xin vui lòng cho biết mức độ thường xuyên chính xác __________)

9. Bạn có ăn các phần khác của cá ngoài thực tế để cá?

- NO
- YES (Xin vui lòng cho biết các bộ phận khác bạn ăn và những cái __________)

10. Bạn có phải một người dân?

- YES (tiếp tục trả lời câu hỏi này và đi đến “PHÂN 3-MÃU TÔC”)
- NO (Xin vui lòng bỏ qua câu hỏi này và đi đến “PHÂN 3-MÃU TÔC”)

(1) Xin liệt kê các loại cá mà bạn thường bắt và ăn. Xin vui lòng đừng bao gồm cá bạn bắt một cách tình cờ, nhưng không bao giờ ăn.

(2) Hãy chọn các tháng bạn thường đánh cá:

- Tháng Một
- Tháng Hai
- Tháng Ba
- Tháng Tư
- Tháng Năm
- Tháng Sáu
- Tháng Bảy
- Tháng Tám
- Tháng Chín
- Tháng Mười
- Tháng Mười Một
- Tháng Mười Hai

(3) Thường xuyên bạn đánh cá bao nhiêu lần trong những tháng này?

__________________ (bao nhiêu lần mỗi tháng)

(4) Bạn làm gì với những con cá bắt được? (Vui lòng chọn tất cả các câu trả lời có liên quan)

- Chỉ tốt ăn
- Gia đình tốt và tốt ăn
- Cung cấp cho / bán cho hàng xóm / công động.
10. Xin liệt kê các mặt hàng thủy sản, bền trong nguyên THEO MUA:

<table>
<thead>
<tr>
<th>Mặt hàng thủy sản</th>
<th>Bữa ăn trong lượng</th>
<th>Làm mỗi tháng</th>
<th>Làm thế nào để lựa chọn được hải sản này?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mùa xuân</td>
<td>mùa hè</td>
<td>mùa đông</td>
</tr>
<tr>
<td>(ví dụ) Striped bass</td>
<td>1</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>
Có kẹo trên BẤN THÔNG TIN. Xin vui lòng để lại mẫu tóc của bạn trong phòng bí, và viết tên hoặc tất tên của bạn vào nó. Cảm ơn bạn!

Làm thế nào để thu thập mẫu tóc?

a. Chọn bồn hình vẽ trên đầu của bạn (xem hình 1). Nếu tóc của bạn không đủ dài, chọn khu vực nhiều hơn.
b. Lấy tóc gần da đầu.
c. Tổng trọng lượng khoảng 0.25 g (0.01 oz), và chiều dài không được vượt quá 1.5 inch (xem hình 2).

Cảm ơn bạn đã hợp tác! Chúng tôi sẽ cung cấp kết quả thủy ngân tóc của bạn trong hai tháng!
APPENDIX 2. Consent Forms
Informed-consent Form

The general nature of this study entitled "Seafood Consumption Questionnaire & Hair Sample Collection for Mercury Analysis" conducted by Xiaoyu Xu has been explained to me. I understand that I will be taken my hair samples, and my participation will take about 5 minutes. I am aware that I must be at least 18 years of age to participate. I know that my responses will be confidential and that my name will not be associated with any result of this study. I also know that I may discontinue my participation at any time. I understand there is no potential risk resulting from my participation in this project.

I am aware that I may report dissatisfactions with any aspect of this experiment to the Chair of the Protection of Human Subjects Committee, Dr. Lee Kirkpatrick, 757-221-3997 or lakirk@wm.edu. My signature below signifies my voluntary participation in this project, and that I have received a copy of this consent form.

__________________________________________  ____________________________________________
Date                                                   Signature

__________________________________________
Print Name
知情同意书（简体）

我已清楚了解这项由徐晓宇进行的调查研究《海鲜食用调查问卷，以及头发汞检测的样品采集》的主旨。我明白我将被询问本人的海鲜食用习惯并提供本人的头发样品。这将花去大约 10 分钟的时间。我了解我的答案是完全保密的并且我的名字不会出现在任何结果中。我有权利拒绝回答任何问题并在任何时间终止我的参与。如有任何不满，我知晓可以联系威廉玛丽大学 Protection of Human Subjects 的主席 Lee Kirkpatrick 先生（电话 757-221-3997；电子邮件 lakirk@wm.edu）。我保证我是 18 或 18 周岁以下的成年人。我的签名象征着我是自愿参与这项研究，并且我已经拿到这份知情同意书的副本。

知情同意书（繁體）

我已清楚瞭解這項由徐曉宇進行的調查研究《海鮮食用調查問卷，以及頭髮汞檢測的樣品採集》的主旨。我明白我將被詢問本人的海鮮食用習慣井提供本人的頭髮用品。這將花去大約10分鐘的時間。我瞭解我的答案是完全保密的並且我的名字不會出現在任何結果中。我有權利拒絕回答任何問題并在任何時間終止我的參與。如有任何不滿，我知曉可以聯繫威廉瑪麗大學Protection of Human Subjects的主席Lee Kirkpatrick先生（電話757-221-3997；電子郵件lakirk@wm.edu）。我保證我是18或18周歲以下的成年人。我的簽名象徵著我是自願參與這項研究，並且我已經拿到這份知情同意書的副本。

_________________________ __________________________
Date/日期/日期                     Signature /签名/簽名

_________________________
Print Name/正楷书写/楷书写
Mẫu thông tin-sự đồng ý

Bàn chät chung cua nghiên cứu này có tura de "báng caú hói vê cống dông người viét tiêu thụ thuy sân & Bồ sưu tập mẫu tóc de phân tích thuy ngân" duốc thực hiện bói Xiaoay Xu đã duốc giải thích cho tôi. Tôi hiểu rằng tôi sẽ được hỏi để trả lời các câu hỏi về mục độ thương xuyên tiêu thụ hãi sán của tôi và lấy mẫu tóc của tôi. Sự tham gia của tôi trong nghiên cứu này cần phải đạt tổng cộng khoảng một giờ. Tôi hiểu rằng những câu trả lời của tôi sẽ duốc giữ bí mật và tên của tôi sẽ không liên kết với bất kỳ kết quả của nghiên cứu này. Tôi biết rằng tôi có thể tự chơi trả lời bất kỳ những câu hỏi và tôi có thể ngừng tham gia bất cứ lúc nào. Các rủi ro từ sự tham gia của tôi trong dự án này đã duốc mô tả với tôi. Tôi biết rằng tôi có thể báo cáo bất mään với bất kỳ khía cạnh của thí nghiệm này đến Chủ tịch Ủy ban Bảo vệ Nhân tướng, Dr. Lee Kirkpatrick, 757-221-3997 or lakirk@wm.edu. Tôi biết rằng tôi phải ít nhất 18 tuổi mới duốc tham gia. Chữ ký của tôi dưới đây chứng nhận sự tham gia tự nguyện của tôi trong dự án này, và tôi đã nhận được một bản sao của giấy chấp thuận này.

Date / Ngày

Signature / Chữ ký

Print Name / In Name
VITA

XIAOYU XU

Born in Changchun, Jilin, China, 1st June 1984. Earned B.S. in Biology and B.A. in Foreign Language from Huazhong University (China) in 2007. Received M.S. in Biochemistry and Molecular Biology from Huazhong University (China) in 2010. Entered doctoral program in College of William and Mary (USA), School of Marine Science in 2013.