EPA/600/R-11/066F
October 2013
www.epa.gov

Fish Tissue Analysis for Mercury and PCBs from a New York City Commercial Fish/Seafood Market

Prepared by:

U.S. Environmental Protection Agency

A collaborative effort between Region 2 and
ORD National Center for Environmental Assessment
ORD National Risk Management Research Laboratory
ORD National Research Exposure Laboratory

This is a Regional Applied Research Effort (RARE)

DISCLAIMER

This document has been reviewed in accordance with U.S. Environmental Protection Agency policy and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Preferred Citation:
U.S. Environmental Protection Agency (EPA). (2013) Fish Tissue Analysis for Mercury and PCBs from a New York City Commercial Fish/Seafood Market, Washington, DC; EPA/600/R-11/066F. Available from: National Technical Information Service, Springfield, VA, and online at: http://www.epa.gov/.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AMF	Asian market fish
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Database Systems
CBOL	Consortium for the Barcoding of Life
CM	commercial market
COXI	cytochrome c oxidase subunit I
CV	coefficient of variation
EPA	U.S. Environmental Protection Agency
EU	European Union
FDA	U.S. Food and Drug Administration
FFM	Fulton Fish Market
HANES	Health and Nutritional Examination Survey
MeHg	methyl mercury
NYCDOHMH	New York City Department of Health and Mental Hygiene
PCB	polychlorinated biphenyls
PCR	polymerase chain reaction
QA/QC	Quality Assurance/Quality Control
QAPP	Quality Assurance Project Plan
R^2	correlation coefficient
RfD	reference dose
SD	standard deviation
SOP	standard operating procedures
TEF	toxic equivalency factors
TSS	total sum of squares

AUTHORS, CONTRIBUTORS, AND REVIEWERS

PRINCIPAL INVESTIGATOR

Mark Maddaloni, DrPH
Region 2 – New York City
U.S. Environmental Protection Agency

PROJECT MANAGER

Cheryl Itkin, MA
ORD National Center for Environmental Assessment – Washington, DC
U.S. Environmental Protection Agency

AUTHORS

Mark Maddaloni, DrPH
Region 2 – New York City
U.S. Environmental Protection Agency

Dennis Santella
Region 2 – New York City
U.S. Environmental Protection Agency

Cheryl Itkin, MA
ORD National Center for Environmental Assessment – Washington, DC
U.S. Environmental Protection Agency

Henry Kahn, DSc
ORD National Center for Environmental Assessment – Washington, DC
U.S. Environmental Protection Agency

Stan Stephansen
Region 2 – New York City
U.S. Environmental Protection Agency

Moses Chang
Region 2 – New York City
U.S. Environmental Protection Agency

Michael Borst
ORD National Risk Management Research Laboratory – Edison, NJ
U.S. Environmental Protection Agency

AUTHORS (continued)

John Bourbon, MS
Region 2 Laboratory – Edison, NJ
U.S. Environmental Protection Agency

Fred Elsen
Region 2 Laboratory – Edison, NJ
U.S. Environmental Protection Agency

John Martinson, MS
ORD National Exposure Research Laboratory – Cincinnati, OH
U.S. Environmental Protection Agency

Maureen O'Neil, MURP
Region 2 – New York City
U.S. Environmental Protection Agency

Cara Henning
ICF International – Durham, NC

REVIEWERS

U.S. EPA Internal Reviewers

Gina Ferreira, MS
Region 2 – New York City
U.S. Environmental Protection Agency

Jacqueline Moya, BS
ORD National Center for Environmental Assessment – Washington, DC
U.S. Environmental Protection Agency

Marla D. Smith, MS
Office of Water – Washington, DC
U.S. Environmental Protection Agency

Rita Schoeny, PhD
Office of Science Policy – Washington, DC
U.S. Environmental Protection Agency

REVIEWERS (continued)

External Reviewers

Henry A. Anderson, MD
Private Consultant
Madison, WI

Gary A. Pascoe, PhD, DABT
Pascoe Environmental Consulting
Port Townsend, WA

Alan H. Stern, DrPH, DABT
Independent Consultant
Metuchen, NJ

ACKNOWLEDGEMENTS

This report was prepared for the U.S. Environmental Protection Agency by ICF International under contract. Additionally, we gratefully acknowledge the programs and individuals mentioned below for their contributions to this project.

The Commercial Fish Market Study was funded through the Regional Applied Research Effort (RARE) Program administered by EPA's Office of Research and Development (ORD). RARE is one approach EPA takes to promote collaboration between the Regional Offices and ORD. The goals of the program are to:

- Provide the regions with near-term research on high-priority, region-specific science needs;
- Improve collaboration between regions and ORD laboratories and centers; and
- Build a foundation for future scientific interaction.

These goals were met through close collaboration between various ORD and Region 2 staff. ORD and Region 2 laboratories located in Edison, New Jersey worked cooperatively to perform sampling and tissue analysis, respectively. ORD's National Exposure Research Lab (NERL) in Cincinnati performed DNA sequencing analysis on the samples in order to assess concordance between market names and taxonomic classification. Carrie Drake, Dynamac Corporation and Stephen Morris, Student Services Contractor contributed to this effort. Thomas O'Connor (EPA ORD NRMRL), Carolyn Esposito (EPA, ORD NRMRL) and Carol Lynnes (EPA, Region 2) provided valuable input to the Quality Assurance Project Plan (QAPP) for the Commercial Fish Market Study. ORD's National Center for Environmental Assessment (NCEA) provided statistical consultation, project management, and funding and contract oversight for data analysis and the development of this report. Overall project quality assurance was managed by Beverly Comfort (ORD NCEA).

Input on study design was provided by Michael Bolger of the Food and Drug Administration (FDA). Numerous individuals, some now retired, from the New York State Department of Agriculture and Markets provided valuable sampling and analytical assistance. Those individuals included Joe Colby, Curt Vincent, and Steve Stitch. Without the benefit of the New York City Department of Health and Mental Hygiene's (NYCDOHMH) Health and Nutritional Examination Survey (HANES), there would have been little impetus to conduct this study. There are many at the NYCDOHMH who assisted in this study, but none more so then Wendy McKelvey. Wendy was involved in both the New York City HANES and a NYCDOHMH companion study of mercury and PCBs in seafood obtained from markets in NYC Asian communities. Wendy provided an enormous amount of input on all aspects of the study design and execution and we would like to think that we repaid her in some small way by

making the services of Region 2's resident ichthyologist, Moses Chang, available to NYCDOHMH to assist in their companion study.

Finally, this study enjoyed a considerable logistic benefit in the form of the New Fulton Fish Market which allowed for "one stop shopping" even though it required three separate trips to acquire the large sample size. The Market Manager, George Maroulis, Director of Security, Ken Klein, and Coop President, Warren Kremin, provided the study team with valuable insights and advice for negotiating our way around a very busy commercial fish market in the wee hours of the morning. In addition, individual fishmongers, too numerous to mention by name, were very generous with sharing their expertise on various aspects of commercial fish markets and the products they purvey. Also we would like to acknowledge PARS Environmental, Inc. for sample acquisition.

EXECUTIVE SUMMARY

The New York City Commercial Market (CM) Seafood Study was undertaken by the Environmental Protection Agency (EPA; the New York Regional Office in collaboration with the Office of Research and Development) to measure mercury (Hg) concentration in the seafood most commonly consumed by residents of the New York City metro area. The goal of this study was to obtain objective information and descriptive statistics on the levels of mercury found in commonly consumed seafood species. The data collected was presented in a manner that allows for informed choices of seafood consumed.

The CM study is one of two complimentary studies conducted as part of an effort to understand and respond to the results of a Health and Nutritional Examination Survey (HANES) conducted by the New York City Department of Health and Mental Hygiene (NYCDOHMH). The NYC HANES included measurements of blood Hg concentration in a probability sample of 1,811 New Yorkers selected to represent the age, gender, and ethnic composition of the adult population (McKelvey et al., 2007). The geometric mean blood Hg (approximately equal to the median) concentration was elevated threefold compared to national estimates. Asians registered unusually high blood Hg, with Chinese New Yorkers registering a geometric mean almost three times that of the overall sample value. Seventy-two percent of Chinese New Yorkers in the NYC HANES had blood Hg attaining the New York State reportable level of 5 µg/L or above, although this is based on a small sub-sample. Citywide, NYC HANES estimated that 1.4 million NYC adults have blood Hg at or above the reportable level. The NYC HANES survey recorded data on the number of meals that included fish but not the amount or type of fish consumed.

An Asian Market Fish (AMF) study was conducted by the NYCDOHMH. The AMF study measured levels of Hg and polychlorinated biphenyls (PCBs) in 282 specimens of 19 species commonly sold in markets that serve the Asian community. Mean Hg levels ranged from below the limit of detection (0.004 mg/g) in tilapia to 0.229 mg/g in tilefish. The highest Hg level (1.150 mg/kg) was measured in a tilefish specimen. Tilefish, canned eel, blackfish, and Spanish mackerel had the highest mean Hg levels. Porgy, yellow croaker, and Buffalo carp were identified as fish with the highest mean PCB levels. The AMF study used the U.S. EPA Reference Dose (RfD) for methyl mercury (MeHg) to calculate the number of 6 oz (170 g) meal servings that a 60 kg women could eat per week (McKelvey et al., 2010).

In the CM study conducted by EPA, samples of 33 commonly consumed species were obtained from the New Fulton Fish Market (Bronx, NY), the largest commercial seafood market in the nation and the source of most of the fresh seafood consumed in the NYC area. Samples from the targeted species list were purchased from vendors operating in the market. For each species selected, multiple specimens (typically three) from the same vendor were combined to form composite samples. All samples were analyzed for total Hg concentrations and PCB measurements were obtained in a limited subset of the samples. Mean Hg concentrations across composite samples for the same market name ranged from as low as 0.0054 mg/kg (shrimp) to as high as 0.42 mg/kg (tuna). The species with the four highest Hg concentrations were tuna,

swordfish, Spanish mackerel, and mahi-mahi, while shellfish tended to have the lowest concentrations.

Measured Hg concentrations were compared to three actions levels (Maine; Florida/European Union [EU]/Canada; U.S. Food and Drug Administration [FDA]) and EPA's level of concern. The derivations and uses of these levels are discussed in this report. None of the measured Hg concentrations in the individual composite samples for any species had concentrations that were higher than the FDA action level; however, 70% or more of tuna and swordfish composite samples exceeded the Maine Action level, the EPA Level of concern, and the Florida/EU/Canada Action Level, and the overall tuna and swordfish mean values exceeded the Maine Action Level and the EPA level of concern.

As was done by NYCDOHMH, EPA used the concentration measurements obtained in this study to estimate mercury intake by adult women of child-bearing age resulting from assumed amounts of fish consumption. Women of child-bearing age were used as the basis of the calculation of Hg intake because they are, as a group, more sensitive than the overall adult population. Thus, values considered protective for this group would be protective of the overall adult population. The permissible daily intake of MeHg was calculated and compared to MeHg intake from fish ingestion. The estimated amount of Hg intake was converted to a number of servings per week that would yield a safe daily intake level of Hg. The number of servings per week for adult women were estimated assuming a body weight of 65 to 67 kg and a serving size of 8 oz fresh weight (or 6 oz cooked weight), and using the U.S. EPA RfD for MeHg of 0.1 µg/kg-day. In addition, it was assumed that a woman eats only a single seafood species in a week.

The CM market species mean composite concentrations were compared to mean concentrations in FDA monitoring data collected from 1995–2004. This comparison showed that the CM species mean concentrations tended to be lower than FDA concentrations. However, the CM mean 0.42 mg/kg for tuna Hg concentrations is within 5% of the more recent (2000–2004) FDA monitoring value (i.e., 0.40 mg/kg). A limited subsample (N = 50) across five species (salmon, crab, tuna, catfish, and mackerel) was also analyzed for 124 different PCB congeners. The PCB analysis was constrained by cost and detailed statistical analysis was not performed on the resulting data which are limited. The species selected for PCB analysis was purposeful in the sense that those species that were included are those suspected of having elevated PCB levels. NYCDOHMH HANES did not analyze biomonitoring data for PCBs. Overall, the composite samples selected for PCB analysis had concentrations within the FDA tolerance level.

This study also made use of recent advances in DNA sequencing technology. "DNA barcoding" has emerged as a useful taxonomic tool that can help overcome some of the issues associated with morphology based identifications. Barcoding uses a short genetic sequence from a standard part of the genome in an attempt to accurately assign a specimen to a given taxon, or ideally, a species. Such an assignment can be made by examining a genomic region that exhibits a high degree of sequence conservation within a species, but appreciable divergence compared to

other species. DNA sequencing of a portion of the cytochrome c oxidase subunit I (coxI) gene was performed by EPA's Office of Research and Development laboratory in Cincinnati. Overall, there was concordance between the DNA-based results and the market names.

1. THE COMMERCIAL FISH MARKET STUDY

The New York City Commercial Market (CM) Seafood Study was undertaken to measure mercury (Hg) concentration in composite samples from seafood species most commonly consumed by New York City residents as represented by specimens obtained from a commercial market. Polychlorinated biphenyl (PCB) measurements were obtained in a limited subset of the study population. Each composite sample was formed by mixing tissue from a number of individual fish specimens into a combined amalgamated sample. The formation of the composite sample was, in effect, a physical averaging of the individual tissue samples and the result of a measurement on the composite sample was an estimate of the average of the specimens in the sample. Composite sample analysis is a well established mechanism for cost effective estimation of means of environmental samples that has a history of use in fish tissue analysis (e.g., Fabrizio et al., 1995; Gilbert, 1987). The U.S. Environmental Protection Agency (EPA) developed a list of the most popular species using regional and national landings, net local and national imports/exports, domestic aquaculture production, nearby surveys of seafood species sold in supermarkets and seafood stores, and the listing of seafood species available for sale by individual CM wholesalers (U.S. EPA, 2008). The New Fulton Fish Market (FFM) was chosen as the site for sample collection because it receives fish from all over the world and is the largest seafood distributor to retailers in the United States.

The CM study is one of two complimentary studies conducted as part of an effort to understand and respond to the results of a Health and Nutritional Examination Survey (HANES) conducted by the New York City Department of Health and Mental Hygiene (NYCDOHMH). The NYC HANES included measurements of blood mercury concentration in a probability sample of 1,811 New Yorkers selected to represent the age, gender, and ethnic composition of the adult population (McKelvey et al., 2007). The geometric mean blood mercury (approximately equal to the median) concentration was elevated threefold compared to national estimates. Asians registered unusually high blood mercury, with Chinese New Yorkers registering a geometric mean almost three times that of the overall sample value. Seventy-two percent of Chinese New Yorkers in the NYC HANES had blood Hg attaining the New York State reportable level of 5 µg/L or above, although this is based on a small sub-sample. Citywide, NYC HANES estimated that 1.4 million NYC adults have blood Hg at or above the reportable level. The NYC HANES survey recorded data on the number of meals that included fish but not the amount or type of fish consumed.

1.1. SAMPLING METHODS

This section briefly describes the sample collection, compositing, and analytic methods used in this study. For a detailed description of the procedures and protocols, you may refer to the *Quality Assurance Project Plan for the Sample Collection, Composite, and Analysis for a Study of Mercury and PCBs in Seafood from the New Fulton Fish Market* (May 2008).

A ranked listing of the most commonly consumed fish species in the NYC metro area was developed by evaluating information from national, regional, and local databases and information sources. Databases of fishery imports, landings, and aquaculture were used to create a ranked listing of species availability, by weight, for the NYC metro area. A regional survey of fish availability in supermarkets, the listing of fish species available for sale at the Fulton Fish Market, and a review of the Mercury Report to Congress were also reviewed to refine the list.

The ranked listing was developed by summing together, by species, the weight of national and local (NYC area) net edible fishery imports. Net imported fish weights were summed together for all forms and cuts of each fish species (i.e., fresh, frozen, dried, smoked, and pickled varieties of whole, gutted, and filleted forms of fish). The latest edible fish import information was obtained from the National Marine Fisheries Service (http://www.st.nmfs.noaa.gov/st1/trade/index.html) and covered the period January–October 2007.

To this initial ranking, weights of National and Regional (Northeast and Middle Atlantic) commercial fish landings were added. Annual landings for the latest commercial fish catches (2006) were obtained from the National Marine Fisheries Service (http://www.st.nmfs.noaa.gov/st1/commercial/landings/annual_landings.html). Weights of domestically produced food sized aquaculture species were obtained for 2005 (the latest year available) from the U.S. Department of Agriculture's Census of Aquaculture report entitled "Summary of Aquaculture Products Sold by Species and Size Category, United States: 2005" (http://www.agcensus.usda.gov/Publications/2002/Aquaculture/index.asp). Weights for fish species from the various data sources were summed together to produce the ranked listing of fish availability in the NYC Metro area.

An adjustment to, and confirmation of, the ranked listing was made by consulting additional local and regional sources. In the report "Fish Availability in Supermarkets and Fish Markets in New Jersey" (Burger et al., 2004), Bluefish was identified as being present in 82.5% of markets surveyed. Bluefish was initially listed as number 45 in the ranked listing of commonly consumed fish, but due to its popularity in NJ supermarkets it was moved up in the rankings and included in the top 30 targeted fish species. Table 4-45 titled "Regional Popularity of Fish and Shellfish Species - East Coast" and Table 4-46 titled "Popularity of Fish/Shellfish Species in Restaurants - By Region - North East" in EPA's "Mercury Report to Congress**,** Volume IV: An Assessment of Exposure to Mercury in the United States" http://www.epa.gov/ttn/oarpg/t3/reports/volume4.pdf) were reviewed and confirmed the inclusion of the fish species identified in the report were included in the final ranked list.

Lastly, the fish species listed for sale by each vendor at the New Fulton Fish Market (FFM) in its "Seafood Products Matrix" (http://www.newfultonfishmarket.com/products_sold.html) was reviewed to confirm that, (1) the most frequently listed species available for sale at the FFM were included in the ranked listing, and (2) that the top 20–30 species in the ranked listing were available for purchase at the FFM. Minor adjustments were made to the ranked listing based on fish availability at the FFM and

conversations with fish wholesalers. For example, herring was identified as being commonly consumed in the NYC Metro area and sold by nine FFM vendors, yet only one vendor at the FFM had herring available for sale on our sampling day. The vendor indicated other distribution channels are used for herring, including the distribution of pickled herring directly from food manufacturers to supermarkets. Therefore, to replace herring, the next most popular fish species was selected for sampling.

Fresh and frozen samples were collected from a variety of wholesalers, and information about the water body of origin was noted where available. Species were identified by wholesaler and species name at the market and were later subjected to DNA analysis (described in Appendix B) and visual inspection to determine the FDA approved market name (market name), common scientific name (common name), and scientific name (i.e., genus and species). Approved market names for seafood sold in Interstate Commerce are identified in "The Seafood List", a guidance document and database accessible at http://www.cfsan.fda.gov/~comm/seaguid7.html that is maintained by the FDA. Some market names included more than one species. The goal was to obtain the required volume needed to do the analyses. The field team targeted collecting three or more individual specimens similar in size per target species from each target wholesaler, for a total of 45 specimens collected per species to yield 15 composite samples per species. More than 20 species were targeted. Actual sample numbers were based on practical availability at the market.

The total length, caudal length, and weight of each specimen were recorded, and composite samples were then created by combining multiple organisms of the same species, roughly equal in size, and from the same wholesaler. Fish composites were prepared from edible fillet portions with removal of skin and any surficial connective tissue or mucus. Shellfish species included only edible tissue (all crab samples include the hepatopancreas, softshell crabs were processed intact while hardshell crabs were shelled prior to processing, and lobster included only muscle tissue). For fish, these composites typically included three individual specimens, while for small shellfish (e.g., clams, oysters) the composites contained up to 200 individual organisms. Occasionally, a composite sample for fish was comprised of two rather than three individual specimens. This was most notable for tuna where 7 of the 15 composite samples contained two specimens. This was due to the large size of the fish and the consequent limited number of individual specimens at a particular vendor. The composite sampling methodology employed provides larger sample that in turn improves the capability of the analytical methods to achieve the target reporting limits and thus better represent fish tissue concentrations.

An analysis sample was then drawn from each composite sample and tested for total Hg using EPA Method 245.1 Revision 3 (cold vapor technique). To help quantify sources of variability, analyses were performed multiple times on a subset of the composite samples in two different ways. In one case, "duplicate" analysis samples were drawn from the same composite sample and analyzed separately. In the other case, the same analysis sample was analyzed using the laboratory testing procedure two or three times ("replicates"). The laboratory reported an analysis sample-specific reporting limit and a Hg concentration if Hg was detected in the assay.

All total Hg concentrations for CM composite samples are reported on a wet-weight basis. The Hg concentrations generally are reported as mg[Hg]/kg[fresh tissue weight] or parts per million (ppm).

1.2. ORGANIZATION OF THIS REPORT

The remainder of this document describes statistical analyses of the distributions of Hg, as measured in composite samples, for each fish and shellfish market name and species. Section 2 provides statistical analyses by market name, which is the name by which consumers typically purchase the fish. In Section 3, the data are examined in more detail to look for trends in Hg concentration by species (presented by "common name"), water body of origin or type of origin, length, caudal length, and weight. Section 4 reports the variance in the data that is attributable to measurement errors based on separate analyses of duplicate and replicate analytic results. The Hg statistics then are placed within a risk framework (subject to caveats discussed in the text) by comparing the mean and distribution of measured Hg concentrations with selected state and federal action levels and by deriving estimated number of servings per week for each species that should ensure that a woman of child-bearing age receives an average daily dose below the EPA RfD for MeHg (see Section 5). The results also are compared to FDA monitoring data to determine differences in the overall trends (see Section 6). Finally, the quality assurance/quality control (QA/QC) measures that were undertaken in the analysis of the data are detailed in Section 7. Appendix A provides the detailed statistical results of the Hg concentration analysis and Appendix B describes the DNA analysis performed on a subset of the samples. A description of the statistical analysis of PCB concentrations is provided in Appendix C as a separate set of analyses of the CM fish tissue. A table of the calculated number of servings is provided in Appendix D and the comments from independent peer reviewers are in Appendix E.

2. RESULTS: MERCURY CONCENTRATIONS ACROSS SPECIES

To understand Hg concentration trends in the CM data, a number of statistical analyses were conducted by market name. The market name was selected as the unit of analysis because it is the name under which consumers generally identify and purchase fish and shellfish. A crosswalk is presented in Section 2.1 (see Table 1) to link market names to species, with water body of origin indicated, when possible, to differentiate species from the Atlantic and Pacific Oceans. Then, Section 2.2 presents the statistical analyses by market name.

2.1. MARKET, COMMON, AND SCIENTIFIC NAMES

Table 1 presents a crosswalk table linking the market names analyzed in the CM study to the scientific name of the one or more species encompassed by the market name. The table is organized taxonomically and also presents one or more common names for each species. The ocean of origin (i.e., Pacific or Atlantic if applicable) also is presented based on the vendor-stated water body of origin for each composite sample from the CM.

Of the 33 market names, 23 referred to a single species. The remaining 10 market names encompassed more than one species: snapper (five different species), flounder/fluke/sole (four different species.), oyster (two different species), squid (three different species), catfish (three different species), shrimp (two different species), whiting (two different species), cod (two different species), bass (two different species), and tuna (two different species). The fish samples collected were split between both Atlantic and Pacific Ocean origins, and included some farmed species.

Table 1. Crosswalk of market names to scientific names

	Order	Family	Market Name	Common Name	Genus	Species	Predominant Location
Phylum Mollusca, Class Bivalvia (bivalves)							
	Ostreoida	Pectinidae	**Scallop**	Sea Scallop	*Placopecten*	*magellanicus*	Atlantic
	Ostreoida	Ostreidae	**Oyster**	Eastern Oyster	*Crassostrea*	*virginica*	Atlantic coast
				Pacific Oyster	*Crassostrea*	*gigas*	Pacific
	Mytiloida	Mytilidae	**Mussel**	Blue Mussel	*Mytilus*	*edulis*	Atlantic coast
	Veneroida	Veneridae	**Clam**	Hardshell Clam/ Quahog/ Northern Clam / Little Neck Clam / Cherry Stone	*Mercenaria*	*mercenaria*	Atlantic coast
Phylum Mollusca, Class Cephalopoda, Subclass Coleoidea, Superorder Decabrachia							
	Teuthida	Loliginidae	**Squid**	Squid	*Illex*	spp.	Pacific
				Longfin Squid	*Loligo*	*pealeii*	Atlantic
	Teuthida	Ommastrephida		Japanese Flying Squid	*Todarodes*	*pacificus*	Pacific
Phylum Arthropoda, Subphylum Crustacea, Class Malacostraca, Subclass Eumlacostraca, Superorder Eucarida							
	Decapoda	Penaeidae	**Shrimp**	White Shrimp	*Litopenaeus*	*vannamei*	Farmed Ecuador, Southeast Asia, and Central America
				Black Tiger Shrimp	*Penaeus*	*monodon*	Farmed India
	Decapoda	Homaridae	**Lobster**	American Lobster	*Homarus*	*americanus*	Atlantic
	Decapoda	Portunidae	**Blue Crab**	Blue Crab; hard and softshell	*Callinectes*	*sapidus*	Atlantic
Phylum Chordata, Subphylum Vertebrata, Class Chondrichthyes, Subclass Elasmobranchii, Superorder Euselachii							
	Rajiformes	Rajidae	**Skate**	Winter Skate	*Leucoraja*	*ocellata*	Atlantic

Table 1. Crosswalk of market names to scientific names (continued)

	Order	Family	Market Name	Common Name	Genus	Species	Predominant Location
Phylum Chordata, Subphylum Vertebrata, Superclass Osteichthyes, Class Actinopterygii, Subclass Neopterygii (rayed fish)							
	Clupeiformes	Clupeidae	**Herring**	Atlantic Herring	*Clupea*	*harengus*	Atlantic
	Gadiformes	Gadidae	**Pollock**	Pollock	*Pollachius*	*virens*	Atlantic
	Gadiformes	Merlucciidae	**Whiting**	Offshore Whiting	*Merluccius*	*albidus*	Atlantic
				Silver Hake	*Merluccius*	*bilinearis*	Atlantic
	Gadiformes	Gadidae	**Cod**	Atlantic Cod	*Gadus*	*morhua*	Atlantic
				Pacific Cod	*Gadus*	*macrocephalus*	Pacific
	Lophiiformes	Lophiidae	**Monkfish**	Monkfish/Goose-fish/Anglerfish	*Lophius*	*americanus*	Atlantic
	Siluriformes	Ictaluidae	**Catfish**	White catfish	*Ameiurus*	*catus*	Atlantic
				Blue catfish	*Ictalurus*	*furcatus*	Atlantic
				Channel catfish	*Ictalurus*	*punctatus*	Atlantic
Phylum Chordata, Subphylum Vertebrata, Superclass Osteichthyes, Class Actinopterygii, Subclass Neopterygii (rayed fish)							
	Perciformes	Cichlidae	**Tilapia**	Tilapia	*Oreochromis*	spp.	Foreign farmed
	Perciformes	Coryphaenidae	**Mahi-mahi**	Mahi-mahi/ Dolphin Fish	*Coryphaena*	*hippurus*	Pacific
	Perciformes	Lutjanidae	**Snapper**	Red Snapper	*Lutjanus*	*campechanus*	Atlantic
				Caribbean Red Snapper	*Lutjanus*	*purpureus*	S Atlantic
				Lane Snapper	*Lutjanus*	*synagris*	Pacific
				Yellowtail Snapper	*Ocyurus*	*chrysurus*	Atlantic
				Vermilion Snapper	*Rhomboplites*	*aurorubens*	Atlantic
	Perciformes	Moronidae	**Bass**	Hybrid Striped Bass	*Morone*	*chrysopes* x *saxatilis*[a]	Atlantic
				Striped Bass	*Morone*	*saxatilis*	Atlantic
	Perciformes	Nototheniidae	**Chilean Sea Bass**	Chilean Sea Bass	*Dissostichus*	*eleginoides*[b]	Pacific

Table 1. Crosswalk of market names to scientific names (continued)

	Order	Family	Market Name	Common Name	Genus	Species	Predominant Location
	Perciformes	Pomatomidae	**Bluefish**	Bluefish	*Pomataomus*	*saltatrix*	N Atlantic
	Perciformes	Sciaenidae	**Croaker**	Atlantic Croaker	*Micropogonias*	*undulates*	N Atlantic
	Perciformes	Scombridae	**Spanish Mackerel**	Spanish Mackerel	*Scomberomorus*	*maculatus*	Atlantic
	Perciformes	Scombridae	**Mackerel**	Atlantic Mackerel	*Scomber*	*scombrus*	Atlantic
	Perciformes	Scombridae	**Tuna**	Yellowfin Tuna	*Thunnus*	*albacares*	Pacific
				Bigeye Tuna	*Thunnus*	*obesus*	Pacific
	Perciformes	Serranidae	**Sea Bass**	Black Sea Bass	*Centropristis*	*striata*	Atlantic
	Perciformes	Serranidae [formerly Sebastidae]	**Ocean Perch**	Ocean Perch	*Serranus* [formerly *Sebastes*]	*scriba* [formerly *marinus*]	N Atlantic
	Perciformes	Sparidae	**Porgy**	Porgy/Scup	*Stenotomus*	*chrysops*	Atlantic
	Salmoniformes	Salmonidae	**Atlantic Salmon**	Atlantic Salmon	*Salmo*	*salar*	Atlantic and Pacific
	Pleuronectiformes	Pleuronectidae	**Halibut**	Pacific Halibut	*Hippoglossus*	*stenolepis*	Pacific
	Pleuronectiformes	Pleuronectidae	**Flounder/ Fluke/ Sole**	Gray Sole	*Glyptocephalus*	*cynoglossus*	Atlantic
				Yellowtail Flounder	*Limanda*	*ferruginea*	Atlantic
				Summer Flounder	*Paralichthys*	*dentatus*	Atlantic
				Blackback Flounder	*Psuedopleuronectes*	*americanus*	Atlantic
	Salmoniformes	Salmonidae	**Rainbow Trout**	Rainbow Trout	*Oncorhynchus*	*Mykiss*	Farmed Idaho
	Perciformes	Xiphiidae	**Swordfish**	Swordfish	*Xiphias*	*gladius*	Atlantic and Pacific

[a]Not recognized as a valid species by the Integrated Taxonomic Information System (http://www.itis.gov/).
[b]DNA evidence (presented in Appendix A) strongly suggests that at least one of these samples is not *Dissostichus eleginoides*, but instead *Dissostichus mawsoni*. Some sources refer to both species as Chilean Sea Bass, but there is not a clear consensus.

2.2. MERCURY CONCENTRATIONS BY MARKET NAME

Table 2 presents statistical descriptors of Hg concentration by market name. This table presents the number of composite samples analyzed for each market name and indicates the number of non-detects and detects. A non-detect refers to a measured Hg concentration that is below the laboratory analysis reporting limit. The percent of samples that were non-detects is also presented in this table. The mean Hg concentration was calculated for all species with at least two composite samples; if only one composite sample was taken, the mean is equal to the single measurement. If there are two or more composite samples, the standard deviation, coefficient of variation, minimum, maximum, and lower and upper confidence limits on the mean also are presented. For market names that have 10 or more composite samples, the empirical 25^{th} percentile, 50^{th} percentile (median), and 75^{th} percentile levels are also presented. Extreme upper and lower percentiles (e.g., the 5^{th} and 95^{th} percentiles) were not calculated because of the relatively small number of samples for each market name. The maximum and minimum concentrations are reported because they provide some information about the potential range of Hg concentrations.

In order to estimate mean Hg concentrations and to provide statistical descriptors of the distribution of Hg concentrations across composite samples for the species with one or more non-detect sample measurements, an assumption must be made about the composite sample Hg concentrations that were below the reporting limit. In the statistical analyses, non-detect Hg concentrations were estimated in two different ways:

- A non-detect was assumed to equal one-half the reporting limit. This method assumes the actual Hg concentration has an equal probability of taking on all values between zero and the reporting limit, so the expected value is one-half the reporting limit. Results presented in the main body of the text use this assumption.
- A non-detect was assumed to equal the reporting limit. This method is a conservative assumption and will yield the highest possible Hg concentrations. Results presented in Appendix A use this assumption.

This was done to provide a sense of the impact of these assumptions on the data analysis. Discussions of the differences between the approaches are provided at appropriate points in the text.

Many of the species have multiple measured Hg concentrations for the same composite sample; these are referred to as “duplicates” or “replicates”. Samples labeled as duplicates indicate that two laboratory analysis samples were drawn from the same composite and analyzed separately. Samples labeled as replicates indicate that multiple measurements of Hg concentration were performed on the same analysis sample. For the purposes of statistical analysis, the duplicates or the replicates were averaged to obtain a single Hg concentration for the respective composite sample. In one special case (Group ID 237, market name Mackerel), three replicates resulted in one Hg non-detect and two detected Hg measurements. This

composite sample was classified as a non-detect to reflect the uncertainty in the overall average; however, the average includes the two detected measurements, along with the assumed non-detect value. The term "aggregated composite samples" hereafter refers to the set of concentration measurements used in the statistical analysis in which duplicates and replicates have been averaged together, non-detects have an assumed value, and all other composite sample measurements are as they were reported in the raw data.

The number of aggregated composite samples varies substantially across market names. Market names have as few as one aggregated composite sample per each market name (Chilean sea bass, halibut, herring, lobster, mahi-mahi, ocean perch, and rainbow trout), to greater than 10 aggregated composite samples per market name (sea bass, cod, blue crab, flounder/fluke/sole, monkfish, snapper, squid, tilapia, and tuna). It should be noted that each composite sample contained more than one organism, so even market names with one or a few composite samples were still effectively averaged over multiple individual organisms. Non-detects occurred more frequently with shellfish (clam, blue crab, mussel, oyster, and scallop), although a few fish had non-detects as well (flounder/fluke/sole, mackerel, and Atlantic salmon).

Looking at standard deviations across composite samples gave an estimate of the variability; however, because there were multiple organisms in each composite sample, it tended to underestimate the population standard deviation. Therefore, for this analysis the population standard deviation was estimated. To make this calculation, it was assumed that in a given species, each composite was made of k fish. In addition, it was assumed that the fish in each composite were statistically independent and the weight of each individual fish used in the sample was approximately equal. If the composites had a mean M, a standard deviation s, and a variance of V, then the individual fish distribution (called here the "estimated population distribution") had a mean of M and a variance of kV (or, equivalently, a standard deviation of $k^{½}s$, where s was the standard deviation across composite samples and equals $V^{½}$). In the CM sample preparation, the number of specimens in each composite in a single species was not always the same, so k actually varied across composite samples; in these cases, the harmonic mean of the numbers of fish in each composite was used to represent k. The harmonic mean was less influenced by large outliers (or small) than the arithmetic mean because in mathematical terms it is a lower bound on the median of a set of values and was a better representation of the sample size in calculation of the variance. In the tuna species, 6 of the 14 composites were composed of two fish rather than three. The harmonic mean of the number of fish in the composite samples was then 2.47 and the standard deviation across the composites was 0.25, so the estimated population standard deviation was $(2.47)^{½} \times 0.25 = 0.39$. For swordfish, all composite samples were composed of three fish, so the estimated population standard deviation was $(3)^{½} \times 0.019 = 0.033$. The upper and lower estimated confidence limits were then calculated assuming a normal distribution and using a sample size of $k \times n$, where n is the number of composite samples.

In Table 2, species are listed in order of decreasing mean Hg concentration. Mean Hg concentrations across composite samples for the same market name range from as low as 0.0054

mg/kg (shrimp) to as high as 0.42 mg/kg (tuna). The species with the four highest Hg concentrations were tuna, swordfish, Spanish mackerel, and mahi-mahi, while shellfish tended to have the lowest concentrations. Where medians could be calculated, they were generally similar to the means. Because medians are less influenced by outliers, similarity between means and medians indicated that the distributions were relatively symmetric and little influenced by outliers in a single direction (e.g., several very high or very low measurements).

The results in Table 2 assume Hg non-detects are equivalent to one-half the reporting limit. To provide a sense of the impact this assumption on the analysis, the data were also analyzed assuming the Hg concentration in samples analyzed as non-detects were equal to the reporting limit and these results are presented in Table A-1 in Appendix A. The results for any market names without non-detects are the same in both tables. For market names that included non-detect concentrations, the percent difference in the mean concentration between tables ranged from 4% for whiting to 72% for shrimp. However, the largest percent difference in mean Hg concentrations between Table 2 (non-detects equal to one-half reporting limit) and Table A-2 (non-detects equal to reporting limit) occurred in species with low Hg concentrations, as expected. The highest absolute change in the mean between Tables 2 and A-2 was only 0.0039 mg[Hg]/kg[fish wet weight].

Table 2. Statistical information by market name, non-detects equal to half the reporting limit[a,b]

Market Name of Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	25th Perc. (mg/kg)	Median (mg/kg)	75th Perc. (mg/kg)	Max. (mg/kg)	Est. Lower 95% C.L. on Mean[d]	Est. Upper 95% C.L. on Mean[d]
	Det.	N.D.	Total												
Tuna	14	0	14	0%	0.42	0.25	0.39	93%	0.043	0.23	0.39	0.57	0.82	0.29	0.55
Swordfish	4	0	4	0%	0.4	0.19	0.33	82%	0.14	N/A	N/A	N/A	0.57	0.22	0.59
Mahi-mahi	1	0	1	0%	0.22	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Spanish Mackerel	3	0	3	0%	0.15	0.045	0.078	51%	0.11	N/A	N/A	N/A	0.2	0.1	0.2
Halibut	1	0	1	0%	0.15	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bluefish	3	0	3	0%	0.15	0.023	0.04	27%	0.12	N/A	N/A	N/A	0.16	0.12	0.17
Chilean Sea Bass	1	0	1	0%	0.13	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pollock	9	0	9	0%	0.13	0.034	0.057	44%	0.079	N/A	N/A	N/A	0.18	0.11	0.15
Monkfish	10	0	10	0%	0.11	0.044	0.069	65%	0.054	0.073	0.095	0.14	0.18	0.08	0.13
Porgy	6	0	6	0%	0.098	0.023	0.04	41%	0.068	N/A	N/A	N/A	0.13	0.079	0.12
Croaker	9	0	9	0%	0.084	0.024	0.043	51%	0.056	N/A	N/A	N/A	0.13	0.069	0.1
Sea Bass	11	0	11	0%	0.075	0.021	0.036	49%	0.03	0.064	0.078	0.087	0.11	0.063	0.088
Lobster	1	0	1	0%	0.069	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Skate	13	1	14	7%	0.06	0.035	0.06	99%	0.005	0.03	0.064	0.081	0.12	0.042	0.078
Flounder / Fluke / Sole	13	2	15	13%	0.051	0.028	0.051	100%	0.0047	0.038	0.049	0.066	0.1	0.037	0.065
Snapper	16	0	16	0%	0.049	0.022	0.039	80%	0.017	0.032	0.044	0.068	0.083	0.038	0.06
Catfish	7	0	7	0%	0.044	0.023	0.041	93%	0.024	N/A	N/A	N/A	0.094	0.026	0.061

Table 2. Statistical information by market name, non-detects equal to half the reporting limit[a,b] (continued)

Market Name of Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	25th Perc. (mg/kg)	Median (mg/kg)	75th Perc. (mg/kg)	Max. (mg/kg)	Est. Lower 95% C.L. on Mean[d]	Est. Upper 95% C.L. on Mean[d]
	Det.	N.D.	Total												
Cod	10	0	10	0%	0.031	0.012	0.019	63%	0.016	0.024	0.027	0.038	0.049	0.024	0.038
Whiting	7	1	8	13%	0.028	0.021	0.051	180%	0.0048	N/A	N/A	N/A	0.075	0.013	0.043
Bass	3	0	3	0%	0.025	0.019	0.033	130%	0.014	N/A	N/A	N/A	0.047	0.0034	0.047
Mackerel	7	1	8	13%	0.022	0.0078	0.017	76%	0.013	N/A	N/A	N/A	0.034	0.017	0.028
Ocean Perch	1	0	1	0%	0.022	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Herring	1	0	1	0%	0.022	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Oyster	6	2	8	25%	0.015	0.014	0.063	410%	0.0046	N/A	N/A	N/A	0.047	0.0059	0.025
Blue Crab	8	3	11	27%	0.015	0.0091	0.03	200%	0.0043	0.007	0.017	0.023	0.029	0.0098	0.021
Tilapia	5	6	11	55%	0.014	0.013	0.023	160%	0.0046	0.0049	0.005	0.021	0.038	0.0069	0.022
Squid	10	2	12	17%	0.014	0.0062	0.017	120%	0.0042	0.011	0.014	0.017	0.024	0.01	0.017
Mussel	5	2	7	29%	0.012	0.0057	0.048	390%	0.0044	N/A	N/A	N/A	0.019	0.0081	0.017
Rainbow Trout	1	0	1	0%	0.012	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Clam	3	4	7	57%	0.0081	0.0058	0.028	350%	0.0043	N/A	N/A	N/A	0.02	0.0038	0.012
Atlantic Salmon	3	6	9	67%	0.0081	0.0055	0.0095	120%	0.0043	N/A	N/A	N/A	0.019	0.0045	0.012
Scallop	1	6	7	86%	0.0055	0.0022	0.0088	160%	0.0043	N/A	N/A	N/A	0.011	0.0038	0.0071
Shrimp	1	6	7	86%	0.0054	0.0025	0.017	310%	0.0042	N/A	N/A	N/A	0.011	0.0036	0.0073

Table 2. Statistical information by market name, non-detects equal to half the reporting limit[a,b] (continued)

Market Name of Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	25th Perc. (mg/kg)	Median (mg/kg)	75th Perc. (mg/kg)	Max. (mg/kg)	Est. Lower 95% C.L. on Mean[d]	Est. Upper 95% C.L. on Mean[d]
	Det.	N.D.	Total												
Total	194	42	236	18%	0.075	0.12	N/A	N/A	0.0042	0.014	0.034	0.083	0.82	0.059	0.091

[a]Det. is the number of samples with results above the reporting limit (referred to here as "detects"), N.D. is the number of samples with results below the reporting limit, total is the total number of samples for the species (excluding replicate and duplicate samples), Perc. N.D. is the percent of the total number of samples which are below the reporting limit, Comp. S.D. is the standard deviation of the mean of the composite sample Hg concentrations for the market name, Est. Pop. S.D. is the estimated population standard deviation, Est. Pop. C.V. is the estimated coefficient of variation calculated as the population standard deviation/mean × 100%, 25th Perc. is the 25th percentile, 75th Perc. is the 75th percentile, est. lower 95% C.L. on mean is the estimated lower 95th percent confidence limit on the mean, and Est. Upper 95% C.L. on mean is the estimated upper 95th percent confidence limit on the mean.

[b]Number of Market Name groups included = 33.

[c]Number of composite samples after averaging the duplicates and replicates for a single composite sample and treating the average as a single point.

[d]Calculated based on the estimated population standard deviation.

NA = Value not available.

Composite samples taken for one market name—tuna—contained an outlier with significantly lower Hg concentrations than in the other tuna samples. This one composite consisted of three replicate analysis samples, with consistently low Hg concentrations. It was impossible to determine from the data collected the reason for the lower measured concentration. The average across these replicates is 0.0427 mg/kg, while the next highest Hg concentration for a tuna composite sample was 0.13 mg/kg. Table 3 presents the effects on the mean, S.D., C.V., and confidence limits of including and excluding this outlier. Without the outlier, the mean Hg concentration is slightly (7%) higher, and the S.D. decreases, reflecting the lower variation across samples when the outlier is removed. Because mean tuna concentration is not strongly sensitive to the outlier, it was not removed for all subsequent analyses.

Table 3. Examination of the effect of outlier data in the tuna species[a,b,c]

Market Name of Species	Number of Composite Samples			Perc. N.D. (%)	Mean (mg/kg)	S.D. (mg/kg)	C.V. (%)	Lower 95% C.L. on Mean	Upper 95% C.L. on Mean
	Det.	N.D.	Total						
Tuna, Including Outliers	14	0	14	0%	0.42	0.25	59%	0.29	0.55
Tuna, Excluding Outliers	13	0	13	0%	0.45	0.23	52%	0.32	0.57

[a]Column names are as defined in Table 2.
[b]Non-detects were assumed to equal half the reporting limit.
[c]The outlier composite sample replicate group had an average measured Hg concentration of 0.0427 mg/kg in fish tissue; the data are too limited to identify a possible reason for the low value.

3. MERCURY CONCENTRATION BY SPECIES, LOCATION, CONDITION, AND SIZE

Differences in habitat (or microhabitat) and diet among different species sold under the same market name (e.g., red and yellowtail snapper sold as snapper) might result in significant differences in Hg tissue residue levels among those species. Different water bodies (e.g., Pacific versus Atlantic) can exhibit lower or higher total Hg contamination of water, sediments, and food webs. Hg concentrations in fish and shellfish were analyzed, therefore, by species (see Section 3.1) and by vendor-stated water-body-of-origin categories (see Section 3.2). To better understand the role of specimen size and age on bioaccumulation of Hg, correlations of Hg concentration and fish or shellfish body mass and length also are presented (see Section 3.3).

3.1. SPECIES

Table 4 presents the summary statistics for each market name broken down by common name (a crosswalk linking the common name to the scientific name can be found in Section 2.1, Table 1). The table includes the 9 of the 33 market names which included two or more species with more than one composite sample per species. Because many species had fewer composite samples than available for market name categories, only the mean, S.D., C.V., maximum, and minimum Hg concentrations are reported in Table 4. The blue crab included both hardshells (condition for most of their annual life cycle) and softshells. Softshell crabs have recently shed their exoskeleton (i.e., have recently molted) to allow growth to a larger size.

In general, little difference was found when comparing species within a single market name. Tests for significance (at the $p = 0.05$ level) revealed no statistically significant differences when comparing the market name mean and the means in the individual common names. Offshore whiting Hg concentrations were higher on average than those for silver hake/whiting by a factor of 2.6, and summer flounder Hg concentrations exceeded those for blackback flounder by a factor of 2.4. For whiting, the organisms sampled from the CM were of similar length and mass for both species (i.e., offshore whiting and silver hake), suggesting size (as a proxy for age) is not responsible for this difference. For flounder/fluke/sole market name, however, the summer flounder sampled tended to be consistently larger than the blackback flounder from the CM. Hardshell blue crabs exhibited higher Hg concentrations than softshell crabs by a factor of 2.1; softshell crabs had multiple non-detects, while all hardshell crab composite samples had detectable Hg concentrations. Softshell crabs also tended to have a significantly higher water content, which would dilute Hg concentrations, than hardshell crabs—particularly hardshells in the few weeks prior to molting when body tissues essentially fill the shell. The conservative assumption that the Hg concentrations in the non-detect samples were equal the full reporting limit, as shown in Table A-3 in Appendix A, does not alter any of the conclusions stated above.

Table 4. Mercury concentrations by species or by condition (e.g., hard- or softshell) within a market name; non-detects equal to half the reporting limit[a,b]

Market Name	Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Catfish	Catfish, Blue	2	0	2	0%	0.037	0.012	0.021	57%	0.028	0.045
	Catfish, Channel	4	0	4	0%	0.048	0.032	0.055	110%	0.024	0.094
	Catfish, White	1	0	1	0%	0.041	N/A	N/A	N/A	N/A	N/A
	All Catfish	7	0	7	0%	0.044	0.023	0.041	93%	0.024	0.094
Cod[d]	Cod, Atlantic	7	0	7	0%	0.031	0.013	0.022	70%	0.016	0.049
	Cod, Pacific	3	0	3	0%	0.030	0.009	0.015	49%	0.024	0.040
	All Cod	10	0	10	0%	0.031	0.012	0.019	63%	0.016	0.049
Blue Crab	Blue Crab/Hardshell	5	0	5	0%	0.021	0.003	0.013	62%	0.017	0.025
	Blue Crab/Softshell	3	3	6	50%	0.010	0.010	0.028	270%	0.004	0.029
	All Crab	8	3	11	27%	0.015	0.009	0.030	200%	0.004	0.029
Flounder/ Fluke/ Sole	Flounder, Blackback	4	1	5	20%	0.031	0.017	0.032	100%	0.005	0.049
	Flounder, Summer	5	0	5	0%	0.073	0.026	0.045	61%	0.045	0.100
	Sole, Gray	4	0	4	0%	0.059	0.012	0.020	34%	0.044	0.071
	All Flounder/Fluke/Sole	13	1	14	13%	0.051	0.028	0.051	100%	0.005	0.100

Table 4. Mercury concentrations by species or by condition (e.g., hard- or softshell) within a market name; non-detects equal to half the reporting limit[a,b] (continued)

Market Name	Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Shrimp	Shrimp, Black Tiger	0	2	2	100%	0.004	0.000	0.001	13%	0.004	0.004
	Shrimp, White	1	4	5	80%	0.006	0.003	0.020	340%	0.004	0.011
	All Shrimp	1	6	7	86%	0.005	0.003	0.017	310%	0.004	0.011
Snapper	Snapper, Caribbean Red	1	0	1	0%	0.039	N/A	N/A	N/A	N/A	N/A
	Snapper, Lane	2	0	2	0%	0.040	0.018	0.031	78%	0.027	0.052
	Snapper, Red	6	0	6	0%	0.057	0.020	0.035	63%	0.031	0.076
	Snapper, Vermilion	3	0	3	0%	0.040	0.037	0.065	160%	0.017	0.083
	Snapper, Yellowtail	4	0	4	0%	0.051	0.022	0.038	76%	0.034	0.082
	All Snapper	16	0	16	0%	0.049	0.022	0.039	80%	0.017	0.083
Squid [d]	Squid, Japanese Flying	3	1	4	25%	0.010	0.004	0.010	92%	0.005	0.015
	Squid, Longfin (Atlantic)	7	0	7	0%	0.017	0.005	0.017	100%	0.012	0.024
	All Squid	10	1	11	17%	0.014	0.006	0.017	120%	0.004	0.024
Tuna	Yellowfin Tuna	7	0	7	0%	0.420	0.280	0.440	100%	0.043	0.800
	Bigeye Tuna	7	0	7	0%	0.410	0.230	0.360	87%	0.130	0.820
	All Tuna	14	0	14	0%	0.420	0.250	0.390	93%	0.043	0.820

Table 4. Mercury concentrations by species or by condition (e.g., hard- or softshell) within a market name; non-detects equal to half the reporting limit[a,b] (continued)

Market Name	Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Whiting	Whiting, Offshore	2	0	2	0%	0.052	0.032	0.062	120%	0.029	0.075
	Whiting/Silver Hake	5	1	6	17%	0.020	0.011	0.028	140%	0.005	0.033
	All Whiting	7	1	8	13%	0.028	0.021	0.051	180%	0.005	0.075

[a]Column names are as defined in Table 2.
[b]Number of Market Names encompassing multiple species or conditions equals 10 of the 33 total Market Names.
[c]Number of composite samples after averaging the duplicates and replicates for a single composite sample and treating the average as a single point.
[d]Species from different oceans.
NA = Value not available because only one composite sample was analyzed.

3.2. WATER BODY OF ORIGIN

Water body (e.g., Atlantic or Pacific) of origin and source type (e.g., farmed or wild) were used to determine if there were any differences in Hg concentrations within a species or among species for a given market name that could be attributed to the water body of origin or the source type for the fish marketed at the CM. Vendor-identified water bodies of origin (included in the CM database) were used to assign composite samples to the following uniform categories: "Atlantic", "Pacific", "Bay", "Sound", "Lake", "River", "Foreign Farmed", and "Unknown". In addition, Atlantic and Pacific water bodies were also further separated into "Wild" and "Farmed" categories. All market names which had at least two composite samples from at least two different water bodies were included in the analysis.

Table 5 shows the seven (out of the 33) market names that included at least two composite samples originating from at least two locations. Because the water body subcategories have fewer composite samples than the market names categories, only mean, S.D., C.V., maximum, and minimum values are reported in Table 5.

Tests for significance (at the $p = 0.05$ level) revealed no statistically significant differences when comparing the market name mean and the means in the individual water bodies of origin. No clear trend was found for concentrations of Hg in organisms caught in the Atlantic versus Pacific Oceans. Wild cod caught from both the Atlantic and Pacific oceans had nearly identical mean Hg concentrations. Longfin squid from the Atlantic Ocean had nearly double the Hg concentration of Japanese flying squid from the Pacific Ocean. Swordfish caught in the Pacific had approximately 50% more Hg than those from the Atlantic Ocean; data on the original size of the fish from which swordfish steaks were cut were inadequate to determine if the effect was due to differences in the average size of fish harvested from each ocean. Note that at this level of categorization, the sample sizes for each group were very small. When the non-detects were assumed to equal the full reporting limit, as shown in Table A-4 of Appendix A, none of the conclusions above were altered.

Table 5. Mercury concentrations by water body of origin within a market name species; non-detects equal to half the reporting limit[a]

Market Name of Species	Water Body or Water Type of Origin	Number of Composite Samples[b]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Clam	Clam, Atlantic, Wild	1	3	4	75%	0.0082	0.0079	0.037	450.0%	0.0043	0.02
	Clam, Farmed	2	0	2	0%	0.0095	0.00071	0.0041	43.0%	0.009	0.01
	Clam, Long Island Sound	0	1	1	100%	0.0047	N/A	N/A	N/A	N/A	N/A
	All Clam	3	4	7	57%	0.0081	0.0058	0.028	350.0%	0.0043	0.02
Cod	Cod, Atlantic, Wild	7	0	7	0%	0.031	0.013	0.022	70.0%	0.016	0.049
	Cod, Pacific, Wild	3	0	3	0%	0.03	0.0086	0.015	49.0%	0.024	0.04
	All Cod	10	0	10	0%	0.031	0.012	0.019	63.0%	0.016	0.049
Mussel	Mussel, Atlantic, Wild	4	1	5	20%	0.013	0.0055	0.046	340.0%	0.0044	0.019
	Mussel, Farmed	1	1	2	50%	0.0097	0.0075	0.064	660.0%	0.0044	0.015
	All Mussel	5	2	7	29%	0.012	0.0057	0.048	390.0%	0.0044	0.019
Salmon	Atlantic Salmon, Wild	0	3	3	100%	0.0044	0.00022	0.00038	8.5%	0.0043	0.0047
	Atlantic Salmon, Farmed	3	3	6	50%	0.0099	0.0061	0.01	110.0%	0.0044	0.019
	All Salmon	3	6	9	67%	0.0081	0.0055	0.0095	120.0%	0.0043	0.019
Snapper	Snapper, Atlantic, Wild	13	0	13	0%	0.05	0.024	0.042	83.0%	0.017	0.083
	Snapper, Pacific, Wild	2	0	2	0%	0.04	0.018	0.031	78.0%	0.027	0.052
	Snapper, Unknown	1	0	1	0%	0.047	N/A	N/A	N/A	N/A	N/A
	All Snapper	16	0	16	0%	0.049	0.022	0.039	80.0%	0.017	0.083

Table 5. Mercury concentrations by water body of origin within a market name species; non-detects equal to half the reporting limit[a] (continued)

Market Name of Species	Water Body or Water Type of Origin	Number of Composite Samples[b]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Squid	Squid, Atlantic, Wild	7	0	7	0%	0.017	0.0048	0.017	100.0%	0.012	0.024
	Squid, Pacific, Wild	3	2	5	40%	0.0092	0.0046	0.0098	110.0%	0.0042	0.015
	All Squid	10	2	12	17%	0.014	0.0062	0.017	120.0%	0.0042	0.024
Swordfish	Swordfish, Atlantic, Wild	2	0	2	0%	0.33	0.26	0.45	140.0%	0.14	0.51
	Swordfish, Pacific, Wild	2	0	2	0%	0.48	0.13	0.22	46.0%	0.39	0.57
	All Swordfish	4	0	4	0%	0.4	0.19	0.33	82.0%	0.14	0.57

[a]Column names are as defined in Table 2.
[b]Number of composite samples after averaging the duplicates and replicates for a single composite sample and treating the average as a single point.
NA = Value not available because only one composite sample was analyzed.

3.3. EFFECTS OF BODY WEIGHT AND LENGTH ON MERCURY CONCENTRATIONS

MeHg in aquatic food webs can bioaccumulate in the consumer organisms over time, suggesting that older fish will tend to have higher tissue MeHg and total Hg concentrations. Both the length and weight of many invertebrates and fish within a single species can act as a proxy for age; however, depending on food availability and water temperature, individuals of the same species may grow at substantially different rates. We examined correlations between total Hg concentrations and body weight, length, and caudal length (fish-only measure)[1] to determine whether significant trends between measures of size and Hg concentrations were present in the data. Only fish weights and lengths that were reported for the entire fish were used in the analysis, and not all the market names could be analyzed since only a fillet or some other partial sample (e.g., steak) was collected from the CM. For fish species, the different lengths and weights for each of the fish in a composite sample were averaged to get the average length and weight for that sample. For shellfish, the total composite weight was divided by the number of organisms in the composite to get the average weight. Shrimp were included in the analysis, although they had been headed prior to collection.

The squares of the correlation coefficients (R^2) between Hg concentrations and the length, caudal length, and weight were calculated, and graphs were generated to visualize the relationships by species. Generally, concentration tends to increase as measures of length and weight increase. However, the correlations and, in turn, R^2 values can be very sensitive to patterns in the data that may result in improperly inflated values and findings of statistical significance that are not meaningful. There were a number of examples of these sorts of issues in the data. In a number of cases, large R^2 values were clearly the result of large separations between data points and/or clusters of data points. In some cases, clusters of species specific data displayed a negative relationship between concentration and size but the overall relationship between clusters was positive. Although the data generally show positive relationships between measures of size and Hg concentration, the results of the tests of significance of the correlations between size and concentration and the graphs of the data were not included because of the many problematic cases.

3.4. WILD VS FARMED RESULTS

Only three species (clams, mussels, and salmon) had results on wild versus farmed samples, precluding a thorough statistical analysis. However, a descriptive qualitative analysis is provided herein. Three of the four samples of wild Atlantic clams had non-detectable concentrations of Hg. The two farmed samples of clams had detectable concentrations of Hg; however the concentration was low for both samples. Four of the five samples of wild Atlantic

[1] The three most common measures of fish length are "standard length" (from tip of the longest jaw or snout to the base of tail fin, specifically, to the end of the hypural bone or caudal peduncle), "total length" (snout to tip of longest tail fin when lobes are pinched together), and "fork length" (snout to the center of the fork in the caudal fin). The lengths of whole fish from the CM were reported as "length", equivalent to total length, and "caudal length", equivalent to the definition of standard length above.

mussels had detectable concentrations of Hg; however, the concentrations were low for all samples. One of the two farmed samples had a detectable concentration of Hg, but the concentration was low. All three samples of wild Atlantic salmon had non-detectable concentrations for Hg. Three of the six samples of farmed Atlantic salmon had detectable concentrations of Hg however, the concentrations were low.

4. ANALYSIS OF MEASUREMENT VARIABILITY

For the analysis of the CM samples, multiple organisms were combined into a composite sample. Next, an analysis sample was drawn from the composite and the laboratory testing procedure was applied to measure the total Hg concentration. Since the composite sample homogenate was not perfectly uniform, there was some variability associated with taking an aliquot of the composite sample for preparation and analysis. In addition, the sample preparation and analysis may have been introduced of variability, such as inherent measurement error or calibration drift.

To help quantify both sources of variability, analyses were performed multiple times on a subset of the composite samples. "Duplicate" analysis samples were drawn from the same composite sample and analyzed separately. In other cases, the same analysis sample was analyzed using the laboratory testing procedure two or three times ("replicates"). In each of these cases, the collection of measurements within a duplicate pair or within a replicate group was referred to as a "duplicate/replicate group". Within the data, there were 24 duplicate groups and 15 replicate groups, and, in general, these groups were for different market name species.

In order to determine the contribution of the measurement variability to the overall Hg variability, the variance within each duplicate and replicate group was calculated as follows:

$$Variance = \frac{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2 + ... + (x_n - \bar{x})^2}{n - 1}$$

where:

x_n	=	Measured concentration for measurement n,
$\bar{x}$	=	Mean of measured concentrations for the replicate/duplicate group, and
n	=	Number of measurements per replicate/duplicate group.

The variance equals the square of the S.D. within the replicate/duplicate group. Table 6 and Table 7 show the variances for the duplicates and replicates, respectively. These variances were calculated using both the raw Hg concentrations and the natural logarithm of the Hg concentrations assuming the non-detects were either all equal to half the reporting limit or all equal to the reporting limit.

Because the duplicate and replicate groups were across many different market name species, it was necessary to confirm that the variances in the measurements did not vary significantly across the different replicate and duplicate groups before performing the full variability analysis. The log-normal distribution is likely to give a better fit than the normal distribution because the data showed evidence of a skew in the positive direction. The Bartlett homogeneity of variance test[2] requires normality to be a valid test; thus, it was applied separately

[2] The Bartlett homogeneity of variance test assumes the individual concentrations or log of concentrations are normally distributed. Non-detects were replaced by half the reporting limit for this test.

to the duplicate and replicate groups after transforming the concentrations using the natural logarithm.

For the duplicates, the Bartlett test indicated the differences in the variances were not significant in log space (*p*-value 0.87). For the replicates, the analysis found significant non-homogeneity in the natural logarithm of the concentrations. However, an inspection of the data revealed that the highest and lowest variances were for samples 001.C and 237.C, each of which include non-detects. Because the true values of the non-detects were uncertain, and hence the variances are uncertain, these two replicate groups were excluded from the analysis. After removing these measurements, the Bartlett test was not significant in log space (*p*-value 0.77). Thus, the analysis of measurement variability for replicates excluded sample groups 001 and 237.

To analyze the variability, the contributions of the measurement variability to the overall variance amongst all the concentrations in the duplicate and replicate groups were separately calculated. Suppose that the *i*th duplicate or replicate group has n_i measurements x_{ij}, j = 1, 2, … n_i. The total sum of squares (*TSS*) can be written as:

$$TSS = \sum_i \sum_j (x_{ij} - \bar{x})^2 ,$$

where:

$$Grand\ mean = \bar{x} = \sum_i \sum_j x_{ij} / \sum_i n_i .$$

This total sum of squares can then be broken into the sum of squares between groups ($SS_{between}$) plus the sum of the squares within groups (SS_{within}):

$$TSS = SS_{between} + SS_{within}.$$

The sum of squares within groups accounts for variation within a single group and is given by the formula:

$$SS_{within} = \sum_i \sum_j (x_{ij} - \bar{x}_i)^2 ,$$

where $\bar{x}_i$ denotes the mean of the n_i measurements on the *i*'th duplicate or replicate group:

$$\bar{x}_i = \sum_j x_{ij} / n_i .$$

The sum of squares between groups accounts for the variation across groups and is given by the formula:

$$SS_{between} = \sum_i n_i (\bar{x}_i - \bar{x})^2 .$$

The degrees of freedom (*df*) is defined as:

$$df = \sum_i (n_i - 1)$$

so that the variance within groups is defined as:

$$Variance\ within\ groups = SS_{within} / df$$

and the S.D. within groups is the square root of the variance within the group. Finally, the proportion of the variance explained by the variability within the duplicates or replicates is defined as:

$$Prop\ of\ Variance = \frac{Variance\ \ within\ \ groups}{\left(\frac{TSS}{N-1}\right)},$$

where N is the total number of measurements across all groups.[3]

Table 8 and Table 9 show the variability analysis for the duplicate groups and replicate groups (respectively) using both the raw and log concentrations and assuming non-detects are either all equal to half the reporting limit or all equal to the reporting limit. The calculations in log space are shown in bold since the data are positively skewed and the lognormal distribution is likely a better characterization of their distribution. The analysis indicates that the proportion of the variance of a sample explained by the variation among the duplicates or replicates of that sample is a very small (less than 1%) portion of the overall variance across all measurements in all groups. In part, this result stems from the fact that many different species are included in the analysis and the variance in concentration (and log concentration) is large across the groups from different species.

To determine the effect of limiting the analysis to a single species, the calculations were repeated for the tuna market name species which included four replicate groups and three duplicate groups. Table 10 shows the variability analysis for the tuna duplicate and replicate groups in both raw and log space, with the log space calculations shown in bold. In this case, the proportion of the variance explained by the measurement variability in the duplicates remains close to 1% (1.2%) for the log space calculations. For the replicate groups, the proportion of the variance explained by the measurement variability was still below 1%; however, this analysis includes the replicate group identified as an outlier in Section 2. When this replicate group was excluded, the proportion of the variance explained by the measurement variability was again below 1% (0.22%, not shown). This analysis suggests that even restricting the analysis to a single species, the variability associated with the composite sampling procedure and the

[3] This proportion of variance equation is an approximation to a much more complicated component of variance calculation. Similar results can be obtained using the sum of squares within groups divided by the total sum of squares, which gives the proportion of the sum of squares explained.

laboratory testing procedure was small. In fact, the variability was minimal and demonstrated the high level of quality control that was achieved in generating the data.

Table 6. Variance within individual composite sample duplicates[a]

Composite Sample Number	Number of Duplicates	Market Name	Non-Detects Equal to Half the Reporting Limit			Non-Detects Equal to the Reporting Limit		
			Mean	Variance Using Concentration	Variance Using Log of Concentration	Mean	Variance Using Concentration	Variance Using Log of Concentration
4	2	Cod	2.6×10^{-02}	4.5×10^{-06}	6.9×10^{-03}	2.6×10^{-02}	4.5×10^{-06}	6.9×10^{-03}
15	2	Shrimp	4.3×10^{-03}	1.1×10^{-08}	6.0×10^{-04}	8.7×10^{-03}	4.5×10^{-08}	6.0×10^{-04}
26	2	Monkfish	1.5×10^{-01}	5.0×10^{-05}	2.4×10^{-03}	1.5×10^{-01}	5.0×10^{-05}	2.4×10^{-03}
37	2	Tuna	4.1×10^{-01}	0.0	0.0	4.1×10^{-01}	0.0	0.0
48	2	Flounder/Fluke/Sole	2.6×10^{-02}	0.0	0.0	2.6×10^{-02}	0.0	0.0
59	2	Atlantic Salmon	4.7×10^{-03}	1.1×10^{-08}	5.1×10^{-04}	9.4×10^{-03}	4.5×10^{-08}	5.1×10^{-04}
70	2	Monkfish	1.5×10^{-01}	0.0	0.0	1.5×10^{-01}	0.0	0.0
81	2	Blue Crab	2.2×10^{-02}	2.0×10^{-06}	4.1×10^{-03}	2.2×10^{-02}	2.0×10^{-06}	4.1×10^{-03}
92	2	Bluefish	1.6×10^{-01}	0.0	0.0	1.6×10^{-01}	0.0	0.0
103	2	Flounder/Fluke/Sole	7.1×10^{-02}	8.0×10^{-06}	1.6×10^{-03}	7.1×10^{-02}	8.0×10^{-06}	1.6×10^{-03}
114	2	Catfish	4.5×10^{-02}	0.0	0.0	4.5×10^{-02}	0.0	0.0
125	2	Porgy	8.6×10^{-02}	8.5×10^{-05}	1.2×10^{-02}	8.6×10^{-02}	8.5×10^{-05}	1.2×10^{-02}
136	2	Flounder/Fluke/Sole	4.5×10^{-02}	5.0×10^{-07}	2.5×10^{-04}	4.5×10^{-02}	5.0×10^{-07}	2.5×10^{-04}
146	4	Lobster	7.3×10^{-02}	1.8×10^{-05}	3.9×10^{-03}	7.3×10^{-02}	1.8×10^{-05}	3.9×10^{-03}
158	2	Tuna	5.0×10^{-01}	5.0×10^{-05}	2.0×10^{-04}	5.0×10^{-01}	5.0×10^{-05}	2.0×10^{-04}
169	2	Blue Crab	2.5×10^{-02}	5.0×10^{-07}	8.3×10^{-04}	2.5×10^{-02}	5.0×10^{-07}	8.3×10^{-04}
180	2	Whiting	7.5×10^{-02}	1.3×10^{-05}	2.3×10^{-03}	7.5×10^{-02}	1.3×10^{-05}	2.3×10^{-03}
191	2	Mussel	1.6×10^{-02}	0.0	0.0	1.6×10^{-02}	0.0	0.0

Table 6. Variance within individual composite sample duplicates[a] (continued)

Composite Sample Number	Number of Duplicates	Market Name	Non-Detects Equal to Half the Reporting Limit			Non-Detects Equal to the Reporting Limit		
			Mean	Variance Using Concentration	Variance Using Log of Concentration	Mean	Variance Using Concentration	Variance Using Log of Concentration
202	2	Tuna	6.8×10^{-01}	8.0×10^{-04}	1.7×10^{-03}	6.8×10^{-01}	8.0×10^{-04}	1.7×10^{-03}
213	2	Croaker	5.8×10^{-02}	5.0×10^{-07}	1.5×10^{-04}	5.8×10^{-02}	5.0×10^{-07}	1.5×10^{-04}
224	2	Cod	2.7×10^{-02}	1.2×10^{-05}	1.8×10^{-02}	2.7×10^{-02}	1.2×10^{-05}	1.8×10^{-02}
235	2	Mackerel	2.1×10^{-02}	5.0×10^{-07}	1.2×10^{-03}	2.1×10^{-02}	5.0×10^{-07}	1.2×10^{-03}
246	2	Monkfish	1.1×10^{-01}	0.0	0.0	1.1×10^{-01}	0.0	0.0
257	2	Scallop	1.1×10^{-02}	5.0×10^{-07}	4.5×10^{-03}	1.1×10^{-02}	5.0×10^{-07}	4.5×10^{-03}
		Minimum Variance		0.0	0.0		0.0	0.0
		Maximum Variance		8.0×10^{-04}	1.8×10^{-02}		8.0×10^{-04}	1.8×10^{-02}

[a]Duplicates refer to multiple samples drawn from the same composite.

Table 7. Variance within individual composite sample replicates[a]

Composite Sample Number	Number of Replicates	Market Name	Non-Detects Equal to Half the Reporting Limit			Non-Detects Equal to the Reporting Limit		
			Mean	Variance Using Concentration	Variance Using Log of Concentration	Mean	Variance Using Concentration	Variance Using Log of Concentration
001.C	3	Flounder/Fluke/Sole	4.8×10^{-03}	3.3×10^{-09}	1.4×10^{-04}	9.6×10^{-03}	1.3×10^{-08}	1.4×10^{-04}
021.C	3	Squid	2.4×10^{-02}	4.0×10^{-06}	7.0×10^{-03}	2.4×10^{-02}	4.0×10^{-06}	7.0×10^{-03}
041.C	2	Oyster	2.0×10^{-02}	5.0×10^{-07}	1.3×10^{-03}	2.0×10^{-02}	5.0×10^{-07}	1.3×10^{-03}
058.C	3	Tuna	4.3×10^{-02}	2.3×10^{-06}	1.3×10^{-03}	4.3×10^{-02}	2.3×10^{-06}	1.3×10^{-03}
076.C	3	Flounder/Fluke/Sole	5.7×10^{-02}	9.3×10^{-06}	2.9×10^{-03}	5.7×10^{-02}	9.3×10^{-06}	2.9×10^{-03}
094.C	3	Ocean Perch	2.2×10^{-02}	2.3×10^{-06}	4.6×10^{-03}	2.2×10^{-02}	2.3×10^{-06}	4.6×10^{-03}
111.C	3	Tuna	8.2×10^{-01}	7.0×10^{-04}	1.0×10^{-03}	8.2×10^{-01}	7.0×10^{-04}	1.0×10^{-03}
129.C	3	Sea Bass	6.1×10^{-02}	4.1×10^{-05}	1.2×10^{-02}	6.1×10^{-02}	4.1×10^{-05}	1.2×10^{-02}
147.C	3	Lobster	6.7×10^{-02}	1.4×10^{-05}	3.3×10^{-03}	6.7×10^{-02}	1.4×10^{-05}	3.3×10^{-03}
165.C	3	Monkfish	8.0×10^{-02}	3.2×10^{-05}	5.0×10^{-03}	8.0×10^{-02}	3.2×10^{-05}	5.0×10^{-03}
183.C	3	Tuna	1.9×10^{-01}	1.0×10^{-04}	2.8×10^{-03}	1.9×10^{-01}	1.0×10^{-04}	2.8×10^{-03}
201.C	3	Tuna	8.0×10^{-01}	4.3×10^{-04}	6.8×10^{-04}	8.0×10^{-01}	4.3×10^{-04}	6.8×10^{-04}
219.C	3	Skate	7.4×10^{-02}	9.0×10^{-05}	1.7×10^{-02}	7.4×10^{-02}	9.0×10^{-05}	1.7×10^{-02}
237.C	3	Mackerel	1.6×10^{-02}	9.4×10^{-05}	7.8×10^{-01}	1.7×10^{-02}	5.1×10^{-05}	2.4×10^{-01}
255.C	3	Skate	2.7×10^{-02}	4.0×10^{-06}	5.5×10^{-03}	2.7×10^{-02}	4.0×10^{-06}	5.5×10^{-03}
Minimum Variance				3.3×10^{-09}	1.4×10^{-04}		1.3×10^{-08}	1.4×10^{-04}
Maximum Variance				7.0×10^{-04}	7.8×10^{-01}		7.0×10^{-04}	2.4×10^{-01}

[a]Replicates refer to multiple laboratory analyses performed on the same composite sample.

Table 8. Variability analysis of composite sample duplicates

	Non-Detects Equal to Half the Reporting Limit		Non-Detects Equal to the Reporting Limit	
	Using Composite Concentrations	**Using Log of Composite Concentrations**	**Using Composite Concentrations**	**Using Log of Composite Concentrations**
Total Sum of Squares (TSS)	1.3	**80**	1.3	**69**
Sum of Squares Between Groups (BSS)	1.3	**80**	1.3	**69**
Sum of Squares Within Groups (WSS)	1.1×10^{-03}	**6.8×10^{-02}**	1.1×10^{-03}	**6.8×10^{-02}**
Total Degrees of Freedom Within Groups	26	**26**	26	**26**
Variance Within Groups	4.2×10^{-05}	**2.6×10^{-03}**	4.2×10^{-05}	**2.6×10^{-03}**
Standard Deviation Within Groups	6.4×10^{-03}	**5.1×10^{-02}**	6.4×10^{-03}	**5.1×10^{-02}**
Proportion of Variance Explained by Variability Within Groups	0.15%	**0.16%**	0.15%	**0.19%**

Bold indicates the calculations in log space since the data are positively skewed and the lognormal distribution is likely a better characterization of their distribution.

Table 9. Variability analysis of composite sample replicates[a]

	Non-Detects Equal to Half the Reporting Limit		Non-Detects Equal to the Reporting Limit	
	Using Composite Concentrations	**Using Log of Composite Concentrations**	**Using Composite Concentrations**	**Using Log of Composite Concentrations**
Total Sum of Squares (TSS)	2.9	**53**	2.9	**53**
Sum of Squares Between Groups (BSS)	2.9	**53**	2.9	**53**
Sum of Squares Within Groups (WSS)	2.9×10^{-03}	**1.3×10^{-01}**	2.9×10^{-03}	**1.3×10^{-01}**
Total Degrees of Freedom Within Groups	25	**25**	25	**25**
Variance Within Groups	1.1×10^{-04}	**5.1×10^{-03}**	1.1×10^{-04}	**5.1×10^{-03}**
Standard Deviation Within Groups	1.1×10^{-02}	**7.1×10^{-02}**	1.1×10^{-02}	**7.1×10^{-02}**
Proportion of Variance Explained by Variability Within Groups	0.15%	**0.35%**	0.15%	**0.35%**

[a]Excludes samples 001.C and 237.C due to the presence of non-detects.
Bold indicates the calculations in log space since the data are positively skewed and the lognormal distribution is likely a better characterization of their distribution.

Table 10. Variability analysis of tuna replicate and duplicate groups

	Duplicates		Replicates	
	Using Composite Concentrations	**Using Log of Composite Concentrations**	**Using Composite Concentrations**	**Using Log of Composite Concentrations**
Total Sum of Squares (TSS)	0.077	**0.26**	1.5	**18**
Sum of Squares Between Groups (BSS)	0.076	**0.26**	1.5	**18**
Sum of Squares Within Groups (WSS)	8.5×10^{-04}	**1.9×10^{-03}**	2.5×10^{-03}	**1.2×10^{-02}**
Total Degrees of Freedom Within Groups	3	**3**	8	**8**
Variance Within Groups	2.8×10^{-04}	**6.5×10^{-04}**	3.1×10^{-04}	**1.4×10^{-03}**
Standard Deviation Within Groups	1.7×10^{-02}	**2.5×10^{-02}**	1.8×10^{-02}	**3.8×10^{-02}**
Proportion of Variance Explained by Variability Within Groups	1.8%	**1.2%**	0.23%	**0.09%**

Bold indicates the calculations in log space since the data are positively skewed and the lognormal distribution is likely a better characterization of their distribution.

5. COMPARISON OF CM DATA TO RISK METRICS

5.1. FISH ADVISORIES AS A COMMUNICATOR OF RISK

Fish advisories to the public, either at the state or federal (e.g., EPA, FDA) level, provide a narrative statement that is reasonably easy for the public to understand and to follow (i.e., implement). For sport or game fish, the states generally post fish advisories by species or for specific bodies of water or regions of the state as needed. For commercially marketed fish, the advisories usually specify several categories of fish consumption frequency (e.g., two servings per week, one serving per week, one serving per month, never consume) for specific population subgroups (e.g., children, women of childbearing age) and which fish market names fall into each consumption frequency category.

Hg is of particular concern as a developmental neurotoxicant; therefore, Hg fish advisories generally target women of childbearing age, nursing infants, and young children. The most recent United States federal fish advisory for Hg is the 2004 EPA and FDA joint recommendation (see text box below), although FDA might still be receiving comments on its new risk/benefit analysis[4] to support an updated advisory that attempts to balance the developmental health benefits of consuming fish against the risks from Hg consumption.

2004 EPA and FDA Advice for:

- Women Who Might Become Pregnant;
- Women Who are Pregnant;
- Nursing Mothers;
- Young Children

By **following these three recommendations** for selecting and eating fish or shellfish, women and young children will receive the benefits of eating fish and shellfish and be confident that they have reduced their exposure to the harmful effects of Hg.

1. Do not eat Shark, Swordfish, King Mackerel, or Tilefish because they contain high levels of Hg.
2. Eat up to 12 ounces (2 average meals) a week of a variety of fish and shellfish that are lower in Hg.
    - Five of the most commonly eaten fish that are low in Hg are shrimp, canned light tuna, salmon, pollock, and catfish.
    - Another commonly eaten fish, albacore ("white") tuna has more Hg than canned light tuna. So, when choosing your two meals of fish and shellfish, you may eat up to 6 ounces (one average meal) of albacore tuna per week.
3. Check local advisories about the safety of fish caught by family and friends in your local lakes, rivers, and coastal areas. If no advice is available, eat up to 6 ounces (one average meal) per week of fish you catch from local waters, but don't consume any other fish during that week.

Follow these same recommendations when feeding fish and shellfish to your young child, but serve smaller portions. Source: http://www.fda.gov/bbs/topics/news/2004/NEW01038.html.

[4] http://www.cfsan.fda.gov/~dms/mehg109.html.

Some states with substantial coastlines also issues fish advisories for commercially marketed fish (e.g., FL, ME), but most appear not to do so. New York State has not issued any fish advisories for commercially marketed fish, but has issued species-specific freshwater fish advisories for specific freshwater bodies.

There are several elements to a risk assessment to support development of fish advisories:

- Assumed meal size (i.e., 6 oz cooked or 8 oz fresh weight for women in the EPA/FDA advisory for Hg as MeHg);
- Assumed fish ingestion frequency (e.g., number of meals per week);
- Assumptions concerning which parts of fish are eaten (e.g., fillet without skin);
- Monitoring data for chemical concentrations in the fish as marketed (i.e., data adequacy given spatial and temporal variation and species diversity);
- Assumed body weights of the human receptors (e.g., 65 kg for women);
- Toxicity reference value(s) of concern (e.g., EPA RfD for MeHg); and
- Sensitive lifestages or subpopulations (e.g., pregnant women - developing fetus).

Additional issues are associated with developing fish advisories in narrative form to assist the public in making informed decisions about purchasing and consuming fish:

- The identification of species of concern by market name and adequacy of coverage by those names (e.g., the “Spanish Mackerel” in the CM survey is biologically similar to the “King Mackerel” in the EPA-FDA fish advisory, yet is not named in the latter fish advisory);
- Providing a conditional narrative that recognizes variation in fish consumption habits (e.g., how one can select different numbers of servings of fish per week depending on combinations of fish in the lower and higher contamination categories); and
- Accounting for families that also consume self-caught freshwater or estuarine fish, particularly in regions of the country with relatively high freshwater contamination levels (e.g., Hg in New England lakes).

To provide a basis of comparison with health-based criteria for Hg concentrations in fish obtained from the CM, the CM fish tissue concentrations are shown within a risk context using two types of health-based criteria:

1. "Action Levels" expressed as Hg or MeHg concentrations in fish on a wet-weight basis. This rapidly identifies the fish types that show sufficient Hg residues to merit action by the agency that published the level depending on their risk management policies.

2. "Allowed Servings per Week" to minimize the possibility that total Hg or MeHg ingestion by women of child-bearing age would exceed the EPA RfD of 0.1 mg/kg-day MeHg. This information may assist in developing narrative fish advisories for customers of the CM.

5.2. COMPARISON OF CM CONCENTRATIONS TO ACTION LEVELS

Table 11 lists several international, U.S. federal, and U.S. state agency standards or guidelines for total Hg and MeHg residue levels in fish that may trigger an agency action (e.g., issuing fish advisories, reducing Hg concentrations in effluents). This table is organized by the value of the "action level" (in mg [Hg or MeHg]/kg [edible portion fish, wet weight]) and includes attributes of the level important to its ancillary descriptive information.

Note that the action levels generally are specified as mg [MeHg]/kg [fish edible portion]; in some cases, however, they are specified on the basis of total Hg. The "edible" portion of ray-finned[5] fish generally is the fillet (skinless) (U.S. EPA, 2000a,b), while the edible portion of other fish may be called other names (e.g., "wings" for skate; "tail" for monkfish, "steak" for tuna) and the edible portion of shellfish also varies by species (e.g., muscle of scallop; entire body of clam and mussel).

Fish monitoring programs generally analyze *total Hg* to minimize costs or to maximize the number of samples that can be analyzed. EPA recommends that States and Tribes monitoring fish for Hg levels assume that all Hg in the fish sampled is present as MeHg (U.S. EPA, 2009). The proportion of total Hg present as MeHg for predatory, or game fish, is high, more than 90% in most studies (U.S. EPA, 2001, 2009b). The ratio of MeHg to total Hg generally is less for lower trophic level fish (e.g., trophic-level three fish, approximately 80%; U.S. EPA, 2009b), and even lower for shellfish (e.g., 49% for oysters from estuaries in South Carolina, [Kawaguchi et al., 1999]; 35% in blue crabs [Ward et al., 1979]). The EPA considers it reasonable for States and authorized Native American Tribes to implement the MeHg "Fish Tissue Residue Criterion" for ambient waters by analyzing fish tissue samples for total Hg first. They may analyze for MeHg as they may deem necessary. Note that the only governmental entity for which we identified a different guideline for total Hg than for MeHg is Japan (0.4 and 0.3 mg/kg, respectively). Based on the information in Table 11, we selected four action levels against which to compare measured total Hg concentrations in fish from the CM, from lowest to highest: 0.2 mg/kg (Maine), 0.3 mg/kg (EPA), 0.5 mg/kg (Florida/EU/Canada), and 1.0 mg/kg (FDA).

[5] See Table 2, "Class Actinopterygii, Subclass Neopterygii (rayed fish)" for a list of the CM fish in this category.

Table 11. International, U.S. federal, and U.S. state MeHg/Hg action levels

Action Level (mg/kg)[a]	Agency, Office	Description	Comments	Ref.
1.0 MeHg	US Food and Drug Administration (FDA)[b], CFSAN[b]	Action Level for edible portion	Compliance policy guides, Sec. 540.600 Fish, Shellfish, Crustaceans and Other Aquatic Animals	(1)
1.0 MeHg	FAO/WHO Codex Alimentarious Commission	Guideline levels for predatory fish	For shark, swordfish, tuna, pike	(2)
0.5 Total Hg	European Union (EU)	Standard for all commercially sold fish	Total Hg, with some exceptions	(2)
0.5 Total Hg	Canadian Food Inspection Agency	Standard for all commercially-sold fish except shark, swordfish, and fresh/frozen tuna (next column)	Fish advisory for shark, swordfish, and fresh/frozen tuna: pregnant women, women of child-bearing age and young children limit consumption to no more than one meal/month; other adults no more than one meal/week	(3)
0.5 Total Hg	Florida Dept. of Environmental Protection	"Safe for unlimited consumption"	Method/assumptions for derivation of value not readily available	(4)
0.5 MeHg	FAO/WHO Codex Alimentarious	Guideline levels for non-predatory fish	Guideline for fish other than shark, swordfish, tuna, pike	(2)
0.4 Total Hg	Japan	Guideline for total Hg in fish	Note Japan sets different guidelines for Total Hg and for MeHg; information based on secondary citation	(2)
0.3 MeHg	US Environmental Protection Agency (EPA), Office of Water	Human Health Criteria: MeHg Fish Tissue Criterion	Freshwater and estuarine fish, adults of general population (70 kg), 70% U.S. EPA RfD apportioned to this source (30% from marine fish and shellfish)	(5,6)
0.3 MeHg	Japan	Guideline for MeHg fish	Note Japan sets different guidelines for Total Hg and for MeHg; Information based on secondary citation	(2)
0.2 MeHg	Maine State Bureau of Health	Fish Tissue Action Level [for women who are pregnant]	Adult females, 60 kg, average long-term fish ingestion rate 32 g/day	(7)

[a]Units of mg[Hg]/kg[fish fresh (wet) weight].
[b]U.S. FDA = U.S. Food and Drug Administration; CFSAN = Center for Food Safety and Nutrition.

References:

(1) U.S. FDA (2000). *Action Levels for Poisonous or Deleterious Substances in Human Food and Animal Feed, Industry Activities Booklet.* August. Accessed 04/14/09 at: http://www.cfsan.fda.gov/~lrd/fdaact.html#merc.

(2) UNEP (United Nations Environment Program) and WHO (World Health Organization) (2008). *Guidance for Identifying Populations at Risk from Mercury Exposure.* UNEP DTIE Chemicals Branch and WHO Department of Food Safety, Zoonoses and Foodborne Diseases. Geneva, Switzerland. August. Accessed on 04/14/09 at: http://www.who.int/foodsafety/publications/chem/mercuryexposure.pdf.

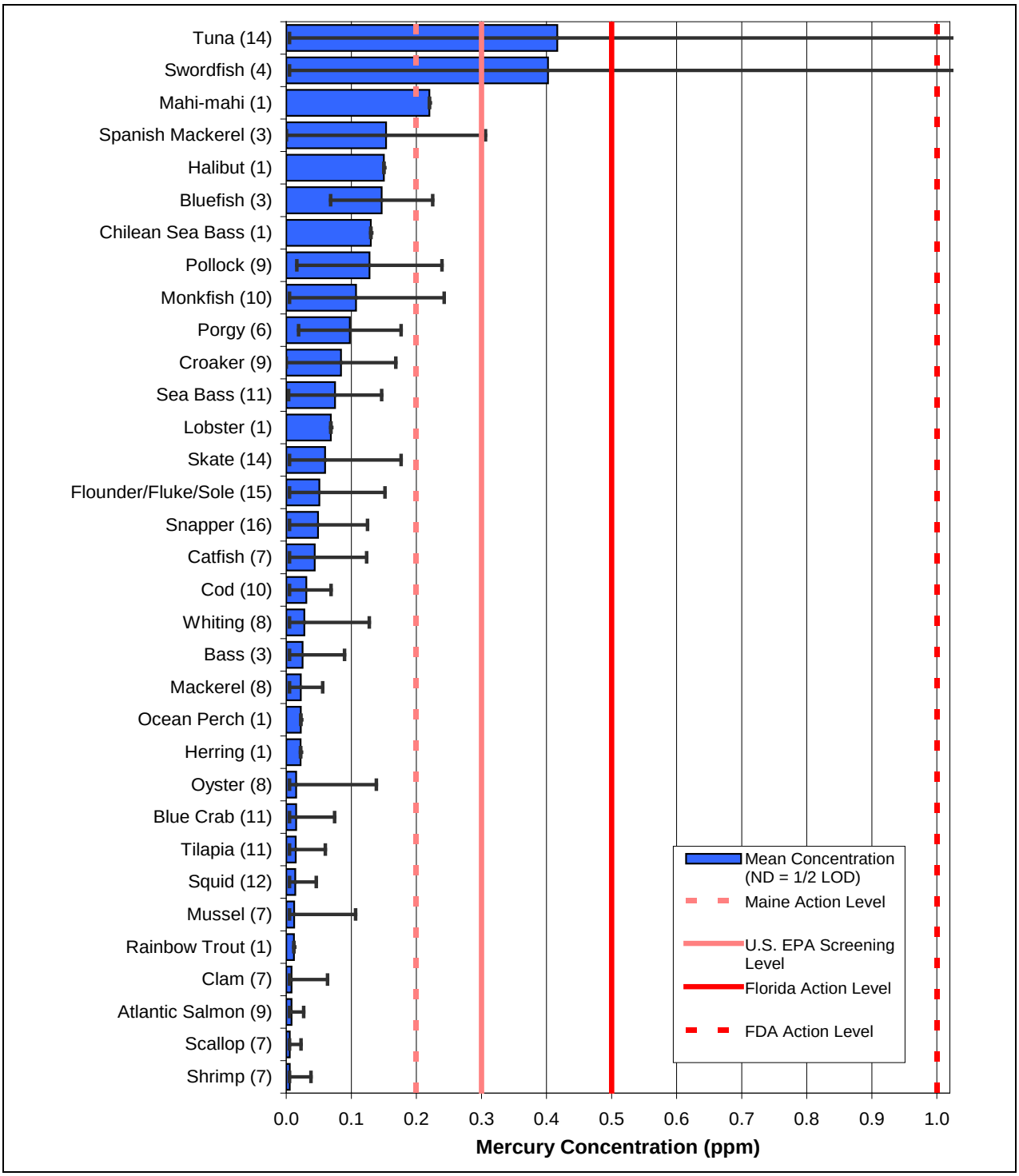
(3) International Food Law News – FAO/WHO/WTP/Codex – 2006, April 24–28, 2006, *38th Session of the Codex Committee on Food Additives and Contaminants*, The Hague, Netherlands. Accessed on 04/14/09 at: http://www.reading.ac.uk/foodlaw/news/in-06014.htm.

(4) Health Canada (2007). *Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption.* Bureau of Chemical Safety, Food Directorate, Health Products and Food Branch, Ottawa, Ontario, Canada. Accessed on 04/14/09 at: http://www.hc-sc.gc.ca/fn-an/pubs/mercur/merc_fish_poisson-eng.php#1.

(5) FDEP (Florida Department of Environmental Protection) (2006). *Fish Consumption Health Advisories* (last updated October 19, 2006). Accessed on 04/14/09 at: http://www.dep.state.fl.us/labs/mercury/docs/fhapre.htm.

(6) U.S. EPA (U.S. Environmental Protection Agency) (2001). Human Health Criteria: Methylmercury Fish Tissue Criterion. Accessed on 04/14/09 at: http://www.epa.gov/waterscience/criteria/methylmercury/document.html.

(7) BMH (Maine Bureau of Health) 2001. *Maine Bureau of Health Fish Tissue Action Levels.* Accessed on 04/14/09 at: http://www.maine.gov/dhhs/eohp/fish/documents/Action%20Levels%20Writeup.pdf.



[a]Error bars are ± 2 estimated population standard deviations from the mean, if a standard deviation could be determined.
[b]Numerical value beside market name indicates the sample size (after averaging the duplicates/replicates), including non-detects.

Figure 1. Mean mercury concentrations ± two estimated population standard deviation for all fish market names and typical action levels[a,b].

Figure 1 shows the mean Hg concentrations in each CM market name category compared to the four action levels, where non-detects have been assumed to equal one-half the reporting limit. The error bars indicate ± two estimated population S.D. wherever S.D. values could be calculated. Two S.D. from the mean encompass approximately 95% of the predicted composite concentrations, assuming the distributions are normal. The numbers in parentheses indicate the number of composite samples (including non-detects) that were averaged to estimate the mean Hg concentration for the fish market name category.

The mean Hg concentrations in swordfish and tuna exceeded the Maine action level and U.S. EPA level of concern, and the Hg concentration in the single mahi-mahi composite sample was larger than the Maine action level. The Hg concentrations across composite samples for both tuna and swordfish exhibited wide distributions, indicating that a given composite sample may or may not have had Hg concentrations above one or more action levels.

To better investigate the relationship between individual composite samples and the action levels, Figure 3 in Appendix A plots the Hg concentrations in individual composite samples, ordered from high to low, against the four action levels selected for comparison (horizontal red lines) for each market name category. The concentrations used in Figure 3 (see Appendix A) assume that the non-detects are half the reporting limit. All composite samples which are non-detects are indicated with an "(ND)". None of the total Hg concentrations for a composite sample for any of the market name categories exceeded the FDA action level for MeHg. However, 4 of the 14 composite samples (30%) for tuna were larger than the Florida/EU/Canadian action level of 0.5 mg/kg, and one of those four composite samples was from bigeye tuna while three were from yellowfin tuna. A total of 10 (70%) of the composite tuna samples exceeded the EPA screening level and the Maine action level. For swordfish (market name), two of the four composite samples (50%) were above the Florida/EU/Canadian action level of 0.5 mg/kg and three (75%) were larger than the EPA guideline and the Maine action level. The single mahi-mahi composite sample Hg concentration was above the Maine action level, while one of the three Spanish mackerel composite samples had a measured Hg concentration equal to the Maine action level.

5.3. ESTIMATES OF THE NUMBER OF SERVINGS PER WEEK OF CM FISH FOR ADULT FEMALES OF CHILD-BEARING AGE

For each fish market name, we estimated the maximum number of servings (generally equivalent to "meals") that a woman of child-bearing age/pregnant woman could consume per week and not exceed a reference toxicity value. A woman of child-bearing age was used to represent the sensitive population in adults. There are three U.S. federal reference toxicity values for MeHg, expressed as a daily dose normalized to body weight that each Agency considers to present minimal risk of adverse developmental effects when mothers are chronically exposed to MeHg:

EPA RfD	0.1 μg/kg-day
Agency for Toxic Substances and Disease Registry (ATSDR) Minimal Risk Level (MRL)	0.3 μg/kg-day
FDA Action Level	0.5 μg/kg-day

The FDA is in the process of reviewing its action level for MeHg. For the servings-per-week analysis, the EPA RfD of 0.1 μg [MeHg]/kg-day was used as the reference toxicity value. The number of fish servings per week (*SpW*) was calculated to be equal to or less than the value estimated by the following equation:

$$SpW \leq \frac{RfD \times BW}{C_{Hg} \times FSS} \times 7 \ days / week$$

where:

SpW = servings per week, often referred to as meal frequency (meals/week),
RfD = EPA reference dose (mg/kg-day) for MeHg,
BW = female body weight (kg),
FSS = fish serving size (kg/serving), and
C_{Hg} = concentration of total Hg in edible portion of fish (mg/kg wet weight).

For body weight (*BW*), a range from 65 to 67 kg was used. Various values, generally between 60 and 70 kg, have been used by federal and state agencies to protect women of child-bearing age and pregnant women from developmental toxicants. EPA has recommended several values in the past: 65 kg for women (U.S. EPA, 1993); 65 kg for adult females and 64 kg for women of reproductive age (U.S. EPA, 1997, 1999); 67 kg (U.S. EPA, 2000c, p. 4–19); and 66 kg for non-lactating and non-pregnant women between the ages of 15 and 44 (i.e., women of child-bearing age), lactating women, and pregnant women (U.S. EPA, 2004b). EPA's RfD for MeHg is based on the 67 kg body weight recommended by EPA's Office of Water (U.S. EPA, 2000c) and by EPA's IRIS (www.epa.gov/iris). A value of 65 kg, on the other hand, would yield slightly more conservative results than using 67 kg in the equation listed above.

For the fish serving size (*FSS*), we assumed EPA's recommended value of 8 oz (227 g) fresh weight fish (U.S. EPA, 2000b, 2004a). EPA considers that value equivalent to 6 oz cooked fish, which is the basis of the joint EPA-FDA advisory illustrated in Section 5.1 (U.S. EPA, 2000a, 2004a). The meal size therefore was set to 0.227 kg/meal.

The concentration of total Hg in fish was set equal to two different values for each market name category individually: (a) the mean Hg concentration for the category, and (b) the mean plus two population S.D. of the mean for each category. As discussed above, the concentrations within two population S.D. of the mean encompass approximately 95% of the estimated population sample concentrations, assuming the distributions are normal. For this analysis, the non-detects are assumed to equal half the reporting limit.

Table 12 shows the estimated number of servings (meals) per week for an adult female of child-bearing age that result in intake at or below the EPA level of concern (i.e., the EPA RfD). The calculations were based on mean and upper 95% confidence limits on mean mercury concentrations and are listed by species market name in order of decreasing mean Hg concentration for the market name category. The upper confidence limit is a statistical upper bound on the actual value of the mean given the data. Use of the upper confidence limit as the basis for the serving calculation is, in general, a conservative approach since it is greater than the mean and thus assumes greater consumption. In many cases, however, the number of servings based on the upper confidence limit is the same as that based on the mean. The calculations yield estimates that indicate CM tuna, swordfish, and mahi-mahi cannot be eaten on a weekly basis without incurring an average daily intake above the EPA RfD. (Note that the tuna values are based on fresh or frozen tuna samples, no canned tuna samples were analyzed.) The estimate for mahi-mahi, however, is based on a single composite sample and thus is relatively less certain.

Converting to meals per 30-day month, as shown in Table 13, tuna and swordfish could be eaten twice a month and mahi-mahi could be eaten three times a month and not exceed the threshold. Using the conservative estimate of Hg concentration (i.e., the mean + two S.D.), Spanish mackerel, bluefish, Pollock, and monkfish also should not be eaten weekly. Detailed results of the calculations used to determine the results shown in Table 12 are provided in Appendix D. These conclusions regarding serving frequency are generally in line with the current EPA-FDA advisory discussed in Section 5.1, although the latter discusses meal frequency for canned “light” and canned albacore tuna and does not discuss fresh or frozen tuna (e.g., tuna steaks, tuna used for sushi).

The analysis indicates that those who eat up to seven meals a week of fish or shellfish should select a diet of low mercury species, such as salmon or scallops. Seven or more servings per week are under the conservative measure of Hg concentration (mean + two S.D). A more sophisticated analysis would also incorporate consumption of multiple species in a given week, although that is beyond the scope of this report. It should be noted that the meal estimates discussed herein are based on the mercury concentrations measured in a seafood sample obtained from a commercial market in New York City. These meal estimates are not intended to be generalizable to the nation’s seafood supply.

Table 12. Estimated number of fish servings per week for an adult female of child-bearing age based on means and upper 95% confidence limits on mercury concentrations by species[a]

(1) Market Name of Species	(2) Mean Mercury (mg/kg)	(3) Number of servings per Week based on Mean Mercury that result in intake at or below the EPA level of concern[b]	(4) 95% Upper Confidence Limit on Mean Mercury (mg/kg)	(5) Number of servings per Week based on 95% upper confidence limit on Mean Mercury that result in intake at or below the EPA level of concern[b]
Tuna	0.42*	0	0.55	0
Swordfish	0.40*	0	0.59	0
Mahi-Mahi	0.22*	0	NA	NA
Spanish Mackerel	0.15	1	0.20	1
Halibut	0.15	1	NA	1
Bluefish	0.15	1	0.17	1
Chilean Sea Bass	0.13	1	NA	NA
Pollock	0.13	1	0.15	1
Monkfish	0.11	1	0.13	1
Porgy	0.098	2	0.12	1
Croaker	0.084	2	0.10	2
Sea Bass	0.075	2	0.088	2
Lobster	0.069	2	NA	NA
Skate	0.060	3	0.078	2
Flounder	0.051	3	0.065	3
Snapper	0.049	4	0.060	3
Catfish	0.044	4	0.061	3
Cod	0.031	6	0.038	5
Whiting	0.028	7	0.043	4
Bass	0.025	8	0.047	4
Mackerel	0.022	9	0.028	7

Table 13. Estimated number of fish servings per week for an adult female of child-bearing age based on means and upper 95% confidence limits on mercury concentrations by species[a] (continued)

(1) Market Name of Species	(2) Mean Mercury (mg/kg)	(3) Number of servings per Week based on Mean Mercury that result in intake at or below the EPA level of concern[b]	(4) 95% Upper Confidence Limit on Mean Mercury (mg/kg)	(5) Number of servings per Week based on 95% upper confidence limit on Mean Mercury that result in intake at or below the EPA level of concern[b]
Ocean Perch	0.022	9	NA	NA
Herring	0.022	9	NA	NA
Oyster	0.015	13	0.025	8
Blue Crab	0.015	13	0.021	9
Tilapia	0.014	14	0.022	9
Squid	0.014	14	0.017	11
Mussel	0.012	16	0.017	11
Rainbow Trout	0.012	16	NA	NA
Clam	0.0081	24	0.012	16
Atlantic Salmon	0.0081	24	0.012	16
Scallop	0.0055	36	0.0071	28
Shrimp	0.0054	37	0.0073	27

[a] Serving values calculated using the following exposure assumptions: Serving size = 8 oz of fish fresh weight, Adult female weight = 65 kg, RfD for MeHg = 1×10^{-4} mg/kg-day (U.S. EPA IRIS database), and the person consumes only the one type of fish or shellfish.

[b] Weekly value calculated using the equation for *SpW* in Section 5.3.

*These concentrations yield values indicating less than one serving a week results in intake at or below the EPA level of concern. Servings per 30 day month of these species that result in intake at or below the EPA level of concern are provided in Table 13.

NA = Value not available because only one composite sample was analyzed so that a standard deviation required for the calculation could not be calculated.

Table 13. Estimated number of fish servings per 30 day month for an adult female of child-bearing age based on means and upper 95% confidence limits on mercury concentrations for three high mercury species

(1) Market Name of Species	(2) Mean Mercury (mg/kg)	(3) Number of servings per *30 Day MONTH* based on Mean Mercury that result in intake at or below the EPA level of concern[a]	(4) 95% Upper Confidence Limit on Mean Mercury (mg/kg)	(5) Number of servings per *30 Day MONTH* based on 95% upper confidence limit on Mean Mercury that result in intake at or below the EPA level of concern[a]
Tuna	0.42	2	0.55	1
Swordfish	0.40	2	0.59	1
Mahi-Mahi	0.22	3	NA	NA

[a]Value per 30 day month calculated using the equation for *SpW* in Section 5.3, using 30 days rather than 7.
NA = Value not available because only one composite sample was analyzed so that a standard deviation required for the calculation could not be calculated.

6. COMPARISON OF CM DATA TO FDA MONITORING DATA

To further assist in evaluating Hg concentrations measured for composite samples of seafood from the CM, we provide a comparison of Hg concentrations in fish from the CM with Hg concentrations for the same market name categories of fish as monitored by the FDA from 2000 to 2004 and from 1990 to 2004. This comparison indicates which market names sold in the CM may be more or less contaminated with Hg than the fish monitored by FDA which are used to develop national fish advisories for commercially marketed fish.

FDA monitoring data for Hg concentrations in fish are available for download from the FDA website. For each record in the FDA database, the sample description includes the market name/common name analyzed, a Hg concentration in mg/kg, and the year of analysis. Measurements of Hg or MeHg concentrations in fish downloaded from the database were limited to the years 1995 through 2004. The market names listed in the database were examined and were then grouped to conform to the CM market names. Table 14 lists the FDA monitoring "names" that were included under each CM market name, where only market names and FDA monitoring names that matched are listed. Means and S.D. were calculated for the FDA monitoring data for two different periods: 1995–2004 to capture as many species as possible and 2000–2004 to focus on the most recent data only.

Table 14. Crosswalk between CM market name and FDA monitoring name

CM Market Name	FDA Monitoring "Sample Description"	CM Market Name	FDA Monitoring "Sample Description"
BASS	FRESHWATER: BASS	SALMON	SALMON
	FRESHWATER: BASS LARGEMOUTH		SALMON (MIXED SPECIES)
BLUEFISH	BLUEFISH		SALMON ATLANTIC
CATFISH	CATFISH		SALMON COHO
CLAM	CLAM		SALMON KING (FARMED)
COD	COD		SALMON PINK
	COD ALASKAN		SALMON SOCKEYE
	COD BLACK	SCALLOP	SCALLOP
	COD GREY	SEA BASS	SEA BASS
	COD PACIFIC		SEA BASS BLACK
CRAB	CRAB		SEA BASS SPOTTED
	CRAB BLUE		SEA BASS STRIPED
CROAKER	CROAKER ATLANTIC		SEA BASS WHITE
	CROAKER WHITE	SEA BASS CHILEAN	SEA BASS CHILEAN
FLOUNDER/FLUKE/SOLE	FLATFISH: FLOUNDER	SHRIMP	SHRIMP
	FLATFISH: PLAICE ALASKAN		SHRIMP PINK
	FLATFISH: PLAICE AMERICAN		SHRIMP ROCK
	FLATFISH: SOLE	SNAPPER	SNAPPER
	FLATFISH: SOLE DOVER		SNAPPER RED
	FLATFISH: SOLE PETRALE	SW0RDFISH	SW0RDFISH
	FLATFISH: SOLE REX	TILAPIA	TILAPIA
	FLATFISH: SOLE YELLOWFIN	RAINBOW TROUT	TROUT FRESHWATER
HALIBUT	HALIBUT		TROUT FRESHWATER (FARMED)
LOBSTER	LOBSTER		TROUT RAINBOW (FRESHWATER)
	LOBSTER SPINY	TUNA	TUNA
MACKEREL	MACKEREL		TUNA FR/FZN
MAHI MAHI	MAHI MAHI		TUNA FR/FZN ALBACORE
OYSTER	OYSTER		TUNA FR/FZN BIGEYE
PERCH OCEAN	PERCH OCEAN		TUNA FR/FZN SKIPJACK
POLLOCK	POLLOCK		TUNA FR/FZN YELLOWFIN
		WHITING	WHITING

Table 15, below, provides a comparison between the means and S.D. in the CM composite samples and the FDA monitoring data by species. The percent differences in the mean Hg concentrations for CM fish compared with the FDA data as follows:

$$Per\ Diff = \frac{(CM - FDA)}{FDA} \times 100\%$$

so that a negative number indicates the CM mean Hg concentrations are smaller than the FDA means. The species were broken into four different categories based on trophic level and taxonomy. The four categories were (1) "Higher Trophic Level Fish"; (2) "Intermediate Trophic Level Fish"; (3) "Lower Trophic Level Fish, Salmonids, and Squid"; and (4) "Shellfish". Market names were assigned to one of the four categories using professional judgment and considering the FDA monitoring concentrations for Hg and basic biological characteristics of the group. As discussed previously, the CM data were based on composite samples which each contained multiple individual organisms, and the measured composite Hg concentration approximated a mean concentration across the individual fish. Thus, comparing the mean across composite samples was roughly equivalent to comparing the mean concentrations across all fish that made up the composites.

In general, the CM mean concentrations were lower than the FDA monitoring means when looking at both the most recent FDA data and the data dating back to 1990. One exception to this trend was for Pollock, which had much higher concentrations in the nine CM composite samples than those in the 62 FDA samples. The CM species with the largest Hg concentrations—tuna—had composite sample mean concentrations that were in fairly good agreement with the FDA mean concentrations. However, the CM swordfish mean Hg concentrations were 60–70% smaller than the FDA mean concentrations.

Figure 2 provides a comparison of the mean Hg concentrations between the CM and FDA measurements in graphical form, where the species were broken into four categories by trophic levels. The error limits shown are one S.D. above the mean, where the CM population S.D. were the estimated population standard deviations calculated from the standard deviations across the composite samples. In nearly all species, the mean CM concentration lied within one S.D. from the mean FDA concentrations; the only exception were bluefish and swordfish, where the CM mean concentrations were more than one S.D. lower than the FDA mean concentrations.

Table 15. Comparisons of commercial market measured concentrations to FDA monitoring data[a,b]

	Commercial Fish Market			FDA, 2000–2004			Percent Difference	FDA, 1995–2004			Percent Difference
	Num. Comp. Samp.	Mean	Est. Pop. S.D.	Num. Samp.	Mean	S.D.		Num. Samp.	Mean	S.D.	
Higher Trophic Level Fish											
Tuna	14	0.42	0.39	99	0.40	0.24	4.7%	290	0.35	0.27	19%
Swordfish	4	0.40	0.33	17	1.2	0.65	-67%	620	0.98	0.51	-59%
Mahi-mahi	1	0.22	N/A	2	0.43	0.035	-48%	22	0.18	0.10	22%
Spanish Mackerel	3	0.15	0.078	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Halibut	1	0.15	N/A	14	0.23	0.10	-35%	46	0.25	0.23	-40%
Bluefish	3	0.15	0.04	51	0.34	0.13	-57%	52	0.34	0.13	-56%
Chilean Sea Bass	1	0.13	N/A	39	0.38	0.37	-66%	40	0.39	0.36	-66%
Monkfish	10	0.11	0.069	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Croaker	9	0.084	0.043	35	0.072	0.036	16%	50	0.14	0.11	-38%
Intermediate Trophic Level Fish											
Pollock	9	0.13	0.057	20	0.0032	0.0037	4000%	51	0.038	0.12	240%
Porgy	6	0.098	0.04	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Sea Bass	11	0.075	0.036	33	0.13	0.08	-43%	46	0.22	0.23	-66%
Skate	14	0.060	0.06	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Flounder / Fluke / Sole	15	0.051	0.051	1	0.015	N/A	240%	23	0.045	0.049	13%
Snapper	16	0.049	0.039	37	0.20	0.29	-75%	43	0.19	0.27	-74%
Catfish	7	0.044	0.041	1	0.10	N/A	-56%	23	0.049	0.084	-11%
Cod	10	0.031	0.019	19	0.084	0.062	-63%	39	0.095	0.080	-67%
Bass	3	0.025	0.033	N/A	N/A	N/A	N/A	4	0.31	0.16	-92%
Mackerel	8	0.022	0.017	N/A	N/A	N/A	N/A	3	0.090	0.085	-75%

Table 15. Comparisons of commercial market measured concentrations to FDA monitoring data[a,b] (continued)

	Commercial Fish Market			FDA, 2000–2004			Percent Difference	FDA, 1990–2004			Percent Difference
	Num. Comp. Samp.	Mean	Est. Pop. S.D.	Num. Samp.	Mean	S.D.		Num. Samp.	Mean	S.D.	
Lower Trophic Level Fish, Salmonids, and Squid											
Whiting	8	0.028	0.051	N/A	N/A	N/A	N/A	2	0	0	N/A
Ocean Perch	1	0.022	N/A	N/A	N/A	N/A	N/A	6	0.005	0.012	350%
Herring	1	0.022	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tilapia	11	0.014	0.023	N/A	N/A	N/A	N/A	9	0.01	0.023	45%
Squid	12	0.014	0.017	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Rainbow Trout	1	0.012	N/A	30	0.077	0.15	-84%	34	0.072	0.14	-83%
Atlantic Salmon	9	0.0081	0.0095	1	0.015	N/A	-46%	26	0.026	0.057	-69%
Shellfish											
Lobster	1	0.069	N/A	10	0.22	0.048	-69%	25	0.15	0.099	-54%
Oyster	8	0.015	0.063	4	0.014	0.010	7.9%	38	0.013	0.042	23%
Blue Crab	11	0.015	0.03	4	0.049	0.012	-69%	51	0.054	0.12	-72%
Mussel	7	0.012	0.048	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Clam	7	0.0081	0.028	N/A	N/A	N/A	N/A	6	0	0	N/A
Scallop	7	0.0055	0.0088	N/A	N/A	N/A	N/A	1	0	N/A	N/A
Shrimp	7	0.0054	0.017	N/A	N/A	N/A	N/A	24	0.0050	0.013	10%

[a]FDA Monitoring Data downloaded from FDA Website.
[b]Num. Comp. Samp. is the number of composite samples in the CM data; Num. Samp. is the number of individual samples in the FDA data; Est. Pop. S.D. is the estimated population standard deviation calculated from the composite samples.
N/A = Value is not available.

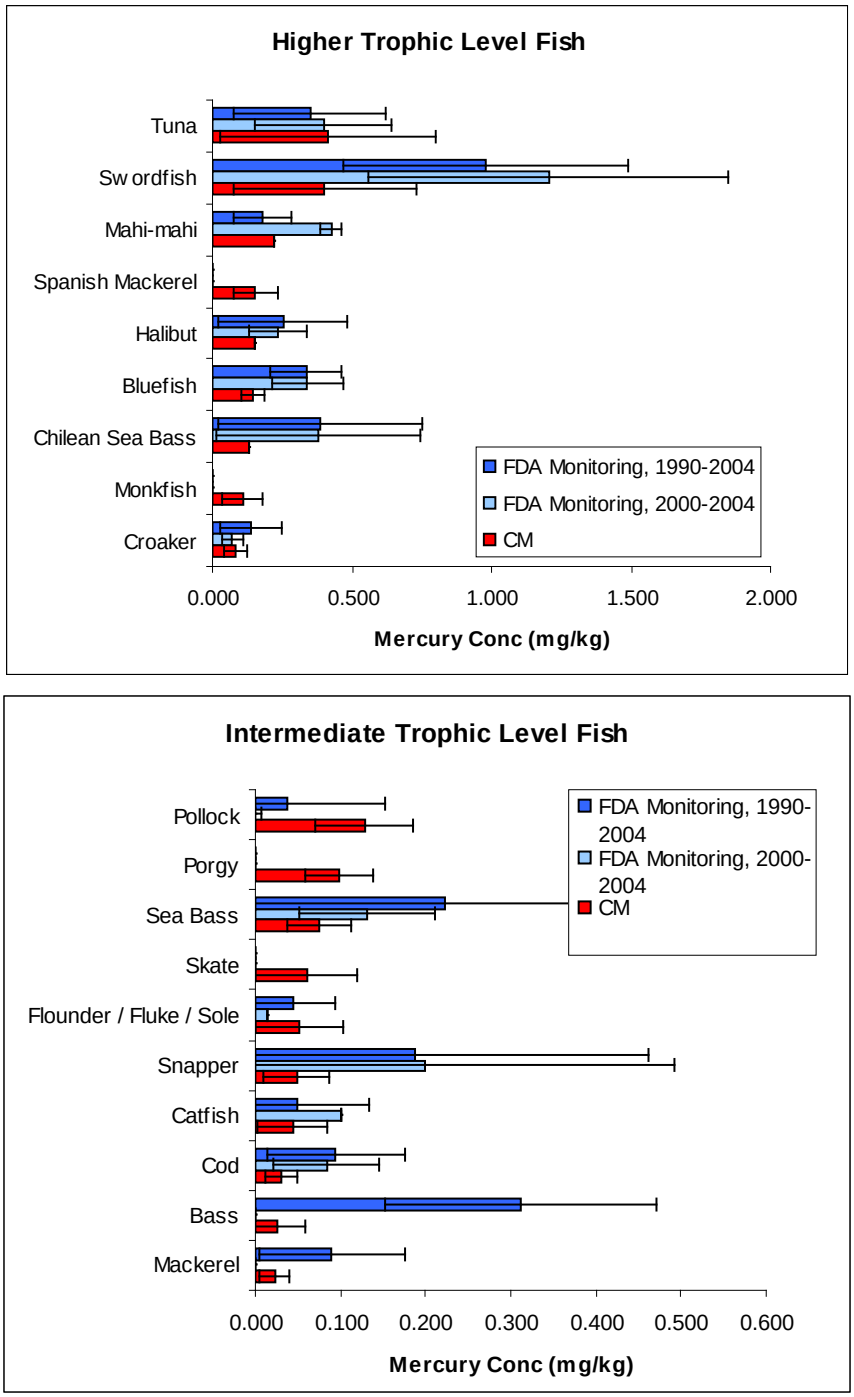


Figure 2. Bar charts comparing the commercial market mercury concentrations to the FDA monitoring data concentrations: Bars are sample means, error limits are one standard deviation above the mean.

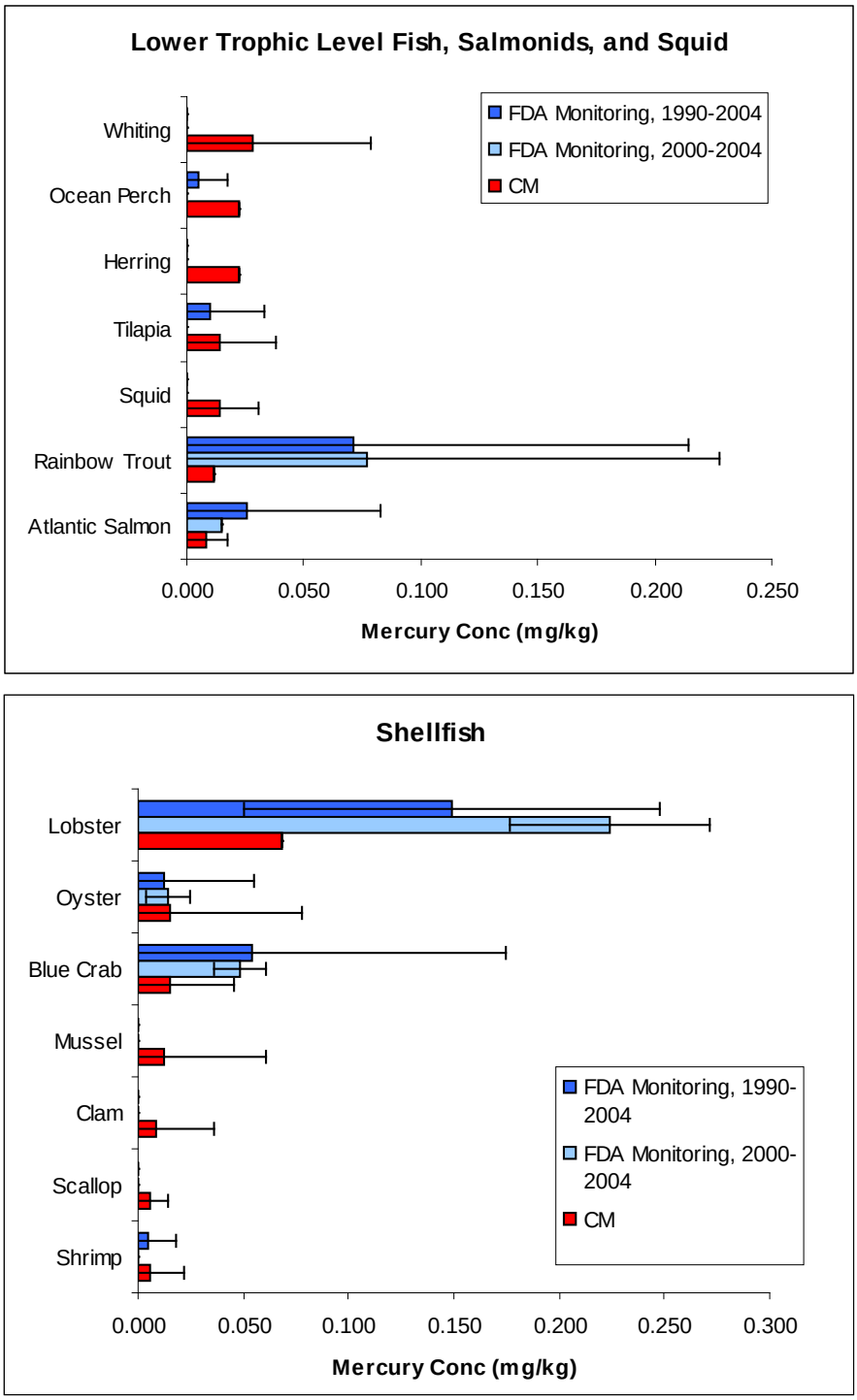


Figure 2. Bar charts comparing the commercial market mercury concentrations to the FDA monitoring data concentrations: Bars are sample means, error limits are one standard deviation above the mean. (continued)

7. QUALITY ASSURANCE/QUALITY CONTROL PROCEDURES

Sample collection, composite preparation, and tissue analyses for this project were conducted in accordance with the approved project's Quality Assurance Project Plan (QAPP) entitled, *Quality Assurance Project Plan for Sample Collection, Composite, and Analysis for a Study of Mercury and PCBs in Seafood from the New Fulton Fish Market (May 2008).* Adherence to the QAPP that was developed specifically for this study and to appropriate laboratory Standard Operating Procedures (SOPs) were evaluated during technical system audits. The Quality Assurance team consisted of a project QA manager (PQAM) who coordinated with each QA manager responsible for oversight of each project task. A quality assurance audit of each laboratory portion of the project was conducted by the QA manager associated with each laboratory. Data validation was accomplished through comparison of paper records from field record forms, chain-of-custody records, processing forms, and logs with output from computer files and internal QC checks on the study's database. The project's data manager maintained oversight of the data collected and analyzed and linked the laboratory results in a single project database.

All laboratory QC procedures set forth in Section 14 of both SOP's (C-104 for PCB's, and C-110 for Mercury) were followed. The analytical laboratory that conducted the mercury and PCB analyses used the LIMS system and exported the results to the project data manager for loading into the project database. For quality control of the DNA analyses, please refer to Appendix B.

A sample from a small piece of tissue from each of the individual specimens was shipped for DNA typing to verify the fish species. One sample tissue per composite was DNA typed and the others were archived. For species that were obtained from the FFM in fillet form, DNA typing was performed on all three fillets that comprised the composite. In addition, a tissue archive sample from each individual specimen as well as a composite archive sample from each of the composite samples was created and archived. The samples that were used for duplicate analysis were selected according to a systematic random sampling procedure.

Sample analyses conducted for this report were performed in accordance with the *Quality Assurance Project Plan for Data Analysis of Mercury and PCBs in Seafood from the New Fulton Fish Market (2009).* This included a check of the underlying raw data, validation of data transfer, an assessment of measurement error, and verification of the calculations.

Section 4 of this report describes the assessment of measurement error conducted using the results of the duplicate samples. The measurement of error variance was calculated for each of the samples using the formula in Section 4.

7.1. QA/QC OF THE DATA DELIVERY

The following steps were taken to ensure accurate delivery and understanding of the database contents:

1. Ensuring that all data fields listed in the *Data_Description* sheet existed in the appropriate table.
2. Ensuring that each field contained values that were all of the correct field type (text, double precision numbers, integers, or dates).
3. Ensuring that fields that could take on only certain discrete values contained only those values (e.g., "Detected" must be "Y" or "N", "QC_Type" must be "Screen" or "Quant").
4. Verifying that all laboratory results reported were for the analyte Mercury
5. Ensuring that all sample groups were represented on each of the three data tabs.
6. Checking that all text fields contained entries that could be interpreted by the analysts.
7. Checking that all numerical fields contained values that lied within expected ranges and with consistent significant figures, given the reported units and reported species.
8. Checking that all reported Hg levels indicated as "detected" had values above the reported detection limit.

Following the database review, the data were considered usable for further data analysis with the following issues noted and resolved:

- In the "Reporting_Limit" column in *Lab_Mercury_Results*, approximately one-third of the values were equal to the method reporting limit listed in the sample collection and analysis QAPP (0.02 mg/kg), while the other two-thirds were approximately half this value. It was confirmed that these were sample-specific reporting limits and were determined using the sample weight and the lowest calibration point on the analytical calibration curve. For the analysis, sample-specific reporting limits were used to estimate non-detected Hg concentrations.

- Upon investigating the "Reporting_Limit" in the *Lab_Mercury_Results* sheet as part of step 7, one value was flagged. The entry on row 217 (Sample 028.C, Lab Sample Name AK04148) listed a reporting limit of exactly zero, while all other reporting limits were above zero as expected. It was confirmed that the value was above zero but was not entered in the database because a detectable amount of Hg was found in the sample. Because analysis of this particular sample yielded a detectable Hg level, the reporting limit was not needed for the analysis.

- Upon investigating the "Result_Qualifier" and "Lab_Result_Qualifier" columns in *Lab_Mercury_Results* as part of step 6, all entries were observed to be blank. No entries could indicate:
    - Case 1. Data were censored prior to transmission into the Excel spreadsheet.
    - Case 2. None of the data were transferred for that particular data field—in which case these fields would need to be updated.
    - Case 3. All of the cells turned out to be blank for Hg.

- It was confirmed that a qualifier was not needed since only values that were above the reporting limit were entered into the database. Based on this assumption, Case 3 applies.

- In the *Lab_Mercury_Results* tab, all entries in the "Basis" column say "N/A". The possible entries in this column are "wet", "dry", and "N/A". The results were actually wet weight basis.

- In the *Field_Lab_Processing* sheet, it was unclear what the entries in the "WholePart" column indicate (e.g., "Whole" and "Partial"). It was confirmed that "Whole" meant the whole entire fish, or most of the entire fish, was collected as a sample specimen at the market. "Partial" indicated only a part or portion of the fish, such as a fillet or part of a loin, was collected at the market, prior to homogenization.

- In the *Field_Collection* sheet, the entries in the "Form" column indicated the size of the original sample (e.g., "Whole gutted", "Whole", and "Fillet"). It was confirmed that an entry could say "Whole" even if it has been gutted.

To check the species identification as part of step 6, autofilters were applied to all columns, then the rows in the *Lab_Mercury_Results* sheet were sorted by family, genus, and species. Spelling of family, genus, and species names was checked, and the data were sorted again after correcting spelling. The following corrections were made:

Lonigo > *Loligo* for Atlantic squid genus,
pealei > *pealeii* for Atlantic squid species,
Pluronectidae > Pleuronectidae for several flatfish records,
Merlucciiidae > Merlucciidae for whiting family,
Sebastidae > Serranidae for ocean perch family,
Sebastes > *Serranus* for ocean perch genus, and
marinus > *scriba* for ocean perch species.

It was noted that *Morone chrysopes* × *saxatilis* is listed as an "invalid" species name in the Integrated Taxonomy Information System (ITIS) used by EPA and other federal agencies. The currently valid genus and species name in ITIS for the ocean perch is *Serranus scriba*, not *Sebastes marinus*, although this was not necessary for analysis.

7.2. QA/QC OF THE ANALYSIS

Commercially available software was used for data analysis. The algorithms used in the calculations were verified for accuracy prior to their use for sample data analysis. Calculation reviews focused on correct transcription of equations and correct input ranges/data sources in addition to evaluation of the statistical approach, compliance with the QAPP, technical validity, and reasonableness of the results.

A two-pronged approach was used to check the calculations performed to estimate the statistics and risk-based estimates for this report. First, each calculation was performed independently by two different analysts. The analysts worked from a common template but did not discuss the analysis prior to beginning the analysis. They used independent analytical tools, including a combination of Microsoft Access®, Microsoft Excel®, and Visual Basic for Applications®. Each calculation was then compared to ensure the calculations agreed to within 0.01%. Next, the QA Officer examined all the calculations, checked for adherence to significant figures, and determined satisfactory completion of the analysis. The QA Officer also examined the report to check for accuracy and internal consistency.

8. REFERENCES

Burger, J; Stern, AH; Dixon, C; Jeitner, C; Shukla, S; Burke, S; Gochfeld, M (2004). Fish availability in supermarkets and fish markets in New Jersey. Sci Total Environ 333(1–3):89–97.

Fabrizio, MC; Frank, AM; Savino, JF (1995). Procedures for Formation of Composite Samples from Segmented Populations. Environ Sci Technol 29(5):1137–1144.

Gilbert, RO (1987). Statistical Methods for Environmental Pollution Monitoring, New York, NY: Wiley and Sons.

Kawaguchi, T; Porter, D; Bushek, D; Jones, B (1999). Mercury in the American oyster *Crassostrea virginica*, in high salinity salt marsh estuaries in South Carolina, USA and the public health concern for the future. Mar Pollut Bull 38:324–327.

McKelvey, W; Gwynn, RC; Jeffery, N; Kass, D; Thorpe, LE; Garg, RK; Palmer, CD; Parsons, PJ (2007). A biomonitoring study of lead, cadmium, and mercury in the blood of New York City adults. Environ Health Perspect 115(10):1435–1441.

McKelvey, W; Chang, M; Arnason, J; Jeffrey, N; Kricheff, J; Kass, D (2010). Mercury and polychlorinated biphenyls in Asian market fish: A response to results from mercury biomonitoring in New York City. Environ Res 110(7):650ket .

U.S. EPA (U.S. Environmental Protection Agency) (1993). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1, Fish Sampling and Analysis, Second Edition. Office of Water, Washington, D.C. EPA 823-R-93-002.

U.S. EPA (U.S. Environmental Protection Agency) (1997). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 2, Risk Assessment and Fish Consumption Limits, Second Edition. Office of Water, Washington, D.C. EPA 823-B-97-009.

U.S. EPA (U.S. Environmental Protection Agency) (1999). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1, Fish Sampling and Analysis, Third Edition, DRAFT. Office of Water, Washington, D.C. EPA 823-R-99-007.

U.S. EPA (U.S. Environmental Protection Agency) (2000a). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1, Fish Sampling and Analysis, Third Edition. Office of Water, Washington, D.C. EPA 823-B-00-007. Available at: http://www.epa.gov/waterscience/fish/advice/volume1/.

U.S. EPA (U.S. Environmental Protection Agency) (2000b). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 2, Risk Assessment and Fish Consumption Limits, Third Edition. Office of Water, Washington, D.C. EPA 823-B-00-008. Available at: http://www.epa.gov/waterscience/fish/advice/volume2/.

U.S. EPA (U.S. Environmental Protection Agency) (2000c). Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health Final. Office of Water, Washington, D.C. EPA 822-B-00-004. Available at: http://www.epa.gov/waterscience/criteria/humanhealth/method/index.html.

U.S. EPA (U.S. Environmental Protection Agency) (2001). Water Quality Criterion for the Protection of Human Health: Methylmercury. Office of Water, Washington, D.C. EPA 823-R-01-001. Available at: http://www.epa.gov/waterscience/criteria/methylmercury/pdf/mercury-criterion.pdf.

U.S. EPA (U.S. Environmental Protection Agency) (2004a). Technical Memorandum: Origin of 1 Meal/Week Noncommercial Fish Consumption Rate in National Advisory for Mercury. March 11, 2004. Office of Water, Washington, D.C. EPA 823-B-00-007. Available at: http://www.epa.gov/waterscience/fish/advice/1-meal-per-week.pdf.

U.S. EPA (U.S. Environmental Protection Agency) (2004b). Estimated Per Capita Water Ingestion and Body Weight in the United States – An Update. Office of Water, Office of Science and Technology, Washington, D.C. EPA-822-R-00-001. Available at: http://www.epa.gov/waterscience/criteria/drinking/percapita/2004.pdf.

U.S. EPA (U.S. Environmental Protection Agency) (2008). Quality Assurance Project Plan for Sample Collection, Composite, and Analysis for a Study of Mercury and PCBs in Seafood from the New Fulton Fish Market. Office of Research and Development, Washington, D.C.

U.S. EPA (U.S. Environmental Protection Agency) (2009a). Quality Assurance Project Plan for Data Analysis of Mercury and PCBs in Seafood from the New Fulton Fish Market. Office of Research and Development, Washington, D.C.

U.S. EPA (U.S. Environmental Protection Agency) (2009b). Guidance for Implementing the January 2001 Methylmercury Water Quality Criterion. Office of Water, Office of Science and Technology. Washington, D.C. EPA 823-R-09-002. Accessed February 14: http://www.epa.gov/waterscience/criteria/methylmercury/guidance-final.html.

Ward, DR; Nickelson II, R; Finne, G (1979). Relationship between methylmercury and total mercury in blue crabs (*Callinectes sapidus*). J Food Sci 44(3):920–921.

APPENDIX A. DETAILED STATISTICAL ANALYSIS RESULTS

Table A-1. Statistical information by market name, non-detects equal to the reporting limit[a,b]

Market Name of Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	25th Perc. (mg/kg)	Median (mg/kg)	75th Perc. (mg/kg)	Max. (mg/kg)	Est. Lower 95% C.L. on Mean[d]	Est. Upper 95% C.L. on Mean [D]
	Det.	N.D.	Tot.												
Tuna	14	0	14	0%	0.42	0.25	0.39	93%	0.043	0.23	0.39	0.57	0.82	0.29	0.55
Swordfish	4	0	4	0%	0.40	0.19	0.33	82%	0.14	N/A	N/A	N/A	0.57	0.22	0.59
Mahi-mahi	1	0	1	0%	0.22	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Mackerel, Spanish	3	0	3	0%	0.15	0.045	0.078	51%	0.11	N/A	N/A	N/A	0.2	0.1	0.2
Halibut	1	0	1	0%	0.15	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Bluefish	3	0	3	0%	0.15	0.023	0.04	27%	0.12	N/A	N/A	N/A	0.16	0.12	0.17
Bass, Chilean Sea	1	0	1	0%	0.13	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pollock	9	0	9	0%	0.13	0.034	0.057	44%	0.079	N/A	N/A	N/A	0.18	0.11	0.15
Monkfish	10	0	10	0%	0.11	0.044	0.069	65%	0.054	0.073	0.095	0.14	0.18	0.08	0.13
Porgy	6	0	6	0%	0.098	0.023	0.040	41%	0.068	N/A	N/A	N/A	0.13	0.079	0.12
Croaker	9	0	9	0%	0.084	0.024	0.043	51%	0.056	N/A	N/A	N/A	0.13	0.069	0.1
Bass, Sea	11	0	11	0%	0.075	0.021	0.036	49%	0.03	0.064	0.078	0.087	0.11	0.063	0.088
Lobster	1	0	1	0%	0.069	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Skate	13	1	14	7%	0.060	0.034	0.059	97%	0.0099	0.03	0.064	0.081	0.12	0.042	0.078
Flounder/ Fluke/Sole	13	2	15	13%	0.051	0.027	0.049	96%	0.0093	0.038	0.049	0.066	0.1	0.038	0.065
Snapper	16	0	16	0%	0.049	0.022	0.039	80%	0.017	0.032	0.044	0.068	0.083	0.038	0.06

Table A-1. Statistical information by market name, non-detects equal to the reporting limit[a,b] (continued)

Market Name of Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	25th Perc. (mg/kg)	Median (mg/kg)	75th Perc. (mg/kg)	Max. (mg/kg)	Est. Lower 95% C.L. on Mean[d]	Est. Upper 95% C.L. on Mean [D]
	Det.	N.D.	Tot.												
Catfish	7	0	7	0%	0.044	0.023	0.041	93%	0.024	N/A	N/A	N/A	0.094	0.026	0.061
Cod	10	0	10	0%	0.031	0.012	0.019	63%	0.016	0.024	0.027	0.038	0.049	0.024	0.038
Whiting	7	1	8	13%	0.029	0.020	0.049	170%	0.0096	N/A	N/A	N/A	0.075	0.014	0.043
Bass	3	0	3	0%	0.025	0.019	0.033	130%	0.014	N/A	N/A	N/A	0.047	0.0034	0.047
Mackerel	7	1	8	13%	0.023	0.0077	0.017	74%	0.013	N/A	N/A	N/A	0.034	0.017	0.028
Perch, Ocean	1	0	1	0%	0.022	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Herring	1	0	1	0%	0.022	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Tilapia	5	6	11	55%	0.017	0.011	0.02	110%	0.0092	0.0097	0.0099	0.021	0.038	0.011	0.023
Oyster	6	2	8	25%	0.017	0.013	0.059	350%	0.0092	N/A	N/A	N/A	0.047	0.0078	0.025
Crab, Blue	8	3	11	27%	0.016	0.0077	0.025	150%	0.0085	0.009	0.017	0.023	0.029	0.012	0.021
Squid	10	2	12	17%	0.015	0.0051	0.014	93%	0.0084	0.011	0.014	0.017	0.024	0.012	0.017
Mussel	5	2	7	29%	0.014	0.0037	0.031	230%	0.0087	N/A	N/A	N/A	0.019	0.011	0.016
Trout, Rainbow	1	0	1	0%	0.012	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Salmon, Atlantic	3	6	9	67%	0.011	0.0035	0.006	54%	0.0086	N/A	N/A	N/A	0.019	0.0089	0.013
Clam	3	4	7	57%	0.011	0.0042	0.02	190%	0.0085	N/A	N/A	N/A	0.02	0.0075	0.014
Scallop	1	6	7	86%	0.0094	0.00074	0.0029	31%	0.0086	N/A	N/A	N/A	0.011	0.0089	0.0099
Shrimp	1	6	7	86%	0.0093	0.0009	0.0061	66%	0.0084	N/A	N/A	N/A	0.011	0.0086	0.01

Table A-1. Statistical information by market name, non-detects equal to the reporting limit[a,b] (continued)

Market Name of Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	25th Perc. (mg/kg)	Median (mg/kg)	75th Perc. (mg/kg)	Max. (mg/kg)	Est. Lower 95% C.L. on Mean[d]	Est. Upper 95% C.L. on Mean [D]
	Det.	N.D.	Tot.												
Total	194	42	236	18%	0.076	0.12	N/A	N/A	0.0084	0.014	0.034	0.083	0.82	0.06	0.092

[a]Det. is the number of samples with results above the reporting limit (referred to here as "detects"), N.D. is the number of samples with results below the reporting limit, Tot. is the total number of samples for the species (excluding replicate and duplicate samples), Perc. N.D. is the percent of the total number of samples which are below the reporting limit, Comp. S.D. is the standard deviation of the mean of the composite sample Hg concentrations for the market name, Est. Pop. S.D. is the estimated population standard deviation, Est. Pop. C.V. is the estimated coefficient of variation calculated as the population standard deviation/mean × 100%, 25th Perc. is the 25th percentile, 75th Perc is the 75th percentile, Est. Lower 95% C.L. on mean is the estimated lower 95th percent confidence limit on the mean, and Est. Upper 95% C.L. on Mean is the estimated upper 95th percent confidence limit on the mean.

[b]Number of Market Name groups included = 33.

[c]Number of composite samples after averaging the duplicates and replicates for a single composite sample and treating the average as a single point.

[d]Calculated based on the estimated population standard deviation.

N/A = Value not available.

Table A-2. Mercury concentrations by species or by condition (e.g., hard- or softshell) within a market name; non-detects equal to the reporting limit[a,b]

Market Name	Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Catfish	Catfish, Blue	2	0	2	0%	0.037	0.012	0.021	57%	0.028	0.045
	Catfish, Channel	4	0	4	0%	0.048	0.032	0.055	110%	0.024	0.094
	Catfish, White	1	0	1	0%	0.041	N/A	N/A	N/A	N/A	N/A
	All Catfish	7	0	7	0%	0.044	0.023	0.041	93%	0.024	0.094
Cod[d]	Cod, Atlantic	7	0	7	0%	0.031	0.013	0.022	70%	0.016	0.049
	Cod, Pacific	3	0	3	0%	0.030	0.009	0.015	49%	0.024	0.040
	All Cod	10	0	10	0%	0.031	0.012	0.019	63%	0.016	0.049
Blue Crab	Blue Crab/Hardshell	5	0	5	0%	0.021	0.003	0.013	62%	0.017	0.025
	Blue Crab/Softshell	3	3	6	50%	0.012	0.008	0.024	190%	0.009	0.029
	All Crab	8	3	11	27%	0.016	0.008	0.025	150%	0.009	0.029
Flounder/ Fluke/ Sole	Flounder, Blackback	4	1	5	20%	0.032	0.015	0.028	88%	0.010	0.049
	Flounder, Summer	5	0	5	0%	0.073	0.026	0.045	61%	0.045	0.100
	Sole, Gray	4	0	4	0%	0.059	0.012	0.020	34%	0.044	0.071
	All Flounder/Fluke/Sole	13	2	15	13%	0.051	0.027	0.049	96%	0.009	0.100
Shrimp	Shrimp, Black Tiger	0	2	2	100%	0.009	0.000	0.001	13%	0.008	0.009
	Shrimp, White	1	4	5	80%	0.010	0.001	0.006	64%	0.009	0.011
	All Shrimp	1	6	7	86%	0.009	0.001	0.006	66%	0.008	0.011

Table A-2. Mercury concentrations by species or by condition (e.g., hard- or softshell) within a market name; non-detects equal to the reporting limit[a,b] (continued)

Market Name	Species	Number of Composite Samples[c]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Snapper	Snapper, Caribbean Red	1	0	1	0%	0.039	N/A	N/A	N/A	N/A	N/A
	Snapper, Lane	2	0	2	0%	0.040	0.018	0.031	78%	0.027	0.052
	Snapper, Red	6	0	6	0%	0.057	0.020	0.035	63%	0.031	0.076
	Snapper, Vermilion	3	0	3	0%	0.040	0.037	0.065	160%	0.017	0.083
	Snapper, Yellowtail	4	0	4	0%	0.051	0.022	0.038	76%	0.034	0.082
	All Snapper	16	0	16	0%	0.049	0.022	0.039	80%	0.017	0.083
Squid[d]	Squid, Japanese Flying	3	1	4	25%	0.012	0.003	0.006	47%	0.009	0.015
	Squid, Longfin (Atlantic)	7	0	7	0%	0.017	0.005	0.017	100%	0.012	0.024
	All Squid	10	2	12	17%	0.015	0.005	0.014	93%	0.008	0.024
Tuna	Yellowfin Tuna	7	0	7	0%	0.420	0.280	0.440	100%	0.043	0.800
	Bigeye Tuna	7	0	7	0%	0.410	0.230	0.360	87%	0.130	0.820
	All Tuna	14	0	14	0%	0.420	0.250	0.390	93%	0.043	0.820
Whiting	Whiting, Offshore	2	0	2	0%	0.052	0.032	0.062	120%	0.029	0.075
	Whiting/Silver Hake	5	1	6	17%	0.021	0.009	0.025	120%	0.010	0.033
	All Whiting	7	1	8	13%	0.029	0.020	0.049	170%	0.010	0.075

[a]Column names are as defined in Table A-1.
[b]Number of Market Names encompassing multiple species or conditions equals 10 of the 33 total Market Names.
[c]Number of composite samples after averaging the duplicates and replicates for a single composite sample and treating the average as a single point.
[d]Species from different oceans.
N/A = Value not available because only one composite was analyzed.

Table A-3. Mercury concentrations by water body of origin within a market name species; non-detects equal to the reporting limit[a]

Market Name of Species	Water Body or Water Type of Origin	Number of Composite Samples[b]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Clam	Clam, Atlantic, Wild	1	3	4	75%	0.011	0.0057	0.027	230.0%	0.0085	0.02
	Clam, Farmed	2	0	2	0%	0.0095	0.00071	0.0041	43.0%	0.009	0.01
	Clam, Sound	0	1	1	100%	0.0094	N/A	N/A	N/A	N/A	N/A
	All Clam	3	4	7	57%	0.011	0.0042	0.02	190.0%	0.0085	0.02
Cod	Cod, Atlantic, Wild	7	0	7	0%	0.031	0.013	0.022	70.0%	0.016	0.049
	Cod, Pacific, Wild	3	0	3	0%	0.03	0.0086	0.015	49.0%	0.024	0.04
	All Cod	10	0	10	0%	0.031	0.012	0.019	63.0%	0.016	0.049
Mussel	Mussel, Atlantic, Wild	4	1	5	20%	0.014	0.0037	0.031	220.0%	0.0088	0.019
	Mussel, Farmed	1	1	2	50%	0.012	0.0045	0.038	320.0%	0.0087	0.015
	All Mussel	5	2	7	29%	0.014	0.0037	0.031	230.0%	0.0087	0.019
Salmon	Atlantic Salmon, Wild	0	3	3	100%	0.0089	0.00043	0.00075	8.5%	0.0086	0.0094
	Atlantic Salmon, Farmed	3	3	6	50%	0.012	0.0038	0.0066	53.0%	0.0088	0.019
	All Salmon	3	6	9	67%	0.011	0.0035	0.006	54.0%	0.0086	0.019
Snapper	Snapper, Atlantic, Wild	13	0	13	0%	0.05	0.024	0.042	83.0%	0.017	0.083
	Snapper, Pacific, Wild	2	0	2	0%	0.04	0.018	0.031	78.0%	0.027	0.052
	Snapper, Unknown	1	0	1	0%	0.047	N/A	N/A	N/A	N/A	N/A
	All Snapper	16	0	16	0%	0.049	0.022	0.039	80.0%	0.017	0.083
Squid	Squid, Atlantic, Wild	7	0	7	0%	0.017	0.0048	0.017	100.0%	0.012	0.024
	Squid, Pacific, Wild	3	2	5	40%	0.011	0.0025	0.0054	49.0%	0.0084	0.015
	All Squid	10	2	12	17%	0.015	0.0051	0.014	93.0%	0.0084	0.024

Table A-3. Mercury concentrations by water body of origin within a market name species; non-detects equal to the reporting limit[a] (continued)

Market Name of Species	Water Body or Water Type of Origin	Number of Composite Samples[b]			Perc. N.D. (%)	Mean (mg/kg)	Comp. S.D. (mg/kg)	Est. Pop. S.D. (mg/kg)	Est. Pop. C.V. (%)	Min. (mg/kg)	Max. (mg/kg)
		Det.	N.D.	Total							
Swordfish	Swordfish, Atlantic, Wild	2	0	2	0%	0.33	0.26	0.45	140.0%	0.14	0.51
	Swordfish, Pacific, Wild	2	0	2	0%	0.48	0.13	0.22	46.0%	0.39	0.57
	All Swordfish	4	0	4	0%	0.4	0.19	0.33	82.0%	0.14	0.57

[a]Column names are as defined in Table A-1.
[b]Number of composite samples after averaging the duplicates and replicates for a single composite sample and treating the average as a single point.
N/A = Value not available because only one composite was analyzed.

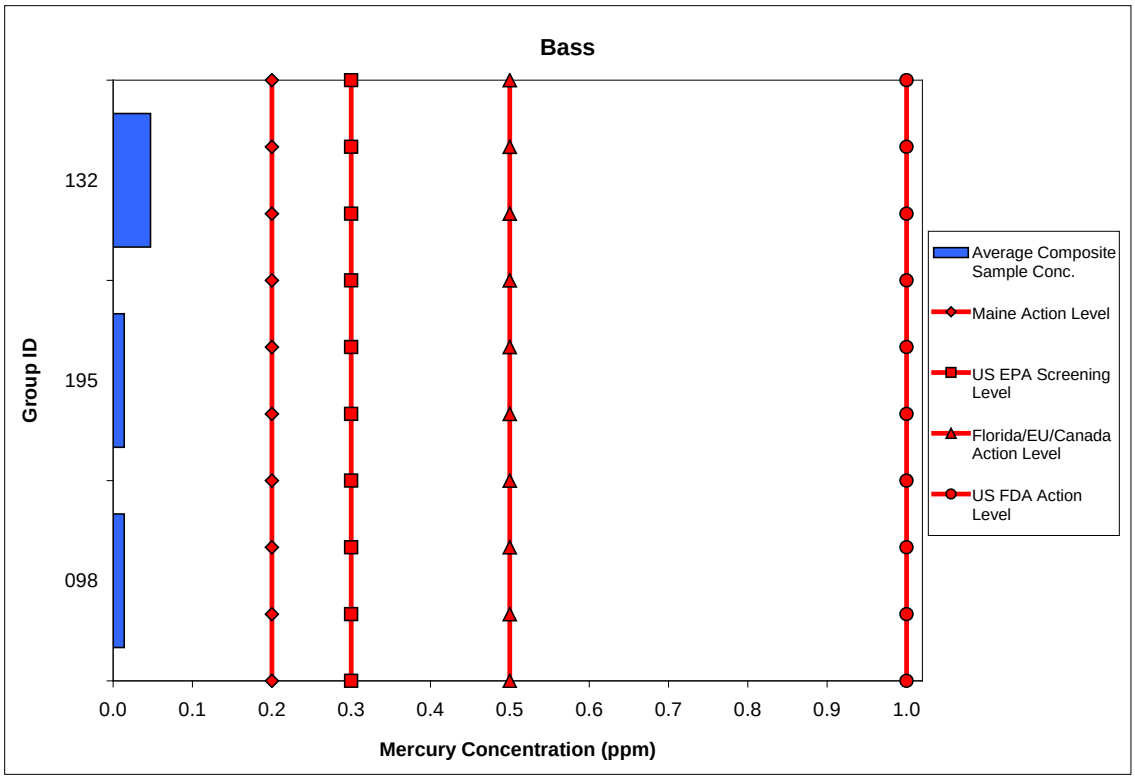


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels.

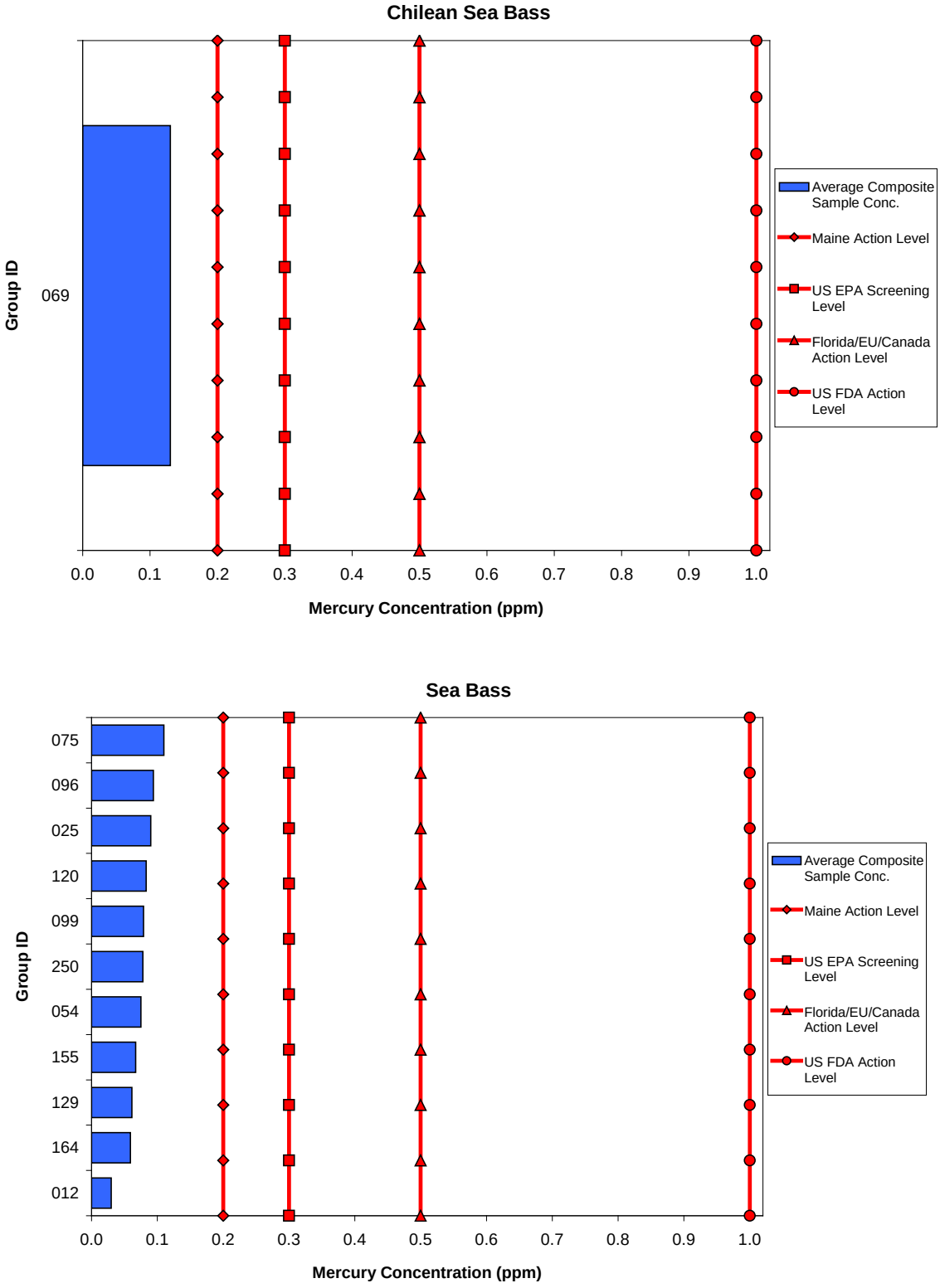


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

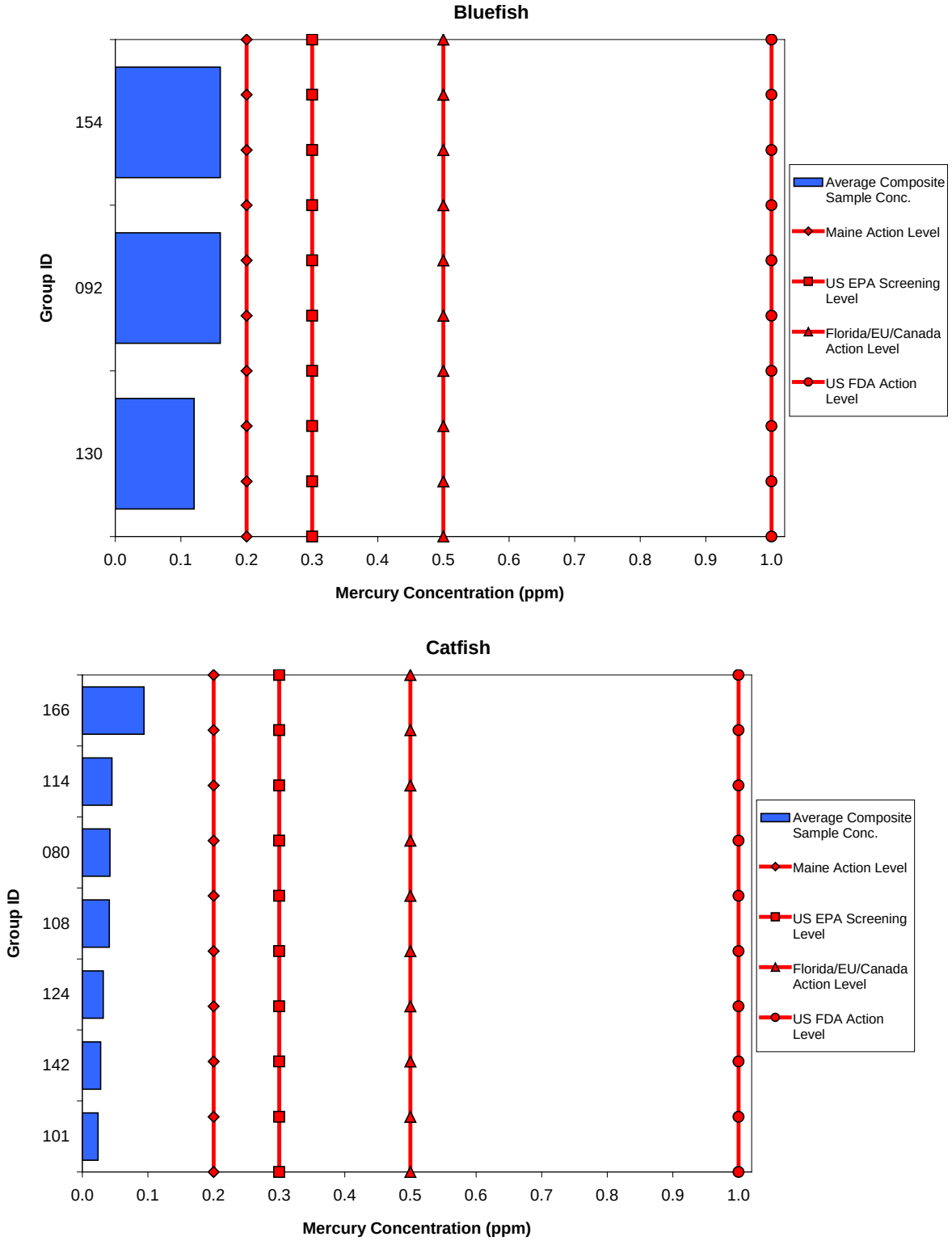


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

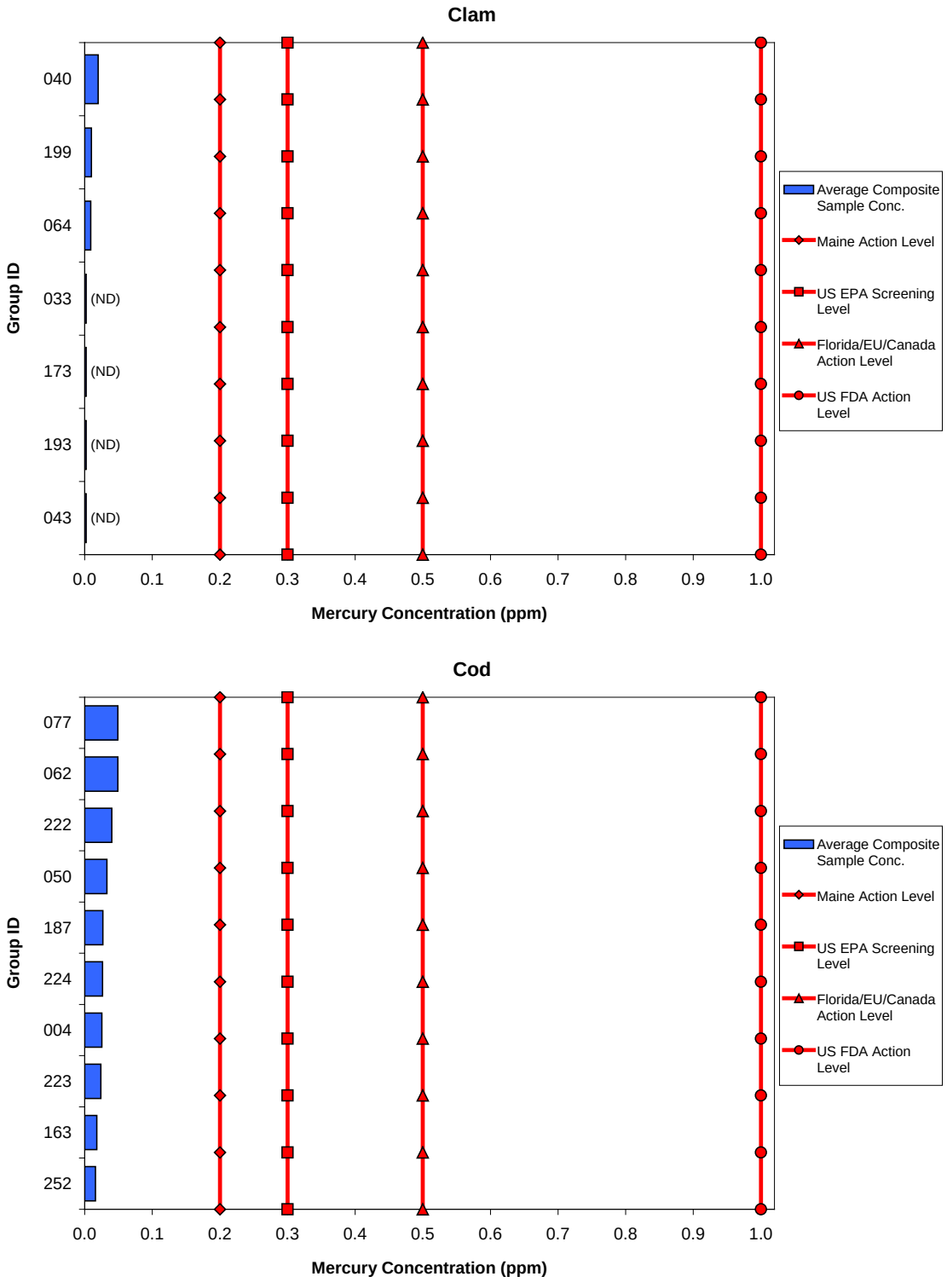


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

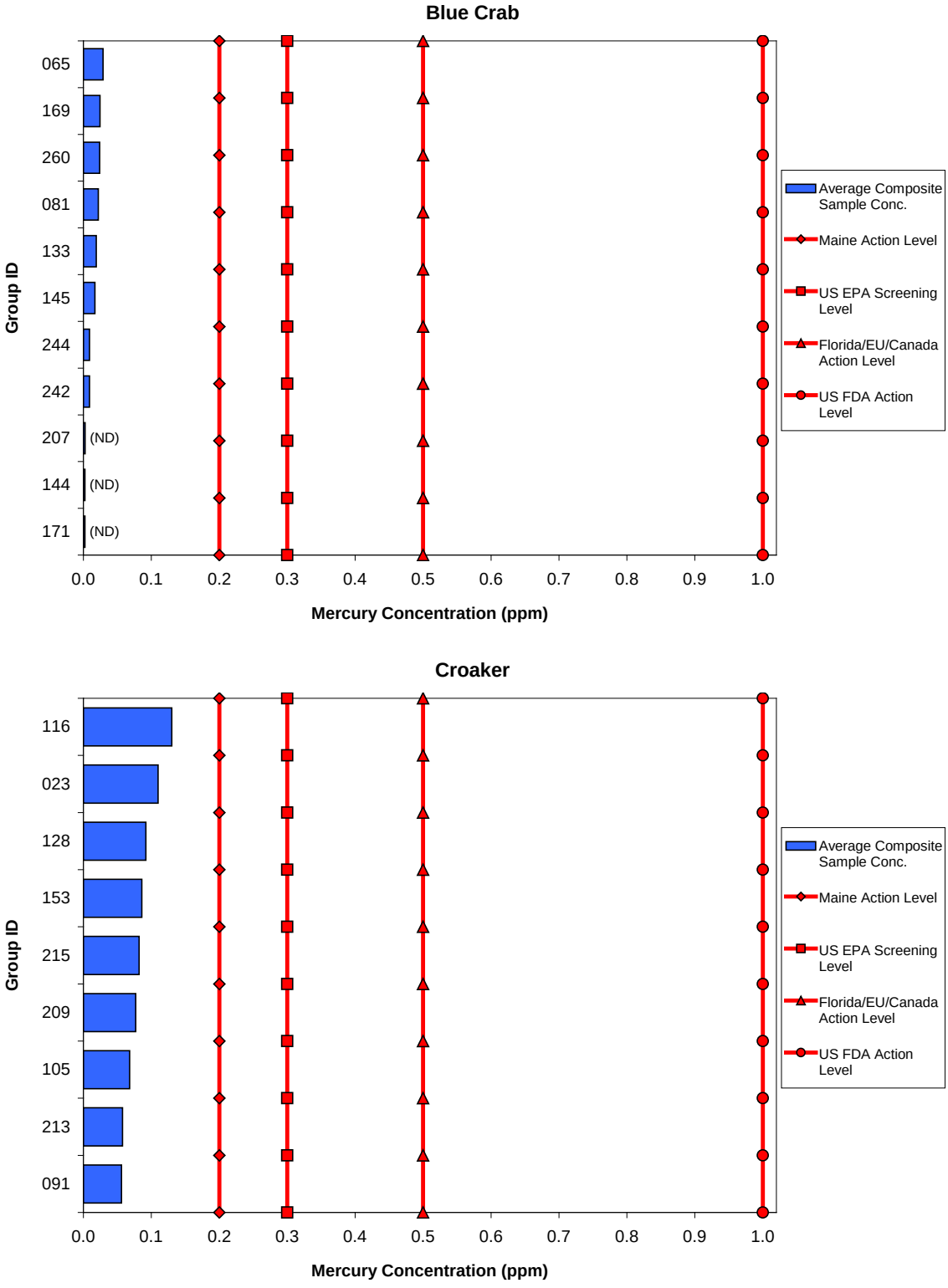


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

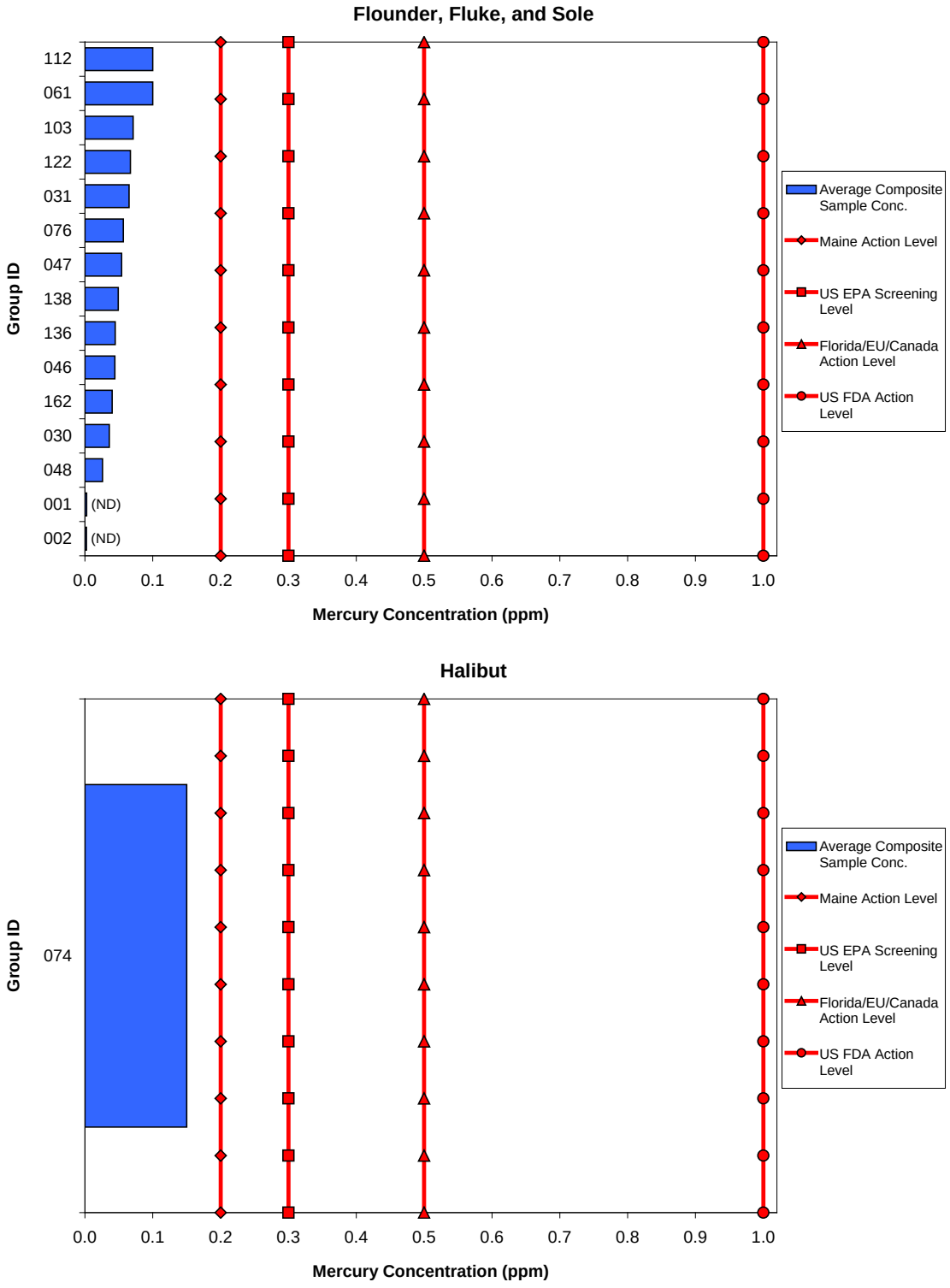


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

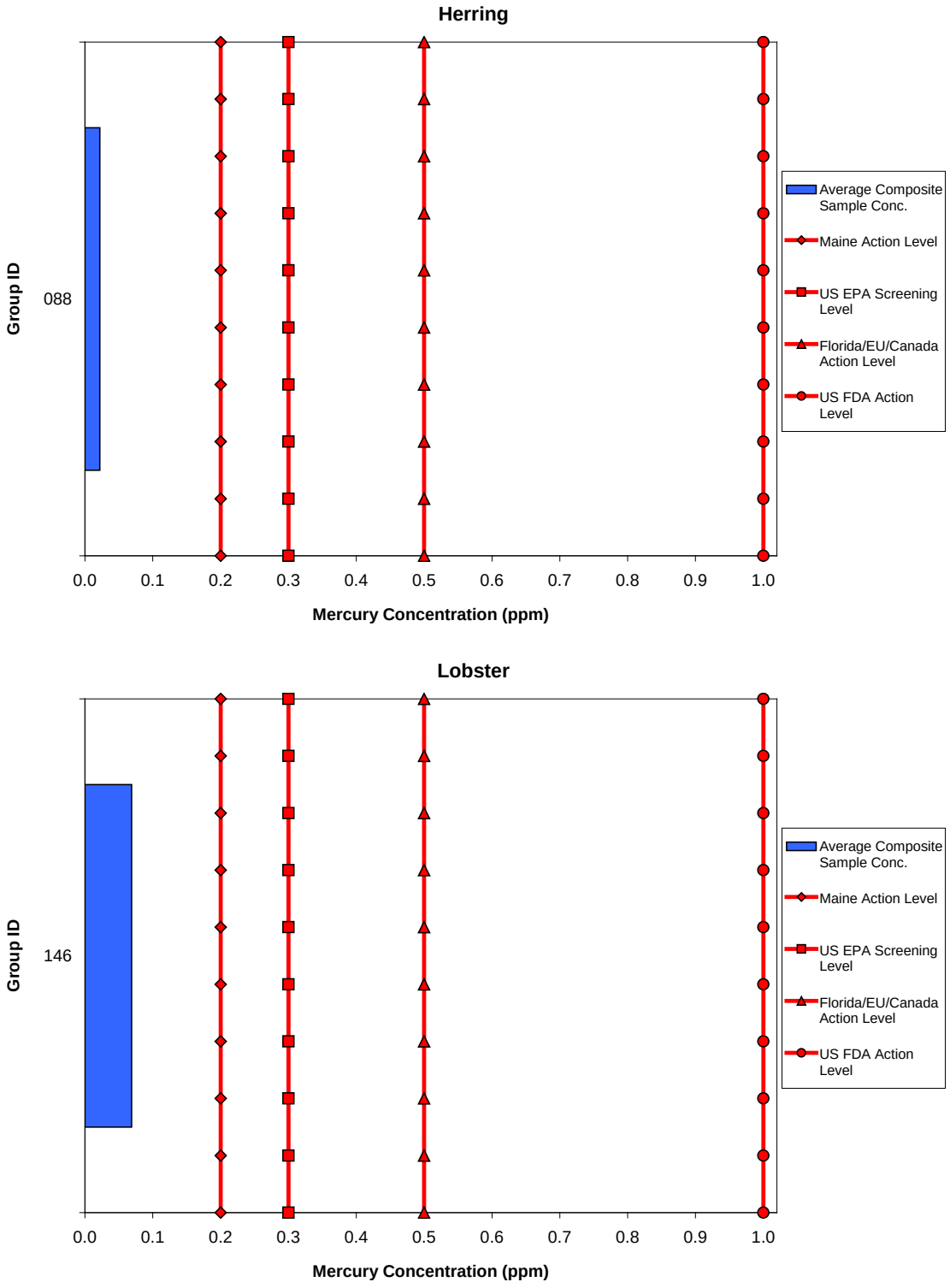
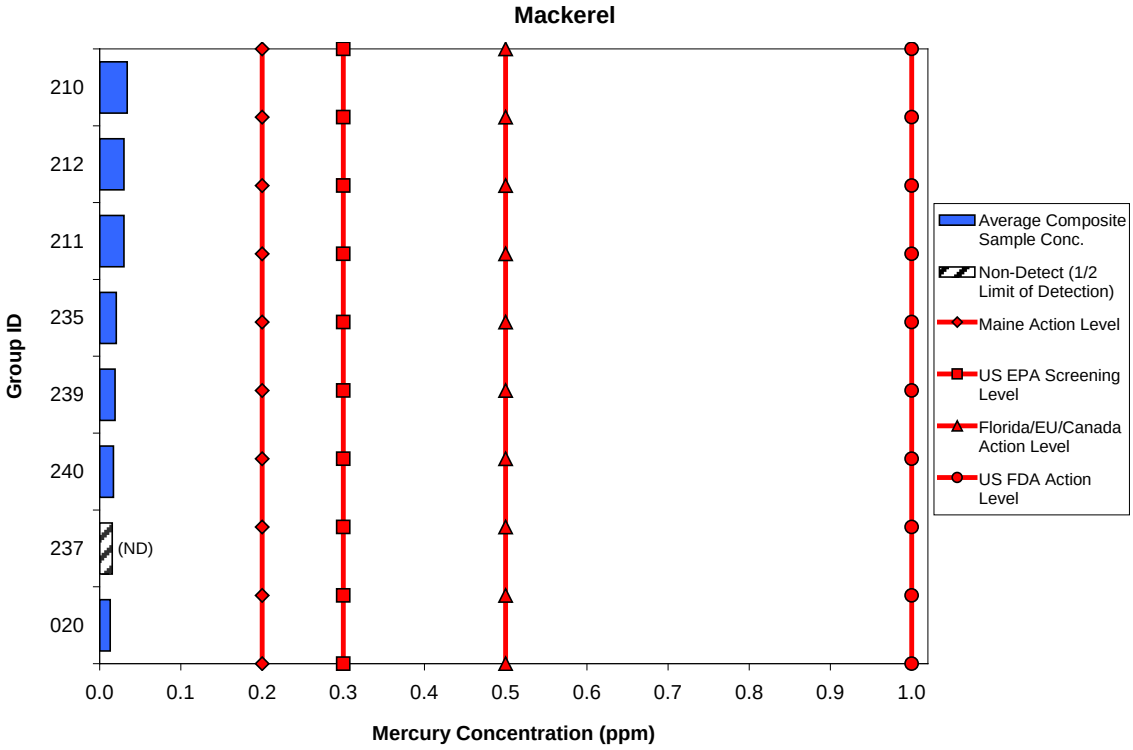


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)



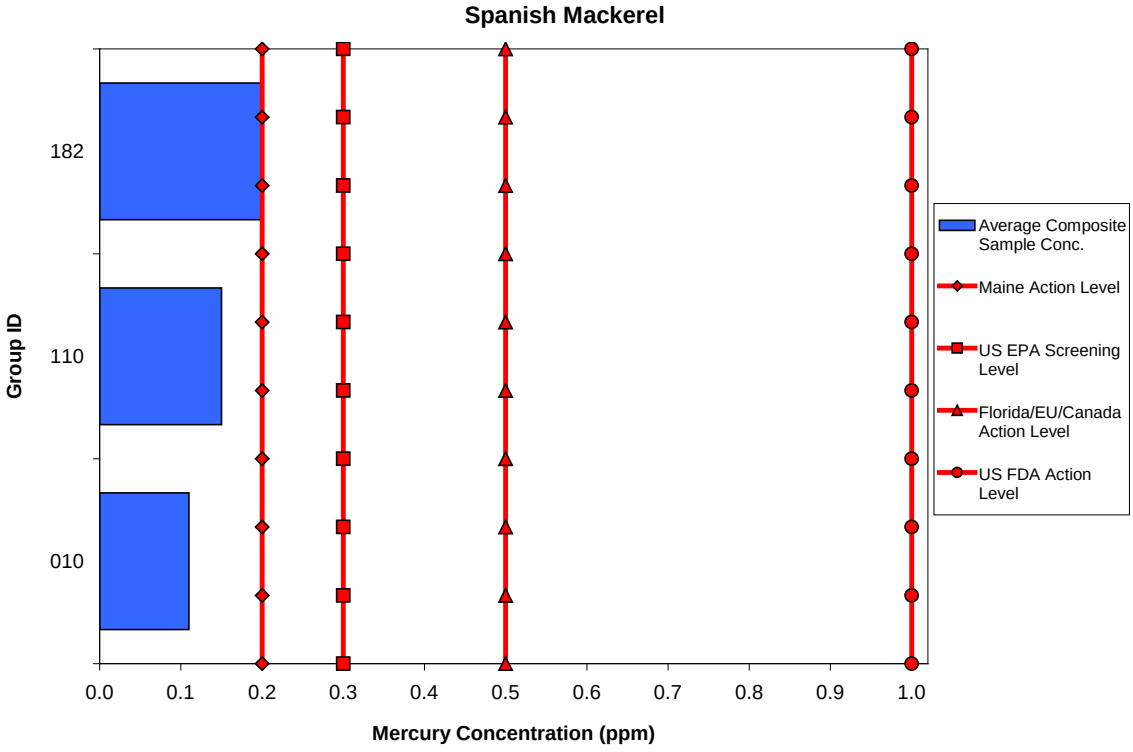


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

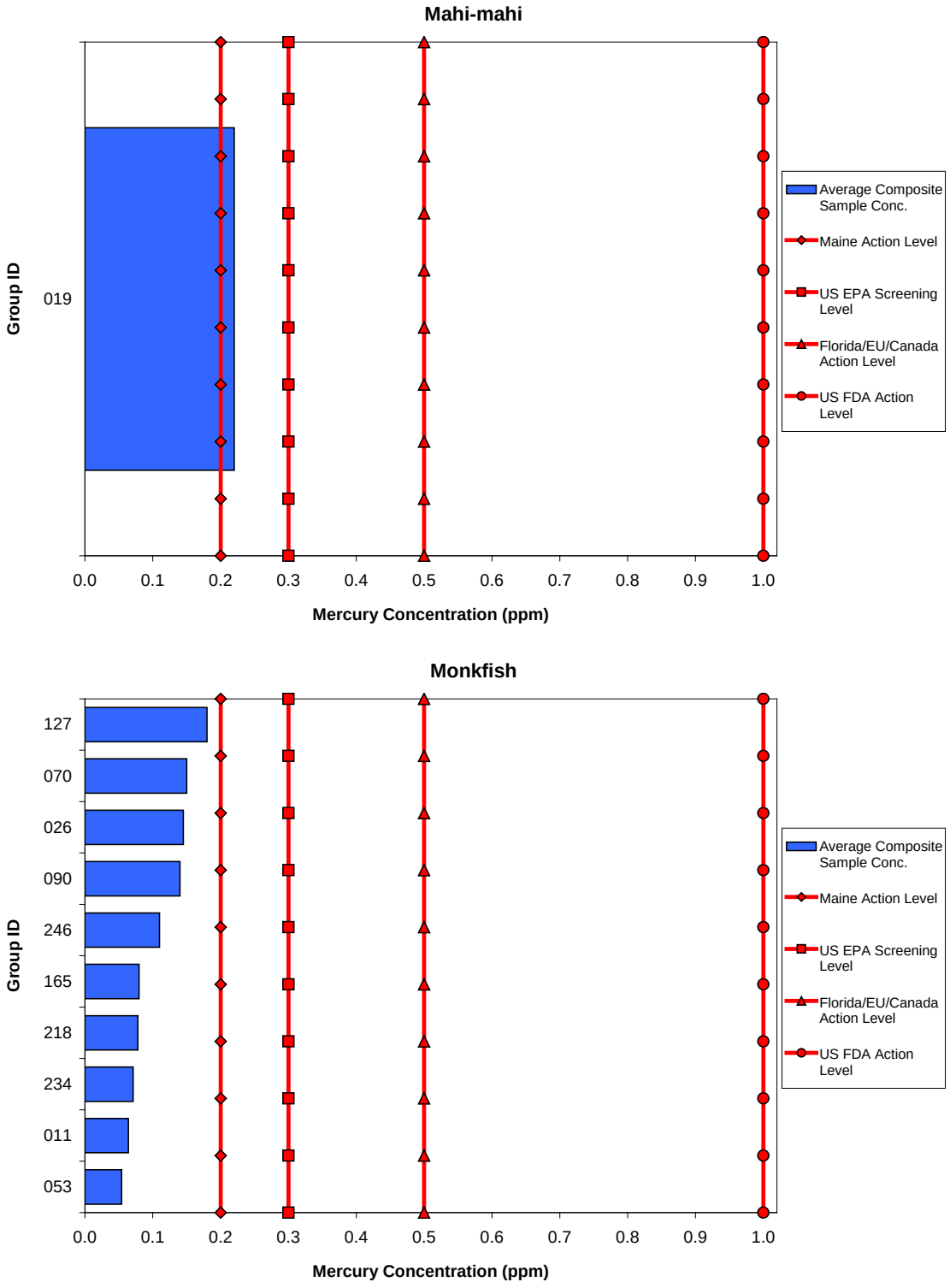


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

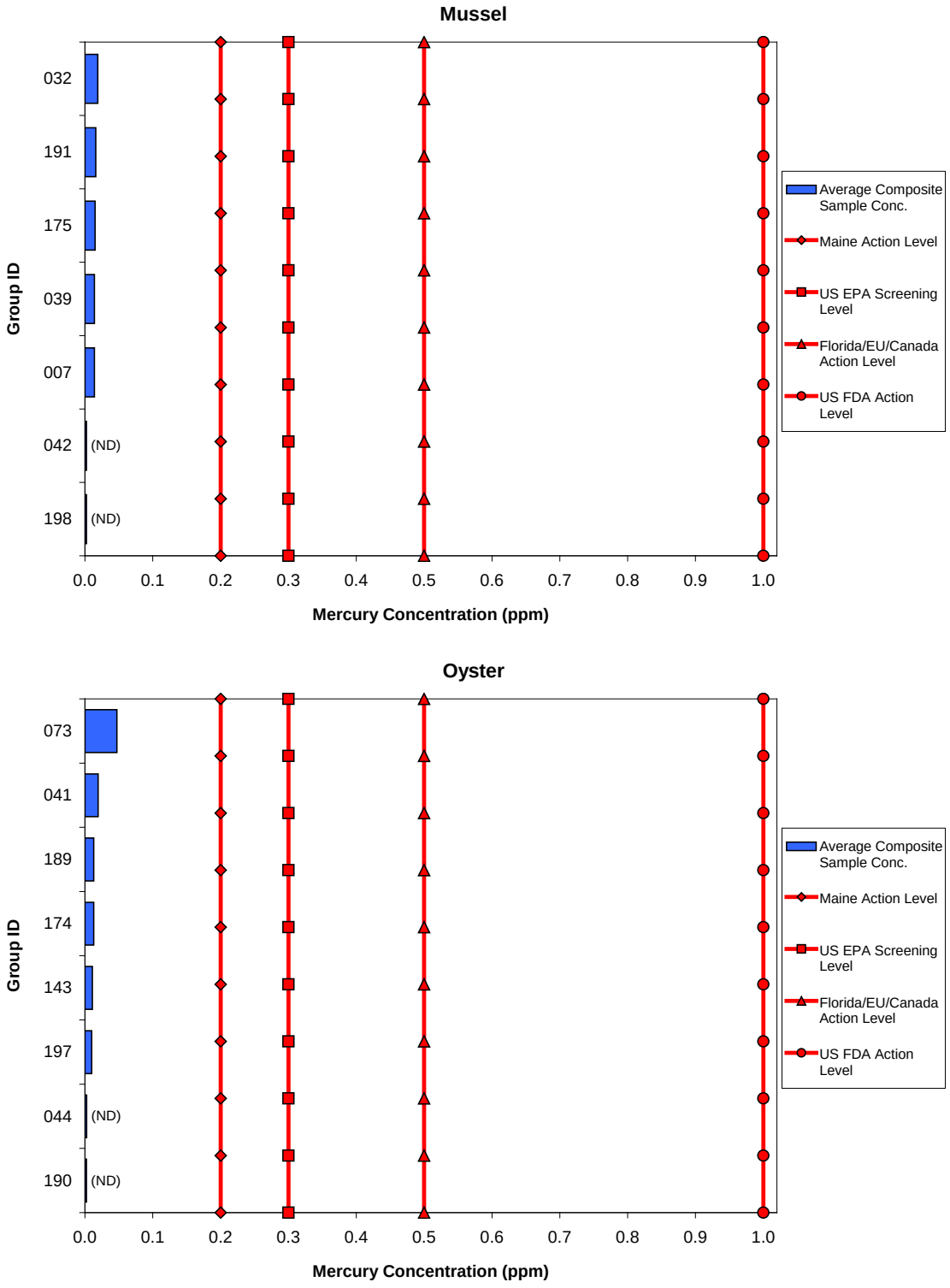


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

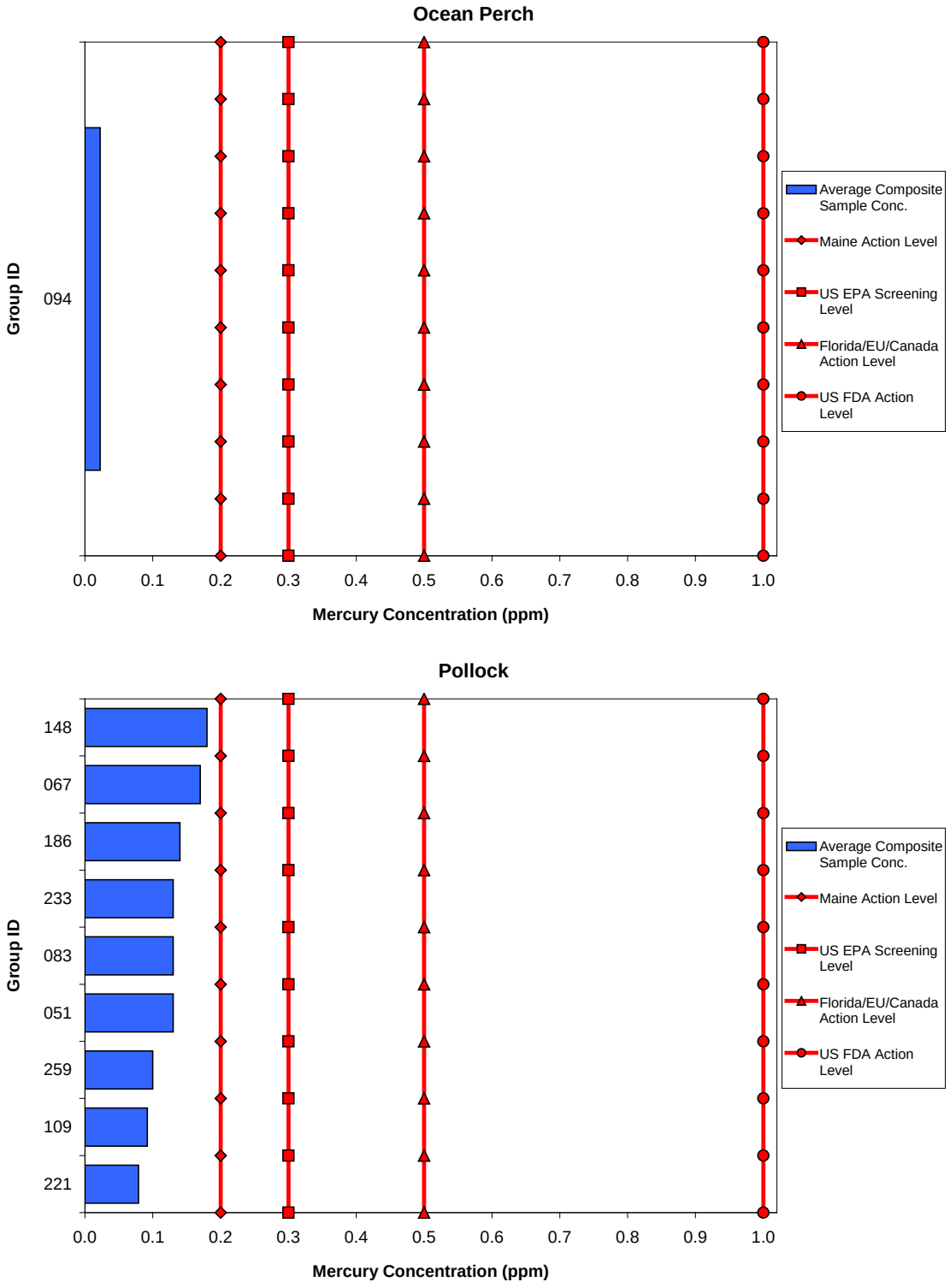


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

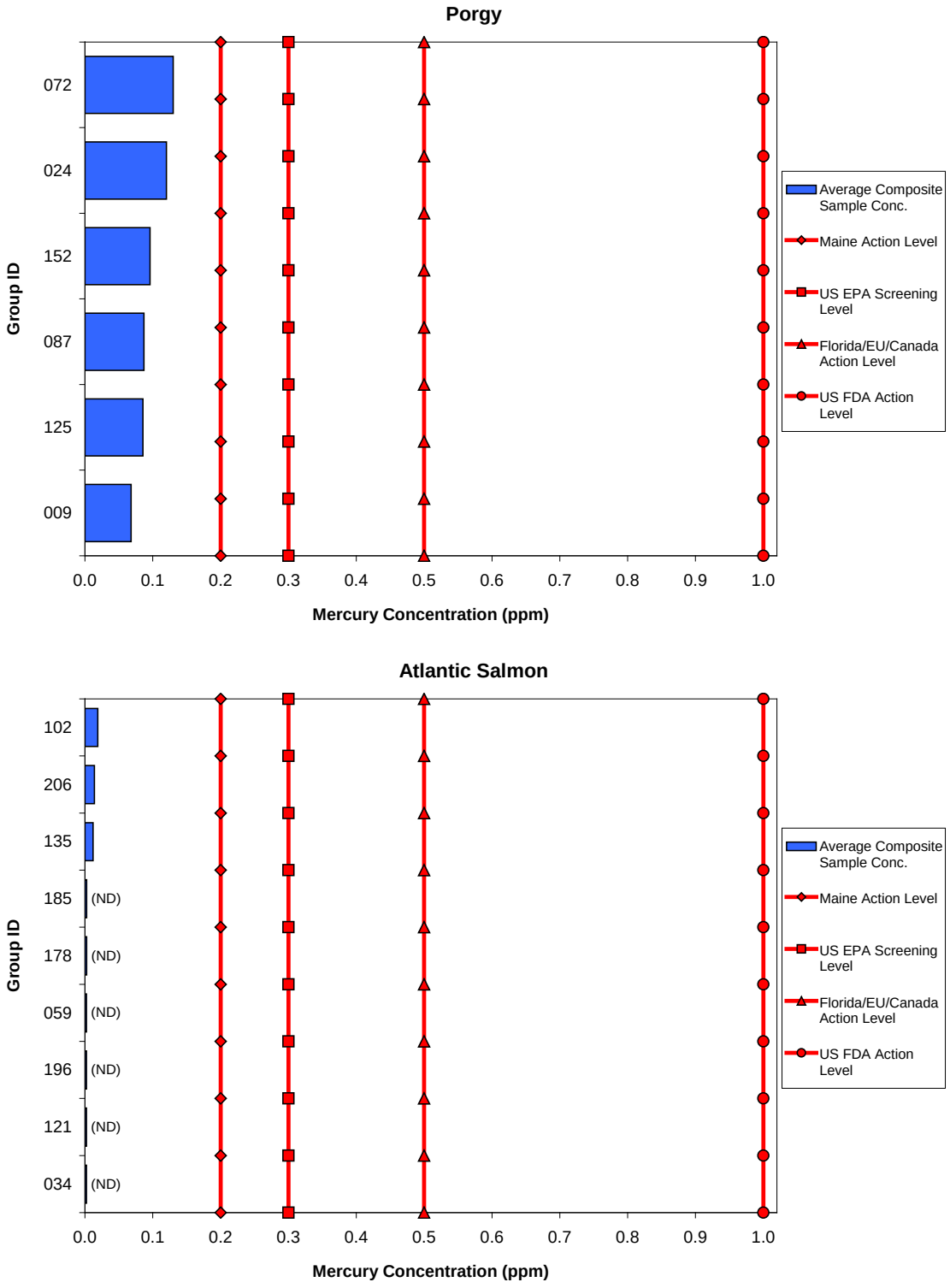


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

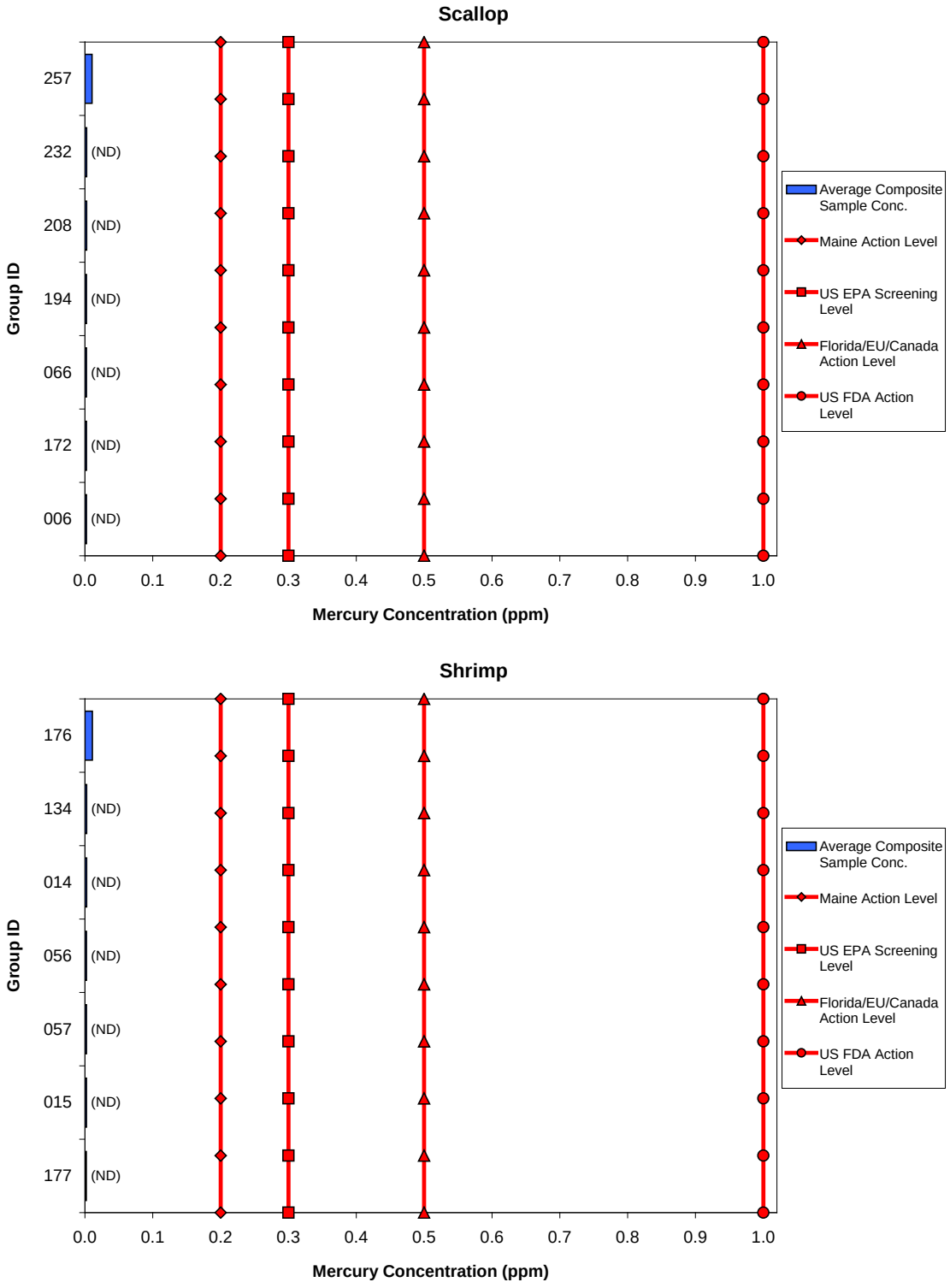


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

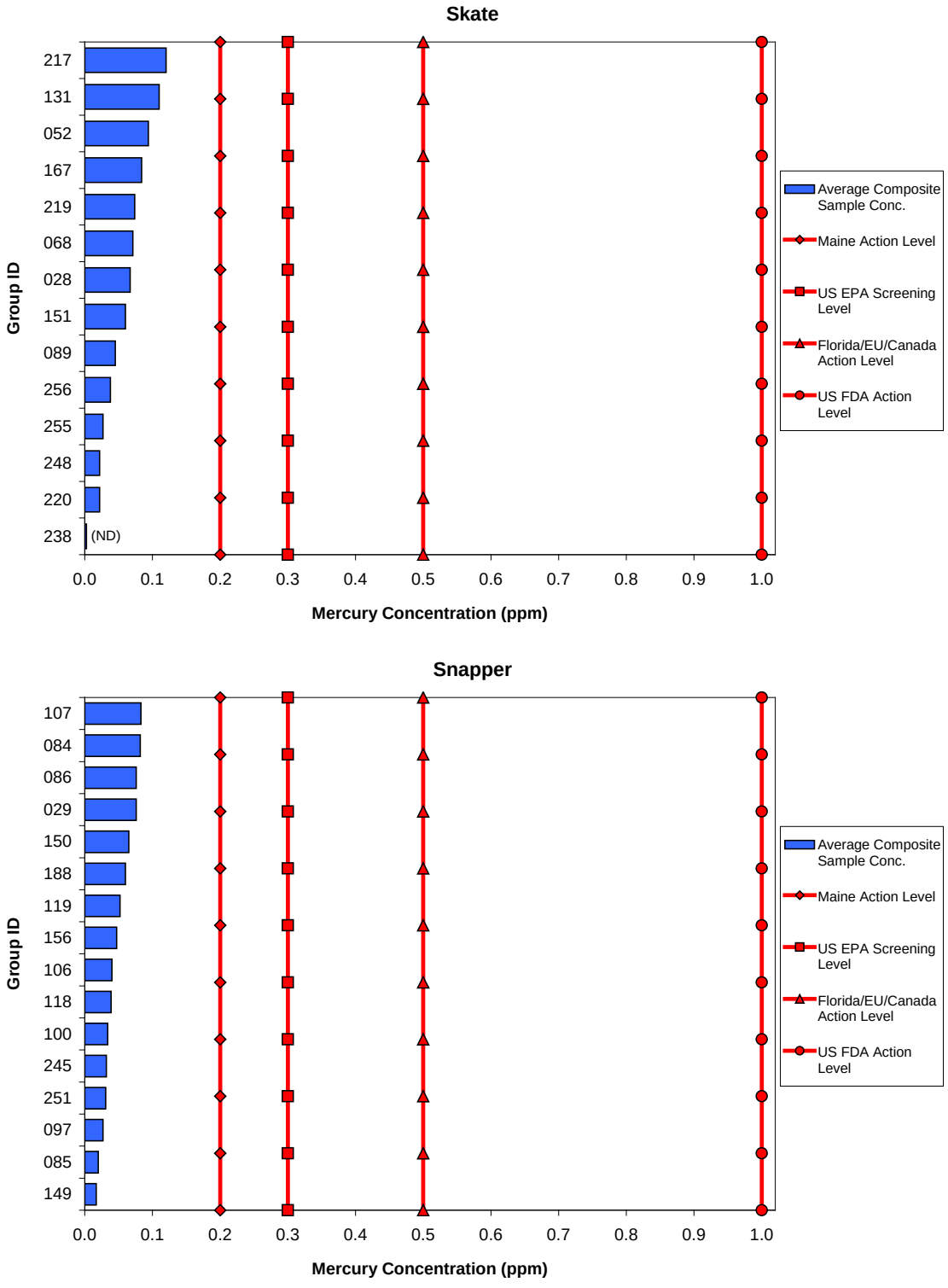


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

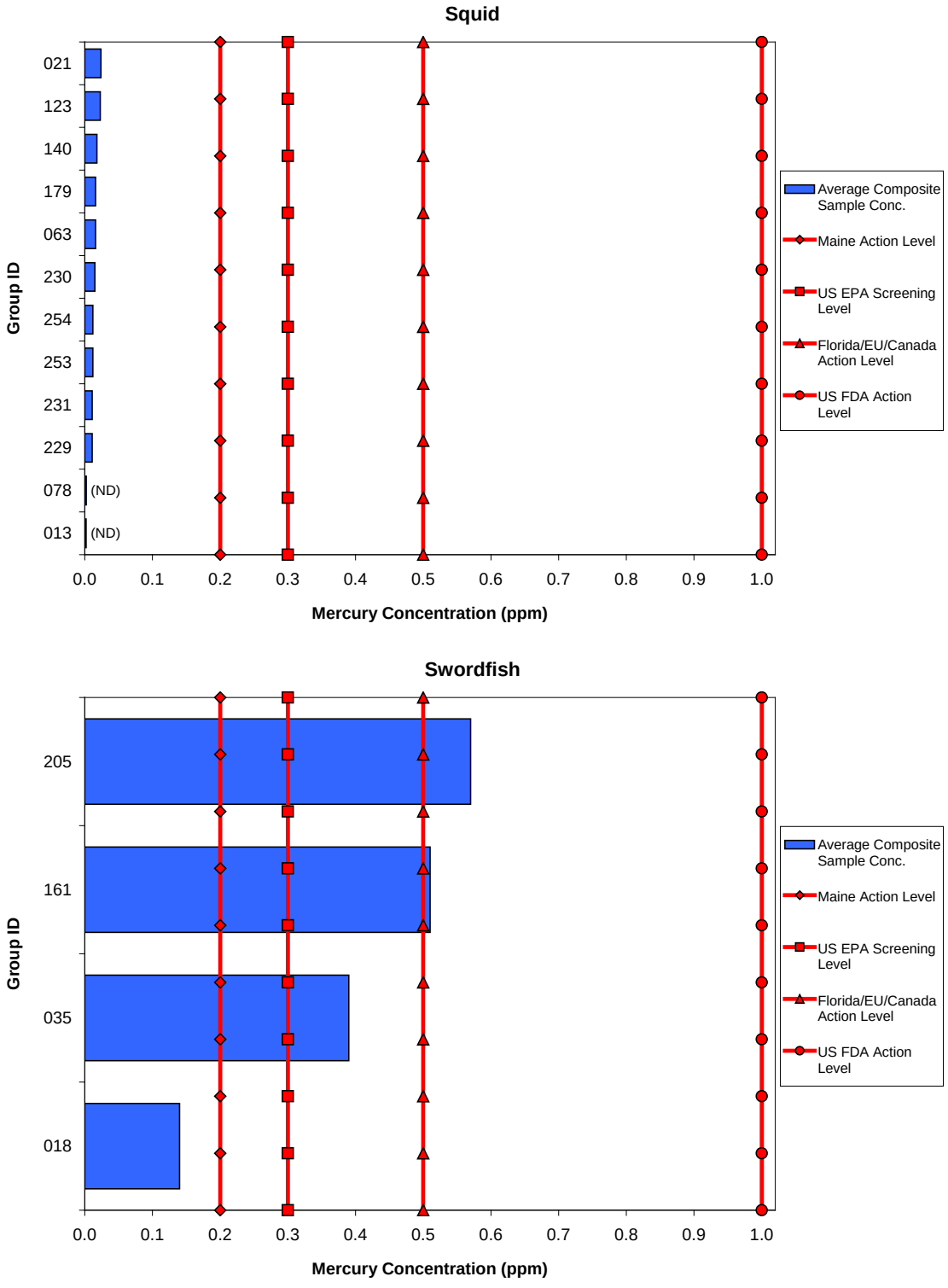


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

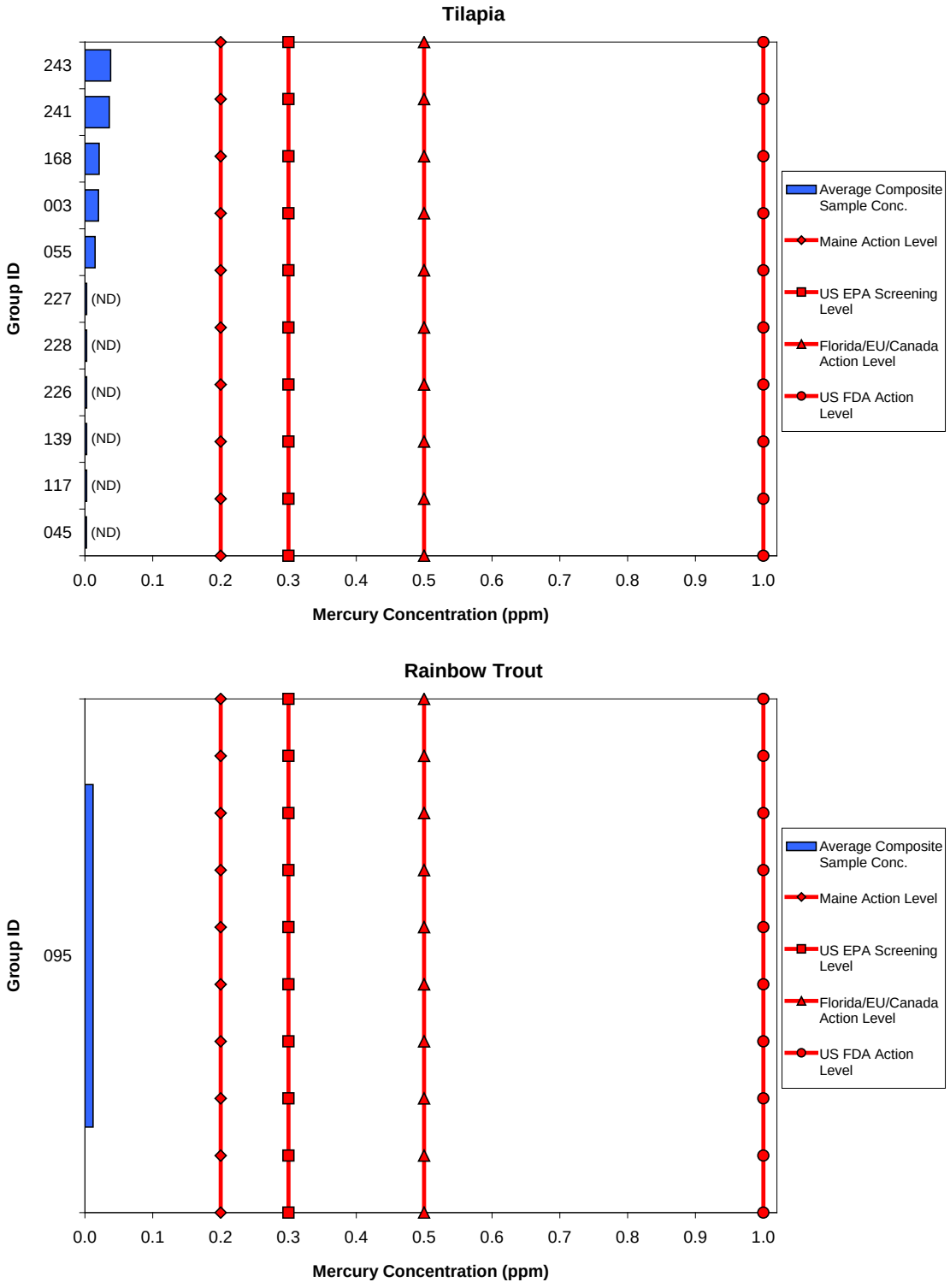


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

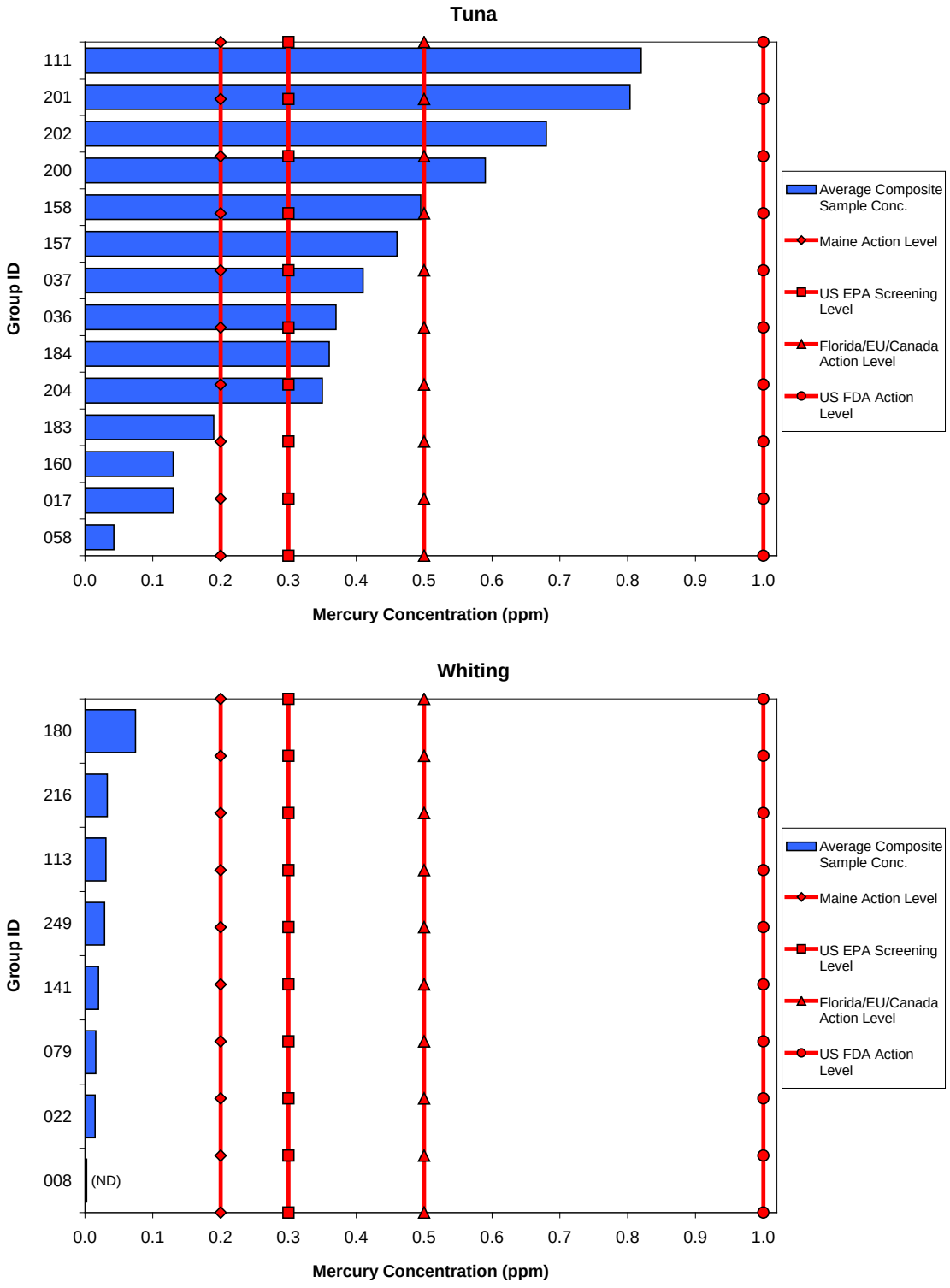


Figure A-1. Measured average mercury concentrations by composite sample vs. selected state and federal action levels. (continued)

Table A-4. Information about each composite sample group used in the analysis

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
1	Flounder/ Fluke/ Sole	Flounder, Blackback	Partial	fillet	Atlantic off Nova Scotia	Wild	Atlantic, Wild	48.3		783.8	13	669.7
2	Flounder/ Fluke/ Sole	Flounder, Yellowtail	Partial	fillet	Atlantic	Wild	Atlantic, Wild			746.5	16	689
3	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm				6	1,084.8
4	Cod	Cod, Atlantic	Partial	fillet	Atlantic: Maine	Wild	Atlantic, Wild				3	970
6	Scallop	Scallop, Sea	Whole	shellfish, shelled	Atlantic	Wild	Atlantic, Wild				18	818.9
7	Mussel	Mussel, Blue	Whole	shellfish	Atlantic	Wild	Atlantic, Wild				155	608.2
8	Whiting	Whiting/ Silver Hake	Whole	whole	Atlantic	Wild	Atlantic, Wild	27.0	23.0	142.6	12	368.6
9	Porgy	Porgy/ Scup	Whole	whole	Atlantic	Wild	Atlantic, Wild	36.3	29.0	875.7	3	530.8
10	Spanish Mackerel	Spanish Mackerel	Whole	whole	Atlantic	Wild	Atlantic, Wild	62.0	51.7	1,475.9	3	595.7
11	Monkfish	Monkfish/ Goosefish	Partial	headed and gutted	Atlantic	Wild	Atlantic, Wild	49.3	37.7	1,576.3	3	596.3
12	Sea Bass	Bass, Black Sea	Whole	whole	Atlantic	Wild	Atlantic, Wild	36.3	29.0	849.2	3	358.6
13	Squid	Squid	Partial	tubes U5	Pacific	Wild	Pacific, Wild	23.7		219.2	3	370.7
14	Shrimp	Shrimp, White	Partial	headed	Farm (Pacific)	Farmed	Farm				75	603.8
15	Shrimp	Shrimp, Black Tiger	Partial	headed	Farm	Farmed	Farm				40	616.3
17	Tuna	Tuna, Bigeye	Partial	fillet / loin	Pacific	Wild	Pacific, Wild				3	624.6
18	Swordfish	Swordfish	Partial	loin	Atlantic	Wild	Atlantic, Wild				3	723.5
19	Mahi-mahi	Dolphin/ Mahi-mahi	Partial	gutted	Atlantic	Wild	Atlantic, Wild	110.0			2	408.6

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
20	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic	Wild	Atlantic, Wild	31.5	27.3	248.4	6	357.2
21	Squid	Squid, Longfin	Whole	whole	Atlantic	Wild	Atlantic, Wild	38.7		113.6	12	695.5
22	Whiting	Whiting/ Silver Hake	Whole	whole	Atlantic	Wild	Atlantic, Wild	28.6	24.1	164.4	8	272.8
23	Croaker	Croaker, Atlantic	Whole	whole	Atlantic	Wild	Atlantic, Wild	32.3	26.8	453.8	6	481.1
24	Porgy	Porgy/ Scup	Whole	whole	Atlantic	Wild	Atlantic, Wild	32.7	26.3	658.6	3	271.9
25	Sea Bass	Bass, Black Sea	Whole	whole	Atlantic	Wild	Atlantic, Wild	33.7	27.0	635.6	3	257.7
26	Monkfish	Monkfish/ Goosefish	Partial	headed	Atlantic	Wild	Atlantic, Wild	48.0	36.0	1,569.1	1	960.2
28	Skate	Skate, Winter	Partial	wings	Atlantic	Wild	Atlantic, Wild	38.5		908.3	2	565.8
29	Snapper	Snapper, Red	Whole	whole, gutted	Gulf	Wild	Atlantic, Wild	34.3	27.7	658.0	3	418.1
30	Flounder/ Fluke/ Sole	Flounder, Blackback	Whole	whole	Atlantic	Wild	Atlantic, Wild	33.0	27.0	473.8	3	209.2
31	Flounder/ Fluke/ Sole	Sole, Gray	Whole	whole	Atlantic	Wild	Atlantic, Wild	41.3	34.7	474.6	3	207.1
32	Mussel	Mussel, Blue	Whole	shellfish	Atlantic	Wild	Atlantic, Wild			746.5	66	164.2
33	Clam	Quahog, Northern/ Little Neck Clam	Whole	shellfish	Sound	Wild	Sound				19	178.4
34	Atlantic Salmon	Atlantic Salmon	Whole	whole, gutted	Atlantic	Wild	Atlantic, Wild	66.0	55.7	2,549.9	3	649.1
35	Swordfish	Swordfish	Partial	loin	Pacific	Wild	Pacific, Wild			39,812.3	3	646.3
36	Tuna	Tuna, Yellowfin	Partial	loin	Pacific	Wild	Pacific, Wild			31,725.4	2	433.5
37	Tuna	Tuna, Bigeye	Partial	loin	Pacific	Wild	Pacific, Wild			20,528.2	40	616.3
39	Mussel	Mussel, Blue	Whole	shellfish	Atlantic	Wild	Atlantic, Wild			963.8	45	333.9

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
40	Clam	Quahog, Northern/ Cherrystone Clam	Whole	shellfish	Atlantic	Wild	Atlantic, Wild				11	328.7
41	Oyster	Oyster, Eastern	Whole	shellfish	Long Island Sound, Conn. Side	Wild	Sound				9	150.2
42	Mussel	Mussel, Blue	Whole	shellfish	Atlantic	Wild	Atlantic, Wild			889.5	47	286.7
43	Clam	Quahog, Northern/ Little Neck Clam	Whole	shellfish	Atlantic	Wild	Atlantic, Wild				21	159
44	Oyster	Oyster, Eastern	Whole	shellfish	Galveston Bay	Farmed	Farm				22	277.5
45	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm				6	618
46	Flounder/ Fluke/ Sole	Sole, Gray	Whole	whole	Atlantic	Wild	Atlantic, Wild	41.7	35.0	497.3	3	160.4
47	Flounder/ Fluke/ Sole	Flounder, Summer	Whole	whole	Atlantic	Wild	Atlantic, Wild	47.0	39.7	1,283.2	3	648.3
48	Flounder/ Fluke/ Sole	Flounder, Blackback	Whole	whole	Atlantic	Wild	Atlantic, Wild	35.7	27.3	553.0	3	360
50	Cod	Cod, Atlantic	Whole	Whole, gutted	Atlantic	Wild	Atlantic, Wild	62.0	53.0	1,954.9	3	600.8
51	Pollock	Pollock	Partial	headed and gutted	Atlantic	Wild	Atlantic, Wild	70.0	56.0	3,639.0	3	601.1
52	Skate	Skate, Winter	Partial	wings	Atlantic	Wild	Atlantic, Wild	30.7		699.8	3	615.9
53	Monkfish	Monkfish/ Goosefish	Partial	tails	Atlantic	Wild	Atlantic, Wild	40.3	30.7	846.0	3	634.8
54	Sea Bass	Bass, Black Sea	Whole	whole (round)	Atlantic	Wild	Atlantic, Wild	33.7	25.7	557.5	3	318.6
55	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm				3	1,084.8
56	Shrimp	Shrimp, White	Partial	headed	Farm (Pacific)	Farmed	Farm				40	605.1

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
57	Shrimp	Shrimp, White	Partial	headed and deveined	Farm	Farmed	Farm				89	623.4
58	Tuna	Tuna, Yellowfin	Partial	loin	Pacific	Wild	Pacific, Wild				3	799.6
59	Atlantic Salmon	Atlantic Salmon	Partial	fillet	N. Pacific	Wild	Pacific, Wild				3	649.1
61	Flounder/ Fluke/ Sole	Flounder, Summer	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	48.7	41.3	1,424.1	3	610.8
62	Cod	Cod, Atlantic	Partial	headed and gutted	N. Atlantic	Wild	Atlantic, Wild	62.7		3,664.6	3	631.7
63	Squid	Squid, Longfin	Partial	whole	N. Atlantic	Wild	Atlantic, Wild	32.5		43.3	13	312
64	Clam	Quahog, Northern/ Little Neck Clam	Whole	shellfish	Farm	Farmed, Cherry Stone Creek	Farm			1,373.9	33	216
65	Blue Crab	Blue Crab/ Softshell	Whole	softshell	N. Atlantic	Wild	Atlantic, Wild			922.0	11	786
66	Scallop	Scallop, Sea	Whole	shelled, dry	N. Atlantic	Wild	Atlantic, Wild				18	864.9
67	Pollock	Pollock	Partial	headed and gutted	N. Atlantic	Wild	Atlantic, Wild	65.7		3,580.1	3	626.2
68	Skate	Skate, Winter	Partial	wings	N. Atlantic	Wild	Atlantic, Wild	36.7		837.7	3	605.4
69	Chilean Sea Bass	Chilean Sea Bass	Whole	steak (headed and gutted)	Pacific	Wild	Pacific, Wild	17.0		392.9	3	624.4
70	Monkfish	Monkfish/ Goosefish	Partial	Tail (headed and gutted)	N. Atlantic	Wild	Atlantic, Wild	53.3		2,090.0	3	634.1
72	Porgy	Porgy/ Scup	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	37.0	29.7	819.7	3	495.8
73	Oyster	Oyster, Eastern	Whole	shellfish	Farm	Farmed (Running Channel)	Farm			2,207.5	34	177.1
74	Halibut	Halibut, Pacific	Partial	headed and gutted	N. Pacific	Wild	Pacific, Wild	71.5		45,17.1	2	436.4

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
75	Sea Bass	Bass, Black Sea	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	52.3	41.0	2,007.0	3	634
76	Flounder/ Fluke/ Sole	Sole, Gray	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	40.7	33.7	517.9	3	250.8
77	Cod	Cod, Atlantic	Partial	headed and gutted	N. Atlantic	Wild	Atlantic, Wild	62.0		6,179.7	2	616.4
78	Squid	Squid, Japanese Flying	Partial	tube	Pacific	Wild	Pacific, Wild			601.4	5	543.3
79	Whiting	Whiting/ Silver Hake	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	28.0		145.6	10	371.1
80	Catfish	Catfish, Channel	Whole	whole	Great Lakes	Wild	Lake	43.3	35.5	751.2	3	349.3
81	Blue Crab	Blue Crab/ Hardshell	Whole	hardshell	N. Atlantic	Wild	Atlantic, Wild	20.0		4,101.4	21	601.8
83	Pollock	Pollock	Partial	headed and gutted	N. Atlantic	Wild	Atlantic, Wild	68.3		3,439.8	3	639
84	Snapper	Snapper, Yellowtail	Partial	gutted	S. Atlantic	Wild	Atlantic, Wild	38.8	28.0	521.1	3	324.5
85	Snapper	Snapper, Vermilion	Partial	gutted	S. Atlantic	Wild	Atlantic, Wild	35.1	27.5	486.6	3	455.4
86	Snapper	Snapper, Red	Partial	gutted	S. Atlantic	Wild	Atlantic, Wild	37.6	29.1	677.7	3	472.7
87	Porgy	Porgy/ Scup	Partial	gutted	N. Atlantic	Wild	Atlantic, Wild	31.4	25.4	549.3	3	261.9
88	Herring	Herring, Atlantic	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	17.0	13.9	54.2	11	128.6
89	Skate	Skate, Winter	Partial	wings	N. Atlantic	Wild	Atlantic, Wild	42.8		883.0	3	591.4
90	Monkfish	Monkfish/ Goosefish	Partial	tail (headed and gutted)	N. Atlantic	Wild	Atlantic, Wild	50.7		1,513.3	3	602.2
91	Croaker	Croaker, Atlantic	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	38.0	31.3	701.5	3	300.9
92	Bluefish	Bluefish	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	68.7	55.0	2,893.4	3	597.7

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
94	Ocean Perch	Ocean Perch	Whole	fillet	China (for filleting, N. Atlantic fish)	Wild	Atlantic, Wild	16.0		61.0	10	608.8
95	Rainbow Trout	Rainbow Trout	Partial	gutted	Farm	Farmed	Farm	36.3	32.0	571.5	3	574.4
96	Sea Bass	Bass, Black Sea	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	40.7	32.0	1,005.6	3	597.3
97	Snapper	Snapper, Lane	Partial	gutted	Pacific	Wild	Pacific, Wild	42.7	34.7	1,075.2	3	615.8
98	Bass	Bass, Hybrid Striped	Whole	whole	Farm	Farmed	Farm	30.2	24.5	396.7	3	201.1
99	Sea Bass	Bass, Black Sea	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	34.3	26.7	558.6	3	284.2
100	Snapper	Snapper, Yellowtail	Partial	gutted	N. Atlantic	Wild	Atlantic, Wild	39.7	28.3	481.3	3	417.9
101	Catfish	Catfish, Channel	Whole	whole	Unknown	Wild	Unknown	44.3	39.3	979.7	3	517.3
102	Atlantic Salmon	Atlantic Salmon	Partial	gutted	Atlantic	Farmed	Farm	87.7	74.3	7,565.7	3	634.3
103	Flounder/ Fluke/ Sole	Sole, Gray	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	38.7	32.0	424.1	3	375.6
105	Croaker	Croaker, Atlantic	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	32.0	27.3	454.0	3	371.9
106	Snapper	Snapper, Yellowtail	Whole	whole	S. Atlantic	Wild	Atlantic, Wild	36.0	26.7	527.3	3	507.8
107	Snapper	Snapper, Vermilion	Whole	whole	S. Atlantic	Wild	Atlantic, Wild	32.3	25.3	451.7	3	396.6
108	Catfish	Catfish, White	Whole	whole	Farm	Farmed	Farm	36.7	31.0	795.0	3	487.2
109	Pollock	Pollock	Partial	headed & gutted	N. Atlantic	Wild	Atlantic, Wild	68.0		3,344.1	3	604.4
110	Spanish Mackerel	Spanish Mackerel	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	42.0	35.0	422.4	3	401

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
111	Tuna	Tuna, Bigeye	Partial	loin	Pacific or Indian	Wild	Pacific, Wild			71,289.0	3	389.6
112	Flounder/ Fluke/ Sole	Flounder, Summer	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	42.0	36.0	795.2	3	615
113	Whiting	Whiting/ Silver Hake	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	28.0	24.0	139.9	10	407.8
114	Catfish	Catfish, Blue	Whole	whole	Farm	Farmed	Farm	44.0	37.3	888.8	3	544.5
116	Croaker	Croaker, Atlantic	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	34.3	28.3	557.4	3	308.2
117	Tilapia	Tilapia	Partial	fillet	Unknown	Wild	Unknown				3	476.8
118	Snapper	Snapper, Caribbean Red	Partial	gutted	S. Atlantic	Wild	Atlantic, Wild	40.0	32.0	833.6	3	657.6
119	Snapper	Snapper, Lane	Partial	gutted	Pacific	Wild	Pacific, Wild	43.0	35.3	1,138.8	3	618.2
120	Sea Bass	Bass, Black Sea	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	42.3	34.0	1,236.8	3	653.3
121	Atlantic Salmon	Atlantic Salmon	Partial	gutted	N. Pacific	Wild	Pacific, Wild	69.7	60.7	3,403.1	3	601.3
122	Flounder/ Fluke/ Sole	Flounder, Summer	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	43.7	37.7	922.4	3	655.5
123	Squid	Squid, Longfin	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	45.0		162.6	8	679
124	Catfish	Catfish, Channel	Whole	whole	Unknown	Wild	Unknown	50.3	42.7	1,595.4	3	614.5
125	Porgy	Porgy/ Scup	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	29.0	23.7	386.6	3	282.6
127	Monkfish	Monkfish/ Goosefish	Partial	tail (headed and gutted)	Hampton Bays	Wild	Bay	51.0		1,536.2	3	597.2
128	Croaker	Croaker, Atlantic	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	33.3	28.3	543.7	3	311.9
129	Sea Bass	Bass, Black Sea	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	33.3	27.3	561.9	3	313
130	Bluefish	Bluefish	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	49.3	42.3	1,007.1	3	621.2
131	Skate	Skate, Winter	Partial	wings	N. Atlantic	Wild	Atlantic, Wild	32.0		735.3	3	608.8

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
132	Bass	Bass, Striped	Whole	whole	Chesapeake Bay	Wild	Bay	64.7	55.7	2,644.4	3	622.4
133	Blue Crab	Blue Crab/ Hardshell	Whole	hardshell	N. Atlantic	Wild	Atlantic, Wild	17.0			19	616.4
134	Shrimp	Shrimp, White	Partial	headed	Farm (Pacific)	Farmed	Farm				37	603
135	Atlantic Salmon	Atlantic Salmon	Partial	fillet	Farm (Pacific)	Farmed	Farm				3	622.4
136	Flounder/ Fluke/ Sole	Flounder, Summer	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	38.0	29.7	591.0	3	478.6
138	Flounder/ Fluke/ Sole	Flounder, Blackback	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	38.0	31.7	871.4	3	615.2
139	Tilapia	Tilapia	Whole	whole	Farm	Farmed	Farm	33.0	27.7	861.3	3	604.4
140	Squid	Squid, Longfin	Whole	whole	Atlantic	Wild	Atlantic, Wild	21.0		90.4	15	659.5
141	Whiting	Whiting/ Silver Hake	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	31.7	27.7	288.5	3	213.7
142	Catfish	Catfish, Blue	Whole	whole	Delaware River	Wild	River	44.7	38.3	905.7	3	605.3
143	Oyster	Oyster, Eastern	Whole	shellfish	Farm	Farmed	Farm			8,137.3	46	587
144	Blue Crab	Blue Crab/ Softshell	Whole	softshell	N. Atlantic or Delaware Bay	Wild	Atlantic, Wild	13.0		81.5	11	903.1
145	Blue Crab	Blue Crab/ Hardshell	Whole	hardshell	N. Atlantic	Wild	Atlantic, Wild				18	579.6
146	Lobster	Lobster, American	Whole	shellfish	N. Atlantic	Wild	Atlantic, Wild	43.0		614.5	3	468.2
148	Pollock	Pollock	Partial	headed and gutted	N. Atlantic	Wild	Atlantic, Wild	60.7		2,605.1	3	628.9
149	Snapper	Snapper, Vermilion	Partial	whole	N. Atlantic	Wild	Atlantic, Wild	29.7	24.0	333.4	3	314.4
150	Snapper	Snapper, Red	Partial	whole	N. Atlantic	Wild	Atlantic, Wild	42.0	35.0	1,123.9	3	607.7
151	Skate	Skate, Winter	Partial	wings	N. Atlantic	Wild	Atlantic, Wild	41.7		754.2	3	597.5
152	Porgy	Porgy/ Scup	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	35.7	30.0	807.3	3	375.5

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
153	Croaker	Croaker, Atlantic	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	32.3	28.0	446.4	3	301.7
154	Bluefish	Bluefish	Whole	whole	N. Atlantic	Wild	Atlantic, Wild	59.0	51.0	1,969.0	3	611.4
155	Sea Bass	Bass, Black Sea	Whole	whole	Long Island Sound	Wild	Sound	35.7	30.0	696.9	3	507.6
156	Snapper	Snapper, Yellowtail	Partial	whole	N. Pacific or Gulf of Mexico	Wild	Unknown	26.0	19.0	203.3	4	325
157	Tuna	Tuna, Bigeye	Partial	loin	Pacific, western	Wild	Pacific, Wild				2	555.9
158	Tuna	Tuna, Bigeye	Partial	loin	Pacific, western	Wild	Pacific, Wild				2	566.1
160	Tuna	Tuna, Yellowfin	Partial	loin	Pacific, western	Wild	Pacific, Wild				2	318.5
161	Swordfish	Swordfish	Partial	loin	Atlantic, North	Wild	Atlantic, Wild				3	686.4
162	Flounder/ Fluke/ Sole	Flounder, Blackback	Whole	whole	Bay of Fundy	Wild	Bay	36.0	30.3	638.7	3	316.9
163	Cod	Cod, Atlantic	Partial	gutted	Atlantic, North	Wild	Atlantic, Wild	63.7	55.3	2,712.7	3	605.2
164	Sea Bass	Bass, Black Sea	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	33.0	27.0	532.0	3	356.6
165	Monkfish	Monkfish/ Goosefish	Partial	tail (headed and gutted)	Atlantic, North	Wild	Atlantic, Wild	39.3		762.0	3	609.8
166	Catfish	Catfish, Channel	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	46.7	40.7	1,022.5	3	467.3
167	Skate	Skate, Winter	Partial	wings	Atlantic, North	Wild	Atlantic, Wild	40.0		722.3	3	517.9
168	Tilapia	Tilapia	Partial	fillets	Farm	Farmed	Farm				3	576
169	Blue Crab	Blue Crab/ Hardshell	Whole	hardshell	Atlantic, North	Wild	Atlantic, Wild			106.1	29	640

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
171	Blue Crab	Blue Crab/ Softshell	Whole	softshell	Atlantic, North	Wild	Atlantic, Wild			112.3	5	649.5
172	Scallop	Scallop, Sea	Whole	shelled, dry	Atlantic, North	Wild	Atlantic, Wild				10	603.6
173	Clam	Quahog, Northern/ Little Neck Clam	Whole	shellfish	Atlantic, North	Wild	Atlantic, Wild				50	608.9
174	Oyster	Oyster, Pacific	Whole	shellfish, shelled	Farm	Farmed	Farm				29	604.1
175	Mussel	Mussel, Blue	Whole	shellfish	Farm	Farmed	Farm				206	600.4
176	Shrimp	Shrimp, White	Partial	headed	Farm	Farmed	Farm				38	608.2
177	Shrimp	Shrimp, Black Tiger	Partial	headed	Farm	Farmed	Farm				39	600.2
178	Atlantic Salmon	Atlantic Salmon	Partial	gutted	Atlantic, North	Farmed	Farm	72.7	66.7	3,978.6	3	607.2
179	Squid	Squid, Longfin	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	23.0		69.4	14	640.2
180	Whiting	Whiting, Offshore	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	47.0	43.3	828.8	3	585.3
182	Spanish Mackerel	Spanish Mackerel	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	47.3	39.0	580.5	3	535.1
183	Tuna	Tuna, Bigeye	Partial	loin, from belly	Pacific	Wild	Pacific, Wild	33.3		530.2	3	598.9
184	Tuna	Tuna, Bigeye	Partial	loin, from belly	Pacific	Wild	Pacific, Wild	26.3		787.5	3	590.8
185	Atlantic Salmon	Atlantic Salmon	Partial	gutted	West Coast farm	Farmed	Farm	77.7	69.3	5,790.7	3	604.6
186	Pollock	Pollock	Partial	fillet	Atlantic, North	Wild	Atlantic, Wild	32.5		870.3	2	404.8
187	Cod	Cod, Atlantic	Partial	fillet	Atlantic, North	Wild	Atlantic, Wild	32.0		432.6	2	446.2

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
188	Snapper	Snapper, Red	Partial	gutted	Atlantic, South	Wild	Atlantic, Wild	35.3	29.3	573.8	3	573.1
189	Oyster	Oyster, Eastern	Whole	shellfish	Farm, Atlantic, North	Farmed	Farm			2,448.4	23	407.3
190	Oyster	Oyster, Eastern	Whole	shellfish	Atlantic, North	Wild	Atlantic, Wild			2,694.5	17	238.4
191	Mussel	Mussel, Blue	Whole	shellfish	Atlantic, North	Wild	Atlantic, Wild			1,799.8	153	521.9
193	Clam	Quahog, Northern/ Little Neck Clam	Whole	shellfish	Atlantic, North	Wild	Atlantic, Wild			1,022.7	44	616.4
194	Scallop	Scallop, Sea	Whole	shelled, dry	Atlantic, North	Wild	Atlantic, Wild			871.1	12	594.2
195	Bass	Bass, Hybrid Striped	Whole	whole	Kent Farm	Farmed	Farm	34.7	29.0	732.1	3	148.4
196	Atlantic Salmon	Atlantic Salmon	Partial	fillet	Farmed	Farmed	Farm	40.0		742.4	3	611.1
197	Oyster	Oyster, Eastern	Whole	shellfish	Delaware Bay, Farmed	Farmed	Farm			3,919.7	32	565
198	Mussel	Mussel, Blue	Whole	shellfish	Atlantic, North, Farm	Farmed	Farm			826.3	44	289
199	Clam	Quahog, Northern/ Top Neck Clam	Whole	shellfish	Atlantic, North, Farm	Farmed	Farm			2,906.2	33	564.2
200	Tuna	Tuna, Yellowfin	Partial	loin	Pacific, North	Wild	Pacific, Wild	13.3		495.3	3	612.1
201	Tuna	Tuna, Yellowfin	Partial	loin	Pacific	Wild	Pacific, Wild	12.5		529.0	2	604.9
202	Tuna	Tuna, Yellowfin	Partial	loin	Pacific, North	Wild	Pacific, Wild	13.0		547.7	3	621.8
204	Tuna	Tuna, Yellowfin	Partial	loin	Pacific, North	Wild	Pacific, Wild	13.3		495.0	3	625.5

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
205	Swordfish	Swordfish	Partial	loin	Pacific	Wild	Pacific, Wild	15.0		442.9	3	610
206	Atlantic Salmon	Atlantic Salmon	Partial	fillet, on site	Farm	Farm	Farm	48.7		1,513.9	3	606.5
207	Blue Crab	Blue Crab/ Softshell	Whole	softshell	Atlantic, North	Wild	Atlantic, Wild			66.3	8	654.4
208	Scallop	Scallop, Sea	Whole	shelled, dry	Atlantic, North	Wild	Atlantic, Wild			855.5	32	585.3
209	Croaker	Croaker, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	35.0	30.3	566.6	3	319.7
210	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	33.5	29.5	333.6	4	299.3
211	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	33.5	29.8	334.0	4	325.4
212	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	32.0	28.0	368.1	4	278.8
213	Croaker	Croaker, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	31.7	27.0	417.5	3	240.2
215	Croaker	Croaker, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	34.7	30.3	526.8	3	358.6
216	Whiting	Whiting/ Silver Hake	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	27.0		140.2	10	446.7
217	Skate	Skate, Winter	Partial	wings	Atlantic, North	Wild	Atlantic, Wild	37.7		445.1	3	325.9
218	Monkfish	Monkfish/ Goosefish	Partial	tail (headed and gutted)	Atlantic, North	Wild	Atlantic, Wild	37.7		781.7	3	602.2
219	Skate	Skate, Winter	Partial	wings	Atlantic, North	Wild	Atlantic, Wild	37.7		379.3	3	255.8
220	Skate	Skate, Winter	Partial	wings, skin off	Atlantic, North	Wild	Atlantic, Wild	30.7		631.7	3	614.7
221	Pollock	Pollock	Partial	fillet	Atlantic, North	Wild	Atlantic, Wild	49.3		1,573.4	3	603.6
222	Cod	Cod, Pacific	Partial	fillet	Pacific, North	Wild	Pacific, Wild	34.3		740.0	3	630.7

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
223	Cod	Cod, Pacific	Partial	fillet	Pacific, North	Wild	Pacific, Wild	34.0		802.9	3	603.7
224	Cod	Cod, Pacific	Partial	fillet	Pacific, North	Wild	Pacific, Wild	38.3		902.1	3	628.4
226	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm	22.3		324.6	3	604.1
227	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm	23.7		343.0	3	659
228	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm	22.7		361.1	3	621.9
229	Squid	Squid, Japanese Flying	Partial	tubes	Pacific, North	Wild	Pacific, Wild	17.0		103.9	5	428.7
230	Squid	Squid, Japanese Flying	Partial	tubes	Pacific, North	Wild	Pacific, Wild	17.0		103.6	5	452.1
231	Squid	Squid, Japanese Flying	Partial	tubes	Pacific, North	Wild	Pacific, Wild	17.0		94.2	5	421.3
232	Scallop	Scallop, Sea	Whole	shelled, dry	Atlantic, North	Wild	Atlantic, Wild	3.0		839.3	27	616
233	Pollock	Pollock	Partial	headed and gutted	Atlantic, North	Wild	Atlantic, Wild	64.7		3,302.8	3	632.4
234	Monkfish	Monkfish/ Goosefish	Partial	tail (headed and gutted)	Atlantic, North	Wild	Atlantic, Wild	36.3		701.2	3	614.8
235	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	29.0	26.0	202.4	5	337.7
237	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	31.0	28.0	239.7	5	342.4
238	Skate	Skate, Winter	Partial	wings	Atlantic, North	Wild	Atlantic, Wild	31.7		743.9	3	596.2
239	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	30.0		216.6	5	411.2
240	Mackerel	Mackerel, Atlantic	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	29.0		215.0	6	511.7
241	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm	19.3		209.2	3	503.4

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
242	Blue Crab	Blue Crab/ Softshell	Whole	softshell	Atlantic, North	Wild	Atlantic, Wild			54.6	9	390.1
243	Tilapia	Tilapia	Partial	fillet	Farm	Farmed	Farm	18.7		206.1	3	486.6
244	Blue Crab	Blue Crab/ Softshell	Whole	softshell	Atlantic, North	Wild	Atlantic, Wild			55.8	11	507.5
245	Snapper	Snapper, Red	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	47.3	37.7	1,706.7	3	600
246	Monkfish	Monkfish/ Goosefish	Partial	tail (headed and gutted)	Atlantic, North	Wild	Atlantic, Wild	37.7		779.5	3	589.1
248	Skate	Skate, Winter	Partial	wings	Atlantic, North	Wild	Atlantic, Wild	40.3		1,175.1	3	601.7
249	Whiting	Whiting, Offshore	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	35.0		271.5	5	450.4
250	Sea Bass	Bass, Black Sea	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	39.7	33.0	842.2	3	575.2
251	Snapper	Snapper, Red	Partial	gutted	Atlantic, South	Wild	Atlantic, Wild	37.0	30.3	599.8	3	575.9
252	Cod	Cod, Atlantic	Partial	gutted	Atlantic, North	Wild	Atlantic, Wild	58.0	51.7	1,942.4	3	646.4
253	Squid	Squid, Longfin	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	18.0		41.9	17	448.4
254	Squid	Squid, Longfin	Whole	whole	Atlantic, North	Wild	Atlantic, Wild	18.0		40.0	21	529
255	Skate	Skate, Winter	Partial	wings	Atlantic, North	Wild	Atlantic, Wild	37.3		888.4	3	595.3
256	Skate	Skate, Winter	Partial	wings	Atlantic, North	Wild	Atlantic, Wild	38.7		769.8	3	600.1
257	Scallop	Scallop, Sea	Whole	shelled, dry	Atlantic, North	Wild	Atlantic, Wild	4.0		724.8	11	627.4
259	Pollock	Pollock	Partial	headed and gutted	Atlantic, North	Wild	Atlantic, Wild	64.7		3,178.3	3	597.8

Table A-4. Information about each composite sample group used in the analysis (continued)

Sam. Grp.	Market Name	Common Name	Whole or Partial	Form of Sample	Water Body Notes	Wild or Farmed	Water Body (Uni-form)	Avg. Total Length (cm)	Avg. Caudal Length (cm)	Avg. Whole Fish Wgt. (g)	Num. in Comp.	Weight of Comp. (g)
260	Blue Crab	Blue Crab/ Hardshell	Whole	hardshell	Atlantic, North	Wild	Atlantic, Wild			116.5	9	209.4

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
001.C	1		LR-1	Flounder/Fluke/Sole	Flounder, Blackback		N	0.0095
001.C	1		LR-2	Flounder/Fluke/Sole	Flounder, Blackback		N	0.0095
001.C	1		LR-3	Flounder/Fluke/Sole	Flounder, Blackback		N	0.0097
002.C	2			Flounder/Fluke/Sole	Flounder, Yellowtail		N	0.0093
003.C	3			Tilapia	Tilapia	0.02	Y	0.01
004.C	4			Cod	Cod, Atlantic	0.027	Y	0.01
005.C	4	004.C		Cod	Cod, Atlantic	0.024	Y	0.01
006.C	6			Scallop	Scallop, Sea		N	0.0086
007.C	7			Mussel	Mussel, Blue	0.014	Y	0.0087
008.C	8			Whiting	Whiting/Silver Hake		N	0.0096
009.C	9			Porgy	Porgy/Scup	0.068	Y	0.01
010.C	10			Spanish Mackerel	Spanish Mackerel	0.11	Y	0.01
011.C	11			Monkfish	Monkfish/Goosefish	0.064	Y	0.01
012.C	12			Sea Bass	Bass, Black Sea	0.03	Y	0.01
013.C	13			Squid	Squid		N	0.0084
014.C	14			Shrimp	Shrimp, White		N	0.0096
015.C	15			Shrimp	Shrimp, Black Tiger		N	0.0085
016.C	15	015.C		Shrimp	Shrimp, Black Tiger		N	0.0088
017.C	17			Tuna	Tuna, Bigeye	0.13	Y	0.01
018.C	18			Swordfish	Swordfish	0.14	Y	0.01
019.C	19			Mahi-mahi	Dolphin/Mahi-mahi	0.22	Y	0.01
020.C	20			Mackerel	Mackerel, Atlantic	0.013	Y	0.009
021.C	21		LR-1	Squid	Squid, Longfin	0.026	Y	0.0096
021.C	21		LR-2	Squid	Squid, Longfin	0.022	Y	0.0088

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
021.C	21		LR-3	Squid	Squid, Longfin	0.024	Y	0.0085
022.C	22			Whiting	Whiting/Silver Hake	0.015	Y	0.0093
023.C	23			Croaker	Croaker, Atlantic	0.11	Y	0.01
024.C	24			Porgy	Porgy/Scup	0.12	Y	0.01
025.C	25			Sea Bass	Bass, Black Sea	0.09	Y	0.01
026.C	26			Monkfish	Monkfish/Goosefish	0.15	Y	0.01
027.C	26	026.C		Monkfish	Monkfish/Goosefish	0.14	Y	0.01
028.C	28			Skate	Skate, Winter	0.067	Y	0
029.C	29			Snapper	Snapper, Red	0.076	Y	0.01
030.C	30			Flounder/Fluke/Sole	Flounder, Blackback	0.036	Y	0.01
031.C	31			Flounder/Fluke/Sole	Sole, Gray	0.065	Y	0.01
032.C	32			Mussel	Mussel, Blue	0.019	Y	0.0094
033.C	33			Clam	Quahog, Northern/Little Neck Clam		N	0.0094
034.C	34			Atlantic Salmon	Atlantic Salmon		N	0.0086
035.C	35			Swordfish	Swordfish	0.39	Y	0.01
036.C	36			Tuna	Tuna, Yellowfin	0.37	Y	0.01
037.C	37			Tuna	Tuna, Bigeye	0.41	Y	0.01
038.C	37	037.C		Tuna	Tuna, Bigeye	0.41	Y	0.01
039.C	39			Mussel	Mussel, Blue	0.014	Y	0.0088
040.C	40			Clam	Quahog, Northern/Cherrystone Clam	0.02	Y	0.01
041.C	41		LR-1	Oyster	Oyster, Eastern	0.019	Y	0.0097
041.C	41		LR-2	Oyster	Oyster, Eastern	0.02	Y	0.01
042.C	42			Mussel	Mussel, Blue		N	0.0088
043.C	43			Clam	Quahog, Northern/Little Neck Clam		N	0.0085
044.C	44			Oyster	Oyster, Eastern		N	0.0099

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
045.C	45			Tilapia	Tilapia		N	0.0092
046.C	46			Flounder/Fluke/Sole	Sole, Gray	0.044	Y	0.01
047.C	47			Flounder/Fluke/Sole	Flounder, Summer	0.054	Y	0.01
048.C	48			Flounder/Fluke/Sole	Flounder, Blackback	0.026	Y	0.01
049.C	48	048.C		Flounder/Fluke/Sole	Flounder, Blackback	0.026	Y	0.01
050.C	50			Cod	Cod, Atlantic	0.033	Y	0.01
051.C	51			Pollock	Pollock	0.13	Y	0.01
052.C	52			Skate	Skate, Winter	0.094	Y	0.01
053.C	53			Monkfish	Monkfish/Goosefish	0.054	Y	0.01
054.C	54			Sea Bass	Bass, Black Sea	0.075	Y	0.01
055.C	55			Tilapia	Tilapia	0.015	Y	0.0099
056.C	56			Shrimp	Shrimp, White		N	0.009
057.C	57			Shrimp	Shrimp, White		N	0.0087
058.C	58		LR-1	Tuna	Tuna, Yellowfin	0.044	Y	0.0092
058.C	58		LR-2	Tuna	Tuna, Yellowfin	0.041	Y	0.0092
058.C	58		LR-3	Tuna	Tuna, Yellowfin	0.043	Y	0.0091
059.C	59			Atlantic Salmon	Atlantic Salmon		N	0.0095
060.C	59	059.C		Atlantic Salmon	Atlantic Salmon		N	0.0092
061.C	61			Flounder/Fluke/Sole	Flounder, Summer	0.1	Y	0.02
062.C	62			Cod	Cod, Atlantic	0.049	Y	0.02
063.C	63			Squid	Squid, Longfin	0.016	Y	0.0094
064.C	64			Clam	Quahog, Northern/Little Neck Clam	0.009	Y	0.0089
065.C	65			Blue Crab	Blue Crab/Softshell	0.029	Y	0.02
066.C	66			Scallop	Scallop, Sea		N	0.0089
067.C	67			Pollock	Pollock	0.17	Y	0.02

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
068.C	68			Skate	Skate, Winter	0.071	Y	0.02
069.C	69			Chilean Sea Bass	Chilean Sea Bass	0.13	Y	0.02
070.C	70			Monkfish	Monkfish/Goosefish	0.15	Y	0.02
071.C	70	070.C		Monkfish	Monkfish/Goosefish	0.15	Y	0.02
072.C	72			Porgy	Porgy/Scup	0.13	Y	0.02
073.C	73			Oyster	Oyster, Eastern	0.047	Y	0.02
074.C	74			Halibut	Halibut, Pacific	0.15	Y	0.02
075.C	75			Sea Bass	Bass, Black Sea	0.11	Y	0.02
076.C	76		LR-1	Flounder/Fluke/Sole	Sole, Gray	0.054	Y	0.0091
076.C	76		LR-2	Flounder/Fluke/Sole	Sole, Gray	0.06	Y	0.0085
076.C	76		LR-3	Flounder/Fluke/Sole	Sole, Gray	0.056	Y	0.0093
077.C	77			Cod	Cod, Atlantic	0.049	Y	0.02
078.C	78			Squid	Squid, Japanese Flying		N	0.0092
079.C	79			Whiting	Whiting/Silver Hake	0.016	Y	0.0098
080.C	80			Catfish	Catfish, Channel	0.042	Y	0.02
081.C	81			Blue Crab	Blue Crab/Hardshell	0.023	Y	0.0093
082.C	81	081.C		Blue Crab	Blue Crab/Hardshell	0.021	Y	0.0089
083.C	83			Pollock	Pollock	0.13	Y	0.02
084.C	84			Snapper	Snapper, Yellowtail	0.082	Y	0.02
085.C	85			Snapper	Snapper, Vermilion	0.02	Y	0.0096
086.C	86			Snapper	Snapper, Red	0.076	Y	0.02
087.C	87			Porgy	Porgy/Scup	0.087	Y	0.02
088.C	88			Herring	Herring, Atlantic	0.022	Y	0.0087
089.C	89			Skate	Skate, Winter	0.045	Y	0.02
090.C	90			Monkfish	Monkfish/Goosefish	0.14	Y	0.02

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
091.C	91			Croaker	Croaker, Atlantic	0.056	Y	0.02
092.C	92			Bluefish	Bluefish	0.16	Y	0.02
093.C	92	092.C		Bluefish	Bluefish	0.16	Y	0.02
094.C	94		LR-1	Ocean Perch	Ocean Perch	0.022	Y	0.0096
094.C	94		LR-2	Ocean Perch	Ocean Perch	0.024	Y	0.0088
094.C	94		LR-3	Ocean Perch	Ocean Perch	0.021	Y	0.0092
095.C	95			Rainbow Trout	Rainbow Trout	0.012	Y	0.0095
096.C	96			Sea Bass	Bass, Black Sea	0.094	Y	0.02
097.C	97			Snapper	Snapper, Lane	0.027	Y	0.0093
098.C	98			Bass	Bass, Hybrid Striped	0.014	Y	0.0097
099.C	99			Sea Bass	Bass, Black Sea	0.079	Y	0.02
100.C	100			Snapper	Snapper, Yellowtail	0.034	Y	0.02
101.C	101			Catfish	Catfish, Channel	0.024	Y	0.0095
102.C	102			Atlantic Salmon	Atlantic Salmon	0.019	Y	0.0099
103.C	103			Flounder/Fluke/Sole	Sole, Gray	0.069	Y	0.02
104.C	103	103.C		Flounder/Fluke/Sole	Sole, Gray	0.073	Y	0.02
105.C	105			Croaker	Croaker, Atlantic	0.068	Y	0.02
106.C	106			Snapper	Snapper, Yellowtail	0.04	Y	0.02
107.C	107			Snapper	Snapper, Vermilion	0.083	Y	0.02
108.C	108			Catfish	Catfish, White	0.041	Y	0.02
109.C	109			Pollock	Pollock	0.092	Y	0.02
110.C	110			Spanish Mackerel	Spanish Mackerel	0.15	Y	0.02
111.C	111		LR-1	Tuna	Tuna, Bigeye	0.85	Y	0.0096
111.C	111		LR-2	Tuna	Tuna, Bigeye	0.8	Y	0.0092
111.C	111		LR-3	Tuna	Tuna, Bigeye	0.81	Y	0.0091

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
112.C	112			Flounder/Fluke/Sole	Flounder, Summer	0.1	Y	0.02
113.C	113			Whiting	Whiting/Silver Hake	0.031	Y	0.02
114.C	114			Catfish	Catfish, Blue	0.045	Y	0.02
115.C	114	114.C		Catfish	Catfish, Blue	0.045	Y	0.02
116.C	116			Croaker	Croaker, Atlantic	0.13	Y	0.02
117.C	117			Tilapia	Tilapia		N	0.0097
118.C	118			Snapper	Snapper, Caribbean Red	0.039	Y	0.02
119.C	119			Snapper	Snapper, Lane	0.052	Y	0.02
120.C	120			Sea Bass	Bass, Black Sea	0.083	Y	0.02
121.C	121			Atlantic Salmon	Atlantic Salmon		N	0.0086
122.C	122			Flounder/Fluke/Sole	Flounder, Summer	0.067	Y	0.02
123.C	123			Squid	Squid, Longfin	0.023	Y	0.0089
124.C	124			Catfish	Catfish, Channel	0.032	Y	0.02
125.C	125			Porgy	Porgy/Scup	0.079	Y	0.02
126.C	125	125.C		Porgy	Porgy/Scup	0.092	Y	0.02
127.C	127			Monkfish	Monkfish/Goosefish	0.18	Y	0.02
128.C	128			Croaker	Croaker, Atlantic	0.092	Y	0.02
129.C	129		LR-1	Sea Bass	Bass, Black Sea	0.064	Y	0.0086
129.C	129		LR-2	Sea Bass	Bass, Black Sea	0.066	Y	0.009
129.C	129		LR-3	Sea Bass	Bass, Black Sea	0.054	Y	0.0093
130.C	130			Bluefish	Bluefish	0.12	Y	0.02
131.C	131			Skate	Skate, Winter	0.11	Y	0.02
132.C	132			Bass	Bass, Striped	0.047	Y	0.02
133.C	133			Blue Crab	Blue Crab/Hardshell	0.019	Y	0.0097
134.C	134			Shrimp	Shrimp, White		N	0.0098

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
135.C	135			Atlantic Salmon	Atlantic Salmon	0.012	Y	0.0095
136.C	136			Flounder/Fluke/Sole	Flounder, Summer	0.045	Y	0.02
137.C	136	136.C		Flounder/Fluke/Sole	Flounder, Summer	0.044	Y	0.02
138.C	138			Flounder/Fluke/Sole	Flounder, Blackback	0.049	Y	0.02
139.C	139			Tilapia	Tilapia		N	0.0097
140.C	140			Squid	Squid, Longfin	0.018	Y	0.0083
141.C	141			Whiting	Whiting/Silver Hake	0.02	Y	0.009
142.C	142			Catfish	Catfish, Blue	0.028	Y	0.0092
143.C	143			Oyster	Oyster, Eastern	0.011	Y	0.0081
144.C	144			Blue Crab	Blue Crab/Softshell		N	0.0088
145.C	145			Blue Crab	Blue Crab/Hardshell	0.017	Y	0.0098
146.C	146			Lobster	Lobster, American	0.073	Y	0.02
147.C	146	146.C	LR-1	Lobster	Lobster, American	0.07	Y	0.0086
147.C	146	146.C	LR-2	Lobster	Lobster, American	0.069	Y	0.0092
147.C	146	146.C	LR-3	Lobster	Lobster, American	0.063	Y	0.0088
148.C	148			Pollock	Pollock	0.18	Y	0.02
149.C	149			Snapper	Snapper, Vermilion	0.017	Y	0.0092
150.C	150			Snapper	Snapper, Red	0.065	Y	0.02
151.C	151			Skate	Skate, Winter	0.06	Y	0.02
152.C	152			Porgy	Porgy/Scup	0.096	Y	0.02
153.C	153			Croaker	Croaker, Atlantic	0.086	Y	0.02
154.C	154			Bluefish	Bluefish	0.16	Y	0.02
155.C	155			Sea Bass	Bass, Black Sea	0.067	Y	0.02
156.C	156			Snapper	Snapper, Yellowtail	0.047	Y	0.02
157.C	157			Tuna	Tuna, Bigeye	0.46	Y	0.02

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
158.C	158			Tuna	Tuna, Bigeye	0.5	Y	0.02
159.C	158	158.C		Tuna	Tuna, Bigeye	0.49	Y	0.02
160.C	160			Tuna	Tuna, Yellowfin	0.13	Y	0.02
161.C	161			Swordfish	Swordfish	0.51	Y	0.02
162.C	162			Flounder/Fluke/Sole	Flounder, Blackback	0.04	Y	0.02
163.C	163			Cod	Cod, Atlantic	0.018	Y	0.0091
164.C	164			Sea Bass	Bass, Black Sea	0.059	Y	0.02
165.C	165		LR-1	Monkfish	Monkfish/Goosefish	0.086	Y	0.0084
165.C	165		LR-2	Monkfish	Monkfish/Goosefish	0.075	Y	0.0088
165.C	165		LR-3	Monkfish	Monkfish/Goosefish	0.078	Y	0.0096
166.C	166			Catfish	Catfish, Channel	0.094	Y	0.02
167.C	167			Skate	Skate, Winter	0.084	Y	0.02
168.C	168			Tilapia	Tilapia	0.021	Y	0.0088
169.C	169			Blue Crab	Blue Crab/Hardshell	0.024	Y	0.0094
170.C	169	169.C		Blue Crab	Blue Crab/Hardshell	0.025	Y	0.0088
171.C	171			Blue Crab	Blue Crab/Softshell		N	0.0085
172.C	172			Scallop	Scallop, Sea		N	0.0086
173.C	173			Clam	Quahog, Northern/Little Neck Clam		N	0.0088
174.C	174			Oyster	Oyster, Pacific	0.013	Y	0.0097
175.C	175			Mussel	Mussel, Blue	0.015	Y	0.0085
176.C	176			Shrimp	Shrimp, White	0.011	Y	0.0087
177.C	177			Shrimp	Shrimp, Black Tiger		N	0.0084
178.C	178			Atlantic Salmon	Atlantic Salmon		N	0.0098
179.C	179			Squid	Squid, Longfin	0.016	Y	0.0101
180.C	180			Whiting	Whiting, Offshore	0.077	Y	0.02

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
181.C	180	180.C		Whiting	Whiting, Offshore	0.072	Y	0.02
182.C	182			Spanish Mackerel	Spanish Mackerel	0.2	Y	0.02
183.C	183		LR-1	Tuna	Tuna, Bigeye	0.18	Y	0.0084
183.C	183		LR-2	Tuna	Tuna, Bigeye	0.2	Y	0.0094
183.C	183		LR-3	Tuna	Tuna, Bigeye	0.19	Y	0.0095
184.C	184			Tuna	Tuna, Bigeye	0.36	Y	0.02
185.C	185			Atlantic Salmon	Atlantic Salmon		N	0.01
186.C	186			Pollock	Pollock	0.14	Y	0.02
187.C	187			Cod	Cod, Atlantic	0.027	Y	0.0093
188.C	188			Snapper	Snapper, Red	0.06	Y	0.02
189.C	189			Oyster	Oyster, Eastern	0.013	Y	0.0097
190.C	190			Oyster	Oyster, Eastern		N	0.0092
191.C	191			Mussel	Mussel, Blue	0.016	Y	0.009
192.C	191	191.C		Mussel	Mussel, Blue	0.016	Y	0.009
193.C	193			Clam	Quahog, Northern/Little Neck Clam		N	0.0085
194.C	194			Scallop	Scallop, Sea		N	0.0094
195.C	195			Bass	Bass, Hybrid Striped	0.014	Y	0.0093
196.C	196			Atlantic Salmon	Atlantic Salmon		N	0.0088
197.C	197			Oyster	Oyster, Eastern	0.01	Y	0.0091
198.C	198			Mussel	Mussel, Blue		N	0.0087
199.C	199			Clam	Quahog, Northern/Top Neck Clam	0.01	Y	0.0084
200.C	200			Tuna	Tuna, Yellowfin	0.59	Y	0.02
201.C	201		LR-1	Tuna	Tuna, Yellowfin	0.78	Y	0.0088
201.C	201		LR-2	Tuna	Tuna, Yellowfin	0.82	Y	0.0091
201.C	201		LR-3	Tuna	Tuna, Yellowfin	0.81	Y	0.0091

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
202.C	202			Tuna	Tuna, Yellowfin	0.7	Y	0.02
203.C	202	202.C		Tuna	Tuna, Yellowfin	0.66	Y	0.02
204.C	204			Tuna	Tuna, Yellowfin	0.35	Y	0.02
205.C	205			Swordfish	Swordfish	0.57	Y	0.02
206.C	206			Atlantic Salmon	Atlantic Salmon	0.014	Y	0.0088
207.C	207			Blue Crab	Blue Crab/Softshell		N	0.01
208.C	208			Scallop	Scallop, Sea		N	0.0098
209.C	209			Croaker	Croaker, Atlantic	0.077	Y	0.0093
210.C	210			Mackerel	Mackerel, Atlantic	0.034	Y	0.0094
211.C	211			Mackerel	Mackerel, Atlantic	0.03	Y	0.0092
212.C	212			Mackerel	Mackerel, Atlantic	0.03	Y	0.0095
213.C	213			Croaker	Croaker, Atlantic	0.057	Y	0.02
214.C	213	213.C		Croaker	Croaker, Atlantic	0.058	Y	0.02
215.C	215			Croaker	Croaker, Atlantic	0.082	Y	0.02
216.C	216			Whiting	Whiting/Silver Hake	0.033	Y	0.0099
217.C	217			Skate	Skate, Winter	0.12	Y	0.02
218.C	218			Monkfish	Monkfish/Goosefish	0.078	Y	0.02
219.C	219		LR-1	Skate	Skate, Winter	0.083	Y	0.0088
219.C	219		LR-2	Skate	Skate, Winter	0.074	Y	0.0095
219.C	219		LR-3	Skate	Skate, Winter	0.064	Y	0.0089
220.C	220			Skate	Skate, Winter	0.022	Y	0.0089
221.C	221			Pollock	Pollock	0.079	Y	0.02
222.C	222			Cod	Cod, Pacific	0.04	Y	0.02
223.C	223			Cod	Cod, Pacific	0.024	Y	0.0095
224.C	224			Cod	Cod, Pacific	0.024	Y	0.0096

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
225.C	224	224.C		Cod	Cod, Pacific	0.029	Y	0.0099
226.C	226			Tilapia	Tilapia		N	0.0097
227.C	227			Tilapia	Tilapia		N	0.0099
228.C	228			Tilapia	Tilapia		N	0.0097
229.C	229			Squid	Squid, Japanese Flying	0.011	Y	0.0084
230.C	230			Squid	Squid, Japanese Flying	0.015	Y	0.0088
231.C	231			Squid	Squid, Japanese Flying	0.011	Y	0.009
232.C	232			Scallop	Scallop, Sea		N	0.01
233.C	233			Pollock	Pollock	0.13	Y	0.02
234.C	234			Monkfish	Monkfish/Goosefish	0.071	Y	0.02
235.C	235			Mackerel	Mackerel, Atlantic	0.02	Y	0.0092
236.C	235	235.C		Mackerel	Mackerel, Atlantic	0.021	Y	0.0095
237.C	237		LR-1	Mackerel	Mackerel, Atlantic		N	0.0091
237.C	237		LR-2	Mackerel	Mackerel, Atlantic	0.019	Y	0.0087
237.C	237		LR-3	Mackerel	Mackerel, Atlantic	0.023	Y	0.0086
238.C	238			Skate	Skate, Winter		N	0.0099
239.C	239			Mackerel	Mackerel, Atlantic	0.019	Y	0.0096
240.C	240			Mackerel	Mackerel, Atlantic	0.017	Y	0.0084
241.C	241			Tilapia	Tilapia	0.036	Y	0.02
242.C	242			Blue Crab	Blue Crab/Softshell	0.009	Y	0.009
243.C	243			Tilapia	Tilapia	0.038	Y	0.0093
244.C	244			Blue Crab	Blue Crab/Softshell	0.009	Y	0.0087
245.C	245			Snapper	Snapper, Red	0.032	Y	0.0088
246.C	246			Monkfish	Monkfish/Goosefish	0.11	Y	0.02
247.C	246	246.C		Monkfish	Monkfish/Goosefish	0.11	Y	0.02

Table A-5. Mercury concentrations by composite sample number used in analysis (prior to averaging duplicates and replicates) (continued)

Sample Number	Sample Group	Duplicate Of	Replicate ID	Market Name	Common Name	Hg Concentration (mg/kg)	Detected	Reporting Limit (mg/kg)
248.C	248			Skate	Skate, Winter	0.022	Y	0.0089
249.C	249			Whiting	Whiting, Offshore	0.029	Y	0.009
250.C	250			Sea Bass	Bass, Black Sea	0.078	Y	0.0099
251.C	251			Snapper	Snapper, Red	0.031	Y	0.0094
252.C	252			Cod	Cod, Atlantic	0.016	Y	0.0096
253.C	253			Squid	Squid, Longfin	0.012	Y	0.0094
254.C	254			Squid	Squid, Longfin	0.012	Y	0.0086
255.C	255		LR-1	Skate	Skate, Winter	0.029	Y	0.0086
255.C	255		LR-2	Skate	Skate, Winter	0.027	Y	0.0088
255.C	255		LR-3	Skate	Skate, Winter	0.025	Y	0.009
256.C	256			Skate	Skate, Winter	0.038	Y	0.0092
257.C	257			Scallop	Scallop, Sea	0.011	Y	0.0088
258.C	257	257.C		Scallop	Scallop, Sea	0.01	Y	0.0099
259.C	259			Pollock	Pollock	0.1	Y	0.0091
260.C	260			Blue Crab	Blue Crab/Hardshell	0.024	Y	0.0095

APPENDIX B. SUMMARY OF DNA BARCODING ANALYSIS

B.1. INTRODUCTION

Traditionally biological specimens have been identified using morphological features. For many species a trained technician can make routine identifications using morphological features (taxonomic keys), but in many cases an experienced professional taxonomist is needed. Even an experienced professional may not be able to identify a specimen in all cases. For example, a specimen may be damaged or represent an immature stage of development, and thus may lack key characters that allow it to be correctly placed taxonomically. Over the last 8 years, "DNA barcoding" has emerged as a commonly employed tool that can help overcome some of the issues associated with morphology based identifications. Barcoding uses a short genetic sequence from a standard part of the genome (the total hereditary information of an organism, encoded within its double-stranded DNA) in an attempt to accurately assign a specimen to a given taxon, ideally, a species. Such an assignment can be made by examining a genomic region (i.e., DNA sequence of base pairs made up of A's, C's, G's, and T's - see explanation below) that exhibits a high degree of sequence conservation within a species, but appreciable divergence compared to other species. An ~650 base pair region of the mitochondrial gene cytochrome c oxidase subunit 1 (coxI) has been found to commonly exhibit the requisite conservation/divergence, and has been adopted as the standard "barcode" region for animals. Barcoding is a valuable new tool in the taxonomist's toolbox that can supplement his or her expert knowledge, and also provides a way for non-experts to make identifications (see http://www.barcodeoflife.org/what-is-dna-barcoding).

Select samples from the FFM project had their COI barcode region sequenced as part of the species identification process for the project. Barcoding was considered useful because the sources of the fish specimens consisted of different commercial fishers with uncertain quality of handling of the specimens and they were not from a scientific survey. Additionally, there was concern that the handling of the fish specimens by the commercial fishers could result in damage to the fish exterior that would make it difficult to identify to species level based on morphology alone. In general it was believed that the use of DNA barcodes could help assure the quality of specimen identification, and thus might impact the study results as a whole.

In order to use DNA barcodes to classify "unknown" specimens, the barcode sequences from unknown specimens must be compared to "known" reference sequences. In 2005, the Consortium for the Barcoding of Life (CBOL, see http://www.barcoding.si.edu), an international consortium whose mission is to promote the exploration and development of DNA barcoding, proposed a standard to be applied to sequences that are to be considered reference barcodes. The standard was designed to apply to reference sequences deposited in the public domain, in International Nucleotide Sequence Database Collaboration (INSDC) databases (GenBank, at the US's National Center of Biotechnology Information [NCBI], the European Molecular Biology Laboratory [EMBL], and the DNA Data Bank of Japan [DDBJ] [Wheeler et al., 2000; Benson et al., 2000]). Background information about the initiative and the entire proposed standard can be

found at http://barcoding.si.edu/PDF/DWG_data_standards-Final.pdf. The details of the standard are beyond the scope of this write-up; the point is that there is a significant and ongoing international effort in this area and many species can now be reliably identified based on their barcode sequence.

The University of Guelph in Ontario, Canada, under the leadership of Dr. Paul Hebert, has established a publically available database and data management tool known as "BOLD Systems" (Barcode of Life Database Systems; Ratnasingham and Hebert, 2007). Researchers the world over have uploaded data into BOLD via the internet, making it a primary repository of barcode data. BOLD also has an internet submission form that allows a sequence generated from an "unknown" specimen to be submitted and compared to entries in BOLD (see http://www.boldsystems.org/index.php/IDS_OpenIdEngine). Ideally the unknown sequence can be assigned as belonging to a particular species based on its similarity to sequences in the BOLD.

At the time of the FFM project, there were essentially three "levels" of the BOLD database to which a test sequence could be compared. The most "stringent" level compared the test specimen's sequence only to BOLD "reference" sequences, which were the sequences in BOLD that met the CBOL reference barcode criteria described in the proposed standard discussed above. If no "matches" of adequate similarity for species identification (roughly >98% sequence identity) were found at that level, the search could be widened and allowed comparison of the test sequence to reference and non-reference species records in BOLD. Non-reference species records did not have all the associated elements required to be considered reference sequences, but were still assigned to a particular species. If a test sequence was assigned to a species based on its matching non-reference records, the result was considered more uncertain. Finally, if no matches were found at either of the first two levels, a test sequence could be compared to every record in BOLD, including those that were not classified down to the species level. At this level of comparison, a test sequence would most likely be assigned as belonging to a particular genus or family, but not a species. The other database queried for this project was GenBank. Details about the specific process used during the FFM project to assign barcode-based taxonomy will be presented later. The following paragraphs summarize, in basic terms, the process used to obtain DNA barcode sequences, and provide additional conceptual information about what they represent.

B.2. GENERATION OF DNA BARCODE SEQUENCES

The analytical process used to produce a barcode sequence starts by extracting and purifying the DNA from a small aliquot (~20 mg) of tissue from a specimen, and then subjecting the extracted DNA to an amplification process known as polymerase chain reaction (PCR). In PCR, specific "primers" are used to target a particular genetic region, which is copied over and over and over, resulting in geometric growth in the number of copies of the targeted region until millions of copies of it are present in the final PCR product. The primers can target any region; the primers used for the current project ('dgLCO1490', 'dgHCO2198'; Meyer, 2003) target the

barcoding region of COI. Following the PCR, an aliquot of the solution containing the amplified target is subjected to a "sequencing reaction" in which fluorescent dyes are associated with the nucleotides making up the target DNA, so that its sequence can be determined on an instrument designed for that purpose.

DNA is a double-stranded molecule. Each strand is composed long chains of four specific bases (nucleotides; adenine (A), guanine (G), cytosine (C), and thymine (T)) which encode the hereditary information of an organism. For example, one strand of DNA, over a very small stretch, might be composed of nucleotides in the order CTTAGGTGCA, and the ordered letters representing the nucleotide bases are called the "sequence" of that stretch of DNA. Importantly, the two strands making up a DNA molecule are complementary, due to the specific pattern of hydrogen bonding that occurs between specific pairs of the four nucleotide bases; A and T form stable hydrogen bonds with each other, and so do C and G. Based on this pattern of hydrogen bonding, the complementary DNA strand for the example sequence above would be GAATCCACGT. Figure B-1 illustrates schematically how the example complementary nucleotide sequences would line-up in the double-stranded stretch of DNA containing them.

Figure B-1. Schematic representation of complementary double-stranded DNA.

The DNA barcode for an organism is the specific, ordered sequence of the nucleotide bases (the A's, C's, G's, and T's) that are present in its COI barcode region. The sequencing process was carried out on each of the complementary DNA strands so that the sequence of each was determined independent of its complement. Due to their complementary nature, the two sequences were then overlaid to confirm, with very high confidence, the identity of each position in the resulting barcode sequence. The complementary nature of the strands also allows the barcode sequence to be represented by a single sequence (strand). As previously described, once a specimen's barcode sequence is determined, it can be compared to barcode sequences in public databases (e.g., GenBank, BOLD), and based on its similarity to reference sequences in the database(s), the specimen from which the sequence originated can be "assigned" to a species (in most cases), or possibly to a higher taxonomic level (e.g., genus, family), depending on how similar the specimen's barcode sequence is to the reference sequences.

B.3. SAMPLING AND ANALYSIS OF FULTON FISH MARKET SAMPLES

The following paragraphs discuss some of the logistical and analytical aspects related to the processing of the Fulton Market samples collected for DNA analysis. Tissue samples for DNA analysis were collected at the Region 2 laboratory in Edison, New Jersey. Approximately 2 g of muscle tissue were taken from individual specimens designated for DNA analysis and also from mixed specimen "super-composite" samples. Sampling tools were wiped clean with ethanol between samples to prevent cross-contamination. Samples were stored frozen at -20°C in "cryo-vials" until they were shipped to the DNA laboratory in coolers containing frozen ice packs (results of a "test" shipment of two samples in April 2008 demonstrated that this form of preservation for shipping allowed successful sequencing).

Five overnight shipments were sent from Edison to the National Exposure Research Laboratory's Ecosystems Exposure Research Division (NERL/EERD) in Cincinnati between June 12 and August 7, 2008; a total of 556 samples were shipped. Upon arrival in Cincinnati, samples were visually inspected for signs of thawing and other problems, and verified against the enclosed chain-of-custody forms. Samples were logged into a spreadsheet (Sample Log.xls) located in the Fulton project's network directory in Cincinnati, and stored at -20°C until analysis. No adverse sample conditions were noted in the sample log or on the custody sheets; these forms were placed in the project lab notebook following sample receipt and inspection. At the conclusion of the project, the Cincinnati sample log was verified as being correct and complete with respect to the records from the shipping lab.

As described in Section 1.1, typically, three individual fish of the same species were collected from a vendor to make up a composite sample for Hg and possibly PCB analyses. When whole fish (rather than fillets) were collected, only one of the three was processed for DNA sequence analysis (e.g., only sample 1.1 of samples 1.1, 1.2, 1.3 was sequenced), although tissue samples from all three were collected and shipped to Cincinnati. Fillet samples were handled differently. All three samples collected for the Hg analysis were processed for DNA sequencing as visual identification of a species from a fillet sample is often difficult. The final type of sample subjected to DNA analysis was the single aliquots taken from the mixed-tissue super-composite samples. Employing this analysis plan yielded a list of 282 samples for DNA analysis. Six additional samples were added to the analysis list later. The added samples were three pairs of "x.2" and "x.3" samples, which were sequenced so the results could be compared with their corresponding "x.1" results, which had some uncertainty or problems associated with them. The total number of samples to be sequenced was therefore 288, 284 of which were successfully sequenced.

One of the four unreported samples, 222.1, twice produced sequences that appeared to be of bacterial origin, so no further attempts were made to analyze that sample. Attempts to generate results for the other three unreported samples (141.1, 176.1. and 233.1) were never successful, despite repeated attempts at DNA extraction, PCR, and sequencing.

B.4. QUALITY ASSURANCE AND QUALITY CONTROL

PCR and sequencing samples are typically processed in 96-well plates (8 rows × 12 columns), and a plate was considered an analytical "batch" regardless of the number of samples loaded on the plate. During this project, negative controls (no DNA template), positive controls (known specimen), and replicate samples were included for QC. Though not considered a critical omission, negative and/or positive control samples were not included in a few batches; see the explanation below.

Negative controls serve to indicate possible contamination. Reasons why a negative control might produce a sequence include: (1) the sample was contaminated with environmental DNA that carried over from harvest, fish market, or processing; (2) the sample was contaminated with human, fungal, or bacterial DNA at some step between harvest and extraction; (3) the DNA was contaminated with volatilized PCR products from previous PCR runs in the same lab; or (4) samples were mislabeled or mis-ordered. For this project, if a negative control produced a sequence, the sequencing reaction product was re-sequenced to see if the sequence remained or not (i.e., was an artifact of the sequencing process). If the second sequencing run confirmed the presence of a sequence in the negative control well, then the entire batch (plate) of samples associated with that control was rejected and reprocessed. There was only one case where a plate was rejected due to negative control failure as described.

Regarding contamination, it should also be noted that human DNA sequence was found twice, however, repeating the PCR amplification from the original DNA extract from the two samples in question yielded sequences that were more appropriately assigned to fish species. Based on these results, it is considered most likely that human contamination somehow occurred during the original PCR or sequencing reactions for these samples

Though the QA plan for the project indicated the use of positive controls and replicates in every sample batch, they were only used sporadically. Positive controls were included in the PCR process, but were omitted from sequencing. Because all of the samples included in this project were identified morphologically, the lack of positive controls is not considered critical since all samples' resulting barcode sequences were used to confirm morphology-based identifications. Details about replicate samples that were analyzed can be found in Table B-1, which summarizes batch/plate analyses and the associated QC samples and results.

The minimum acceptable length of each of the two complementary DNA sequences generated for a given specimen was 500 bases, with a minimum overlap of the complementary sequences of 400 bases. (Typically, the total length of the final sequence for any specimen, once the two overlapping sequences were combined, was approximately 665 bases.) There were a few cases where a reported result was based on sequences that did not meet the length criteria. In two cases, one of the individual DNA sequences was <500 bases, and in the other the overlap was <400 bases. In both cases, the overall quality of the individual reads and resulting final sequences and the agreement of the final sequences with reference sequences lead to the decision not to reanalyze the samples. In a third case, a result based on only one of the complementary DNA sequences from a specimen was reported, due to difficulties experienced in sequencing the

second strand. This case represented a "confirmatory" re-analysis (discussed below), and the single sequence agreed with the morphologically-determined species assignment, so further analysis was deemed unnecessary. In the deliverable to Region 2 containing the record for this sample (44.1), the comment field noted that the result was based on a single DNA strand.

Additional QA/QC procedures related to the DNA analysis are described in the Quality Assurance Project Plan (QAPP) for the project.

Table B-1. DNA analysis, reporting, and QC summary

Batch/Plate	# of Samples	# Submitted for Sequencing	# of Samples Reported	# of Negative Controls	Did Negative Control Sequence?	# of Replicates	Replicate % Identity
NY1	30[a]	10[b]	3	1[a]	No	1[a]	100%
NY2	64	64[c]	53	1	No	2[c]	100%, 100%
NY3	42	42	37	1	No	1	100%
NY4.1	58	58	51	1	No	1	100%
NY5	42	42	38	1	Not submitted for sequencing; no band on agarose gel	1	Not submitted for sequencing; appropriate band on agarose gel
NY_Fillets	46	46	40	2	Negative control sequenced in forward direction on first run, however did not sequence on reanalysis, so OK	0	N/A
Extraction and PCR repeats							
NYrepeats#5[d]	15	15	0	1	Negative control sequenced both directions – all results discarded	0	N/A
Nyrepeats#6[e]	16	16	0	1	No	0	N/A
Nyrepeats#7 and NY Grad#1[f]	6/7	6/7	0/2	1/1	Control not submitted for sequencing; very faint band on agarose gel; however not considered critical given that only 2 results reported and both match morph. ID.	0/0	N/A/N/A
Nyrepeats#8	21	21	12	1	Negative control sequenced in forward direction on first attempt, however did not sequence on reanalysis, so OK	0	N/A
TD1290L[g]	2	2	2	0	N/A	2	100%, 100%
PCR only repeats							
NY1.2	30[a]	11[b]	6	1[a]	No	1[a]	100%
NY1.3 (50 degrees)	30[a]	2[b]	0	1[a]	No	1[a]	100%
NY1.4 (Deg. Folmers)	30[a]	15[c]	11	1[a]	No	2[c]	100%, 100%

Table B-1. DNA analysis, reporting, and QC summary (continued)

Batch/Plate	# of Samples	# Submitted for Sequencing	# of Samples Reported	# of Negative Controls	Did Negative Control Sequence?	# of Replicates	Replicate % Identity
NY2and3_repeats	8	6	5	0	N/A	0	N/A
NY1.4and2_repeats	15	15	10	1	No	0	N/A
NYrepeats dg	20	20	10	1	No	1	Replicate failed to produce sequence, so not evaluated
NYChi+repeats	6	6	1	1	Negative control sequenced in reverse direction on first attempt, however did not sequence on reanalysis, so OK	1	100%
NYrepeats#5[d]	9	9	0	1	Negative control sequenced both directions – all results discarded	0	N/A
NYrepeats#5c[d]	24	24	3	1	No	0	N/A

[a]NY1, NY1.2, NY1.3, and NY1.4 were all the same sample extracts amplified with different PCR primers and/or with different PCR conditions in an effort to find the best PCR conditions. Negative controls and replicates in those plates represent the same negative control and replicate extracts.

[b]A total of 19 single samples plus two each of 13.1 and 50.1 (from different PCR plates) were submitted for sequencing from plates NY1, NY1.2, and NY1.3. These 23 total samples were sequenced together. Of these 23 samples, nine were reported. Only one of the two 50.1 samples produced good sequence. Both 13.1 samples produced good sequence, so these results were used as a substitute for the failed replicate of 53.1, which had only one of its two replicate extractions (the second) produce good sequence. Though used as a replicate for QC purposes in this batch (reported as the "task_1290b" deliverable), the 13.1 results from this batch were not reported. Another aliquot of sample 13.1 from plate 1.4 was also analyzed, and the final results from that analysis were the ones reported (in the "task_1290c" deliverable).

[c]NY2 and NY1.4 were submitted for sequencing together, with the negative control and replicate from plate NY2 (sample 150.1) serving as primary QC samples. The first extraction aliquot of sample 53.1 was submitted again as part of plate NY1.4, but is not counted in the # of Samples Reported Column since its result was previously reported, however it is counted as an additional replicate for plates NY2 and NY1.4. It shared 100% identity with the previously reported 53.1.

[d]Plate NYrepeats#5 was a combination of nine samples subject only to re-amplification by PCR and another 15 samples that were both re-extracted and re-amplified. The negative control on this plate sequenced in both directions. All 24 extracts and the negative control were subjected to PCR again, and this time the negative control showed no sign of contamination. This "clean" plate was submitted for sequencing and was named NYrepeats#5c.

[e]All 16 samples on NYrepeats#6 were confirmatory analyses of samples previously reported.

[f]These were two PCR repeat plates sequenced together.

[g]The analysis of these two samples (129.1 and 164.1) was unique because previous rounds of PCR had resulted in multiple bands (products) appearing on the post-PCR agarose gel. In task1290L, following PCR and gel electrophoresis, the bands of the appropriate size (i.e., the assumed target) were excised from the gel and purified. This purified product was then sequenced and was confirmed to represent the target, and the results reported.

[h]All "repeat" plates may contain combinations of samples which were previously unreported and samples that were previously reported to Region 2 that were being reanalyzed for some reason (such as being identified as a different species genetically compared to the morphologically assigned species designation).

B.5. ASSIGNING DNA-BASED TAXONOMY

The following paragraphs describe the process used on this project to make DNA barcode-based taxonomic assignments. In the simplest terms, a specimen was assigned to a particular species when its barcode sequence strongly matched (approximately >98% identical) the sequence of one or more reference sequences of a single species in GenBank and/or BOLD. However, there were cases where this did not occur. Matches of a lower percentage identity typically resulted in assigning the "unknown" specimen to a genus or family rather than to a species. There are other reasons a specimen may not have been assigned to a particular species, which are discussed below.

Following the generation of sequence data for any given batch of samples, a simple text file was output from the sequence editing program (Sequencher, v4.8, www.genecodes.com) containing a sample identifier and the associated barcode sequence for each sample in the batch. Using a web browser, the file was uploaded and compared to the sequences in GenBank using the "megablast" algorithm (Zheng et al., 2000; a particular version of the Basic Local Alignment Search Tool, BLAST, http://www.ncbi.nlm.nih.gov/blast). The BLAST results returned via the browser report which GenBank sequences most closely match the "query" sequences in the uploaded file. A tab-delimited text file summarizing the results, called a "hit table," was downloaded from the BLAST results page and saved, and then a custom Perl script (computer program; Wall et al., 2000) was used to parse the results file and to retrieve taxonomic information associated with each of the top ten BLAST "hits" for any given input sequence. The taxonomy results were culled from the taxonomy database associated with GenBank (http://www.ncbi.nlm.nih.gov/taxonomy) and augmented with information from the Integrated Taxonomic Information System (ITIS, originally referred to as the Interagency Taxonomic Information System; www.itis.gov). The script also automatically went back to GenBank and examined the full GenBank records of the "hit" sequences to see if they contained the "BARCODE" designation and to see if they were cross-referenced to records in BOLD. In theory, having one or both of these features would provide additional confidence in the veracity of the assigned taxonomy of the "hit" sequence.

Following the compilation and summarization of the BLAST-based results, the next step was to go to the BOLD "species identification" page, http://www.barcodinglife.org/views/idrequest.php. Here, each individual query sequence from the analytical batch was cut and pasted into the webpage form and submitted for species identification using the proprietary BOLD algorithm. As discussed previously, the query sequence was first compared to BOLD reference sequences (the default choice in the web form), but if no species match was returned, the query sequence was compared to the non-reference species database. If there were still no species-level results, then the "entire database" option was used for the query.

Once all the results from both GenBank and BOLD were gathered, a weight-of-evidence approach was used to make a DNA-based taxonomic assignment. When the BOLD and GenBank results agreed (i.e., BOLD assigned a species-level identification that was the same

species associated with the best GenBank results), a sample could be assigned to that species with relatively high confidence. The confidence in such a result was greater if the BOLD "match" was to one or more reference sequences and/or the GenBank/BLAST "hits" were to official "BARCODE" sequences.

In some cases however, there was not such a clear correspondence between the BOLD and GenBank results. For example, a BOLD species identification might be relied on exclusively when there were no corresponding BLAST hits (i.e., COI barcode sequence data for the species in question was present in BOLD, but not available in GenBank). The opposite might also be true, so that a taxonomic assignment for a particular sample might be based only on comparison to sequences found in GenBank. As previously stated, a species-level assignment sometimes was not made because of lower than ideal sequence similarity results, so a genus-level or family-level assignment was made. There were also cases where GenBank and BOLD both provided apparent species level matches, but they did not agree. Finally, there are some groups (e.g., tuna, tilapia, most snappers) where there doesn't appear to be enough divergence in COI barcode region to reliably make a species assignment. In the end, 195 of the 284 successfully sequenced samples were reported to species level. Regardless of the taxonomic level assigned, a column in the deliverables to Region 2 was used to indicate the primary database (BOLD reference, BOLD non-reference, or GenBank) that was used for taxon assignment; GenBank and BOLD were both referenced when the supporting evidence came from both databases and appeared to be independent.

B.6. COMPARISON WITH MORPHOLOGICALLY DERIVED TAXONOMY RESULTS

Samples were initially sequenced and "DNA identified" blindly, and the results submitted to Region 2 in an agreed upon spreadsheet format. (It should be noted that the DNA sequences themselves were not delivered to Region 2. Rather, Region 2 received a single-line record summarizing the results of the "unknown" sequence's comparison to GenBank and BOLD, and its inferred taxonomic classification.) Upon receipt of deliverables, Region 2 compared the sequence-based results to their own morphologically-based taxonomic assignments. In cases where there was disagreement, NERL/EERD was notified, and the samples were subjected to reanalysis to confirm or refute the earlier DNA results. In total, 27 samples were reanalyzed due to such disagreements (or in a few cases for other reasons). In five of these cases, the "DNA ID" was changed after reanalysis and agreed with the morphologically-based result. For two of the modified DNA results, it appeared likely that the initial error was due to two samples' positions being switched on the 96-well processing plate during the original analysis.

As stated above, difficulties experienced when attempting to sequence one of the two DNA strands for another sample (number 44.1) resulted in using a single strand's sequence to assign the sample's taxon, which agreed with the morphologically-based assignment. There is no clear explanation for the disagreement between the DNA-based results and morphologically-

based results for the other two samples. Most likely, cross-contamination occurred somewhere in the sampling or analytical process, which lead to erroneous sequencing results.

Of the remaining 22 reanalyzed samples, one clearly had incorrect DNA results; the photograph for sample 232 was clearly a scallop, however the DNA results twice came up as a cod. It is again assumed there must have been a handling error in the field or lab that caused cross-contamination, perhaps the wrong tissue was placed in the sample vial or the vial was mislabeled. Another case involved a sample representing fish that typically resolved to species level resolving only to genus level based on its initial DNA analysis; the reanalysis did not change this. (Region 2 still assigned this sample to its presumed species based on its morphology.) Another five samples (four fillet samples and one mussel) were re-sequenced due to initial DNA results that were confounded or were of poor quality (including one case evidencing human contamination). The five samples reconciled with their market names based on the reanalysis.

Of the remaining 15 reanalyzed samples, 14 had their final species changed, including one changed from a striped bass to a striped bass hybrid. The fifteenth, though not officially changed, was flagged as potentially being another species (see note b, Table 1). Not only reanalyzed samples were changed however, as the entire process of DNA analysis, reanalysis, and reexamination of photos by Region 2's ichthyologist, Moses Chang, resulted in a total of 57 samples (not considering the three "sub-samples" making up the composite samples), representing 15 species, having their final identification changed (or in some cases where there was initial uncertainty, confirmed). Table B-2 summarizes the changes that were made.

It is important to note that scientific names were changed, but not the market names (though FDA vernacular names often differed). As people purchase fish based on market names, and considering the analysis presented in Section 3.1 of this report, it appears likely that market customers do not face a great risk of obtaining improperly labeled seafood containing higher levels of mercury, however the small sample size for any given species in this study makes it hard to make that assertion with any confidence. Taking that into account and given that there were a significant number of species identifications changed based on the DNA, future studies of this type should still consider the use of DNA barcoding to allow a better examination of the potential for species specific risks. Aside from that consideration, the FDA, in association with the Consortium for the Barcode of Life (CBOL) and its FISH-BOL project (www.fishbol.org) developed a laboratory protocol for DNA barcoding of fish (LIB 4420: DNA-Barcoding for the Species Identification of Fish, July, 2008, updated July 2009 (Yancy, 2008). FDA has since developed an official Standard Operating Procedure (SOP) for DNA barcoding of fish to replace LIB4420, "Single Laboratory Validated Method for DNA-Barcoding for the Species Identification of Fish for FDA Regulatory Compliance" (http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/ucm237391.htm). Along with the development of the SOP, the "FDA Reference Standard Sequence Library for Seafood Identification" has been established (http://www.fda.gov/Food/FoodSafety/Product-SpecificInformation/Seafood/DNAspeciation/ucm238880.htm). As the use of DNA barcodes to

ensure proper identification of seafood is now part of the regulatory landscape, its use in the current study aligned well with the general trends in this arena.

Table B-2. Influenced species identifications in final project database

Sample ID #s	Species Name in Project Database		Common Name in Project Database		Acceptable FDA Market Names	
	Original	Final	Original	Final	Original	Final
13	*Loligo pealii*	*Illex* spp.	Squid	Squid	Squid or Calamari (vernacular; Winter Squid/ Boston Squid/ Longfin Inshore Squid)	Squid or Calamari (vernacular; various depending on species; none in common with *L. pealii*)
78 229 230 231	*Loligo pealii*	*Todarodes pacificus*	Squid	Squid, Japanese Flying	Squid or Calamari (vernacular; Winter Squid/ Boston Squid/ Longfin Inshore Squid)	NA
28 52 68 89 131 151 167 217 219 220 238 248 255 256	*Raja binoculata* (?)	*Leucoraja ocellata*	Skate	Skate, Winter	Skate	Skate

Table B-2. Influenced species identifications in final project database (continued)

Sample ID #s	Species Name in Project Database		Common Name in Project Database		Acceptable FDA Market Names	
	Original	Final	Original	Final	Original	Final
33 43 64 173 193	*Protothaca*	*Mercenaria mercenaria*	Clam, Littleneck	Quahog, Northern/Littleneck Clam	*P. thaca* - Clam, Hardshell or Quahog *P. staminea* - Clam, Littleneck (vernacular; Steamer/ Native Littleneck) *P. tenerrima* - Clam, Littleneck	Clam or Quahog (vernacular; Hardshell/ Littleneck)
40	*Protothaca*	*Mercenaria mercenaria*	Clam, Cherry Stone	Quahog, Northern/ Cherrystone Clam	*P. thaca* - Clam, Hardshell or Quahog *P. staminea* -Clam, Littleneck (vernacular; Steamer/ Native Littleneck) *P. tenerrima* - Clam, Littleneck	Clam or Quahog (vernacular; Hardshell/ Littleneck)
199	*Protothaca*	*Mercenaria mercenaria*	Clam, Top Neck	Quahog, Northern/Top Neck Clam	*P. thaca* - Clam, Hardshell or Quahog *P. staminea* -Clam, Littleneck (vernacular; Steamer/ Native Littleneck) *P. tenerrima* - Clam, Littleneck	Clam or Quahog (vernacular; Hardshell/ Littleneck)
48	*Syacium micrurum* (?)	*Pseudopleuro-nectes americanus*	Flounder/Sole, Channel	Flounder, Blackback	NA	Flounder or Sole (vernacular; Winter Flounder/ Lemon Sole/ Georges Bank Flounder)

Table B-2. Influenced species identifications in final project database (continued)

Sample ID #s	Species Name in Project Database		Common Name in Project Database		Acceptable FDA Market Names	
	Original	Final	Original	Final	Original	Final
138	*Paralichthys dentatus*	*Pseudopleuro-nectes americanus*	Flounder/Sole, Summer	Flounder, Blackback	Flounder or Fluke (vernacular: Plaice/Northern Fluke)	Flounder or Sole (vernacular; Winter Flounder/ Lemon Sole/ Georges Bank Flounder)
162	?	*Pseudopleuro-nectes americanus*	Flounder/Sole, Canadian BB	Flounder, Blackback	NA	Flounder or Sole (vernacular; Winter Flounder/ Lemon Sole/ Georges Bank Flounder)
51 67 83 109 148 186 221 259	*Pollachius pollachius*	*Pollachius virens*	Pollock, Atlantic	Pollock	Pollock (vernacular; Lythe/ Saithe/ Dover Hake/ Grass Whiting/ Greenfish/ Margate Hake)	Pollock (vernacular; Saithe/ Coalfish/ Coley/ Green Cod/ Boston Bluefish)
57	*Penaeus monodon*	*Litopenaeus vannamei*	Shrimp, Black Tiger	Shrimp, White	Shrimp (vernacular; Jumbo Tiger Prawn/ Black Tiger Shrimp)	Shrimp
84 100 106 156	*Ocyurus*? *chrysurus*?	*Ocyurus chrysurus*	Snapper, Yellowtail	Snapper, Yellowtail	Snapper (vernacular: Palu-i'usama)	Snapper (vernacular: Palu-i'usama)
85 107 149	?	*Rhomboplites aurorubens*	Snapper, B-Liner	Snapper, Vermilion	NA	Snapper (vernacular: Beeliner/ Clubhead Snapper/ Night Snapper)

Table B-2. Influenced species identifications in final project database (continued)

Sample ID #s	Species Name in Project Database		Common Name in Project Database		Acceptable FDA Market Names	
	Original	Final	Original	Final	Original	Final
98	*Morone saxatilis*	*Morone chrysops* × *saxatilis*	Striped Bass	Bass, Hybrid Striped	Bass (vernacular; Rockfish/ Striper/ Linesides)	Bass (vernacular; White and Striped Bass Hybrid)
195	*Morone* (?) *saxatilis* (?)	*Morone chrysops* × *saxatilis*	Striped Bass, Hybrid	Bass, Hybrid Striped	Bass	Bass (vernacular; White and Striped Bass Hybrid)
108	*Ictalurus punctatus*	*Ameiurus catus*	Catfish	Catfish, White	Catfish (vernacular; Spotted Cat/ White Cat/ Lake Catfish)	Catfish (vernacular; White Cat/ Channel Cat)
166	*Ictalurus punctatus*	*Ameiurus catus*	Catfish, Channel	Catfish, White	Catfish (vernacular; Spotted Cat/ White Cat/ Lake Catfish)	Catfish (vernacular; White Cat/ Channel Cat)
114 142	*Ictalurus punctatus*	*Ictalurus furcatus*	Catfish	Catfish, Blue	Catfish (vernacular; Spotted Cat/ White Cat/ Lake Catfish)	Catfish (vernacular; Bullhead/ Chuckleheaded Cat/ Mississippi Cat)
174	*Crassostrea virginica* (?)	*Crassostrea gigas*	Oyster	Oyster, Pacific	Oyster (vernacular; Bluepoint/ American Oyster)	Oyster (vernacular; Japanese Oyster/ Pacific Giant Oyster)
180 249	*Merluccius bilinearis*	*Merluccius albidus*	Whiting	Whiting, Offshore	Whiting (vernacular; American Hake/ Silverfish/ Stockfish/ Winter Trout/ Frostfish)	NA
222 223 224	*Gadus morhua*	*Gadus macrocephalus*	Cod	Cod, Pacific	Cod (vernacular; Rock Cod/ Codling/ Scrod Cod)	Cod or Alaska Cod (vernacular; Alaska Cod/ Grey Cod/ True Cod/ Treska)

B.7. RESULT QUALIFIER SCORE

At the conclusion of the DNA sequencing effort, a "qualifier score" was created for each reported sample record to reflect some estimate of the confidence associated with the result. The score also reflected the result's relative taxonomic position, as a given result would start with a qualifier score of 0, 0.5, or 1 depending on whether or not its DNA-based taxonomic level was family, genus, or species, respectively. Without going into detail about the empirically-derived scoring criteria, it can be said that a species-level assignment would generally be expected to be higher scored than a genus-level assignment, and a genus-level assignment higher than a family-level assignment. Various weight-of-evidence criteria such as mentioned above were used to adjust the score. For instance, sequences matching the same taxa in both GenBank and BOLD sequences would be higher scored, with additional weight given when matches were to GenBank "BARCODE" sequences and/or BOLD "reference" sequences. Higher percentage shared identity with database sequences also increased the qualifier score. The qualifier score for any sample ended up somewhere between 0 and 5, where a lower score indicated the sample was more likely assigned to a higher taxonomic level (e.g., family) with less weight of evidence, and a higher score indicating that the sample was probably assigned to a species, with reasonably strong evidence supporting its assignment.

As mentioned above, the qualifier score was derived empirically, and was not intended to be a robust measure of result quality. Rather, it was an attempt to give end-users, particularly those unfamiliar with DNA barcoding, some idea about which results were the best and which might be improved upon. There might not be any easy way to "improve" a given result at the current time however, given that a number of factors are out of the control of a person using a barcode sequence to identify an "unknown" specimen. One attempting identification is always subject to what is available in the reference databases and the quality of the database contents (e.g., Is the species they are trying to identify represented in the databases? Are the sequences in the database correct? Were the specimens the sequences came from identified correctly in the first place?). Even if reference COI barcode sequences are available, this gene region alone does not necessarily resolve all animal species, as was seen in the present study. A detailed description of the calculation of the qualifier score is beyond the scope of this report, however it can be stated that generally more weight was given to results based on sequences that matched GenBank "BARCODE" and/or BOLD "reference" sequences, and by giving more weight to taxonomic assignments that were based on matching independent sequences in both databases.

Table B-3 displays the count and average qualifier score for different taxonomic levels based on the databases used for their assignment. It is worth pointing out that at the species level, GenBank/BOLD non-reference-based results have a higher average qualifier score than GenBank/BOLD reference-based results. This is surprising, as it implies that many BOLD "non-reference" sequences that were matched had more supporting evidence in GenBank than BOLD "reference" sequences that were matched. It is assumed this is the result of chance, however a detailed review to confirm this assumption was not performed.

B.8. ADDITIONAL INFORMATION

More details about any aspect of the DNA barcoding analysis, including specific laboratory procedures, sequence data files, summarized BLAST/BOLD results, deliverable files submitted to Region 2, qualifier score calculation, etc. can be obtained by contacting John Martinson (martinson.john@epa.gov).

Table B-3. Distribution of taxa level assignments based on taxon assignment source database(s) and average result qualifier score

Assigned_taxonomic_level	Taxa_Assignment_Source	Data	Total
family	BOLD non-reference	Average of total score	0.5
		Count of Assigned_taxonomic_level	10
	Genbank/BOLD reference	Average of total score	1.1
		Count of Assigned_taxonomic_level	10
family Average of total score			0.8
family Count of Assigned_taxonomic_level			20
genus	BOLD non-reference	Average of total score	1.0
		Count of Assigned_taxonomic_level	10
	Genbank	Average of total score	0.8
		Count of Assigned_taxonomic_level	1
	Genbank/BOLD non-reference	Average of total score	0.8
		Count of Assigned_taxonomic_level	29
	Genbank/BOLD reference	Average of total score	2.7
		Count of Assigned_taxonomic_level	29
genus Average of total score			1.65
genus Count of Assigned_taxonomic_level			69
species	BOLD non-reference	Average of total score	1.5
		Count of Assigned_taxonomic_level	23
	BOLD reference	Average of total score	2.5
		Count of Assigned_taxonomic_level	43
	Genbank	Average of total score	2.5
		Count of Assigned_taxonomic_level	8
	Genbank/BOLD non-reference	Average of total score	3.8
		Count of Assigned_taxonomic_level	61
	Genbank/BOLD reference	Average of total score	3.3
		Count of Assigned_taxonomic_level	60
species Average of total score			3.0
species Count of Assigned_taxonomic_level			195
Total Average of total score			2.5
Total Count of Assigned_taxonomic_level			284

B.9. ACKNOWLEDGEMENTS

The efforts of Tamara Goyke (NERL/EERD), Stephen Morris (independent student contractor), and Carrie Drake (Dynamac Corp.) are greatly appreciated. They all played key roles and contributed greatly to the success of the effort to generate the DNA sequence data for this project. Suzanne Christ, Erik Pilgrim, John Darling, and Eric Waits are thanked for their helpful review comments.

B.10. REFERENCES

Benson, DA; Karsch-Mizrachi, I; Lipman, DJ; Ostell, J; Rapp, BA; Wheeler, DL (2000). GenBank. Nucleic Acids Res 28(1):15–18.

Meyer, CP (2003). Molecular systematics of cowries (Gastropoda: Cypraeidae) and 538 diversification patterns in the tropics. Biol J Linnean Soc 539(79):401–459.

Ratnasingham, S; Hebert, PDN (2007). BOLD: The Barcode of Life Data System (www.barcodinglife.org). Mol Ecol Notes, doi: 10.1111/j.1471-8286.2006.01678.x.

Wall, L; Christiansen, T; Orwant, J (2000). *Programming Perl, 3rd ed.*, Sebatopol, CA: O'Reilly Media.

Wheeler, DL; Chapper, C; Lash, AE; Leipe, DD; Madden, TL; Schuler, GD; Tatusova, TA; Rapp, BA (2000). Database resources of the National Center for Biotechnology Information. Nucleic Acids Res 28(1):10–14.

Yancy, HF; Fry, FS; Randolph, SC; Deeds, J; Ivanova, NV; Grainger, CM; Hanner, R; Weigt, LA; Driskell, A; Hunt, J; Ormos, A; Hebert, PDN (2008/2009). A Protocol for Validation of DNA-Barcoding for the Species Identification of Fish for FDA Regulatory Compliance, Lab Inform Bull 4420(24), Division of Field Science, Office of Regulatory Affairs, U.S. Food and Drug Administration.

Zhang, Zheng; Schwartz, S; Wagner, L; Miller, W (2000). A greedy algorithm for aligning DNA sequences. J Comput Biol 7(1–2):203–214.

APPENDIX C. PCB ANALYSIS

A subset of the CM composite samples was also analyzed for the presence of polychlorinated biphenyls (PCBs). The PCB analysis was limited to a maximum sample number of 50 due to cost constraints. Given the limited sample size, five species (likely to be high in PCBs) were initially selected for analysis. Two samples (one scallop, one swordfish) selected only for mercury analysis were also analyzed for PCBs. Additionally, Spanish mackerel is listed separately from mackerel. Consequently, the 49 samples selected for the analysis, as shown in Table C-1 represent a total of eight different species instead of five. The analysis tested for 124 different PCB congeners, as shown in Table C-1, and the analysis of each congener had an associated limit of detection. The resulting PCB concentrations from each congener were then summed to represent an estimate of the total PCB in the sample. Three of the samples were analyzed in triplicate as a further test of laboratory analysis precision. Twelve PCB congeners have dioxin-like toxic equivalency factors (TEFs). Not all of the 12 dioxin-like PCB congeners were analyzed, and, consequently, no attempt was made to estimate a dioxin TEF from the PCB sampling analysis.

As shown in Table C-2, many of the congeners resulted in analytical results below the limit of detection. In this case, the limit of detection represents a sample-specific reporting limit. Sample-specific limits take into account both the preparation and analysis effects as well as specifics related to the specific sample matrix such as percent dry weight, grams of sample weighed out, and exact solvent volumes used. These limits are intended to indicate the level at which the method can reliably quantify positive results, where reliability is measured in terms of the method precision and accuracy limits.

When a sample result is below this limit, an assumption must be made about the approximate magnitude before the different congeners are summed to estimate the total PCB concentration. However, because so many of the congeners had non-detected levels of PCBs, adding even one-half the detection limit could severely overestimate the total PCB concentration. In this analysis, species which had no detectable levels in any congeners were not quantitatively analyzed; for species with detected levels in at least one congener for all samples (catfish), the assumption was made that the levels in the other congeners were zero. This assumption tended to underestimate the total PCB concentrations but likely represents the method with the smallest bias. In the three samples examined in triplicate, all congeners returned non-detected levels in all three trials.

Table C-3 shows the total PCB concentrations in the eight catfish samples. The concentrations are presented in two ways: (1) as mass of PCB per mass of fish tissue, and (2) as mass per lipid in the tissue. The measured lipid fraction is used to convert the PCB mass per total sample weight into a mass per lipid value. The current FDA tolerance level for PCBs in fish is 2 ppm fish tissue (U.S. FDA, 2001) or 2,000 μg/kg. In the CM catfish samples, none of

Table C-1. PCB congeners evaluated in analysis

PCB Number				
PCB 1	PCB 41	PCB 105	PCB 165	PCB 205
PCB 4	PCB 44	PCB 110	PCB 167	PCB 206
PCB 5	PCB 45	PCB 115	PCB 170	PCB 208
PCB 6	PCB 46	PCB 117	PCB 172	PCB 209
PCB 7	PCB 48	PCB 122	PCB 173	PCB 13 / PCB 27
PCB 8	PCB 49	PCB 124	PCB 174	PCB 31 / PCB 53
PCB 9	PCB 51	PCB 128	PCB 175	PCB 20 / PCB 33
PCB 10	PCB 52	PCB 130	PCB 176	PCB 47 / PCB 104
PCB 12	PCB 54	PCB 132	PCB 177	PCB 42 / PCB 59
PCB 14	PCB 64	PCB 134	PCB 179	PCB 37 / PCB 103
PCB 15	PCB 67	PCB 135	PCB 180	PCB 63 / PCB 93
PCB 16	PCB 69	PCB 136	PCB 183	PCB 66 / PCB 91
PCB 17	PCB 70	PCB 137	PCB 185	PCB 56 / PCB 84 / PCB 92
PCB 18	PCB 71	PCB 138	PCB 187	PCB 60 / PCB 90 / PCB 101
PCB 19	PCB 73	PCB 141	PCB 189	PCB 83 / PCB 119
PCB 22	PCB 74	PCB 144	PCB 190	PCB 85 / PCB 154
PCB 24	PCB 75	PCB 147	PCB 191	PCB 109 / PCB 123
PCB 25	PCB 77	PCB 149	PCB 193	PCB 118 / PCB 131
PCB 26	PCB 81	PCB 151	PCB 194	PCB 114 / PCB 146
PCB 28	PCB 82	PCB 153	PCB 196	PCB 129 / PCB 178
PCB 29	PCB 87	PCB 156	PCB 197	PCB 171 / PCB 201
PCB 32	PCB 95	PCB 157	PCB 199	PCB 195 / PCB 207
PCB 34	PCB 97	PCB 158	PCB 200	
PCB 35	PCB 99	PCB 163	PCB 202	
PCB 40	PCB 100	PCB 164	PCB 203	

Table C-2. Samples measured for PCBs and the number of congeners with detected and non-detected levels of PCBs

Market Name and Sample Number	Number of Non-Detects	Number of Detects	Market Name and Sample Number	Number of Non-Detects	Number of Detects
Catfish, Sample 1	76	46	Salmon, Atlantic, Sample 1	122	0
Catfish, Sample 2	97	25	Salmon, Atlantic, Sample 2	122	0
Catfish, Sample 3	119	3	Salmon, Atlantic, Sample 3	121	1
Catfish, Sample 4	117	5	Salmon, Atlantic, Sample 4	120	2
Catfish, Sample 5	117	5	Salmon, Atlantic, Sample 5	122	0
Catfish, Sample 6	112	10	Salmon, Atlantic, Sample 6	122	0
Catfish, Sample 7	119	3	Salmon, Atlantic, Sample 7	122	0
Catfish, Sample 8	119	3	Salmon, Atlantic, Sample 8	122	0
Crab, Blue, Sample 1	121	1	Salmon, Atlantic, Sample 9	122	0
Crab, Blue, Sample 2	122	0	Salmon, Atlantic, Sample 10	122	0
Crab, Blue, Sample 3	122	0	Scallop, Sample 1	122	0
Crab, Blue, Sample 4	122	0	Swordfish, Sample 1	122	0
Crab, Blue, Sample 5	122	0	Tuna, Sample 1	122	0
Crab, Blue, Sample 6	122	0	Tuna, Sample 2	122	0
Crab, Blue, Sample 7	122	0	Tuna, Sample 3	122	0
Crab, Blue, Sample 8	122	0	Tuna, Sample 4	122	0
Crab, Blue, Sample 9	122	0	Tuna, Sample 5	122	0
Crab, Blue, Sample 10	122	0	Tuna, Sample 6	122	0
Mackerel, Sample 1	122	0	Tuna, Sample 7	122	0
Mackerel, Sample 2	122	0	Tuna, Sample 8	122	0
Mackerel, Sample 3	122	0	Tuna, Sample 9	122	0
Mackerel, Sample 4	122	0	Tuna, Sample 10	122	0
Mackerel, Sample 5	122	0			
Mackerel, Sample 6	122	0			
Mackerel, Sample 7	122	0			
Mackerel, Spanish, Sample 1	122	0			
Mackerel, Spanish, Sample 2	122	0			

the samples had total PCB concentrations above this tolerance limit when assuming the non-detects are zero. The waterbodies of origin for each sample are also shown, but no clear trends appear to connect the PCB concentrations with the waterbodies of origin.

Table C-4 shows the summary statistics from the eight catfish samples. Because several of the samples contained much larger concentrations, suggesting a skewed distribution, both the mean and standard deviation as well as the geometric mean and geometric standard deviation are shown. The mean was heavily influenced by the largest concentration, suggesting the lognormal distribution was a better approximation for the distribution. Assuming a lognormal distribution with geometric mean and geometric standard deviation as shown, the FDA tolerance level is above the 99th percentile of the distribution represented by the eight samples.

Table C-3. Total PCB concentrations in catfish samples

Sample	Waterbody of Origin	Total PCB µg/kg total weight	Lipid Fraction	Total PCB µg/kg lipid
Sample 1	Lake	600	0.052	11,538
Sample 2	Farm	8.8	0.05	176
Sample 3	Farm	22	0.02	1,100
Sample 4	Farm	24	0.02	1,200
Sample 5	Unknown	190	0.04	4,750
Sample 6	Atlantic	17	0.014	1,214
Sample 7	Unknown	58	0.046	1,261
Sample 8	Farm	10	0.0067	1,493

Table C-4. Summary of total PCB concentrations in catfish samples represented as a lipid weight basis

Species	Mean (µg/kg lipid)	Standard Deviation (µg/kg lipid)	Geometric Mean (µg/kg lipid)	Geometric Standard Deviation	Minimum (µg/kg lipid)	Maximum (µg/kg lipid)
Catfish	116	205	39	4.4	8.8	600

Table C-5 shows the congeners which returned detectable levels in at least one of the catfish samples. The two samples with the highest total PCB concentrations had detectable levels in a wide range of congeners (46 different congeners for Sample 1 and 25 different congeners for Sample 5), while the other samples had detectable levels in relatively few congeners (3–10).

Table C-5. Congeners returning detectable levels of PCBs in catfish samples (µg/kg lipid)

PCB Number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
PCB 44	3.3							
PCB 49	4.9							
PCB 52	5							
PCB 56 / PCB 84 / PCB 92	2.5							
PCB 60 / PCB 90 / PCB 101	6.3				1.8			
PCB 66 / PCB 91	4.5							
PCB 74	2.5							
PCB 81	36							
PCB 95	8.3							
PCB 97	5.1							
PCB 99	13		2.4	3.1	5		4.6	
PCB 105	6.1				1.7			
PCB 114 / PCB 146	5.2				1.9			
PCB 118 / PCB 131	7.5				2.6		2	
PCB 128	8.9				2.3			
PCB 130	4.3							
PCB 135	7.8							
PCB 137	2.1							
PCB 138	59	2.7	4.6	4.8	16	4.6	9	3.1
PCB 141	10				1.8			
PCB 149	51				10			
PCB 153	95	3.8	7.3	8.1	41	8.4	15	5
PCB 156	3.3							
PCB 158	4.1							
PCB 163	15				4.9			
PCB 164	3.2							
PCB 167	2.1							
PCB 170	21				4.7			

Table C-5. Congeners returning detectable levels of PCBs in catfish samples (μg/kg lipid) (continued)

PCB Number	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
PCB 171 / PCB 201	4				1.6			
PCB 172	4.6							
PCB 174	13				3.5			
PCB 177	9.7				3.2			
PCB 179	4							
PCB 180	67		3.8	4.4	12		6	
PCB 183	15				4.3			
PCB 187	40	2.3	3.4	3.7	14	3.9	6.9	2.1
PCB 190	4				1.4			
PCB 194	11				2.3			
PCB 195 / PCB 207	2.5				1.7			
PCB 196	4.9							
PCB 199	9.9				7		2.9	
PCB 202	2.4				4.8			
PCB 203	9.1							
PCB 206	5.3				29		4.8	
PCB 208	1.5				11		2.7	
PCB 209	2						5.1	
Total	600	8.8	22	24	190	17	58	10

Finally, Table C-6 shows the total PCB concentrations and percent lipids in all the samples examined in the analysis. As discussed above, only the catfish, Atlantic salmon, and blue crab species had any samples with detectable levels of PCBs. The detection limits for the individual congeners ranged from 1.005 to 19.6 μg/kg with an average of 2.69 μg/kg. This suggests the analytical techniques have precision which is at least a factor of 100 below the tolerance level for each of the 124 individual congeners. These detection limits appear to be appropriately low to resolve any total concentrations above the tolerance limit. Overall, the particular composite samples from the species selected for PCB analysis appear to have concentrations within the FDA tolerance level.

Table C-6. Total PCBs and percent lipids in all samples

Sample	Market Name	Common Name	Variable	Value (% or µg/kg)	Detect Flag
010.C	Mackerel, Spanish	Mackerel, Spanish	PERCENT LIPIDS	0.82	Y
010.C	Mackerel, Spanish	Mackerel, Spanish	TOTAL PCB CONGENERS FISH TISSUE	--	N
017.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.17	Y
017.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
020.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	1.3	Y
020.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
034.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	8.6	Y
034.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
035.C	Swordfish	Swordfish	PERCENT LIPIDS	2.1	Y
035.C	Swordfish	Swordfish	TOTAL PCB CONGENERS FISH TISSUE	--	N
036.C	Tuna	Tuna, Yellowfin	PERCENT LIPIDS	0.73	Y
036.C	Tuna	Tuna, Yellowfin	TOTAL PCB CONGENERS FISH TISSUE	--	N
037.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.64	Y
037.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
038.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.63	Y
038.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
058.C	Tuna	Tuna, Yellowfin	PERCENT LIPIDS	0.54	Y
058.C	Tuna	Tuna, Yellowfin	PERCENT LIPIDS	0.54	Y
058.C	Tuna	Tuna, Yellowfin	PERCENT LIPIDS	0.54	Y
058.C	Tuna	Tuna, Yellowfin	TOTAL PCB CONGENERS FISH TISSUE	--	N
058.C	Tuna	Tuna, Yellowfin	TOTAL PCB CONGENERS FISH TISSUE	--	N
058.C	Tuna	Tuna, Yellowfin	TOTAL PCB CONGENERS FISH TISSUE	--	N
059.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	10	Y
059.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
060.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	11	Y
060.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	1.5	Y
065.C	Crab, Blue	Crab, Blue/Softshell	PERCENT LIPIDS	1.7	Y
065.C	Crab, Blue	Crab, Blue/Softshell	TOTAL PCB CONGENERS FISH TISSUE	1.5	Y
080.C	Catfish	Catfish, Channel	PERCENT LIPIDS	5.2	Y
080.C	Catfish	Catfish, Channel	TOTAL PCB CONGENERS FISH TISSUE	600	Y
081.C	Crab, Blue	Crab, Blue/Hardshell	PERCENT LIPIDS	1.5	Y

Table C-6. Total PCBs and percent lipids in all samples (continued)

Sample	Market Name	Common Name	Variable	Value (% or µg/kg)	Detect Flag
081.C	Crab, Blue	Crab, Blue/Hardshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
082.C	Crab, Blue	Crab, Blue/Hardshell	PERCENT LIPIDS	1	Y
082.C	Crab, Blue	Crab, Blue/Hardshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
101.C	Catfish	Catfish, Channel	PERCENT LIPIDS	4	Y
101.C	Catfish	Catfish, Channel	TOTAL PCB CONGENERS FISH TISSUE	190	Y
102.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	15	Y
102.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	8.5	Y
108.C	Catfish	Catfish, White	PERCENT LIPIDS	5	Y
108.C	Catfish	Catfish, White	TOTAL PCB CONGENERS FISH TISSUE	8.8	Y
111.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.18	Y
111.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.18	Y
111.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.18	Y
111.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
111.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
111.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
114.C	Catfish	Catfish, Blue	PERCENT LIPIDS	2	Y
114.C	Catfish	Catfish, Blue	TOTAL PCB CONGENERS FISH TISSUE	22	Y
115.C	Catfish	Catfish, Blue	PERCENT LIPIDS	2	Y
115.C	Catfish	Catfish, Blue	TOTAL PCB CONGENERS FISH TISSUE	24	Y
121.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	11	Y
121.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
124.C	Catfish	Catfish, Channel	PERCENT LIPIDS	4.6	Y
124.C	Catfish	Catfish, Channel	TOTAL PCB CONGENERS FISH TISSUE	58	Y
133.C	Crab, Blue	Crab, Blue/Hardshell	PERCENT LIPIDS	4.3	Y
133.C	Crab, Blue	Crab, Blue/Hardshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
135.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	9.4	Y
135.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
142.C	Catfish	Catfish, Blue	PERCENT LIPIDS	0.67	Y
142.C	Catfish	Catfish, Blue	TOTAL PCB CONGENERS FISH TISSUE	10	Y
144.C	Crab, Blue	Crab, Blue/Softshell	PERCENT LIPIDS	1.2	Y
144.C	Crab, Blue	Crab, Blue/Softshell	TOTAL PCB CONGENERS FISH TISSUE	--	N

Table C-6. Total PCBs and percent lipids in all samples (continued)

Sample	Market Name	Common Name	Variable	Value (% or µg/kg)	Detect Flag
145.C	Crab, Blue	Crab, Blue/Hardshell	PERCENT LIPIDS	3.7	Y
145.C	Crab, Blue	Crab, Blue/Hardshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
157.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.25	Y
157.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
158.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.35	Y
158.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
159.C	Tuna	Tuna, Bigeye	PERCENT LIPIDS	0.54	Y
159.C	Tuna	Tuna, Bigeye	TOTAL PCB CONGENERS FISH TISSUE	--	N
160.C	Tuna	Tuna, Yellowfin	PERCENT LIPIDS	0.2	Y
160.C	Tuna	Tuna, Yellowfin	TOTAL PCB CONGENERS FISH TISSUE	--	N
166.C	Catfish	Catfish, Channel	PERCENT LIPIDS	1.4	Y
166.C	Catfish	Catfish, Channel	TOTAL PCB CONGENERS FISH TISSUE	17	Y
169.C	Crab, Blue	Crab, Blue/Hardshell	PERCENT LIPIDS	4.4	Y
169.C	Crab, Blue	Crab, Blue/Hardshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
170.C	Crab, Blue	Crab, Blue/Hardshell	PERCENT LIPIDS	3.6	Y
170.C	Crab, Blue	Crab, Blue/Hardshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
171.C	Crab, Blue	Crab, Blue/Softshell	PERCENT LIPIDS	1.1	Y
171.C	Crab, Blue	Crab, Blue/Softshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
172.C	Scallop	Scallop, Sea	PERCENT LIPIDS	0.11	Y
172.C	Scallop	Scallop, Sea	TOTAL PCB CONGENERS FISH TISSUE	--	N
178.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	9.8	Y
178.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
182.C	Mackerel, Spanish	Mackerel, Spanish	PERCENT LIPIDS	0.27	Y
182.C	Mackerel, Spanish	Mackerel, Spanish	TOTAL PCB CONGENERS FISH TISSUE	--	N
185.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	10	Y
185.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
196.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	6.2	Y
196.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
206.C	Salmon, Atlantic	Salmon, Atlantic	PERCENT LIPIDS	5	Y
206.C	Salmon, Atlantic	Salmon, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
207.C	Crab, Blue	Crab, Blue/Softshell	PERCENT LIPIDS	1.3	Y

Table C-6. Total PCBs and percent lipids in all samples (continued)

Sample	Market Name	Common Name	Variable	Value (% or µg/kg)	Detect Flag
207.C	Crab, Blue	Crab, Blue/Softshell	TOTAL PCB CONGENERS FISH TISSUE	--	N
210.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	1.6	Y
210.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
211.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	3.5	Y
211.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
212.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	1.1	Y
212.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
235.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	2.4	Y
235.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
236.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	2.6	Y
236.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
237.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	2.4	Y
237.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	2.4	Y
237.C	Mackerel	Mackerel, Atlantic	PERCENT LIPIDS	2.4	Y
237.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
237.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N
237.C	Mackerel	Mackerel, Atlantic	TOTAL PCB CONGENERS FISH TISSUE	--	N

C.1. REFERENCES

U.S. FDA (2001). Fish and Fisheries Products Hazards and Controls Guidance, Third Edition. http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/Seafood/FishandFisheriesProductsHazardsandControlsGuide/ucm091998.htm.

APPENDIX D. CALCULATIONS FOR NUMBER OF SERVINGS

Tables D-1 and D-2 show the values used to determine the fish servings values shown in Table 12 in Section 5.3. The serving values calculated using the mean and upper confidence limits for each species are shown in columns (3) and (6), respectively. The calculated values were truncated (i.e., rounded down) to the lower integer values shown in columns (4) and (7), respectively. Rounding down regardless of the decimal in the calculated value is a conservative approach to estimating the number of servings that result in intake at or below the EPA level of concern for mercury intake.

Table D-1. Estimated number of fish servings per week for an adult female of child-bearing age based on means and upper 95% confidence limits on mercury concentrations by species[a]

(1) Market Name of Species	(2) Mean Mercury (mg/kg)	(3) Weekly Servings Value Calculated using Mean Mercury in equation for SpW^b	(4) Number of servings per Week using Mean Mercury that result in intake at or below the EPA level of concern	(5) 95% Upper Confidence Limit on Mean Mercury (mg/kg)	(6) Weekly Servings Value Calculated using 95% Upper Confidence Limit on Mean Mercury in equation for SpW^b	(7) Number of servings per Week using 95% Upper Confidence Limit on Mean Mercury that result in intake at or below the EPA level of concern
Tuna	0.42*	0.477	0	0.55	0.364	0
Swordfish	0.40*	0.501	0	0.59	0.340	0
Mahi-Mahi	0.22*	0.911	0	NA	NA	NA
Spanish Mackerel	0.15	1.336	1	0.20	1.002	1
Halibut	0.15	1.336	1	NA	NA	1
Bluefish	0.15	1.336	1	0.17	1.179	1
Chilean Sea Bass	0.13	1.542	1	NA	NA	NA
Pollock	0.13	1.542	1	0.15	1.336	1
Monkfish	0.11	1.822	1	0.13	1.542	1
Porgy	0.098	2.045	2	0.12	1.670	1
Croaker	0.084	2.386	2	0.10	2.004	2
Sea Bass	0.075	2.673	2	0.088	2.278	2
Lobster	0.069	2.905	2	NA	NA	NA
Skate	0.060	3.341	3	0.078	2.570	2
Flounder	0.051	3.930	3	0.065	3.084	3
Snapper	0.049	4.091	4	0.060	3.341	3
Catfish	0.044	4.555	4	0.061	3.286	3
Cod	0.031	6.466	6	0.038	5.275	5
Whiting	0.028	7.159	7	0.043	4.661	4
Bass	0.025	8.018	8	0.047	4.265	4
Mackerel	0.022	9.111	9	0.028	7.159	7

Table D-1. Estimated number of fish servings per week for an adult female of child-bearing age based on means and upper 95% confidence limits on mercury concentrations by species[a] (continued)

(1) Market Name of Species	(2) Mean Mercury (mg/kg)	(3) Weekly Servings Value Calculated using Mean Mercury in equation for SpW[b]	(4) Number of servings per Week using Mean Mercury that result in intake at or below the EPA level of concern	(5) 95% Upper Confidence Limit on Mean Mercury (mg/kg)	(6) Weekly Servings Value Calculated using 95% Upper Confidence Limit on Mean Mercury in equation for SpW[b]	(7) Number of servings per Week using 95% Upper Confidence Limit on Mean Mercury that result in intake at or below the EPA level of concern
Ocean Perch	0.022	9.111	9	NA	NA	NA
Herring	0.022	9.111	9	NA	NA	NA
Oyster	0.015	13.363	13	0.025	8.018	8
Blue Crab	0.015	13.363	13	0.021	9.545	9
Tilapia	0.014	14.317	14	0.022	9.111	9
Squid	0.014	14.317	14	0.017	11.791	11
Mussel	0.012	16.703	16	0.017	11.791	11
Rainbow Trout	0.012	16.703	16	NA	NA	NA
Clam	0.0081	24.746	24	0.012	16.703	16
Atlantic Salmon	0.0081	24.746	24	0.012	16.703	16
Scallop	0.0055	36.444	36	0.0071	28.231	28
Shrimp	0.0054	37.119	37	0.0073	27.458	27

[a]Serving values calculated using the following exposure assumptions: Serving size = 8 oz of fish fresh weight, Adult female weight = 65 kg, RfD for methyl mercury = 1×10^{-4} mg/kg-day (U.S. EPA IRIS database), and the person consumes only the one type of fish or shellfish.

[b]Weekly value calculated using the equation for SpW in Section 5.3.

*These concentrations yield values indicating less than one serving a week results in intake at or below the EPA level of concern. Servings per 30 day month of these species that result in intake at or below the EPA level of concern are provided in Table D-2.

Table D-2. Estimated number of fish servings per 30 day month for an adult female of child-bearing age based on means and upper 95% confidence limits on mercury concentrations for three high mercury species

(1) Market Name of Species	(2) Mean Mercury (mg/kg)	(3) Servings Value per *30 Day MONTH* Calculated using Mean Mercury [c]	(4) Number of servings per *30 Day MONTH* using Mean Mercury that result in intake at or below the EPA level of concern	(5) 95% Upper Confidence Limit on Mean Mercury (mg/kg)	(6) Servings Value per *30 Day MONTH* Calculated using 95% Upper Confidence Limit on Mean Mercury[a]	(7) Number of servings per *30 Day MONTH* using 95% Upper Confidence Limit on Mean Mercury that result in intake at or below the EPA level of concern
Tuna	0.42	2.045	2	0.55	1.562	1
Swordfish	0.40	2.148	2	0.59	1.456	1
Mahi-Mahi	0.22	3.905	3	NA	NA	NA

[a]Value per 30 day month calculated using the equation for *SpW* in Section 5.3, with 30 days rather than 7.

APPENDIX E. EXTERNAL PEER REVIEW OF EPA'S DRAFT REPORT, FISH TISSUE ANALYSIS FOR MERCURY AND PCBS FROM A NEW YORK CITY COMMERCIAL FISH/SEAFOOD MARKET AND EPA'S RESPONSE TO COMMENTS

EXTERNAL PEER REVIEW OF EPA'S DRAFT REPORT,

FISH TISSUE ANALYSIS FOR MERCURY AND PCBs FROM A NEW YORK CITY COMMERCIAL FISH/SEAFOOD MARKET

Contract No.: EP-C-07-024
Task Order 114

Submitted to:

Cheryl Itkin
U.S. Environmental Protection Agency
Office of Research and Development
National Center for Environmental Assessment
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Submitted by:

Eastern Research Group, Inc.
110 Hartwell Avenue
Lexington, MA 02421-3136

July 25, 2011

Printed on Recycled Paper

QUALITY NARRATIVE STATEMENT

ERG selected reviewers according to selection criteria provided by EPA. EPA confirmed that the scientific credentials of the reviewers proposed by ERG fulfilled EPA's selection criteria. Reviewers conducted the review according to a charge prepared by EPA and instructions prepared by ERG. ERG checked the reviewers' written comments to ensure that each reviewer had provided a substantial response to each charge question (or that the reviewer had indicated that any question[s] not responded to was outside the reviewer's area of expertise). Since this is an independent external review, ERG did not edit the reviewers' comments in any way, but rather transmitted them unaltered to EPA. ERG did, however, format reviewers' comments as needed for consistency in this final peer review summary report.

Contents

Responses to Charge Questions

1. Please comment on the organization and clarity of the report.

Reviewer	Comments	Response to Comments
Anderson	The organization of the report is appropriate and easy to follow. There are a number of approaches that would help with clarity. The report is quite long and presents many tables and figures which are very useful. This makes the executive summary become particularly important. Right now the executive summary is pretty cursory and the key findings of the report need to be highlighted in some kind of order. Having them as bullets would improve the clarity. It might also help if the key findings were highlighted in each section of the report at the front. These could be bullets but listing them and then having the text explain and elaborate would help. For instance, there is a lot of interest in farmed vs wild fish. This is mentioned and information provided in the text and there is not much to say since only three species were farm raised, and only one of those a fin fish. But it might be mentioned that no mercury was detected in the wild Atlantic salmon but found in half of the farmed salmon, even though the levels were very low. Farm vs wild is only included as part of "water body" discussion. If the authors go through each section and select what they view as the key findings, list them in the sections and then carry them into the executive summary that would help the reader focus on key findings.	The Executive Summary (ES) has been re-written to comprehensively address all reviewer comments related to the ES. A new section (3.4) has been added that qualitatively discusses the wild vs farmed results.
	The impetus for the study was the New York City blood mercury report. This study and its findings should probably be summarized a bit better and the fish consumption rates mentioned as well as the mercury prevalence's in the general population and other associated factors. The question that this report does not address is whether these fish monitoring data presented can explain the blood mercury distributions seen? It would help if somehow the authors could translate the various "action levels" presented into what are their blood mercury level equivalents. Or at least discuss how the meal rates reported in Table 14a might translate into blood mercury.	The suggestion to translate "action levels" and species specific seafood meal recommendations in the report (Tables 14 A and B [now Tables 12 and 13]) into an equivalent blood mercury (Hg) concentration is an interesting and insightful thought; however, it would require a significant biokinetic analysis which is beyond the stated goals of this study. It is a subject worth consideration for a future spin-off paper.
	What does the NY 5 ug/L blood translate into as fish	The NYCHANES study notes that NYC residents have both higher blood Hg

	contaminant level and meals per week? Will the Table 14a result in blood mercury less than the NY 5 ug/L value? It is somewhat counter intuitive that none of the composites exceed the FDA action level, and very few the EPA Rfd based level, yet 72% of the NYC Chinese exceed the NY reportable level of 5 ug/L mercury in blood. If these "action levels" are to protect everyone at unlimited fish consumption levels, then these "action levels" don't seem to be protective as far as the NY reportable level is concerned. Some discussion of the basis for the NY reportable level is needed and how it relates to the report's findings. In the executive summary it is mentioned that the NYC fish samples appear to be somewhat lower than the FDA comparisons. That triggers the question of then why do NYC residents have blood mercury levels three times those of the rest of the country? I would suggest that the testing samples are qualitatively similar, but doing a statistical comparison is problematic because of the different sampling structure. In the introduction it is mentioned that the "action levels" used will be discussed further in the report. But all that is included is a table and references. There is no discussion of how they were derived, why they are different and how they are intended to be used. This is a bit of apples vs oranges. What is the level of fish consumption that each assumes? The challenge in understanding this report is how to arrive at a "dose" by navigating between fish tissue concentration, fish consumption rates over a selected time period and the blood mercury levels seen in the NYC study. It appears simple but is complex.	levels than national levels and higher fish consumption rates as well. Text has been added to the introduction section of Section 1 that articulates this information. Derivation of "action levels" is beyond the scope of this study.
Pascoe	The report is well organized and clearly written, with a few exceptions noted below; nonetheless, it should be easily understandable by health professionals and the interested public. The following are editorial comments that either need addressing or may help with clarity. These comments focus on the Executive Summary, which should be both comprehensive yet simplified enough with explanations of technical issues in order to be sufficiently understandable to someone unfamiliar with those issues. Page 1, Executive Summary, first line – Because some states use the same or similar regulatory agency name, EPA should be referred to as the U.S. Environmental Protection Agency upon first use in the Executive Summary and the main body of the report.	No response needed. The Executive Summary (ES) has been re-written to comprehensively address all reviewer comments related to the ES.

	Page 1, Executive Summary, first paragraph – The goal of the study to support the NYC public health message should be clarified with more detail. The development of a large seafood mercury database to provide support for the City's message on public health is highly admirable, and the readers could be more informed of how the data were actually used in formulating the health messages, and what those messages are. The final tables in the report provide recommendations on meals per month that sensitive members of the population (e.g., women of child bearing age) can consume of different types of fish from the Commercial Market, and the development of these recommendations using the mercury seafood data that the study collected should be more strongly linked to the NYC public health messages.	The Executive Summary (ES) has been re-written to comprehensively address all reviewer comments related to the ES. Beyond widely deciminating this report, the onus will be on the NYCDOHMH to incorpoarate the findings of the report into their public health message of seafood consumtion.
	Page 1, Executive Summary, second paragraph – The derivation and meaning of the term "reportable level" is not explained, and may have different meanings to a chemist and a health professional or someone not versed in those fields. The term should be clarified as to whether it is a health-based criterion or a departmental advisory, or an enforceable standard, or whether it refers to concentrations that should be reported to a regulatory agency, and if so the basis for the requirement and the concentration of mercury should be noted.	The Executive Summary (ES) has been re-written to comprehensively address all reviewer comments related to the ES.
	Page 1, Executive Summary, third paragraph, fourth sentence – A comma appears to be missing.	
	Page 2, Executive Summary, third paragraph – The first sentence appears to state that the report estimates the amount of seafood adult women of child bearing age consume, from which the intake of mercury is estimated. This is an incorrect description of the process that is actually used in the report, in that the amount of seafood consumed by adult women of child bearing age is not itself estimated, but instead what is estimated is the amount of seafood consumption that corresponds to a level of concern for exposure to mercury. If the report intended to estimate the amount of seafood that a population or subgroup of a population consumes, an entirely different type of consumption study would be needed. The amount of seafood consumption considered to correspond to a level of concern is independent of actual seafood consumption rates. This distinction is critical primarily because some subpopulations will consume more seafood than others, which is noted at the	The authors disagree with the reviewers comment re: the message communicated in the first sentence of paragraph 3, ES. There is no intent in the study to estimate the amount of seafood women of child bearing age consume. As subsequently noted by the reviewer, the goal of the study is to estimate the allowable number of seafood meals (on a species-specific basis) for women of child bearing age.

	end of the report, and descriptions of seafood consumptions by subpopulations need to be sensitive to such cultural differences. The fourth sentence of this paragraph also alludes to a determination of the amount of seafood that a subpopulation consumes – the permissible daily intake of methyl mercury was not actually compared with the amount of methyl mercury intake from fish ingestion, but rather was used to identify the permissible amount of ingestion of fish that contain varying levels of methyl mercury.	
	Page 2, Executive Summary, fourth paragraph and Section 6 – The report should attempt to provide some explanation of why the NYC CM data were so much lower than the FDA data on mercury in fish tissue, if there is a known or suspected reason.	With the exception of sword fish (which had a small sample size) and bluefish, the study results were not neccesarily "so much lower" than the FDA data. All species with the exception of swordfish and bluefish had sample means that were within one standard deviation of FDA sample means. The results are appropriately described in the Executive Summary (i.e., "tended to be lower than FDA").
	Page 3, Executive Summary, first full paragraph – Additional summary of the rationale for the use of the barcoding would be appropriate here. A rationale is alluded to at the beginning of Appendix B which discusses the problems of taxonomic identification of dead fish. It should be emphasized in the Executive Summary that the barcoding was considered useful because the sources of the fish specimens consisted of different commercial fishers with uncertain quality of handling of the specimens, and the sources were not from a scientific survey and collection from the water bodies from which NYC fish are typically caught. Additionally, there was concern that the handling of the fish specimens by the commercial fishers could result in enough damage to the fish exterior to make it difficult to identify at a species level, as well as the reasons stated in Appendix B. In addition, more summary of the utility of the barcoding to the study should be added to the end of the Executive Summary. Specifically, the first full paragraph of Page 113 mentions that disparities between taxonomic identification and barcoding also resulted in re-examination of the photos of collected specimens by EPA to determine whether they had been mis-identified.	
	Page 31, Section 5.2, third paragraph – This text discusses the comparison of total mercury concentrations in fish with the EPA action level for methyl mercury. The reason given as to why EPA considers it acceptable to compare total mercury concentrations with the action	Revisions were made as per the reviewer's comment. **(**all but the first sentence of Section 5.2, paragraph 3 was removed).

	level for methyl mercury is not entirely correct. EPA (2009) does not state that the comparison is acceptable because the molecular weight of mercury is close to that of methyl mercury, but because most if not sometimes all of the total mercury in fish tissue is actually methyl mercury, as stated in the text of the subsequent paragraph (fourth paragraph of Section 5.2). The text of the third paragraph needs to be changed to reflect the actual reasoning contained in EPe (2009) for the comparison. Page 34 – The reference to Figure 10 in Appendix A should be Figure 3. Page 37 – The reference to Table D-1a appears to refer to Table 14. Page 43, Section 6 has a formatting problem. Page 54 – Two references for EPA 2009 are provided; however, the second reference of EPA (2009) *Guidance for Implementing the January 2001 Methylmercury Water Quality Criterion* has been superseded by an updated document: USEPA. 2010. Guidance for Implementing the January 2001 Methylmercury Water Quality Criterion. April. EPA 823-R-10-001. http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/pollutants/methylmercury/upload/mercury2010.pdf	Editor/corrected. Editor/corrected. Editor/corrected.
Stern	In general, the report is well organized and clearly written. There are some specific points where the text is unclear. These are noted in my specific comments below. The one significant exception to this, as discussed in detail below, is that the full objectives of the report as evinced by the analyses in the report, itself, are not clearly stated. Notwithstanding the overall logical organization and general clarity of the text, however, the report seems to reflect an inconsistent level of technical detail. Some sections are technically quite detailed and appear not to be intended to be generally accessible to the lay readier such as, for example, Section 4, "Analysis of Measurement Variability" and Appendix B, the explanation DNA "barcode" analysis. While Section 5, "Comparison of CM Data to Risk Metrics" is much more accessible. Some thought could be given to the intended audience for this report. The report compares the NYC commercial market Hg	The report is intended to reach a wide audience of readers: from the lay public for end-user advice on selection of species and the frequency of seafood meals, to health professionals for crafting public health messages and for general research purposes. Parts of the report's content are necessarily more complex (e.g., DNA barcoding, statistical analysis). To the extent feasible, the report has employed simplified text in communicating is primary goal: recommendations on types and frequencies of seafood meals. The authors agree that an analysis of

	concentrations by species to the FDA database of Hg concentration by species and gives the implications for consumption frequency advisories relative to the EPA RfD. Further, the report finds that, in general, the concentrations from the NYC commercial market samples are significantly lower on a species-by-species basis. The obvious next logical step is to compare the current FDA/EPA advisory specifics to the meal frequencies derived in the report (Table 14). This is not done and is conspicuous by its absence.	current FDA advisories to seafood meal frequencies cited in this report would be informative, but such an exercise is beyond the stated goals of this study.
	<u>Specific Comments</u>	
	Pg. 11, par. 2 - The text is unclear as to how duplicates and replicates were treated in terms of the value for each composite sample that was actually entered into the overall statistical analysis.	The paragraph that starts at the bottom of page 10 and continues to the top of page 11 describes how duplicate and replicate analyses on the same composite were handled. The last sentence on page 10 reads as follows: "For the purposes of statistical analysis, the duplicates or the replicates were averaged to obtain a single Hg concentration for the respective composite sample." This text is now on the bottom of page 3 of the Final draft1. The description here and on the top of page 4 should clarify the issue raised in the comment. (see also the comment by Pascoe re: use of ProUCL in response to question #4).
	Pg 12, par. 2 - The treatment of non-detects appears to have little overall impact on the estimation of the average Hg concentration across species and on the use of these data for fish consumption advisories (because the non-detects are in the species with the lowest Hg concentrations). It should, however, be noted for the sake of completeness that the comparison between C = ND/2 and C = ND is a biased comparison since it assumes that the true value of the concentration in the case of a non-detect can only be larger than the theoretical average (i.e., ND/2). A more balanced comparison would be among C = ND/4, C = ND/2 and C = ND.	The use of C = ND/2 and C = ND was intended to explore the sensitivity of the descriptive statistics results to legitimate alternative values for the non-detects and the results show, as the reviewer notes, little overall impact on the averages. There was however, no intent to bias the results or to perform an exhaustive analysis of the effects of possible values for measured values reported as non-detect. In fact, the theoretical average of the non-detects is unknown and the distribution of the non-detects is unknown (it could, for example be highly skewed) and non-detect values are not reported. In any case, C = ND/2 is more likely an approximation to the median non-detect value although this also is not known. The text referred to in the comment is not now on page 12 of the Final draft1. There is a considerable amount of discussion with tables in Sections 2, 3,

		and 4 that deals with the non-detects
	Pg. 21, par. 2 - The reference to large R^2 values resulting from "large separations in data points" sound like the effect of influential/outlying observations. If the concern is with such observations unduly influencing the correlation, were non-parametric correlations considered?	There was a general tendency for fish size to be positively related to mercury concentrations regardless of the R^2 values. The distortion in the R^2 values were generally due to large separations in clusters of data points (i.e., not necessarily the effect of individual influential observations) resulting in a graphical pattern that cast doubt on the validity of the usual linear regression type of relationship. Non-parametric correlations were not considered. The discussion [of the R^2 values] is adequate. Generally the problem with the R^2 values is not due to individual influential observations, which is clear from the text and the response to the comment. On-parametric correlations would not remedy this problem.
		Editor reformat/corrected. Subscript on x_i corrected which should help to clarify.
	Pg. 22 (equation) - The equation is not comprehensible as printed although it can be decoded from the variable definitions below.	Text was revised to read: "…and includes ancillary descriptive information."
	Pg. 31, par. 3 - *…and includes attributes of the level important to its meaning or implementation."* This language is unclear.	Agree (Editor revised). Editor: this paragraph should begin with the words "This table…"/corrected.
	Pg. 37, par. 3 - "*Table D-1a"* The primary reference here should probably be to Table 14.	Editor to revise/corrected.

	"Converting to meals per 30-day month as shown in Table 14" The reference here should be to Table 14b.	Text makes sense as worded – no revision needed.
	"Using the conservative estimate of Hg concentration… also should not be eaten weekly." The issue at this point in the text is <u>monthly</u> consumption. Weekly consumption was already addressed.	Note to editor: remove the comma from the next to last line of paragraph 1, Pg 43/corrected. Editor revised/corrected.
	Pg. 43, par. 2 - The format needs to be edited. Par. 3 - The basis for CM-FDA matching should be clarified.	The basis for CM-FDA matching is sufficiently descriptive – no revision needed. Note to editor: Pg 43 paragraph 3 should begin with the words: "Table 15…" Corrected to Table 14. The purpose of Table 15 (now Table 14) is to compare CM market names with FDA monitoring names. Adding the analytical data from Table 2 would make the table unwieldy and cluttered – no revision needed.
	Table 15 - I don't understand why the species detail in the CM data present in Table 2 was not carried forward here.	Agree - Swordfish is also an exception. Note to editor: The reference to Figure 3 in this paragraph is misplaced. The paragraph needs to be revised to reference Table 16/corrected.
	Pg. 45, par. 3 - "…the mean CM concentration…the only exception is bluefish" Swordfish also appears to be an exception.	Text refers to two additional species (other than predetermined group of 5) that were analyzed for PCBs as well as Hg. No revision needed. No revision to text indicated.

	Pg. 117, par. 1 - "Two samples…selected only for mercury analysis were also analyzed for PCBs" I don't understand this. Pg. 119, par. 2 - "The waterbodies of origin for each sample are also shown, but no clear trends appear to connect the PCB concentrations with the waterbodies of origin." Another way to look at the data in Table C-15 is that the farm-raised catfish were uniformly low in PCBs.	

2. Is the data adequate for meeting the objectives of this study?

Reviewer	Comments	Response to Comments
Anderson	It is unclear whether the data meets the object of measuring mercury in commonly consumed seafood species in New York. Certainly the data represents the fresh seafood consumed in the city. What is not addressed is what percentage of seafood consumed is consumed fresh. If there is any data on total seafood from the city or other surveys, reporting that would help place this data in perspective. The data is certainly important and very useful. The data and methods descriptions are very good and comprehensive. It is easy to understand what was done and mostly why. It should be helpful to the NYC "Eat Fish, Choose Wisely" project. The PCB information, although an ancillary activity is very useful and provides a great deal of information that augments the paucity of commercial fish PCB data. Most of the PCB mention is relegated to the appendix C except for brief mention in the executive summary. The critical statement in the executive summary is that the PCBs appear within the FDA tolerance level. For mercury the authors selected several "action levels" to compare to not just the FDA tolerance. Many states, as well as the EPA have "action levels" for PCBs which are, as with mercury, considerably lower than what FDA uses. While the PCBs are all quite low, including the EPA RfD or some of the states' or the Great Lakes Protocol value would be informative. Several states have catfish on their advisories. The highest catfish with PCB appears to be a wild caught lake fish. So for comparative purposes, having the low to high "action levels" used by various entities for PCB would be appropriate.	As the report notes, and the reviewer acknowledges, the limited PCB analysis was an ancillary activity to the main goal of the report. Accordingly, in keeping with the level of effort for this ancillary activity and the overall scope of the report, the text will provide only the FDA Tolerance Level for PCBs in fish.
Pascoe	The data collected by NYC and EPA are fully adequate for meeting the primary objective of the study. Based on the objective of analyzing mercury in the different seafood types	No response needed.

	that are consumed by people in NYC, the report sufficiently characterized mercury in a large suite of seafood types, covering multiple trophic levels and multiple phylogenetic levels. The study collected sufficient numbers of samples to enable statistical analyses among the different categories of seafood for total mercury concentrations, and among species within some of the categories. In Appendix C, the data collected on PCB concentrations in fish specimens were insufficient to perform a similar level of statistical analysis as was performed for mercury, but the data collection was not a primary objective of the study and the data still provide useful information on levels of PCBs that the general fish consuming public might be exposed to. The discussion on page 122 about the adequacy of the detection limits could be expanded to include comparison of total PCBs based on assuming non-detected congener concentrations at the detection limit rather than as zero, which would appear to still result in the concentration of total PCBs based on congeners to be below the FDA action level.	As discussed in the report, due to the many PCB congeners analyzed, assigning a value of ½ the detection limit for non detects "could severely overestimate the total PCB concentration." Assigning a value of zero to non detects "will tend to underestimate the total PCB concentration but likely represents the method with the smallest bias." Accordingly, no revision indicated.
	The report mentions the availability of blood levels of mercury for NYC residents. If studies have been performed that link those blood levels to patterns of seafood consumption and types of fish, particularly for ethnic or cultural subpopulations, some discussion should be considered in the report that would more fully link those studies with the objectives of the CM study.	The NYC HANES study (McKelvey et al., 2007) reported on the patterns of seafood consumption in ethnic/racial subpopulations within NYC noting that New Yorkers of Asian descent had both the highest blood Hg levels and the highest self reported fish consumption. Text was added to Section 1 describing blood Hg levels and seafood consumption patterns.
Stern	The specific objectives of the study do not appear to be stated in the report. This is a shortcoming that should be rectified. The first paragraph of Section 1 states that study was "…undertaken to measure mercury (Hg) concentration in composite samples from seafood species most commonly consumed by New York City residents as represented by specimens obtained from a commercial market." Based on the sections of the report that compare the NY City market Hg concentrations to those from the same species in the FDA database and based on the calculation of the meal frequency	The objective of the study is to give New York City residents information (Hg concentration) on the species of seafood most commonly consumed by area residents so they can make informed choices on the type and frequency of seafood meals that will minimize (i.e., maintain exposure below the RfD) their Hg exposure. As noted previously, a larger exercise to

	by species that would exceed the RfD, it is clear that the objectives of the report go beyond simply measuring Hg concentration in common commercial fish. The apparent objective of the study is to assess the currency and accuracy of the existing FDA/EPA mercury advisory for commercial fish and to recommend possible adjustments based on the more up-to-date NY City commercial market data. To the extent that the objectives are focused on the FDA database and on the FDA/EPA advice *relative to the NYC wholesale market per se*, the study appears to meet those implied objectives both in terms of sampling and statistical analysis.	evaluate the overall adequacy of FDA regulations/advisories re: Hg in fish is not the intended goal of the study and beyond its scope. That said, The Executive Summary and introduction to Chapter 1 has been revised to more clearly state the specific aims of the study.
	However, given the absence of clear statement of objectives, it is not clear to what extent EPA intends the data from the NYC commercial market to be representative of commercial fish Hg data nationwide. Section 1 does state the goal of the study was to measure Hg levels in fish commonly consumed by NYC residents. Nonetheless, the study clearly has broader implications. The NYC commercial fish market is a major intake and distribution point for commercial fish in the eastern U.S. and the NYC wholesale market receives fish caught in global waters. Nonetheless, there are other wholesale commercial fish markets in the eastern U.S. and there are other wholesale markets that serve other parts of the U.S. Thus, it is unclear to what extent EPA intends to generalize the findings of this study to the U.S. as a whole. A clear statement of the objectives of this study should include a discussion of the intent of its focus on the NYC market and the extent to which EPA believes that data from the NYC market can and should be used to generalize to commercial fish nationwide.	As noted above, study objectives have been clarified. The study grew out of the results from the NYC HANES (McKelvey et al., 2007) study which reported that relative to national data New York City adults had higher blood Hg concentration and a greater frequency of seafood meals. The results of the study are not intended to be generalizable to the nation as a whole.

3. Is the selection of the fish species adequate for this study?

Reviewer	Comments	Response to Comments
Anderson	The selection of fish species is adequate for the general NYC population who purchase fresh fish. The sample sizes while not large are sufficient. While analyses of single fish would have been preferred simply because that is what is most descriptive of consumer activity, the use of composites is acceptable. This does make it difficult to directly compare results from other studies. But, the statistical analyses provided show that the findings are consistent with what has been found in other studies. It would be helpful to have a bit more explanation of how the use of composites rather than the same number of samples but of individual fish strengthens the	The basic unit of analysis in the study is a composite sample of fish tissue by species. From the first paragraph in the report (page 4): "The New York City Commercial Market (CM) Seafood Study was undertaken to measure mercury (Hg) concentration in composite samples from seafood species most commonly consumed by New York City residents as represented by specimens obtained from a

	findings. No major commercial species appears to be missing.	commercial market." "Each composite sample was formed by mixing tissue from a number individual fish specimens into a combined amalgamated sample. The formation of the composite sample is, in effect, a physical averaging of the individual tissue samples and the result of a single measurement on the composite sample is an estimate of the average of the specimens in the sample. Composite sample analysis is a well established mechanism for cost effective estimation of means of environmental samples that has a history of use in fish tissue analysis (e.g., Procedures for Formation of Composite Samples from Segmented Populations, Fabrizio et al., *Environ. Sci. Technol.*, 1995, 29 (5), 1137-1144, Gilbert, R.O., Statistical Methods for Environmental Pollution Monitoring, 1987.)." The use of estimates of mean concentrations (derived from measurements on composite samples) supports the objective of assessing consumer behavior over time which is indicative of chronic exposure. Sampling of frozen, canned and dried fish sold in Asian markets and biomonitoring of blood mercury data were not in the scope of this study. This response and the document are responsive to the comment about composite samples. Questions about the Chinese or Asian intake of different species are outside the scope of our study. It has been included in the response. It could be emphasized more as some readers may have a similar reaction.

	Given that the report introduction and background emphasizes the blood mercury levels in the NYC Chinese, what is missing is any sampling of the imported frozen, canned and dried fish that are sold in Chinese/oriental groceries. Some mention of any published studies showing that these fish are similar in contaminant concentration than other fish would help. It is unclear how much of the fish in Chinese diets come from the species sampled in this study. The lack of ethnic specific commercial fish should at least be discussed and might help explain the biomonitoring observations rather than just that for this population frequency of consumption probably accounts for the differences. At least some discussion or at least the recognition of the possible impact of ethnically preferred fish needs to be mentioned. As mentioned in response to #1, it would be good to include the biomonitoring blood mercury data for the general NYC population and not just the Chinese. This study is not ethnic focused.	The original study design, in partnership with the NYCDOHMH, was to do city –wide sampling of seafood with oversampling in predominantly Asian communities to capture cultural differences in species consumption. Ultimately, the studies were bifurcated with the NYCDOHMH focusing exclusively on markets in Asian communities. (see McKelvey, Chang, et al., 2010)
Pascoe	The species selected for the study address multiple types of seafood that are typically consumed by the targeted audience, with the variety ranging from shellfish to squid to various sizes and types of finfish. Since the objective of the study focused on the types of seafood that consumers would typically eat from a commercial market, the variety in the types of seafood that were collected from the market was appropriate and adequate. Because the study was able to analyze fish and shellfish from multiple trophic and phylogenetic levels, it exceeded the needs of the study with regards to variety in seafood types.	No response needed.
Stern	As above, it is not possible to tell the extent to which the selection of fish species is adequate without knowing the overall objective of the report and more specifically, without knowing the extent to which EPA intends to generalize from the data collected from the NYC commercial market. The stated intent of the collection process was to obtain a representative sample of the fish most commonly consumed by NYC residents. The report briefly cites several databases (NMFS fish landings data; "Fish availability in supermarkets…in New Jersey" (Burger et al., 2004, but not specifically identified as such in the report); and the "Seafood Products Matrix" (not further defined)) as the basis for the selection of fish obtained from the NYC market. Given the level of detail in other parts of the report, it is surprising that no information was provided as to how these databases were actually used to determine the market sampling strategy. This information should be included in the report. The list of fish included in the study appears to be appropriate and does, in	

	my subjective assessment, include all or most fish commonly found in NYC markets. Nonetheless, given the nature of the study, documentation of the process seems essential.	

4. Is the use and presentation of the descriptive statistics appropriate?

Reviewer	Comments	Response to Comments
Anderson	The use of descriptive statistics is appropriate and when reported are presented with appropriate caveats about sample size etc and explanations of why certain procedures were or were not used. This was very helpful.	No response needed.
	The description of the analyses of variability is quite good and comprehensive. It is probably too detailed for most readers and all the formulae are quite intimidating. These details might be better included as an appendix and just the results and general methods discussed in the main text. What is also needed is a discussion of the a priori acceptable level of variability or what variability is scientifically acceptable. A clear set of concluding statements are needed. These need to indicate that the laboratory analyses and sample processing methods were assessed for consistency and reliability and found to meet accepted scientific standards. Actually the minimal variability found is quite impressive and shows the rigorous quality control that was utilized.	An appendix would be appropriate but not necessary especially if the comment document is included. Added concluding statements - text to the document on page 19. Thanks again to Anderson for pointing out that the minimal variability found and the demo of good quality control.
	I don't think it is appropriate to add the replicates and duplicates to the primary sample and average them and then assign that value to the sample. This really messes up the variability since they are no longer individual composite results but means. I doubt it makes much practical difference, but I would prefer that the primary sample result be the only result used in the overall analyses. Then all the composite sample results are truly comparable. The replicates and duplicates should be used only for what they were intended to be used for – assessing variability. The report quite effectively presents the impact of various analytic decisions such as how to handle the non-detects - showing that the differences in methods make little impact on conclusions. The same "with and without" approach could be done for the duplicate/replicate averaging impact. Rather than redo all the tables without the replicate/duplicate data included, you could see if it makes a difference and if it does not, simply state that to be the case. Then explain why you believe having some values as means and others not is more robust and statistically appropriate and that is why you chose to "use all the data". But whether it is appropriate and allows use of the statistical procedures used in other tables, I will leave to a statistician to	Composite samples are a physical average of all the individual specimens in the composite. A single analysis of the composite sample is by definition an estimate of the mean of all the individual specimens in the composite and thus composite sample analysis is a cost effective mechanism for obtaining an estimate of the mean of a number of individual specimens. Replicate and duplicate analyses provide valuable information on the content of the composite and assigning the value of the average of replicate and duplicate analyses to the composite is a standard practice. (See, for example: http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/upload/2009_04_23_fish_advice_volume1_v1cover.pdf; http://www.epa.gov/reg3hwmd/risk/eco/faqs/composite.htm.)

	decide.	Individual measurements on composite samples are, by definition, estimates of the mean of the individual specimens in the composite and thus averages of composite sample replicates and duplicates will have somewhat smaller variance compared to individual measurements but the assertion that "This really messes up the variability since they are no longer individual composite results but means" is not correct. In fact, valid estimates of the overall population variance of individual samples can be derived from composite sample measurements. Also, identification of a 'primary' sample is not meaningful in this situation because the analyses were conducted in blind random order.
Pascoe	The selection of statistical tests in Section 2 should include mention of why the US EPA program ProUCL was not used for determining means and other statistical metrics. The ProUCL program includes algorithms to account for non-detected values, with a recommended general approach that uses the distribution of detected values to replace the non-detects, and it can perform various statistical tests. The ProUCL manual recommends using the replacement procedure for non-detects if the sample size is at least six, and for most seafood types in the CM study the number of specimens usually exceeded this minimum value. The reliance on use of one-half the detection limit, the full detection limit, or in some cases a value of zero, for samples that had no detections might not be necessary or of lesser need if ProUCL were used.	The ProUCL statistical software is a comprehensive statistical software package with statistical methods suitable for addressing a wide range of environmental statistical issues. The software was developed primarily to support analyses used evaluate the attainment of cleanup standards for soils and solid media, analysis of groundwater monitoring data at RCRA facilities and analysis of data on chemical concentrations in soil at CERCLA sites and exposure data at hazardous waste sites. The extensive ProCal capabilities were not necessary for this study which was, by intent, a straight forward descriptive analysis of the fish tissue concentration data. In this study, upper confidence limits on the means of the mercury levels in fish were calculated in a straight forward manner using simple estimates of the population standard deviations recovered from the composite sample results under the assumption of approximate normality which is reasonable for the composite sample results.
	Page 11, top partial paragraph – The sample Group ID 237 should be classified as a detect, since two of the three samples	Authors agree.

	had detected values. Referring to the final averaged value as a non-detect seems to be an inaccurate portrayal of the intended result, in that mercury was mostly detected.	
	Page 11, second full paragraph – A definition of harmonic mean would be helpful, since, although a rarely used term, it is becoming increasingly utilized in chemical contamination studies. An additional mention of why it is less influenced by outliers would also be helpful.	We should provide a definition: The harmonic mean is the reciprocal of the arithmetic mean of a number of values. For n values, $x_1,\ldots, x_n$ the harmonic mean $H = \frac{n}{\frac{1}{x1}+\ldots+\frac{1}{xn}}$. The harmonic mean is less influenced by extreme values because by definition (i.e., in mathematical terms) it is a lower bound on the median of a set of values. As stated on page 11 of the report, the harmonic mean was used to represent the number k of fish specimens in a composite and is a conservative choice to represent the number in a composite for the purpose of estimating variance across composite samples. This is illustrated in the tuna example described on page 11. The discussion of the harmonic mean is now on page 4 of the document and reflects this comment response. Some additional words were added.
	Table 4 – The number of composite samples for Flounder/Fluke/Sole and for Squid under both "N.D." and "Total" do not add up.	Flounder/Fluke/Sole numbers were revised to indicate 1 non detect and a total sample size of 14. Squid will be revised to indicate 1 non detect and a total sample size of 11.
	Table 6 – Reason should be provided in a footnote as to why some variances are 0.0.	The reason some variances are 0 is that all the values in the calculation are the same or there is only one value.
Stern	The section on the derivation of the statistical approach is probably more detailed and theoretical than necessary, particularly given that the low Hg concentrations in many species of fish and the low detection limits, makes concerns	The reviewer demonstrates a correct understanding of the use of composite sampling and the objectives of the study, the straightforward nature of the

	about the treatment of non-detects of little practical significance. Nonetheless, the treatment appears reasonable and, in the end, straightforward. The use of composite samples provides for a reasonable estimate of the mean given limited sampling and analytical resources. However, despite the statistical approach that yields an estimate of the standard deviation in the sample, composite samples necessarily preclude accurate assessments of the extent of divergence of individual samples within the composite from the overall mean. This is only partially remedied by comparing the variability among several different composites for the same species that are ultimately combined to estimate the mean. For some contaminants (e.g., PCBs) for which the toxicological concern is largely with long-term average exposure, this is generally not a problem. However, for contaminants, such as MeHg, where short-term elevations during a critical window of development may have toxicological significance, lack of information on the full range of variability across individual samples is more of a concern. While this is a shortcoming of the statistical design of the study, it is recognized that the specific goal of the study was to estimate mean Hg concentrations and the variance around those mean estimates rather than to estimate maximum concentrations or upper percentiles of the overall distribution. In general, the figures in the report are well presented and informative. Figure 2 is well designed but a bit cramped and would benefit from having one category graphic per page. The Figure 3 presentations in Appendix A are particularly informative.	methods employed. We disagree with the assertion that "lack of information on the full range of variability across individual samples is more of a concern" and that "this is a shortcoming of the statistical design of the study." The goal of the study was to obtain estimates of mean concentrations which is consistent with the use of composite sample results and the objective of assessing possible chronic health effects. While obtaining individual fish measurements was not a goal of the study, it is possible to obtain an estimate of the population standard deviation from the composite data and this was done to construct the upper confidence limits on the means. This responds well to the comment and edits to the document are not needed.

5. Please comment on the data summary in Table 14. "Estimated Fish Servings per Week for an Adult Female of Child-bearing age based on Means and Upper 95% Confidence Limits on Mean Mercury Concentrations by Species". This table will draw a lot of attention. Is the table clear and does it provide the appropriate message?

Reviewer	Comments	Response to Comments
Anderson	The table is clear and having whole meals as the metric is far superior to the old table with fractional meals. I would place greater emphasis on the fact that each of these only applies if this is the only fish consumed in a given week and that these are not to be mixed. While it is in the foot note, it is easy to miss. It probably should be in the title of the table. This is an issue in advisories that easily and regularly confuses the public. I would also place this at the front of the text section and not just at the end of the section.	The reviewer points to an important proviso to the table, but moving it from a footnote to the title of the table would be unwieldy.
	However I think the message could be clearer. The upper 95% confidence interval of the mean adds little information and is not well understood as to what it means. On the other hand, presenting the value for two standard deviations from the estimated population mean as a second metric would be far more informative (as in Table 2 p 13 or figure 1 p 35). Most people don't want to know the average fish, but what the level is in the fish they are buying and how likely it is that the fish they will buy is over a given value. So a measure of the range of values is most useful. Providing the two standard deviations from the population mean would provide the information that going to the store and buying a fish you can be nearly certain that it will have less mercury than this value. That is more useful information than how robust is the estimate of the mean. With the mean, half the fish you buy will be above the value and of course half could be below. While over a prolonged period the mean is the best estimate of total dose received, it must be kept in mind that this report and information is targeted to pregnant women and peak fetal vulnerability may be only a short period of time. For most of the fish in the study, these two values (upper 95% confidence limit of the mean and two standard deviations from the estimated population mean) are quite similar and the advice doesn't change much. But for those fish with a high variance it is important. It helps explain why Tuna and Swordfish are "do not eat." And this might also help explain why blood mercury levels were found elevated. If you have a large family, you might be more likely to purchase a larger fish of a species or select a larger fillet. So what you actually purchase may not be random. The data does show that larger (or at least longer) fish have higher concentrations within a species. The mean minimizes that information.	We have a reasonable approach using the confidence limits on the mean. Anderson states the concept that underlies our approach: "While over a prolonged period the mean is the best estimate of total dose received" but is apparently more concerned about individual fish that could have high levels of mercury. This was not our focus and it is stated adequately in the report. Do we have any information on how harmful a single or a few meals of fish with high mercury may be or how this may relate to "peak fetal vulnerability"? Statistically we are on more solid ground making confidence limit statements about the mean with these data than we would be talking about upper percentile estimates based on what in many cases are rather small data sets. Also, Anderson has confused the mean with the median (half above and half below) and 'what you actually purchase may not be random'. Table 2 shows a number of informative statistics but does not include values of two standard deviations from the mean. The upper 95% limit on the means was used because the goal was to estimate average consumption levels that would represent chronic

		exposures. The UCL represents a plausible upper bound on the mean consistent with the observed data. The range of observed values may be of interest but not that informative with regard to the content of a particular future fish purchase. Authors agree to pregnant women as a sensitive subpopulation conservative indicator and should be a point of emphasis. Tuna and sword fish have higher mean levels. If you tend to eat larger fish you will tend to intake more Hg, the mean does not minimize 'that information'.
Pascoe	This table is recognized as a critical summary and presentation of the main points of the study, and as such is clear and appropriate.	No response needed.
Stern	In terms of its presentation, Table 14 is clear and easily interpretable. However, there is an underlying potential for significant uncertainty in the interpretation and application of the results of Table 14 relative to the FDA/EPA guidance to which it implicitly relates. The FDA/EPA advisory language for Hg in commercial fish states that, with a few specific exceptions, 12 oz., equal to two meals, of fish should be eaten per week. Thus, the assumed nominal average serving size is 6 oz. However, due to loss of moisture content with cooking, a 6 oz. cooked serving of fish is approximately equivalent to 8 oz. of purchased raw fish. Since the language in the FDA/EPA advisory does not specify as-purchased, or as-cooked, the actual intended serving size is unclear. Table 14 is based on 8 oz. wet weight (i.e., as-purchased). Presumably, the intended guidance to be derived from Table 14 relates to as-purchased weight. This is appropriate since stores weigh fish on purchase, but consumers generally do not weigh fish once it is cooked. However, this can lead to confusion with respect to comparison of Table 14 to the FDA/EPA guidance. Another issue with Table 14 is that (as stated on pg. 37, paragraph 2) the number of meals per week so as not to exceed the RfD is presented relative to two different estimates of the Hg concentration by species – the mean and the mean + 2 population-sd. However, Table 14 presents the latter category as the 95% upper confidence limit (UCL) on the mean. The 95% UCL of the mean is equal to the standard error of the mean times the t-statistic corresponding to 95% of the t-distribution. Thus, the mean + 2 sd is approximately equivalent to the 98th percentile of the population distribution,	To clarify a point raised by the reviewer, the 8 oz. meal relates to as purchased (uncooked) or wet weight to be consistent with the Hg analysis which was performed on raw specimens. Address the 95 % UCL vs mean + 2 SD issue.

	and not the 95th UCL on the mean. Given the relatively small number of observations, this is not likely to make a large practical difference, but this should be corrected. Also, as discussed above, the implications and intended use of Table 14 as presented in the report are ambiguous. Strictly speaking, Table 14 applies only to consumers who get their commercial fish from the NYC wholesale market. Thus, guidance to be derived from Table 14 would specifically apply only to those consumers. Further, apparent contradictions between the FDA/EPA guidance and Table 14 would not necessarily imply contradictions if the nationwide wholesale fish supply were similarly examined. The nominal specificity of Table 14 should be clearly stated and the implications or lack of implications for the application of the FDA/EPA advisory nationally should also be clearly stated.	As noted previously, the results of this study were not intended to be generalizable to the nation's seafood supply and the recommendations for meal frequency of different seafood species is not intended to be a referendum on FDA regulations and/or guidance.

6. Were the analytic methods for obtaining mercury and PCB concentrations in fish tissue appropriate for this study?

Reviewer	Comments	Response to Comments
Anderson	The laboratory methods used were standard and those widely used. Doing congener specific PCB analyses was informative although no analyses were included looking at whether congener patterns were different by species etc. The variability analyses demonstrated that the analytic procedures were applied consistently and the laboratories used excellent QA/QC practices.	The PCB analysis was ancillary to the primary goal of the study; therefore, analysis of PCB congener patterns across species is beyond the study's scope.
Pascoe	The analytical methods were appropriate for total mercury and PCBs. PCBs were appropriately analyzed as congeners. For mercury, the detection limits were sufficiently low enough for adequate detection in most seafood tissues.	No response needed.
Stern	The analytical method for Hg determination in fish tissue appears to be the standard cold vapor AA method. However, there is no mention of standard reference materials used in calibration or QA. I assume that these were, in fact, used, but this should be stated. With respect to the PCB analyses, the analytical method appears to be relatively standard and the approach of adding total PCB congener concentrations to obtain a composite measure of total PCBs is appropriate for the purpose of comparison to the FDA tolerance limit. However, it should	Text was added to reflect the reviewers comment re: co-planer (dioxin-like) PCB congeners.

	be noted that certain PCB congeners have toxicity that relates to TCDD-TEQ derived measures in addition to their toxicity as measured by total PCBs. The significance and utility of the lipid adjusted metric of PCB concentration should be addressed. Notwithstanding that PCB concentrations are often reported relative to lipid adjustment, from a consumption/exposure standpoint, why would lipid adjustment be useful?	

7. Given that the fish specimens were obtained from commercial venders and some specimens were not whole fish, the EPA implemented DNA analysis for verification of species. Please comment on this approach and how the information is presented in the appendix of the report.

Reviewer	Comments	Response to Comments
Anderson	The use of DNA analyses to confirm fish identities was quite innovative and informative. Yet the only mention of the DNA results is in the executive summary. Appendix B, while very informative, does not interpret the results. The conclusions reached and briefly mentioned in the executive summary need to be enhanced and maybe made a section in the report. Although not directly stated, it does appear that fish are not being mislabeled in a manner that would lead to fish with higher mercury levels being sold as a lower contaminated species or via versa. That is important consumer information. However, it would be useful to see a discussion as the 27 samples that were reanalyzed and 5 were changed and found to agree with the morphologically derived result. What is of interest is what of the 22 others that apparently remained different than the description of the fish. While less than 10% of the samples analyzed, it would be good to know how these differed. Are these really different or is it a method issue?	DNA barcoding section was not moved from the appendices to the body of the report.
Pascoe	The use of barcoding for this study was a fairly novel use of the technique and appropriate to the study. Typical fish sampling studies would rely on fish taxonomists to identify specimens either during field collection or in the laboratory prior to tissue dissection. As mentioned above, the utility of the barcoding exercise could be emphasized more in the report, that difference between taxonomic identification and the barcoding also resulted in EPA's re-examination of the photos of some of the collected specimens to determine whether they had been mis-identified.	Added that Moses Chang, an ichthyologist, was at the commercial fish market during sample acquisition.
Stern	There is clearly a potential for misidentification and mislabeling of fish species at the commercial level. Thus, DNA barcoding was an appropriate approach for positive species identification. It should be noted, however, that the FDA database to which the barcode-identified species are compared is not based on similar species identification. Furthermore, consumers purchase fish based on the vendor's identification that is, likewise, generally not based on DNA barcoding. Thus, while the DNA barcoding carried out in this study is a useful first step in putting fish consumption advisories on a standardized footing, it does not, practically speaking, reduce the uncertainty for comparison with the	No response needed.

	FDA database or for the consumer in purchasing. The description of the barcoding procedure and the reporting of the results of the barcoding in the report are inconsistent with respect to the level of technical detail. Some of the information on the barcoding procedure and interpretation (e.g., the CBOL criteria, the description of the primers and sequence overlap, the description of the result qualifier scores) are quite technical and largely inaccessible to even scientific readers who are not previously acquainted with the terminology and procedures. Other parts (e.g., explanation of DNA structure and base-pairing) are much more straightforward and apparently aimed at a different audience. In the end, all of the method description and data presentation does not lead to a clear discussion of the barcoding findings and their implications.	

Appendix A: Individual Reviewer Comments

COMMENTS SUBMITTED BY
Henry A. Anderson, M.D.
Private Consultant
Madison, WI 53704
608-241-1227
Email: anderha@sbcglobal.net

Henry A. Anderson, M.D.

Fish Tissue Analysis for Mercury and PCBs from a New York City Commercial Fish/Seafood Market

Contract No. EP-C-07-024
Task Order No. 114
June 30, 2011

External Letter Peer Review of EPA's Draft Report

CHARGE QUESTIONS

General note: There are a few typos and what appear to be mislabels in the document. The document needs careful editing to correct and find all these gremlins. For instance on page 34 a "Figure 10 in Appendix A" is mentioned, but there is no Figure 10 in appendix A. I believe this is referring to what is labeled Figure 3. It appears that tables and figures are numbered consecutively from the beginning through appendix A, then B appendix starts numbering again as does C. That is confusing.

1. Please comment on the organization and clarity of the report.

The organization of the report is appropriate and easy to follow. There are a number of approaches that would help with clarity. The report is quite long and presents many tables and figures which are very useful. This makes the executive summary become particularly important. Right now the executive summary is pretty cursory and the key findings of the report need to be highlighted in some kind of order. Having them as bullets would improve the clarity. It might also help if the key findings were highlighted in each section of the report at the front. These could be bullets but listing them and then having the text explain and elaborate would help. For instance, there is a lot of interest in farmed vs wild fish. This is mentioned and information provided in the text and there is not much to say since only three species were farm raised, and only one of those a fin fish. But it might be mentioned that no mercury was detected in the wild Atlantic salmon but found in half of the farmed salmon, even though the levels were very low. Farm vs wild is only included as part of "water body" discussion. If the authors go through each section and select what they view as the key findings, list them in the sections and then carry them into the executive summary that would help the reader focus on key findings.

The impetus for the study was the New York City blood mercury report. This study and its findings should probably be summarized a bit better and the fish consumption rates mentioned as well as the mercury prevalence's in the general population and other associated factors. The question that this report does not address is whether these fish monitoring data presented can explain the blood mercury distributions seen? It would help if somehow the authors could translate the various "action levels"

presented into what are their blood mercury level equivalents. Or at least discuss how the meal rates reported in Table 14a might translate into blood mercury.

What does the NY 5 ug/L blood translate into as fish contaminant level and meals per week? Will the Table 14a result in blood mercury less than the NY 5 ug/L value? It is somewhat counter intuitive that none of the composites exceed the FDA action level, and very few the EPA Rfd based level, yet 72% of the NYC Chinese exceed the NY reportable level of 5 ug/L mercury in blood. If these "action levels" are to protect everyone at unlimited fish consumption levels, then these "action levels" don't seem to be protective as far as the NY reportable level is concerned. Some discussion of the basis for the NY reportable level is needed and how it relates to the report's findings. In the executive summary it is mentioned that the NYC fish samples appear to be somewhat lower than the FDA comparisons. That triggers the question of then why do NYC residents have blood mercury levels three times those of the rest of the country? I would suggest that the testing samples are qualitatively similar, but doing a statistical comparison is problematic because of the different sampling structure.

In the introduction it is mentioned that the "action levels" used will be discussed further in the report. But all that is included is a table and references. There is no discussion of how they were derived, why they are different and how they are intended to be used. This is a bit of apples vs oranges. What is the level of fish consumption that each assumes? The challenge in understanding this report is how to arrive at a "dose" by navigating between fish tissue concentration, fish consumption rates over a selected time period and the blood mercury levels seen in the NYC study. It appears simple but is complex.

2. Is the data adequate for meeting the objectives of this study?

It is unclear whether the data meets the object of measuring mercury in commonly consumed seafood species in New York. Certainly the data represents the fresh seafood consumed in the city. What is not addressed is what percentage of seafood consumed is consumed fresh. If there is any data on total seafood from the city or other surveys, reporting that would help place this data in perspective. The data is certainly important and very useful. The data and methods descriptions are very good and comprehensive. It is easy to understand what was done and mostly why. It should be helpful to the NYC "Eat Fish, Choose Wisely" project.

The PCB information, although an ancillary activity is very useful and provides a great deal of information that augments the paucity of commercial fish PCB data. Most of the PCB mention is relegated to the appendix C except for brief mention in the executive summary. The critical statement in

the executive summary is that the PCBs appear within the FDA tolerance level. For mercury the authors selected several “action levels” to compare to not just the FDA tolerance. Many states, as well as the EPA have “action levels” for PCBs which are, as with mercury, considerably lower than what FDA uses. While the PCBs are all quite low, including the EPA RfD or some of the states’ or the Great Lakes Protocol value would be informative. Several states have catfish on their advisories. The highest catfish with PCB appears to be a wild caught lake fish. So for comparative purposes, having the low to high “action levels” used by various entities for PCB would be appropriate.

3. Is the selection of the fish species adequate for this study?

The selection of fish species is adequate for the general NYC population who purchase fresh fish. The sample sizes while not large are sufficient. While analyses of single fish would have been preferred simply because that is what is most descriptive of consumer activity, the use of composites is acceptable. This does make it difficult to directly compare results from other studies. But, the statistical analyses provided show that the findings are consistent with what has been found in other studies. It would be helpful to have a bit more explanation of how the use of composites rather than the same number of samples but of individual fish strengthens the findings. No major commercial species appears to be missing.

Given that the report introduction and background emphasizes the blood mercury levels in the NYC Chinese, what is missing is any sampling of the imported frozen, canned and dried fish that are sold in Chinese/oriental groceries. Some mention of any published studies showing that these fish are similar in contaminant concentration than other fish would help. It is unclear how much of the fish in Chinese diets come from the species sampled in this study. The lack of ethnic specific commercial fish should at least be discussed and might help explain the biomonitoring observations rather than just that for this population frequency of consumption probably accounts for the differences. At least some discussion or at least the recognition of the possible impact of ethnically preferred fish needs to be mentioned. As mentioned in response to #1, it would be good to include the biomonitoring blood mercury data for the general NYC population and not just the Chinese. This study is not ethnic focused.

4. Is the use and presentation of the descriptive statistics appropriate?

The use of descriptive statistics is appropriate and when reported are presented with appropriate caveats about sample size etc and explanations of why certain procedures were or were not used. This was very helpful.

The description of the analyses of variability is quite good and comprehensive. It is probably too detailed for most readers and all the formulae are quite intimidating. These details might be better included as an appendix and just the results and general methods discussed in the main text. What is also needed is a discussion of the a priori acceptable level of variability or what variability is scientifically acceptable. A clear set of concluding statements are needed. These need to indicate that the laboratory analyses and sample processing methods were assessed for consistency and reliability and found to meet accepted scientific standards. Actually the minimal variability found is quite impressive and shows the rigorous quality control that was utilized.

I don't think it is appropriate to add the replicates and duplicates to the primary sample and average them and then assign that value to the sample. This really messes up the variability since they are no longer individual composite results but means. I doubt it makes much practical difference, but I would prefer that the primary sample result be the only result used in the overall analyses. Then all the composite sample results are truly comparable. The replicates and duplicates should be used only for what they were intended to be used for – assessing variability. The report quite effectively presents the impact of various analytic decisions such as how to handle the non-detects - showing that the differences in methods make little impact on conclusions. The same "with and without" approach could be done for the duplicate/replicate averaging impact. Rather than redo all the tables without the replicate/duplicate data included, you could see if it makes a difference and if it does not, simply state that to be the case. Then explain why you believe having some values as means and others not is more robust and statistically appropriate and that is why you chose to "use all the data". But whether it is appropriate and allows use of the statistical procedures used in other tables, I will leave to a statistician to decide.

5. Please comment on the data summary in Table 14, "Estimated Fish Servings per Week for an Adult Female of Child-bearing age based on Means and Upper 95% Confidence Limits on Mean Mercury Concentrations by Species". This table will draw a lot of attention. Is the table clear and does it provide the appropriate message?

The table is clear and having whole meals as the metric is far superior to the old table with fractional meals. I would place greater emphasis on the fact that each of these only applies if this is the only fish consumed in a given week and that these are not to be mixed. While it is in the foot note, it is easy to miss. It probably should be in the title of the table. This is an issue in advisories that easily and regularly confuses the public. I would also place this at the front of the text section and not just at the end of the section.

However I think the message could be clearer. The upper 95% confidence interval of the mean adds little information and is not well understood as to what it means. On the other hand, presenting the value for two standard deviations from the estimated population mean as a second metric would be far more informative (as in Table 2 p 13 or figure 1 p 35). Most people don't want to know the average fish, but what the level is in the fish they are buying and how likely it is that the fish they will buy is over a given value. So a measure of the range of values is most useful. Providing the two standard deviations from the population mean would provide the information that going to the store and buying a fish you can be nearly certain that it will have less mercury than this value. That is more useful information than how robust is the estimate of the mean. With the mean, half the fish you buy will be above the value and of course half could be below. While over a prolonged period the mean is the best estimate of total dose received, it must be kept in mind that this report and information is targeted to pregnant women and peak fetal vulnerability may be only a short period of time. For most of the fish in the study, these two values (upper 95% confidence limit of the mean and two standard deviations from the estimated population mean) are quite similar and the advice doesn't change much. But for those fish with a high variance it is important. It helps explain why Tuna and Swordfish are "do not eat." And this might also help explain why blood mercury levels were found elevated. If you have a large family, you might be more likely to purchase a larger fish of a species or select a larger fillet. So what you actually purchase may not be random. The data does show that larger (or at least longer) fish have higher concentrations within a species. The mean minimizes that information.

6. Were the analytic methods for obtaining mercury and PCB concentrations in fish tissue appropriate for this study?

The laboratory methods used were standard and those widely used. Doing congener specific PCB analyses was informative although no analyses were included looking at whether congener patterns were different by species etc. The variability analyses demonstrated that the analytic procedures were applied consistently and the laboratories used excellent QA/QC practices.

7. Given that the fish specimens were obtained from commercial venders and some specimens were not whole fish, the EPA implemented DNA analysis for verification of species. Please comment on this approach and how the information is presented in the appendix of the report.

The use of DNA analyses to confirm fish identities was quite innovative and informative. Yet the only mention of the DNA results is in the executive summary. Appendix B, while very informative, does not interpret the results. The conclusions reached and briefly mentioned in the executive summary need to be

enhanced and maybe made a section in the report. Although not directly stated, it does appear that fish are not being mislabeled in a manner that would lead to fish with higher mercury levels being sold as a lower contaminated species or via versa. That is important consumer information. However, it would be useful to see a discussion as the 27 samples that were reanalyzed and 5 were changed and found to agree with the morphologically derived result. What is of interest is what of the 22 others that apparently remained different than the description of the fish. While less than 10% of the samples analyzed, it would be good to know how these differed. Are these really different or is it a method issue?

COMMENTS SUBMITTED BY
Gary A. Pascoe, Ph.D., DABT
Principal, Pascoe Environmental Consulting
Port Townsend, WA 98368
360-385-9977
Email: gpascoe@plympus.net

External Peer Review

Contract No. EP-C-07-024
Task Order No. 114
June 30, 2011

External Letter Peer Review of EPA's Draft Report,
Fish Tissue Analysis for Mercury and PCBs from a New York City Commercial Fish/Seafood Market

1. Please comment on the organization and clarity of the report.

The report is well organized and clearly written, with a few exceptions noted below; nonetheless, it should be easily understandable by health professionals and the interested public. The following are editorial comments that either need addressing or may help with clarity. These comments focus on the Executive Summary, which should be both comprehensive yet simplified enough with explanations of technical issues in order to be sufficiently understandable to someone unfamiliar with those issues.

Page 1, Executive Summary, first line – Because some states use the same or similar regulatory agency name, EPA should be referred to as the U.S. Environmental Protection Agency upon first use in the Executive Summary and the main body of the report.

Page 1, Executive Summary, first paragraph – The goal of the study to support the NYC public health message should be clarified with more detail. The development of a large seafood mercury database to provide support for the City's message on public health is highly admirable, and the readers could be more informed of how the data were actually used in formulating the health messages, and what those messages are. The final tables in the report provide recommendations on meals per month that sensitive members of the population (e.g., women of child bearing age) can consume of different types of fish from the Commercial Market, and the development of these recommendations using the mercury seafood data that the study collected should be more strongly linked to the NYC public health messages.

Page 1, Executive Summary, second paragraph – The derivation and meaning of the term "reportable level" is not explained, and may have different meanings to a chemist and a health professional or someone not versed in those fields. The term should be clarified as to whether it is a health-based criterion or a departmental advisory, or an enforceable standard, or whether it refers to concentrations that should be reported to a regulatory agency, and if so the basis for the requirement and the concentration of mercury should be noted.

Page 1, Executive Summary, third paragraph, fourth sentence – A comma appears to be missing.

Page 2, Executive Summary, third paragraph – The first sentence appears to state that the report estimates the amount of seafood adult women of child bearing age consume, from which the intake of mercury is estimated. This is an incorrect description of the process that is actually used in the report, in that the amount of seafood consumed by adult women of child bearing age is not itself estimated, but instead what is estimated is the amount of seafood consumption that corresponds to a level of concern for exposure to mercury. If the report intended to estimate the amount of seafood that a population or subgroup of a population consumes, an entirely different type of consumption study would be needed. The amount of seafood consumption considered to correspond to a level of concern is independent of actual seafood consumption rates. This distinction is critical primarily because some subpopulations will consume more seafood than others, which is noted at the end of the report, and descriptions of seafood consumptions by subpopulations need to be sensitive to such cultural differences. The fourth sentence of this paragraph also alludes to a determination of the amount of seafood that a subpopulation consumes – the permissible daily intake of methyl mercury was not actually compared with the amount of methyl mercury intake from fish ingestion, but rather was used to identify the permissible amount of ingestion of fish that contain varying levels of methyl mercury.

Page 2, Executive Summary, fourth paragraph and Section 6 – The report should attempt to provide some explanation of why the NYC CM data were so much lower than the FDA data on mercury in fish tissue, if there is a known or suspected reason.

Page 3, Executive Summary, first full paragraph – Additional summary of the rationale for the use of the barcoding would be appropriate here. A rationale is alluded to at the beginning of Appendix B which discusses the problems of taxonomic identification of dead fish. It should be emphasized in the Executive Summary that the barcoding was considered useful because the sources of the fish specimens consisted of different commercial fishers with uncertain quality of handling of the specimens, and the sources were not from a scientific survey and collection from the water bodies from which NYC fish are typically caught. Additionally, there was concern that the handling of the fish specimens by the commercial fishers could result in enough damage to the fish exterior to make it difficult to identify at a species level, as well as the reasons stated in Appendix B. In addition, more summary of the utility of the barcoding to the study should be added to the end of the Executive Summary. Specifically, the first full paragraph of Page 113 mentions that disparities between taxonomic identification and barcoding also resulted in re-examination of the photos of collected specimens by EPA to determine whether they had been mis-identified.

Page 31, Section 5.2, third paragraph – This text discusses the comparison of total mercury concentrations in fish with the EPA action level for methyl mercury. The reason given as to why EPA considers it acceptable to compare total mercury concentrations with the action level for methyl mercury is not entirely correct. EPA (2009) does not state that the comparison is acceptable because the molecular weight of mercury is close to that of methyl mercury, but because most if not sometimes all of the total mercury in fish tissue is actually methyl mercury, as stated in the text of the subsequent paragraph (fourth paragraph of Section 5.2). The text of the third paragraph needs to be changed to reflect the actual reasoning contained in EPA (2009) for the comparison.

Page 34 – The reference to Figure 10 in Appendix A should be Figure 3.

Page 37 – The reference to Table D-1a appears to refer to Table 14.

Page 43, Section 6 has a formatting problem.

Page 54 – Two references for EPA 2009 are provided; however, the second reference of EPA (2009) *Guidance for Implementing the January 2001 Methylmercury Water Quality Criterion* has been superseded by an updated document: USEPA. 2010. Guidance for Implementing the January 2001 Methylmercury Water Quality Criterion. April. EPA 823-R-10-001. http://water.epa.gov/scitech/swguidance/standards/criteria/aqlife/pollutants/methylmercury/upload/mercury2010.pdf.

2. Is the data adequate for meeting the objectives of this study?

The data collected by NYC and EPA are fully adequate for meeting the primary objective of the study. Based on the objective of analyzing mercury in the different seafood types that are consumed by people in NYC, the report sufficiently characterized mercury in a large suite of seafood types, covering multiple trophic levels and multiple phylogenetic levels. The study collected sufficient numbers of samples to enable statistical analyses among the different categories of seafood for total mercury concentrations, and among species within some of the categories.

In Appendix C, the data collected on PCB concentrations in fish specimens were insufficient to perform a similar level of statistical analysis as was performed for mercury, but the data collection was not a primary objective of the study and the data still provide useful information on levels of PCBs that the general fish consuming public might be exposed to. The discussion on page 122 about the adequacy of the

detection limits could be expanded to include comparison of total PCBs based on assuming non-detected congener concentrations at the detection limit rather than as zero, which would appear to still result in the concentration of total PCBs based on congeners to be below the FDA action level.

The report mentions the availability of blood levels of mercury for NYC residents. If studies have been performed that link those blood levels to patterns of seafood consumption and types of fish, particularly for ethnic or cultural subpopulations, some discussion should be considered in the report that would more fully link those studies with the objectives of the CM study.

3. Is the selection of the fish species adequate for this study?

The species selected for the study address multiple types of seafood that are typically consumed by the targeted audience, with the variety ranging from shellfish to squid to various sizes and types of finfish. Since the objective of the study focused on the types of seafood that consumers would typically eat from a commercial market, the variety in the types of seafood that were collected from the market was appropriate and adequate. Because the study was able to analyze fish and shellfish from multiple trophic and phylogenetic levels, it exceeded the needs of the study with regards to variety in seafood types.

4. Is the use and presentation of the descriptive statistics appropriate?

The selection of statistical tests in Section 2 should include mention of why the US EPA program ProUCL was not used for determining means and other statistical metrics. The ProUCL program includes algorithms to account for non-detected values, with a recommended general approach that uses the distribution of detected values to replace the non-detects, and it can perform various statistical tests. The ProUCL manual recommends using the replacement procedure for non-detects if the sample size is at least six, and for most seafood types in the CM study the number of specimens usually exceeded this minimum value. The reliance on use of one-half the detection limit, the full detection limit, or in some cases a value of zero, for samples that had no detections might not be necessary or of lesser need if ProUCL were used.

Page 11, top partial paragraph – The sample Group ID 237 should be classified as a detect, since two of the three samples had detected values. Referring to the final averaged value as a non-detect seems to be an inaccurate portrayal of the intended result, in that mercury was mostly detected.

Page 11, second full paragraph – A definition of harmonic mean would be helpful, since, although a rarely used term, it is becoming increasingly utilized in chemical contamination studies. An additional mention of why it is less influenced by outliers would also be helpful.

Table 4 – The number of composite samples for Flounder/Fluke/Sole and for Squid under both "N.D." and "Total" do not add up.

Table 6 – Reason should be provided in a footnote as to why some variances are 0.0

5. Please comment on the data summary in Table 14, "Estimated Fish Servings per Week for an Adult Female of Child-bearing age based on Means and Upper 95% Confidence Limits on Mean Mercury Concentrations by Species". This table will draw a lot of attention. Is the table clear and does it provide the appropriate message?

This table is recognized as a critical summary and presentation of the main points of the study, and as such is clear and appropriate.

6. Were the analytic methods for obtaining mercury and PCB concentrations in fish tissue appropriate for this study?

The analytical methods were appropriate for total mercury and PCBs. PCBs were appropriately analyzed as congeners. For mercury, the detection limits were sufficiently low enough for adequate detection in most seafood tissues.

7. Given that the fish specimens were obtained from commercial venders and some specimens were not whole fish, the EPA implemented DNA analysis for verification of species. Please comment on this approach and how the information is presented in the appendix of the report.

The use of barcoding for this study was a fairly novel use of the technique and appropriate to the study. Typical fish sampling studies would rely on fish taxonomists to identify specimens either during field collection or in the laboratory prior to tissue dissection. As mentioned above, the utility of the barcoding exercise could be emphasized more in the report, that difference between taxonomic identification and the barcoding also resulted in EPA's re-examination of the photos of some of the collected specimens to determine whether they had been mis-identified.

COMMENTS SUBMITTED BY

Alan H. Stern, Dr.P.H., D.A.B.T.
Independent Consultant
Metchen, NJ 08840
609-633-2374
Email: ahstern1@verizon.net

Review of Fish Tissue Analysis for Mercury and PCBs from a New York City Commercial Fish/Seafood Market
Alan H. Stern, Dr.P.H.

1. Please comment on the organization and clarity of the report.

In general, the report is well organized and clearly written. There are some specific points where the text is unclear. These are noted in my specific comments. The one significant exception to this, as discussed in detail below, is that the full objectives of the report as evinced by the analyses in the report, itself, are not clearly stated. Notwithstanding the overall logical organization and general clarity of the text, however, the report seems to reflect an inconsistent level of technical detail. Some sections are technically quite detailed and appear not to be intended to be generally accessible to the lay readier such as, for example, Section 4, "Analysis of Measurement Variability" and Appendix B, the explanation DNA "barcode" analysis. While Section 5, "Comparison of CM Data to Risk Metrics" is much more accessible. Some thought could be given to the intended audience for this report.

The report compares the NYC commercial market Hg concentrations by species to the FDA database of Hg concentration by species and gives the implications for consumption frequency advisories relative to the EPA RfD. Further, the report finds that, in general, the concentrations from the NYC commercial market samples are significantly lower on a species-by-species basis. The obvious next logical step is to compare the current FDA/EPA advisory specifics to the meal frequencies derived in the report (Table 14). This is not done and is conspicuous by its absence.

2. Is the data adequate for meeting the objectives of this study?

The specific objectives of the study do not appear to be stated in the report. This is a shortcoming that should be rectified. The first paragraph of Section 1 states that study was "…undertaken to measure mercury (Hg) concentration in composite samples from seafood species most commonly consumed by New York City residents as represented by specimens obtained from a commercial market." Based on the sections of the report that compare the NY City market Hg concentrations to those from the same species in the FDA database and based on the calculation of the meal frequency by species that would exceed the RfD, it is clear that the objectives of the report go beyond simply measuring Hg concentration in common commercial fish. The apparent objective of the study is to assess the currency and accuracy of the existing FDA/EPA mercury advisory for commercial fish and to recommend possible adjustments based on the more up-to-date NY City commercial market data. To the extent that the objectives are focused on the

FDA database and on the FDA/EPA advice *relative to the NYC wholesale market per se*, the study appears to meet those implied objectives both in terms of sampling and statistical analysis.

However, given the absence of clear statement of objectives, it is not clear to what extent EPA intends the data from the NYC commercial market to be representative of commercial fish Hg data nationwide. Section 1 does state the goal of the study was to measure Hg levels in fish commonly consumed by NYC residents. Nonetheless, the study clearly has broader implications. The NYC commercial fish market is a major intake and distribution point for commercial fish in the eastern U.S. and the NYC wholesale market receives fish caught in global waters. Nonetheless, there are other wholesale commercial fish markets in the eastern U.S. and there are other wholesale markets that serve other parts of the U.S. Thus, it is unclear to what extent EPA intends to generalize the findings of this study to the U.S. as a whole. A clear statement of the objectives of this study should include a discussion of the intent of its focus on the NYC market and the extent to which EPA believes that data from the NYC market can and should be used to generalize to commercial fish nationwide.

3. Is the selection of the fish species adequate for this study?

As above, it is not possible to tell the extent to which the selection of fish species is adequate without knowing the overall objective of the report and more specifically, without knowing the extent to which EPA intends to generalize from the data collected from the NYC commercial market. The stated intent of the collection process was to obtain a representative sample of the fish most commonly consumed by NYC residents. The report briefly cites several databases (NMFS fish landings data; "Fish availability in supermarkets…in New Jersey" (Burger et al., 2004, but not specifically identified as such in the report); and the "Seafood Products Matrix" (not further defined)) as the basis for the selection of fish obtained from the NYC market. Given the level of detail in other parts of the report, it is surprising that no information was provided as to how these databases were actually used to determine the market sampling strategy. This information should be included in the report. The list of fish included in the study appears to be appropriate and does, in my subjective assessment, include all or most fish commonly found in NYC markets. Nonetheless, given the nature of the study, documentation of the process seems essential.

4. Is the use and presentation of the descriptive statistics appropriate?

The section on the derivation of the statistical approach is probably more detailed and theoretical than necessary, particularly given that the low Hg concentrations in many species of fish and the low detection limits, makes concerns about the treatment of non-detects of little practical significance. Nonetheless, the

treatment appears reasonable and, in the end, straightforward. The use of composite samples provides for a reasonable estimate of the mean given limited sampling and analytical resources. However, despite the statistical approach that yields an estimate of the standard deviation in the sample, composite samples necessarily preclude accurate assessments of the extent of divergence of individual samples within the composite from the overall mean. This is only partially remedied by comparing the variability among several different composites for the same species that are ultimately combined to estimate the mean. For some contaminants (e.g., PCBs) for which the toxicological concern is largely with long-term average exposure, this is generally not a problem. However, for contaminants, such as MeHg, where short-term elevations during a critical window of development may have toxicological significance, lack of information on the full range of variability across individual samples is more of a concern. While this is a shortcoming of the statistical design of the study, it is recognized that the specific goal of the study was to estimate mean Hg concentrations and the variance around those mean estimates rather than to estimate maximum concentrations or upper percentiles of the overall distribution.

In general, the figures in the report are well presented and informative. Figure 2 is well designed but a bit cramped and would benefit from having one category graphic per page. The Figure 3 presentations in Appendix A are particularly informative.

5. Please comment on the data summary in Table 14, "Estimated Fish Servings per Week for an Adult Female of Child-bearing age based on Means and Upper 95% Confidence Limits on Mean Mercury Concentrations by Species". This table will draw a lot of attention. Is the table clear and does it provide the appropriate message?

In terms of its presentation, Table 14 is clear and easily interpretable. However, there is an underlying potential for significant uncertainty in the interpretation and application of the results of Table 14 relative to the FDA/EPA guidance to which it implicitly relates. The FDA/EPA advisory language for Hg in commercial fish states that, with a few specific exceptions, 12 oz., equal to two meals, of fish should be eaten per week. Thus, the assumed nominal average serving size is 6 oz. However, due to loss of moisture content with cooking, a 6 oz. cooked serving of fish is approximately equivalent to 8 oz. of purchased raw fish. Since the language in the FDA/EPA advisory does not specify as-purchased, or as-cooked, the actual intended serving size is unclear. Table 14 is based on 8 oz. wet weight (i.e., as-purchased). Presumably, the intended guidance to be derived from Table 14 relates to as-purchased weight. This is appropriate since stores weigh fish on purchase, but consumers generally do not weigh fish once it is cooked. However, this can lead to confusion with respect to comparison of Table 14 to the FDA/EPA guidance.

Another issue with Table 14 is that (as stated on pg. 37, paragraph 2) the number of meals per week so as not to exceed the RfD is presented relative to two different estimates of the Hg concentration by species – the mean and the mean + 2 population-sd. However, Table 14 presents the latter category as the 95% upper confidence limit (UCL) on the mean. The 95% UCL of the mean is equal to the standard error of the mean times the t-statistic corresponding to 95% of the t-distribution. Thus, the mean + 2 sd is approximately equivalent to the 98^{th} percentile of the population distribution, and not the 95^{th} UCL on the mean. Given the relatively small number of observations, this is not likely to make a large practical difference, but this should be corrected.

Also, as discussed above, the implications and intended use of Table 14 as presented in the report are ambiguous. Strictly speaking, Table 14 applies only to consumers who get their commercial fish from the NYC wholesale market. Thus, guidance to be derived from Table 14 would specifically apply only to those consumers. Further, apparent contradictions between the FDA/EPA guidance and Table 14 would not necessarily imply contradictions if the nationwide wholesale fish supply were similarly examined. The nominal specificity of Table 14 should be clearly stated and the implications or lack of implications for the application of the FDA/EPA advisory nationally should also be clearly stated.

6. Were the analytic methods for obtaining mercury and PCB concentrations in fish tissue appropriate for this study?

The analytical method for Hg determination in fish tissue appears to be the standard cold vapor AA method. However, there is no mention of standard reference materials used in calibration or QA. I assume that these were, in fact, used, but this should be stated.

With respect to the PCB analyses, the analytical method appears to be relatively standard and the approach of adding total PCB congener concentrations to obtain a composite measure of total PCBs is appropriate for the purpose of comparison to the FDA tolerance limit. However, it should be noted that certain PCB congeners have toxicity that relates to TCDD-TEQ derived measures in addition to their toxicity as measured by total PCBs.

The significance and utility of the lipid adjusted metric of PCB concentration should be addressed. Notwithstanding that PCB concentrations are often reported relative to lipid adjustment, from a consumption/exposure standpoint, why would lipid adjustment be useful?

7. Given that the fish specimens were obtained from commercial venders and some specimens were not whole fish, the EPA implemented DNA analysis for verification of species. Please comment on this approach and how the information is presented in the appendix of the report.

There is clearly a potential for misidentification and mislabeling of fish species at the commercial level. Thus, DNA barcoding was an appropriate approach for positive species identification. It should be noted, however, that the FDA database to which the barcode-identified species are compared is not based on similar species identification. Furthermore, consumers purchase fish based on the vendor's identification that is, likewise, generally not based on DNA barcoding. Thus, while the DNA barcoding carried out in this study is a useful first step in putting fish consumption advisories on a standardized footing, it does not, practically speaking, reduce the uncertainty for comparison with the FDA database or for the consumer in purchasing.

The description of the barcoding procedure and the reporting of the results of the barcoding in the report are inconsistent with respect to the level of technical detail. Some of the information on the barcoding procedure and interpretation (e.g., the CBOL criteria, the description of the primers and sequence overlap, the description of the result qualifier scores) are quite technical and largely inaccessible to even scientific readers who are not previously acquainted with the terminology and procedures. Other parts (e.g., explanation of DNA structure and base-pairing) are much more straightforward and apparently aimed at a different audience.

In the end, all of the method description and data presentation does not lead to a clear discussion of the barcoding findings and their implications.

Specific Comments

Pg. 11, par. 2 - The text is unclear as to how duplicates and replicates were treated in terms of the value for each composite sample that was actually entered into the overall statistical analysis.

Pg 12, par. 2 - The treatment of non-detects appears to have little overall impact on the estimation of the average Hg concentration across species and on the use of these data for fish consumption advisories (because the non-detects are in the species with the lowest Hg concentrations). It should, however, be noted for the sake of completeness that the comparison between C = ND/2 and C = ND is a biased comparison since it assumes that the true value of the concentration in the case of a non-detect can only

be larger than the theoretical average (i.e., ND/2). A more balanced comparison would be among C = ND/4, C = ND/2 and C = ND.

Pg. 21, par. 2 - The reference to large R^2 values resulting from "large separations in data points" sound like the effect of influential/outlying observations. If the concern is with such observations unduly influencing the correlation, were non-parametric correlations considered?

Pg. 22 (equation) - The equation is not comprehensible as printed although it can be decoded from the variable definitions below.

Pg. 31, par. 3 - *"…and includes attributes of the level important to its meaning or implementation."* This language is unclear.

Pg. 37, par. 3 - *"Table D-1a"* The primary reference here should probably be to Table 14.

"Converting to meals per 30-day month as shown in Table 14" The reference here should be to Table 14b.

"*Using the conservative estimate of Hg concentration…also should not be eaten weekly."* The issue at this point in the text is <u>monthly</u> consumption. Weekly consumption was already addressed.

Pg. 43, par. 2 - The format needs to be edited.

Par. 3 - The basis for CM-FDA matching should be clarified.

Table 15 - I don't understand why the species detail in the CM data present in Table 2 was not carried forward here.

Pg. 45, par. 3 - *"…the mean CM concentration…the only exception is bluefish"* Swordfish also appears to be an exception.

Pg. 117, par. 1 - *"Two samples…selected only for mercury analysis were also analyzed for PCBs"* I don't understand this.

Pg. 119, par. 2 - *"The waterbodies of origin for each sample are also shown, but no clear trends appear to connect the PCB concentrations with the waterbodies of origin."* Another way to look at the data in Table C-15 is that the farm-raised catfish were uniformly low in PCBs.