Project Title:

Risks of expanding the blue catfish fishery as a population control strategy: influence of ecological factors on fish contaminant burdens

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Abstract

Expansion of the fishery has been proposed as a possible tool for controlling burgeoning blue catfish populations in the Chesapeake Bay watershed and lessening attendant ecological impacts. However, contaminant burdens in edible fish tissues may present toxicological risks to human consumers. Accordingly, we determined concentrations of multiple contaminants known to pose human health concerns (i.e., mercury, chlorinated and brominated organic micropollutants) in fillets from blue catfish greater than 300 mm from three Chesapeake Bay tributaries: the James, Rappahannock and Potomac rivers. Fish from these locales were exposed to differing levels of point- and non-point sources of pollutants. Blue catfish from the upper Potomac and upper James exhibited greater fillet burdens of most contaminants than conspecifics from the lower James or Rappahannock rivers. However, despite high human population densities in the area, mercury levels were lower in Potomac blue catfish fillets. Fish sex and $\delta^{15}N$ values (as a surrogate for trophic position) had minimal influences on contaminant

fillet burdens in blue catfish of the sizes examined in this study. Potomac catfish exhibited distinctly greater $\delta^{15}N$ values, suggestive of feeding at a higher trophic level or ingestion of prey items with higher $\delta^{15}N$ signatures. For most contaminants, pollutant burdens increased with fish size. Fillet % lipid was positively related to lipophilic organic pollutant concentrations, but not to total mercury. Our contaminant burden results support existing VA and MD advisories regarding regional fish consumption, i.e. concentrations of PCBs and Hg in blue catfish fillets from some locales pose risks to human health, and this risk varies with fish consumption rate. Based on the Hg and PCBs concentrations we observed, the majority of blue catfish sampled surpassed existing EPA recommended limits for unrestricted human consumption. Furthermore, river-segment specific consumption advisories are necessary as contaminant types and concentrations varied within rivers. Within river segments, fish length and weight were useful predictors of concentrations of most contaminants. Consideration of % lipid content improved predictions of fat-soluble organic pollutants, but not Hg. However, % lipid is not a measure that is readily usable by anglers or consumers to inform or limit their contaminant exposure.

Introduction

Blue catfish (*Ictalurus furcatus*) were first introduced to Virginia waters in the 1970s in an effort to establish recreational fisheries for this popular catfish. In subsequent decades, blue catfish have not only become well-established in rivers that were initially stocked (James, York, and Rappahannock river systems), but they have also spread to other systems (e.g. Potomac, Piankatank, Patuxent, and Nanticoke rivers) within the Bay watershed (Schloesser et al. 2011). Their increasing populations and life history characteristics (e.g. omnivorous diet, long lifespan exceeding 20 years, and large size) exacerbate the threat to native species such as alosines, and compromise the effectiveness fisheries management actions (MacAvoy et al. 2000 & 2009; Schloesser et al. 2011).

One possible avenue for controlling blue catfish population size is through increased harvests. However, the above mentioned species characteristics may also facilitate greater

bioaccumulation of toxic chemicals. Indeed, limited monitoring of blue catfish contaminant loads in Virginia previously revealed levels posing human health concerns in some locales. For example, polychlorinated biphenyl (PCB) levels exceeding the current Virginia Department of Health (VDH) concentration threshold (50 ng/g wet weight) associated with consumption advisories (VDH 2004. See Appendices) were observed in blue catfish from sections of the James, Dan, Bannister, Appomattox, and Rappahannock rivers (http://www.vdh.virginia.gov/Epidemiology/dee/PublicHealthToxicology/Advisories/). Furthermore, it was previously observed that mercury (Hg) levels in Pamunkey River blue catfish exceeded the VA advisory level (>500 ng/g; http://www.vdh.virginia.gov/Epidemiology/dee/PublicHealthToxicology/Advisories/). Mercury loads in other catfish species from Chesapeake Bay tidal tributaries also exhibited levels above the health advisory value, underscoring concerns about mercury concentrations in blue catfish from those systems. If increasing harvest of blue catfish is promoted as a management tool to control population size, then a more thorough understanding of contaminant loads is critical.

PCBs and mercury are the contaminants most commonly triggering fish consumption advisories in the U.S. in general and in the Chesapeake Bay watershed in particular. While their reported tissue burdens have remained relatively constant in recent years, and even decreased in some species, allowable levels for these contaminants have dropped dramatically as our knowledge regarding health effects thresholds has increased. Other contaminants are also of concern, notably legacy chlorinated pesticides, e.g. chlordanes and DDTs. In addition, emerging contaminants such as brominated diphenyl ethers (PBDE) flame retardants, and antibacterial agents (e.g. triclosan) may present new health threats to humans and wildlife.

PCBs were used commercially in the U.S. as multicomponent congener mixtures known as Aroclors. The compositions of these mixtures are altered during passage through the environment due to differential transport, degradation, and bioaccumulation of the constituents. Thus field studies of PCBs must be based on evaluation of individual congener levels, which then can be summed to provide an accurate estimate of total PCBs. Limited data

on contaminant loads in blue catfish collected from several Virginia and Maryland rivers have shown PCB levels above human health advisory limits established by the US EPA, the Maryland Department of Environment (MDE) and Virginia Department of Environmental Quality (VDEQ)/Health (VDH) (see Appendices). Although a recent study of mercury in blue catfish from Chesapeake Bay tributaries found total mercury concentrations in fish fillets below the VDEQ threshold (range: 26.4 – 125 ng/g wet weight; mean: 52.3 ng/g), that study was based on a limited number of fish ranging from 325 mm to 595 mm total length from estuarine portions of the James (n=12), York (n=12), and Rappahannock (n=11) river systems (Xu et al. 2013). A number of rivers have not been sampled at all for contaminants in blue catfish. However, pollutant levels in other catfish species in some of these systems exceeded advisory limits (e.g. http://www.mde.state.md.us/programs/Marylander/CitizensInfoCenterHome/Documents/www.mde.state.md.us/assets/document/Maryland%20Fish%20Advisories%202011.pdf).

Habitat use, ecology, and fish metabolic capabilities may influence contaminant loads. As blue catfish grow, they become increasingly piscivorous; this shift occurs at approximately 300 mm fork length (Schloesser et al. 2011). Such dietary changes may enhance bioaccumulation of contaminants. Local environmental conditions and individual characteristics, such as sex, may also impact growth rates and contaminant burdens. Hence, a better understanding of the role of these factors is needed to devise effective and prudent advisories.

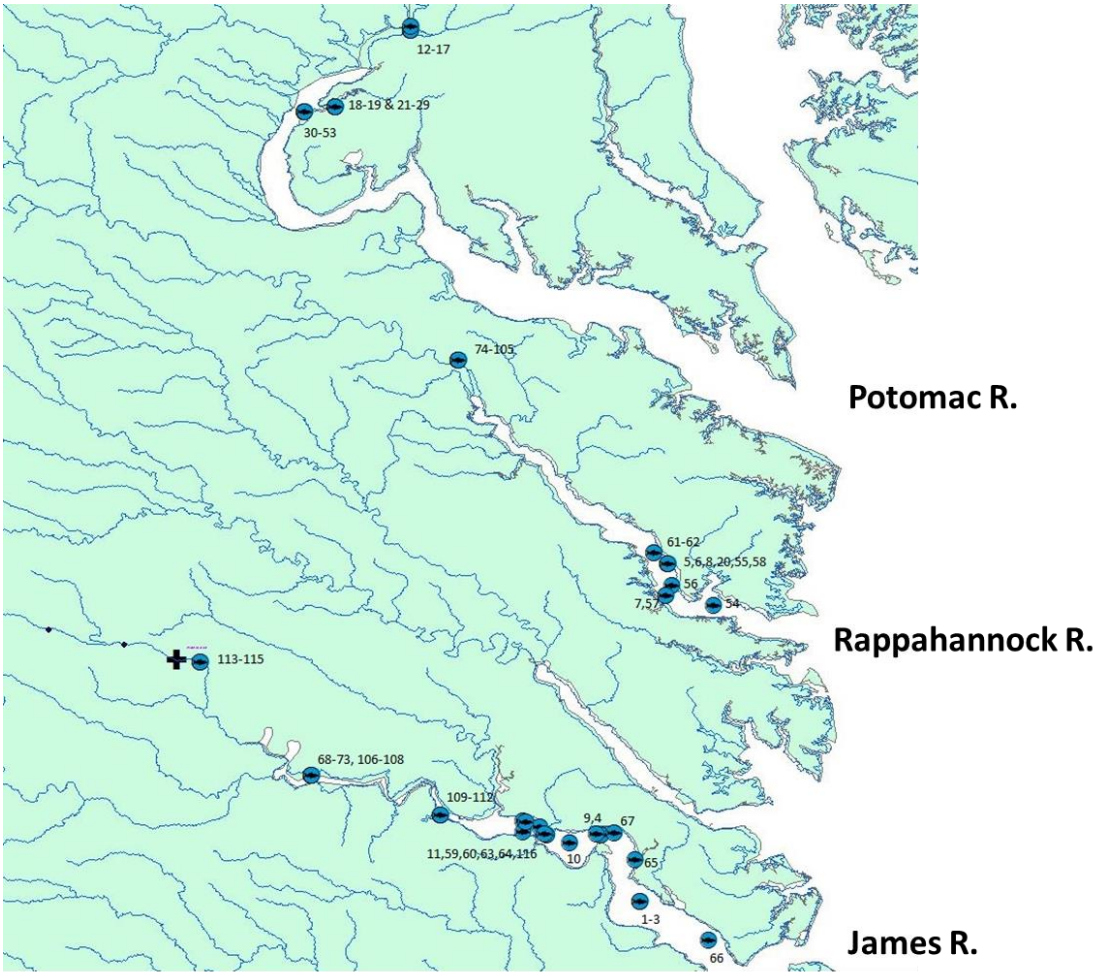
Accordingly, the objective of this study was to develop predictive tools, appropriate for Chesapeake Bay tributaries, for estimating the relationship between contaminant burdens and an easily measured parameter, blue catfish size. In this way, risks to consumers may be better managed. The results of these analyses were interpreted relative to current state and EPA advisory limits for human consumption. Fish fillet contaminant burdens were also evaluated with respect to fish sex, tissue lipid content and stable isotope profile. Blue catfish from three major Chesapeake Bay river systems (James, Rappahannock, and Potomac) were sampled across the size range likely to support a fishery (>300 mm fork length). For comparability, we

applied analytical methods used by VIMS researchers in previous efforts supporting VDEQ fish consumption advisories. Examination of multiple river systems permitted a more comprehensive evaluation to assure observed contaminant/tissue relationships were representative of blue catfish in the Chesapeake Bay watershed. Sex of the fish was also noted, as females may off-load contaminants through the release of eggs (Rypel et al., 2007). Uptake of the targeted contaminants is primarily via the diet and levels generally increase with trophic position. Traditional diet studies demonstrate recent feeding activities and trophic position, whereas stable isotopes can effectively determine long-term trophic position (MacAvoy et al. 2000 & 2009). Accordingly, stable isotope ratios (i.e. as $\delta^{15}N$ and $\delta^{13}C$) were also evaluated in fillets as these have been found to be valuable tools for gauging trophic position of aquatic organisms (Thomas and Cahoon, 1993).

Methods

Fish collection and processing

Due to budgetary limitations, blue catfish collection was coordinated between existing sampling programs at VIMS (T. Tuckey and M. Fabrizio), VDGIF (R. Greenlee), VCU (G. Garman), and MD DNR (M. Groves). Fish were collected from the James and Rappahannock rivers in VA and the Potomac River in MD. Collections targeted fish between 300 and 600 mm fork length (FL) to include sizes likely to be encountered in a fishery and retained by anglers for consumption. Additional specimens longer than the targeted size range were included from the James River to provide an indication of contaminant loads in larger individuals. Muscle tissue (fillet) was resected from each fish. Typically, the entire fillet from a single side of the fish was taken and homogenized. For small fish both sides were taken to ensure adequate tissue for analysis. For large fish, a vertical subsection from one side was removed, and a section of belly fat proportional to that present for the entire side of the fish was included. Some of the fish analyzed here were used for multiple studies and were filleted prior to receipt at VIMS. Unfortunately sex of those fish was not recorded. After tissue resection, individual fillet samples were lyophilized using a Virtis Genesis freeze-drier. Drying improves extraction efficiency of organic contaminants and is also preferred for stable isotope determinations.



Locations where blue catfish were sampled from VA and MD waters in 2011 and 2012. Numbers refer to individual fish identification numbers from the various locations in the three rivers studied. For some statistical analyses the samples were parsed into five groups: upper Potomac (PU); upper Rappahannock (RU: 74-105); lower Rappahannock (RL: remainder); upper James (JU: 109-112 & those upstream); and the lower James (JL: remainder).

Contaminant analyses

A surrogate standard (suite of ^{13}C-PCB congeners covering tri- to decachlorinated congeners) was added to freeze-dried tissue samples. The extraction, purification and chromatographic detection methods have been previously published (Chen et al. 2009). Blanks

(consisting of pre-extracted sodium sulfate) were analyzed coincident with each sample queue to assess possible lab introduction of contaminants.

Surrogate-spiked, dried samples were subjected to accelerated solvent extraction (Dionex ASE 200). For each sample an aliquot of extract (10% of total) was removed, the solvent evaporated, and the residual weighed to estimate sample extractable lipid content (i.e. % lipid). Due to the presence of co-extracted interfering compounds in fish (e.g. lipids), size exclusion (Phenomenex Envirosep ABC 350 mm x 21.2 mm and matching guard column 60 mm x 21.2 mm) and normal phase adsorption (Isoelute 2 g silica gel solid phase extraction columns) liquid chromatography cleanup steps were applied to the remainder of the extracts. Decachlorodiphenyl ether (DCDE) was then added as the internal quantitation standard. Extract volumes were then reduced to ~0.2 ml under a purified nitrogen stream. Purified extracts were analyzed by gas chromatography/mass spectrometry (GC/MS; Varian CP-3800 GC, Saturn 2200 ion trap MS and CP-8400 autosampler) to identify and quantitate PCBs. Multi-level calibration curves were generated comparing the response of the internal standard to authentic analyte standards. Data were generated for individual congeners and these were summed for total PCBs. The following PCB congeners were determined (listed in order of GC elution): 19, 18, 17, 15, 16/32, 26, 25, 28/31, 53, 33/20, 22, 45, 46, 52, 49, 47/48, 44, 42/59, 41/64, 40, 67, 63, 74, 70, 95, 66, 91, 56/60, 92, 84, 101/90, 99, 83, 97, 87/115, 85, 136, 110, 77, 82, 151, 135, 107, 149, 123, 118, 134, 131/165, 146, 153/132, 105, 141/179, 137, 176, 130, 163/164, 138/158, 178, 129, 175, 187, 183, 128, 167, 185, 174, 177, 171, 156, 201, 173, 172, 180/193, 191, 200, 170/190, 199, 208, 195, 194, 206, 209. Non-PCB chlorinated pollutants (listed in the Appendices along with the surrogates used for method recovery correction) were analyzed in these same extracts by GC/MS (Varian 3400 CX GC, Saturn 4D MS and 8200cx GC autosampler) similarly. The GC column used in both cases was a DB-5MS (Agilent Technologies; 60 m x 0.32 mm ID, 0.25 µm film thickness). Data were corrected for the recoveries of the surrogate standard of homologous chlorination or the standard eluting closest to the target analyte in the chromatographic run.

The PBDE analysis method has been previously published (La Guardia et al., 2012). Briefly, extracts were solvent exchanged to methanol and analytes therein separated by ultra-performance liquid chromatography (UPLC: Acquity UPLC, Waters Corporation, Milford, MA, U.S.A.) operated in the gradient mode (methanol/water program), equipped with a C_{18} UPLC analytical column (Acquity UPLC BEH C18, 1.7 µm, 2.1 mm × 150 mm, Waters Corp.). Analytes were ionized by atmospheric pressure photoionization (APPI). The dopant (acetone) was introduced at 150 µL/min by a liquid chromatography pump (LC-20AD, Shimadzu Corporation, Kyoto, Japan), and product ions were detected by a triple quadrupole mass spectrometer (3200 QTrap, AB Sciex, Framingham, MA, U.S.A.) operated in the multiple reaction monitoring (MRM) mode. Congeners examined were: BDE-28, 47, 99, 100, 153, 154 and 183. Quantitation limits were 1 ng/g (wet weight) or better for individual congeners.

Blanks analyzed concurrently with fish fillet samples exhibited concentrations of the target analytes below the limits of quantification. Mean recoveries of ^{13}C-labeled PCB surrogate standards for the fish fillets were good, ranging from 75% to 103% with standard deviations ranging from 28% to 35%.

Mercury analysis was performed by cold vapor atomic absorption spectroscopy. Fish samples (~ 0.5 g of freeze dried tissue) were weighed to the nearest 0.1 mg directly into 55 mL Teflon microwave digestion vessels. Samples were digested in 15 mL of concentrated “Trace metal grade” nitric acid in accordance with EPA Method 3052 and recommendations provided by CEM, Inc. for microwave digestion of tissues in the MARS Express System (CEM Corp., Mathews, NC). The resulting digests were cooled, transferred to 100 mL volumetric flasks, diluted with deionized water, and transferred to polyethylene bottles for storage prior to analysis. Acid blanks and standard reference materials (NRC-DOLT 4 and NRC-DORM 3) were treated similarly.

Stable isotope analyses

For isotope (δ^{15} N and δ^{13} C) determinations, 1 mg aliquots of lyophilized fish fillets were packed into tin capsules at VIMS. Fillets were not pre-extracted to remove lipids. These samples were analyzed by the Stable Isotope Facility at University of California at Davis (http://stableisotopefacility.ucdavis.edu/13cand15n.html).

Multiple reference materials were run by UC Davis to evaluate analysis accuracy: i.e. bovine liver, USGS-41 (enriched L-glutamic acid), USGS-40 (un-enriched L-glutamic acid) and nylon 5d. Briefly, analyses were conducted on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS; Sercon Ltd., Cheshire, UK). Samples were combusted at 1000°C in a reactor packed with chromium oxide and silvered copper oxide. Following combustion, oxides were removed in a reduction reactor (reduced copper at 650°C). The helium carrier then flowed through a water trap (magnesium perchlorate) and an optional CO_2 trap (for N-only analyses). N_2 and CO_2 were separated on a Carbosieve GC column (65°C, 65 mL/min) before entering the IRMS.

Data analysis

We used linear models to predict contaminant concentrations in blue catfish from each river using fish size, sex, and location as predictors; individual contaminants were log-transformed prior to consideration in the model. Because we did not have age data from blue catfish collected from the Potomac River, and because fisheries are primarily managed on the basis of size, we chose to model contaminant levels using fish length instead of age. Contaminant levels for each tributary and section thereof were compared to VA and US EPA consumption advisory limits. Analysis of variance and regressions were performed using SPSS (Version 20). Using a General Linear Model (GLM) procedure, we tested a number of null hypotheses about the effects of factors on the means of various groupings of a single dependent variable (contaminant concentration). Interactions between factors were also investigated. The effects of covariates (e.g. fish length) and covariate interactions with factors were likewise included.

Multivariate ordination techniques were used to better visualize the relationship among groups of fish from the various tributaries sampled, and to consider all the contaminants simultaneously. Contaminant levels were standardized using root-root transformations to reduce the effects of the largest observations. A Bray-Curtis similarity matrix was constructed to evaluate contaminant loads of individual fish and the resulting ordination was examined for correlations with explanatory factors, namely, fish length, and stable isotope results, using Non-Metric Multidimensional Scaling (NMDS). These analyses were performed using R (R Core Team 2012).

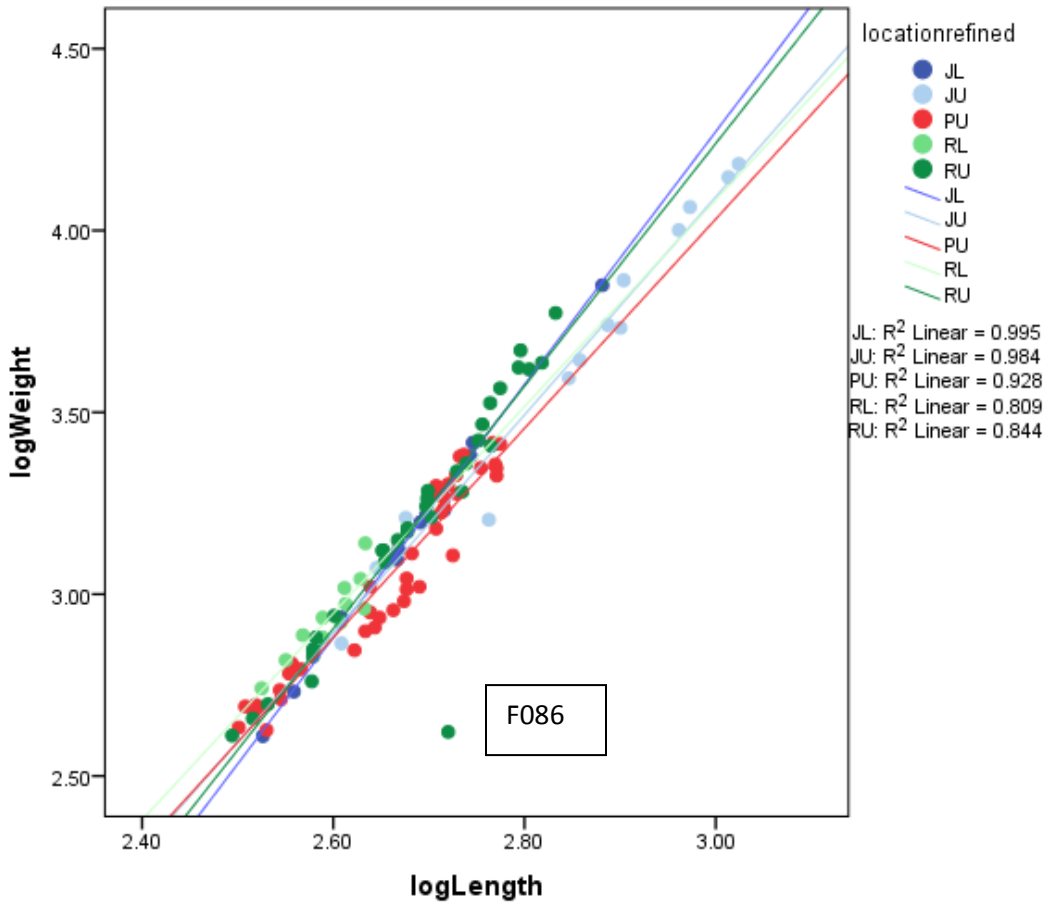
Results

Biological characteristics of fish sampled.

A total of 31 blue catfish from the James, 44 from the Rappahannock, and 41 from the Potomac River were processed for contaminants in this study. Samples were obtained opportunistically from several ongoing field studies in VA and MD. Hence, size distributions may not reflect those in the rivers. The largest blue catfish obtained were from the James River (1057 mm FL, mean = 585.9 mm FL). Blue catfish from the Potomac (mean = 464.0 mm FL) and Rappahannock rivers (mean = 469.4 mm FL) were somewhat smaller.

Mean (SE), range, and median of blue catfish length, weight, and age from the James, Rappahannock, and Potomac rivers collected during 2011 and 2012. Otoliths were not available from blue catfish from the Potomac River.

Location	*n*	Fork Length (mm)	Weight (g)	Age (yr)
James River	31	585.9 (36.97)	3640.3 (730.45)	10.2 (0.66)
		336 - 1057	407 - 15250	5 - 18
		513	1702	9.5
Rappahannock River	44	469.4 (14.86)	1730.3 (201.78)	10.5 (0.46)
		312 - 680	408 - 5933	5 - 17
		450	1318	9.5
Potomac River	41	464.0 (13.75)	1316.0 (112.11)	NA
		317 - 595	423 - 2611	-
		475	1048	



Plot of log fish weight (g) versus log length (mm) for blue catfish from Chesapeake Bay tributaries. Sample F086 (lower green point) from the upper Rappahannock River exhibited an anomalously low weight for its length. Location abbreviations are as defined previously.

Because fish weight was highly variable in this study, we sought to determine factors associated with this variability. The effect of fish length, sex, $\delta^{15}N$, and location, parsed into upper and lower river sections for the James and Rappahannock rivers, on weight of fish was investigated by ANOVA. Weight, fish length, and $\delta^{15}N$ were log-transformed for this analysis. Sex, log $\delta^{15}N$ and location-sex interaction were not significant (p=0.829, 0.458 and 0.633,

respectively). Location also was not significant (p=0.071); but log length was significant (p <0.001). The model accounted for 93.4% of the total variation in fish weight observed in this study.

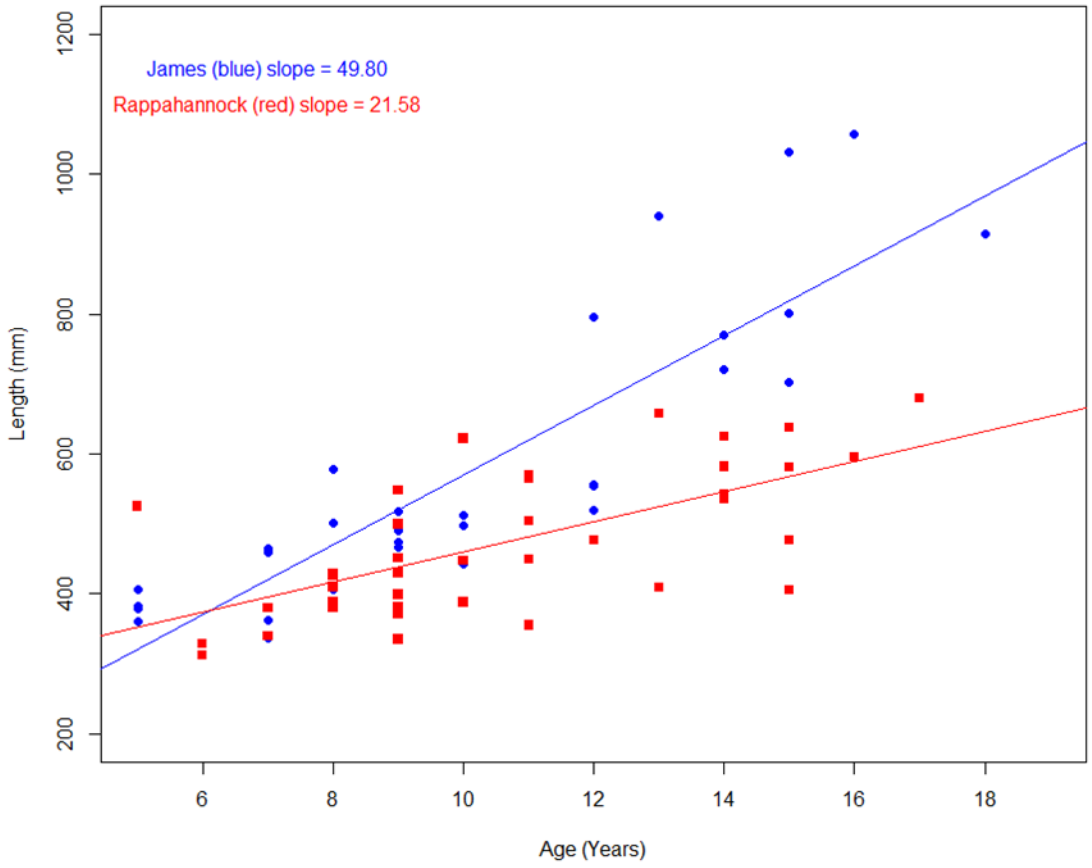
Tests of Between-Subjects Effects

Dependent Variable: logWeight

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13.347[a]	14	.953	117.127	.000
Intercept	.881	1	.881	108.225	.000
logLength	7.446	1	7.446	914.787	.000
log15N	.005	1	.005	.555	.458
locationrefined	.073	4	.018	2.234	.071
sex	.007	3	.002	.295	.829
locationrefined * sex	.028	5	.006	.688	.633
Error	.822	101	.008		
Total	1169.299	116			
Corrected Total	14.169	115			

a. R Squared = .942 (Adjusted R Squared = .934)

Considering each river system in its entirety, age of blue catfish in the James and Rappahannock rivers was linearly related to length at the sizes observed in this study, though the James River fish were larger at age than those from the Rappahannock River (see figure below). This linear growth pattern has been observed for other blue catfish obtained from Chesapeake Bay tributaries (Greenlee and Liem, 2011) and is unlike the sigmoidal growth pattern typically seen in most fishes. Fish age ranged from 5 to 18 years in specimens from the James River and 5 to 17 years in fish from the Rappahannock River. In addition, length-at-age was highly variable within rivers, as evidenced by the variation around the linear model depicted in the figure. As pollutant concentrations are calculated on a tissue weight basis, growth characteristics such as these will influence observed results.

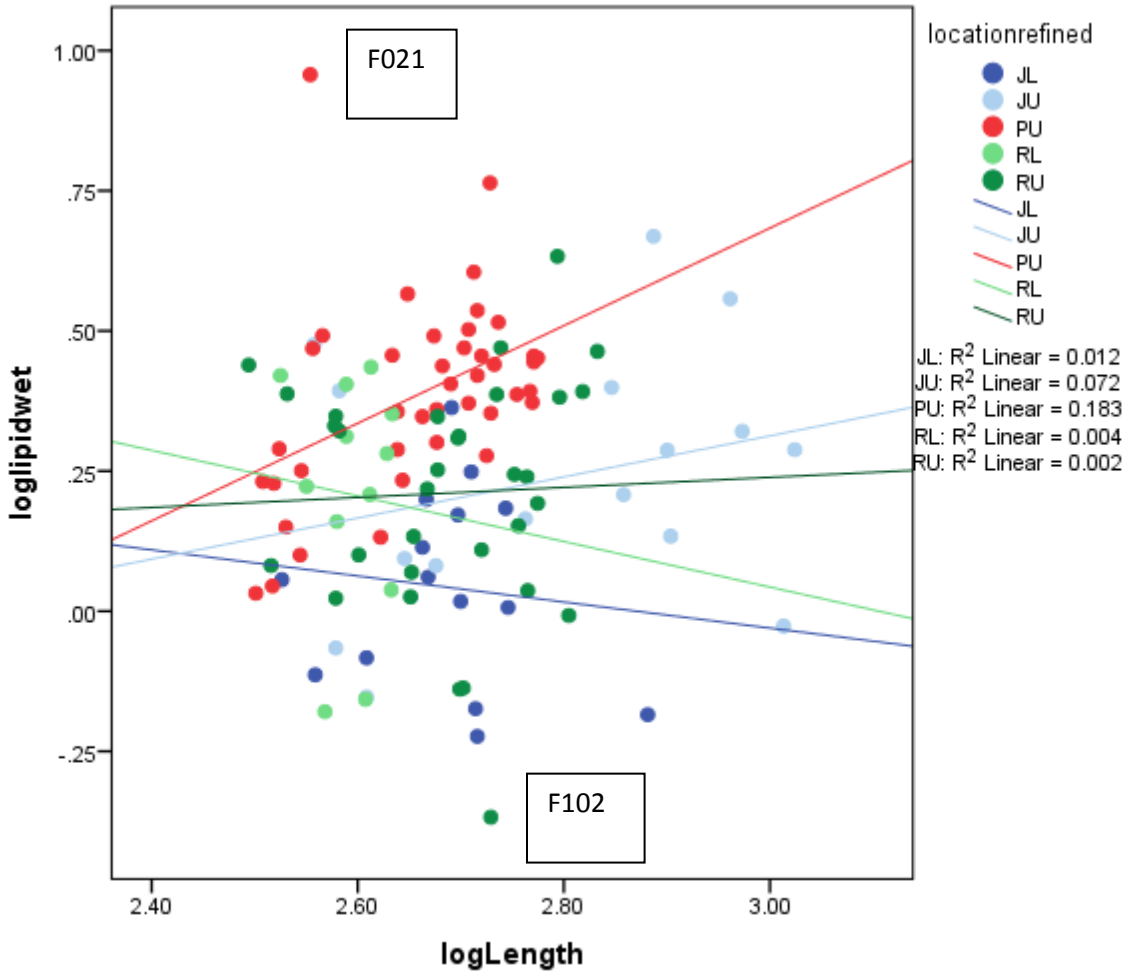


Length- at-age for blue catfish from the James and Rappahannock Rivers.

The bioaccumulative organic pollutants examined in this study are known to partition preferentially into nonpolar tissue lipids. Hence, greater lipid content may translate into higher contaminant burdens when concentrations are expressed on a wet weight basis (as is the norm for contaminant consumption advisories). Thus, an understanding of lipid levels in fillets is in order. For all fish, lipid content ranged from 2.54% to 35.1% (mean 9.85%, SD 4.58).

A significant linear relationship (p =0.045; SPSS GLM) was observed between log fish length (mm) and log % extractable lipid (wet weight basis). However, the relationship differed between river segments and was variable within river segments (see figure below). Fillet lipid levels were highest for Potomac (PU) and lowest for lower James River (JL) fish fillets. Upper

James (JU) lipid levels were similar to Rappahannock fish (RU and RL) levels. Overall, however, fish size was a poor predictor of lipid content in blue catfish (all r^2 values <0.2).



Log % lipid content of fillets (wet weight basis) versus log fish length (mm) for blue catfish. Sample F021 (Potomac River-red data point) was a positive outlier in terms of lipid content. F102 (upper Rappahannock-green data point) exhibited an anomalously low lipid content. Location abbreviations are as defined previously.

A model to investigate the effects of fish length, location, sex, and logδ^{15}N, on the mean log %lipid of fillets indicated that sex and logδ^{15}N were not significant predictors (p=0.335 and 0.066, respectively) of lipid content of blue catfish.

Tests of Between-Subjects Effects

Dependent Variable: loglipidwet

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2.045[a]	13	.157	4.297	.000
Intercept	.030	1	.030	.827	.365
log15N	.127	1	.127	3.460	.066
logLength	.150	1	.150	4.090	.046
locationrefined	1.073	4	.268	7.330	.000
sex	.081	2	.040	1.106	.335
locationrefined * sex	.222	5	.044	1.211	.309
Error	3.733	102	.037		
Total	13.299	116			
Corrected Total	5.778	115			

a. R Squared = .354 (Adjusted R Squared = .272)

Pairwise Comparisons

Dependent Variable: loglipidwet

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
JL	JU	-.101[a,b]	.080	.902	-.330	.127
	PU	-.466[a,*]	.089	.000	-.719	-.212
	RL	-.231[a,*]	.080	.047	-.461	-.002
	RU	-.179[a,b]	.064	.058	-.361	.003
JU	JL	.101[a,b]	.080	.902	-.127	.330
	PU	-.364[a,*]	.109	.012	-.677	-.052
	RL	-.130[a]	.095	.848	-.401	.140
	RU	-.077[a,b]	.070	.958	-.278	.123
PU	JL	.466[b,*]	.089	.000	.212	.719
	JU	.364[b,*]	.109	.012	.052	.677
	RL	.234	.082	.051	-.001	.469
	RU	.287[b,*]	.077	.003	.067	.507
RL	JL	.231[b,*]	.080	.047	.002	.461
	JU	.130[b]	.095	.848	-.140	.401
	PU	-.234	.082	.051	-.469	.001
	RU	.053[b]	.071	.998	-.149	.255
RU	JL	.179[a,b]	.064	.058	-.003	.361
	JU	.077[a,b]	.070	.958	-.123	.278
	PU	-.287[a,*]	.077	.003	-.507	-.067
	RL	-.053[a]	.071	.998	-.255	.149

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (I).

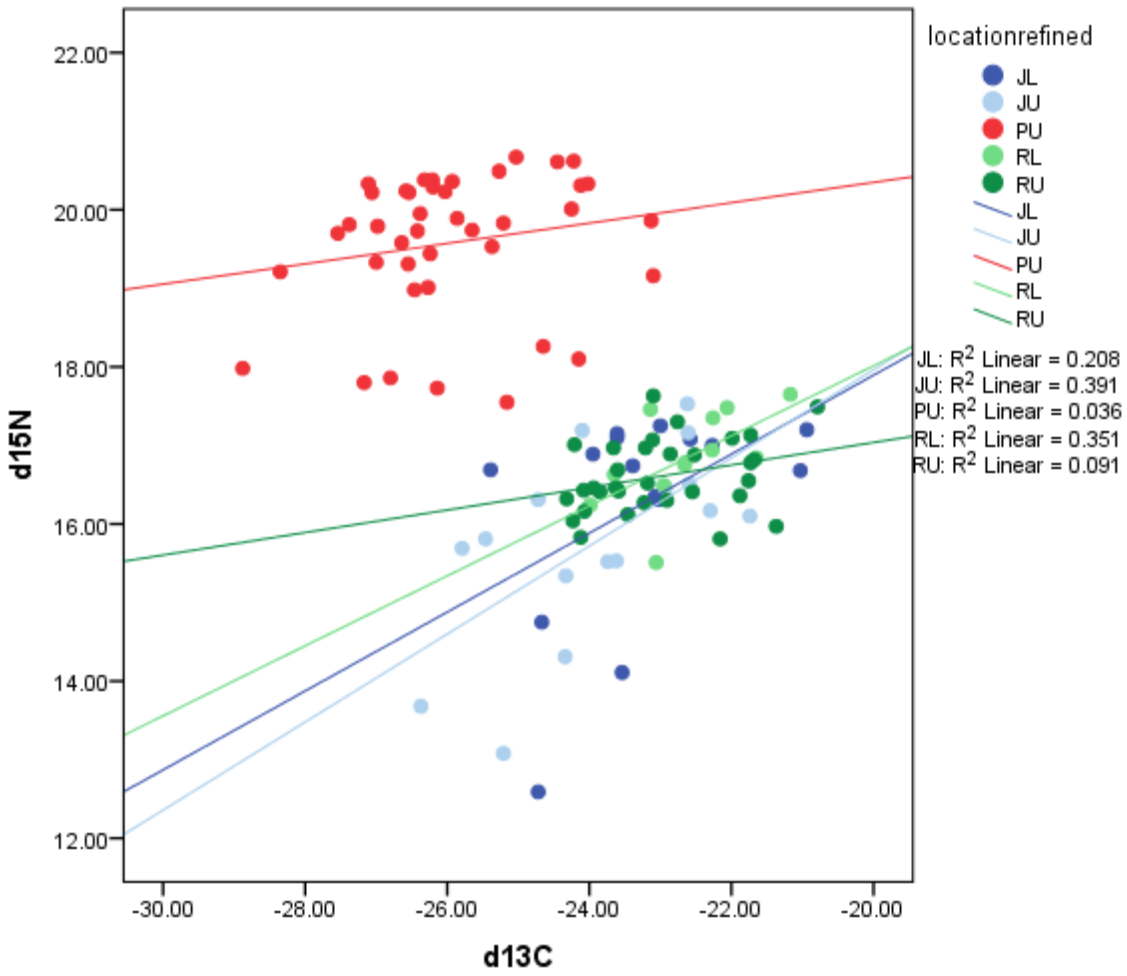
b. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Sidak.

The *post-hoc* Sidak comparison test indicated that mean % lipid content of fillets from fish from the Potomac River was greater than those observed at all other locations except for fish from the lower Rappahannock River (p=0.051). The analysis further indicated that upper and lower James River fillets possessed similar % lipid content; as did fillets from the upper and lower Rappahannock River (RU and RL).

Stable Isotope results

The $\delta^{15}N$ and $\delta^{13}C$ isotope distributions in blue catfish fillets from the James and Rappahannock rivers clustered together (see figure below) and were similar to those reported in Xu et al. (2013). Blue catfish from the Potomac River, however, exhibited generally higher $\delta^{15}N$ (mean 19.6) and lower $\delta^{13}C$ levels than fish from the other river systems. The lower $\delta^{13}C$ values for Potomac River fish may be related to the higher fillet lipid levels in these fish, as discussed previously. The higher $\delta^{15}N$ in the Potomac River fish may be due to these fish feeding at a higher trophic level, or feeding on prey that were themselves exposed to greater ^{15}N content. Regardless, these results suggest that the nutritional status/feeding ecology of Potomac River blue catfish fish differed from that of fish from the Rappahannock and James rivers. Further investigation of feeding behaviors and fish physiology are merited.



$\delta^{15}N$ and $\delta^{13}C$ values in blue catfish fillets from tributaries of the Chesapeake Bay (location abbreviations as defined previously). Fillets were not pre-extracted to remove lipids prior to $\delta^{13}C$ determinations.

Log fish length (mm) and location significantly affected stable nitrogen isotope signatures ($\delta^{15}N$) in blue catfish (see table below), but the assumptions of this linear model may have been violated). Levene's test of equality of error variances was significant (p=0.002), indicating heterogeneity of variance among fish from the various locations. The *post-hoc* Sidak comparison test suggested $\delta^{15}N$ in Potomac River fish exceeded that observed from all other sites, and $\delta^{15}N$ values for fish from the upper James River were similar to $\delta^{15}N$ values for fish from the lower James River, but less than in those from the Rappahannock sites.

Tests of Between-Subjects Effects

Dependent Variable: log15N

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	.171[a]	5	.034	62.481	.000
Intercept	.173	1	.173	317.058	.000
logLength	.004	1	.004	6.905	.010
locationrefined	.168	4	.042	77.030	.000
Error	.060	110	.001		
Total	178.984	116			
Corrected Total	.231	115			

a. R Squared = .740 (Adjusted R Squared = .728)

Pairwise Comparisons

Dependent Variable: log15N

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
JL	JU	.019	.009	.249	-.006	.045
	PU	-.083*	.007	.000	-.104	-.063
	RL	-.022	.009	.175	-.049	.004
	RU	-.011	.007	.803	-.031	.010
JU	JL	-.019	.009	.249	-.045	.006
	PU	-.103*	.008	.000	-.125	-.081
	RL	-.042*	.010	.001	-.070	-.013
	RU	-.030*	.008	.001	-.052	-.008
PU	JL	.083*	.007	.000	.063	.104
	JU	.103*	.008	.000	.081	.125
	RL	.061*	.008	.000	.039	.084
	RU	.073*	.006	.000	.057	.089
RL	JL	.022	.009	.175	-.004	.049
	JU	.042*	.010	.001	.013	.070
	PU	-.061*	.008	.000	-.084	-.039
	RU	.011	.008	.832	-.012	.035
RU	JL	.011	.007	.803	-.010	.031
	JU	.030*	.008	.001	.008	.052
	PU	-.073*	.006	.000	-.089	-.057
	RL	-.011	.008	.832	-.035	.012

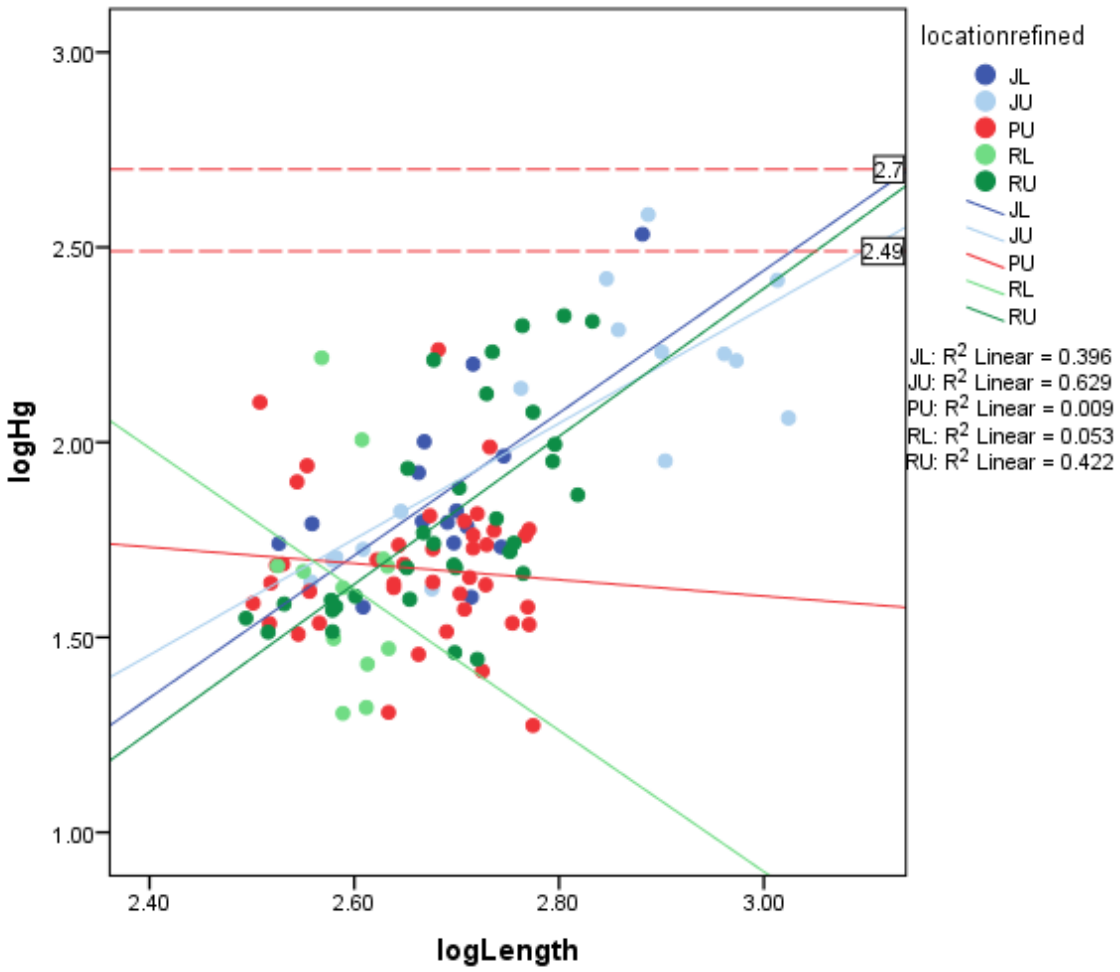
Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Sidak.

Contaminant concentrations

Mercury: Total Hg in fish consists primarily of methylmercury, due to its propensity to bind with sulfur groups in tissue proteins.



Log Hg concentration (ng/g wet weight) versus log fish length (mm) for blue catfish collected from Chesapeake Bay tributaries (location abbreviations as defined previously). The current VDH two meal/month advisory limit is 500 ng/g (log = 2.71) and the corresponding EPA threshold is 310 ng/g (log=2.49). These thresholds are shown as horizontal, dashed red lines. None of the samples surpassed the VDH limit, but two James River fish exceeded the lower EPA threshold. The majority of samples surpassed the EPA unrestricted consumption limit for Hg, i.e. 29 ng/g (log=1.47)

Statistical analysis (SPSS GLM) with log Hg as the dependent variable and log length, log lipid content, $\delta^{15}N$ and sex as predictors indicated that only log length was significant ($p>0.001$). The model predicted about 40% of the total variation in total Hg observed in blue catfish from Chesapeake Bay tributaries. Somewhat surprisingly, in light of the generally higher organic pollutant levels in Potomac River fish (see later discussion) and extent of human development in the area, Hg concentrations were among the lowest in Potomac River fish fillets. Sidak's pairwise comparison test did not identify significant differences in mean Hg concentrations in fish fillets between the sampling locations.

Tests of Between-Subjects Effects

Dependent Variable: logHg

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.087[a]	14	.292	6.465	.000
Intercept	.264	1	.264	5.846	.017
logLength	1.773	1	1.773	39.260	.000
loglipidwet	.028	1	.028	.621	.433
log15N	.004	1	.004	.087	.769
locationrefined	.159	4	.040	.880	.479
sex	.174	2	.087	1.931	.150
locationrefined * sex	.252	5	.050	1.118	.356
Error	4.560	101	.045		
Total	377.438	116			
Corrected Total	8.647	115			

a. R Squared = .473 (Adjusted R Squared = .400)

Pairwise Comparisons

Dependent Variable: logHg

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[c]	95% Confidence Interval for Difference[c]	
					Lower Bound	Upper Bound
JL	JU	.000[a,b]	.089	1.000	-.256	.256
	PU	.156[a]	.111	.832	-.162	.473
	RL	.095[a]	.093	.974	-.170	.360
	RU	.069[a,b]	.073	.986	-.141	.279
JU	JL	.000[a,b]	.089	1.000	-.256	.256
	PU	.156[a]	.128	.922	-.210	.522
	RL	.096[a]	.106	.990	-.208	.399
	RU	.069[a,b]	.078	.991	-.155	.294
PU	JL	-.156[b]	.111	.832	-.473	.162
	JU	-.156[b]	.128	.922	-.522	.210
	RL	-.060	.095	.999	-.332	.211
	RU	-.087[b]	.091	.985	-.348	.174
RL	JL	-.095[b]	.093	.974	-.360	.170
	JU	-.096[b]	.106	.990	-.399	.208
	PU	.060	.095	.999	-.211	.332
	RU	-.026[b]	.079	1.000	-.251	.199
RU	JL	-.069[a,b]	.073	.986	-.279	.141
	JU	-.069[a,b]	.078	.991	-.294	.155
	PU	.087[a]	.091	.985	-.174	.348
	RL	.026[a]	.079	1.000	-.199	.251

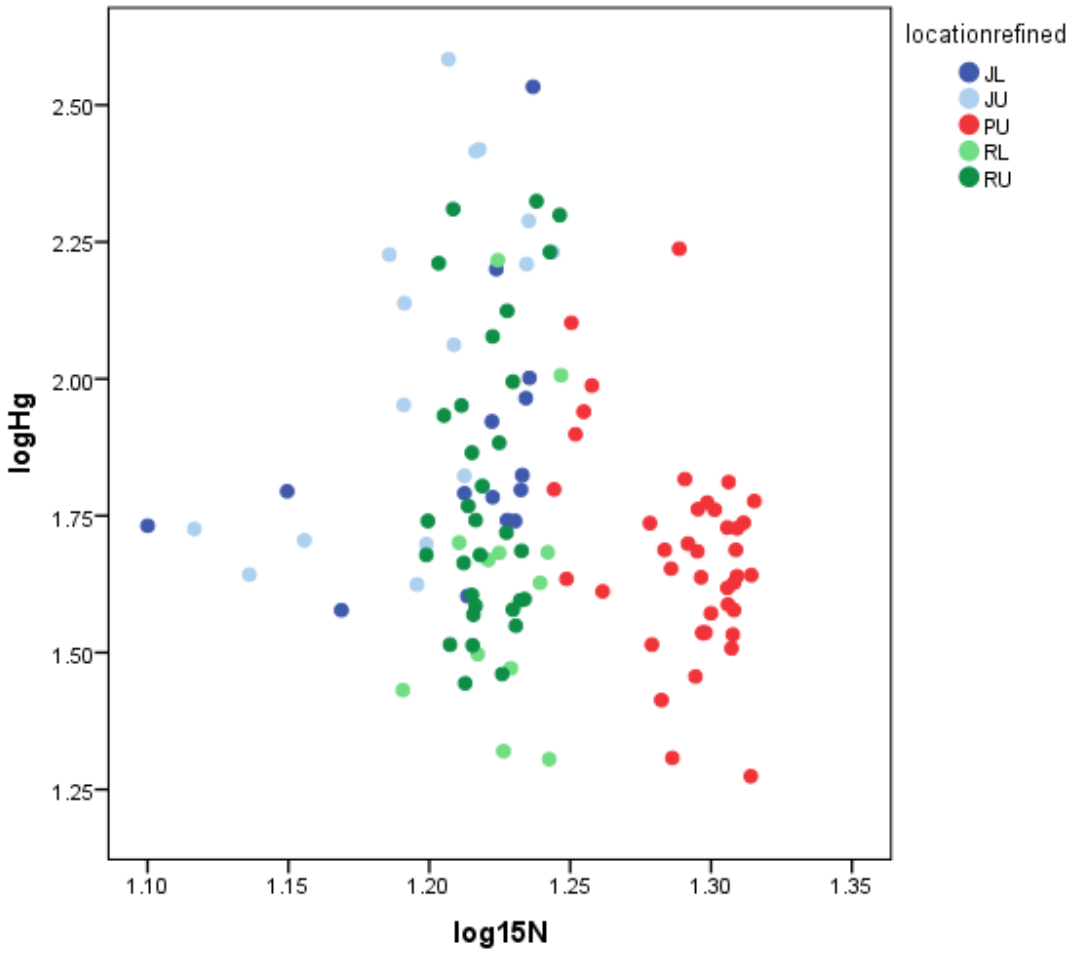
Based on estimated marginal means

a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

c. Adjustment for multiple comparisons: Sidak.

Mercury concentrations in blue catfish fillets increased significantly with increasing fish size for fish in the James and Rappahannock rivers. The concentration of total Hg in James River blue catfish increased by 15.6% with a 10% increase in fish length, whereas total Hg in Rappahannock River blue catfish increased 18% for a similar change in length. There was no significant relationship between fish length and total Hg content of blue catfish from the Potomac River at the sizes we sampled. None of the individual blue catfish contained Hg levels exceeding the current Virginia Department of Health two meal/month fish consumption limit (0.50 ppm or 500 ng/g). It should be noted, however, that many of the existing state Hg advisories for other fish species apply to fishes from smaller tributaries where environmental methylation of Hg and subsequent bioaccumulation may be accentuated.

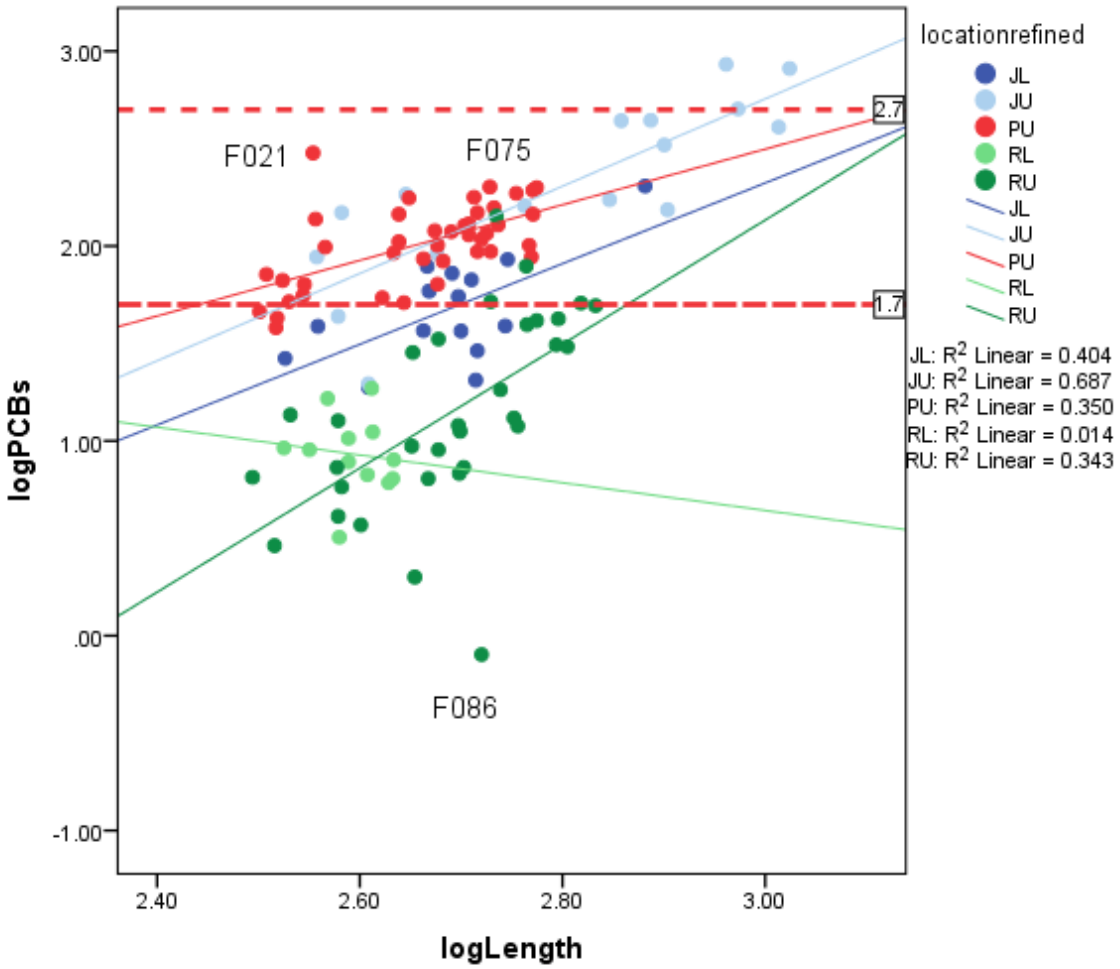


Plot of log Hg versus log $\delta^{15}N$ for blue catfish fillets sampled from Chesapeake Bay tributaries (location abbreviations as defined previously). Potomac River fish fillets exhibited the highest $\delta^{15}N$ values, but the lowest Hg concentrations.

Hg concentrations were expected to increase with trophic level, i.e. rise with increasing $\delta^{15}N$ (Power et al 2002). This was the case here except for Potomac River fillet samples, which exhibited the highest $\delta^{15}N$ values, but lowest Hg concentrations.

Synthetic organic pollutants

PCBs: Among the contaminants we assessed, PCB levels in blue catfish were the most problematic relative to the current VDH two and three meals/month fish consumption advisories (50 and 500 ng/g wet weight, respectively).



Total PCB concentrations relative to the 50 and 500 ng/g wet weight VA consumption limits (horizontal red dashed lines: two meal and zero meals per month; log=1.7 and 2.7, respectively). Several high and low PCB concentration outliers are identified by sample ID. Location abbreviations as defined previously.

Total PCB concentrations in fish fillets varied widely, ranging from 0.8 to 856 µg/kg (wet weight). The lowest fillet PCB concentration was observed from a fish that exhibited the lowest weight at size (F086 outlier). The overall mean PCB concentration among all fish sampled was 97.4 µg/kg (SD 136). PCB congener patterns as a percent of total PCBs in blue catfish fillets among fish from the three rivers were similar. However, contributions of the more chlorinated congeners were higher in James River fish (e.g. PCB 180 to PCB 209). The largest fish sampled were from the James River (>800 mm) and this may have played a role.

GLM analysis (SPSS results: see table below) indicated that fish length, fillet %lipid and sampling location affected tissue PCB concentrations (P<0.001), but that fillet $\delta^{15}N$ and fish sex were not significant predictors (P=0.318 and 0.090, respectively). However, model assumptions may have been violated as Levene's test of equality of error variances was significant (p<0.001). The Sidak *post-hoc* test indicated that mean PCB levels in fish obtained from the lower James River were similar to concentrations from fish from the upper James and Potomac rivers, but higher than fish from either the upper or lower Rappahannock River. PCB concentrations in fish from the upper and lower Rappahannock River were similar and the lowest observed. The mean PCB concentration for Potomac River fish was greater than mean PCB concentrations for Rappahannock fish and similar to James River fish.

Tests of Between-Subjects Effects

Dependent Variable: logPCBs

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	33.410[a]	14	2.386	33.260	.000
Intercept	1.263	1	1.263	17.603	.000
logLength	3.584	1	3.584	49.952	.000
log15N	.072	1	.072	1.009	.318
loglipidwet	1.062	1	1.062	14.805	.000
locationrefined	13.372	4	3.343	46.592	.000
sex	.354	2	.177	2.465	.090
locationrefined * sex	.052	5	.010	.146	.981
Error	7.247	101	.072		
Total	358.598	116			
Corrected Total	40.656	115			

a. R Squared = .822 (Adjusted R Squared = .797)

Pairwise Comparisons

Dependent Variable: logPCBs

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
JL	JU	-.260[a,b]	.113	.210	-.582	.063
	PU	-.092[a]	.140	.999	-.492	.309
	RL	.680[a,*]	.117	.000	.346	1.014
	RU	.664[a,b,*]	.092	.000	.400	.928
JU	JL	.260[a,b]	.113	.210	-.063	.582
	PU	.168[a]	.161	.972	-.293	.630
	RL	.939[a,*]	.134	.000	.557	1.322
	RU	.924[a,b,*]	.099	.000	.641	1.206
PU	JL	.092[b]	.140	.999	-.309	.492
	JU	-.168[b]	.161	.972	-.630	.293
	RL	.771[*]	.119	.000	.429	1.113
	RU	.756[b,*]	.115	.000	.427	1.084
RL	JL	-.680[b,*]	.117	.000	-1.014	-.346
	JU	-.939[b,*]	.134	.000	-1.322	-.557
	PU	-.771[*]	.119	.000	-1.113	-.429
	RU	-.016[b]	.099	1.000	-.299	.268
RU	JL	-.664[a,b,*]	.092	.000	-.928	-.400
	JU	-.924[a,b,*]	.099	.000	-1.206	-.641
	PU	-.756[a,*]	.115	.000	-1.084	-.427
	RL	.016[a]	.099	1.000	-.268	.299

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Sidak.

PCB concentrations increased significantly with fish length, and all but one (F086-upper Rappahannock River) blue catfish exceeded the EPA unrestricted consumption advisory limit (> 1.5 ng/g; PCB Table). A 10% increase in fish length for blue catfish from the James and Rappahannock rivers resulted in a 30% increase in PCB levels, whereas a 10% increase in fish length in the Potomac River increased PCB levels by 14.6%.

Three large James River fish surpassed the VDH “zero meals” (>500 µg/kg) advisory level and 18 fish fell within the “two 8 oz meals/month” (50 µg/kg < x <500 µg/kg) advisory level. Hence, 68% of the James River fish sampled (21 of 31) met or exceeded the two or 8 oz meals/month PCB concentration limitation. No Potomac River fish fell into the “consume zero meals” category, but 93% were in the “two 8 oz meals/month” limit category. For the Rappahannock River only 9% were in the “two 8 oz meals/month” limit category. Fish fillet

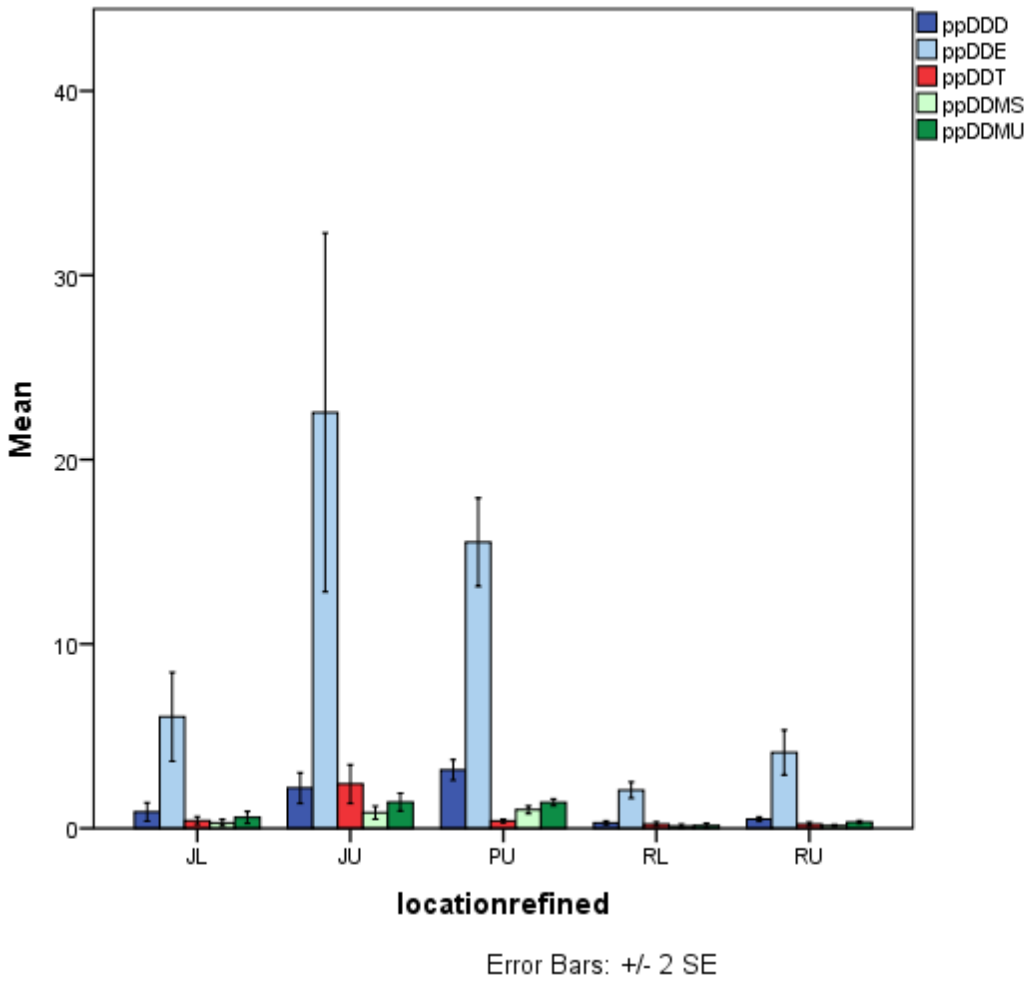
F021 from the Potomac and F075 from the Rappahannock exhibited unexpectedly high PCB levels relative to their size. Fish F021 exhibited the highest % lipid content (35.1%) of all fillets tested; while F075 contained 13.2% lipid.

All but three blue catfish from the Potomac River exceeded the EPA four meals/month advisory threshold (59 ng/g). Similarly, many blue catfish less than 600 mm FL from the James River had PCB levels above this limit. All James River fish larger than 600 mm FL exceeded it. In the Rappahannock River, only two individuals exceeded the 4 meals/month advisory limit.

Note that the EPA cancer risk endpoints (16 and 12 ng/g for two and three meal/month) are lower than the non-cancer risk endpoints.

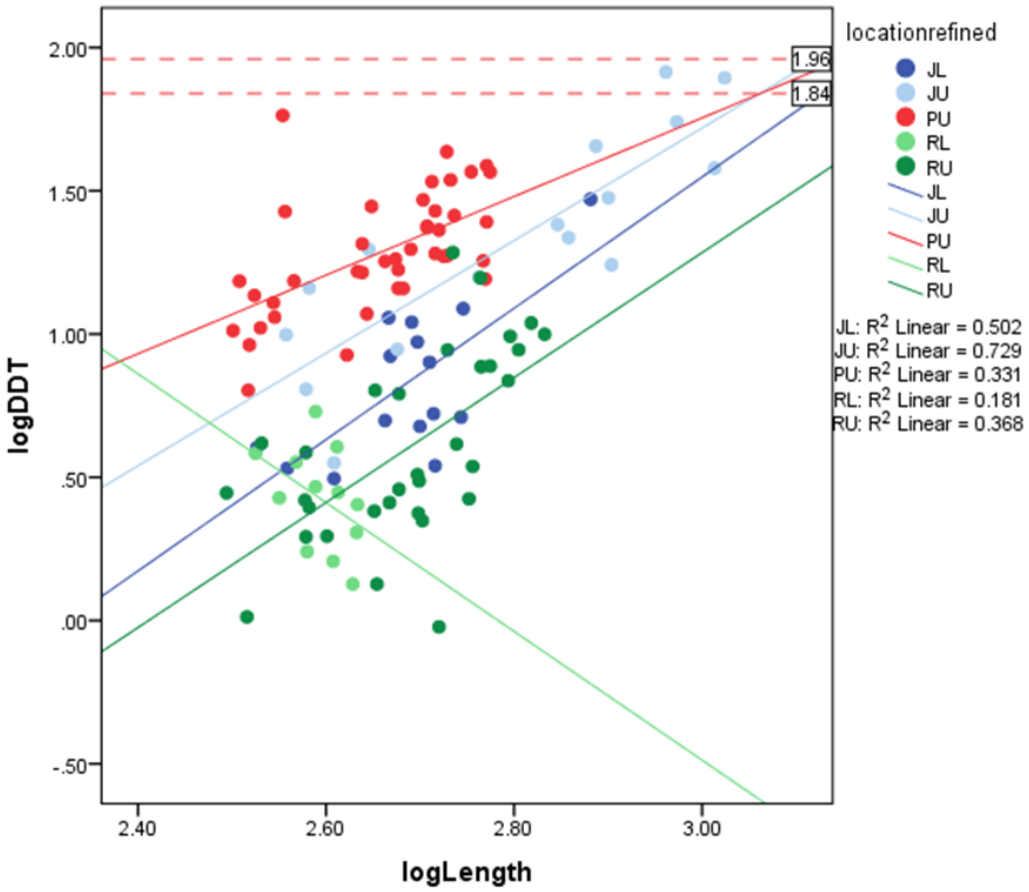
Organochlorine pesticides

DDTs: Summed DDTs consisted of the total of p,p' and o, p' isomers of DDT; plus the DDT degradates DDE, DDD, p,p'-DDMS and p,p'-DDMU. The major component was p,p'-DDE, followed by p,p'-DDD. The o,p isomers were infrequently encountered, as is typically the case. As seen in the below figure, concentrations were generally highest for fish from the upper James and Potomac rivers and lowest for fish from the Rappahannock and lower James rivers.



Concentrations of DDT components (ng/g wet weight) for blue catfish sampled from Chesapeake Bay tributaries in 2011 and 2012 (location abbreviations as defined previously).

In general, summed DDTs in blue catfish fillets increased significantly with length, but fish from the lower Rappahannock were an exception, possibly because the range of sizes obtained from this location was limited.



Concentrations of log summed DDTs (ng/g wet weight) versus log length (mm) for blue catfish sampled from Chesapeake Bay tributaries (location abbreviations as defined previously). The EPA two and three meal per month limits are 92 and 69 ng/g (log equivalents 1.96 and 1.84, respectively - dashed horizontal red lines). The EPA unrestricted consumption limit is 8.6 ng/g (log=0.93).

Fish sampling location, length, and fillet %lipid were significant factors predicting the variation in log summed DDT levels in blue catfish ($p<0.001$ for all, see table below), but model assumptions may have been violated. Levene's Test of equality of error variances was significant ($p=0.048$). This model appeared to account for 67% of the total variation in log DDT concentrations. $\delta^{15}N$ and sex were not significant predictors ($P=0.998$ and 0.394, respectively) of total DDT concentration. Sidak *post-hoc* pairwise comparisons suggested that only summed DDT concentrations in fillets from the upper Rappahannock River differed statistically (0.05 level), being lower than concentrations in fillets from the upper James and Potomac rivers,

Tests of Between-Subjects Effects

Dependent Variable: sumDDTs

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	17795.824[a]	14	1271.130	17.390	.000
Intercept	1151.175	1	1151.175	15.749	.000
loglipidwet	1700.877	1	1700.877	23.269	.000
log15N	.001	1	.001	.000	.998
logLength	3534.665	1	3534.665	48.357	.000
locationrefined	3460.552	4	865.138	11.836	.000
sex	137.291	2	68.645	.939	.394
locationrefined * sex	152.945	5	30.589	.418	.835
Error	7382.610	101	73.095		
Total	49620.533	116			
Corrected Total	25178.434	115			

a. R Squared = .707 (Adjusted R Squared = .666)

Pairwise Comparisons

Dependent Variable: sumDDTs

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
JL	JU	-8.519[a,b]	3.598	.181	-18.818	1.779
	PU	-7.234[a]	4.465	.682	-20.012	5.545
	RL	3.500[a]	3.726	.986	-7.165	14.166
	RU	7.702[a,b]	2.949	.099	-.738	16.141
JU	JL	8.519[a,b]	3.598	.181	-1.779	18.818
	PU	1.286[a]	5.145	1.000	-13.441	16.013
	RL	12.020[a]	4.264	.056	-.183	24.223
	RU	16.221[a,b,*]	3.152	.000	7.200	25.243
PU	JL	7.234[b]	4.465	.682	-5.545	20.012
	JU	-1.286[b]	5.145	1.000	-16.013	13.441
	RL	10.734	3.814	.057	-.181	21.649
	RU	14.936[b,*]	3.667	.001	4.441	25.430
RL	JL	-3.500[b]	3.726	.986	-14.166	7.165
	JU	-12.020[b]	4.264	.056	-24.223	.183
	PU	-10.734	3.814	.057	-21.649	.181
	RU	4.201[b]	3.162	.874	-4.850	13.252
RU	JL	-7.702[a,b]	2.949	.099	-16.141	.738
	JU	-16.221[a,b,*]	3.152	.000	-25.243	-7.200
	PU	-14.936[a,*]	3.667	.001	-25.430	-4.441
	RL	-4.201[a]	3.162	.874	-13.252	4.850

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

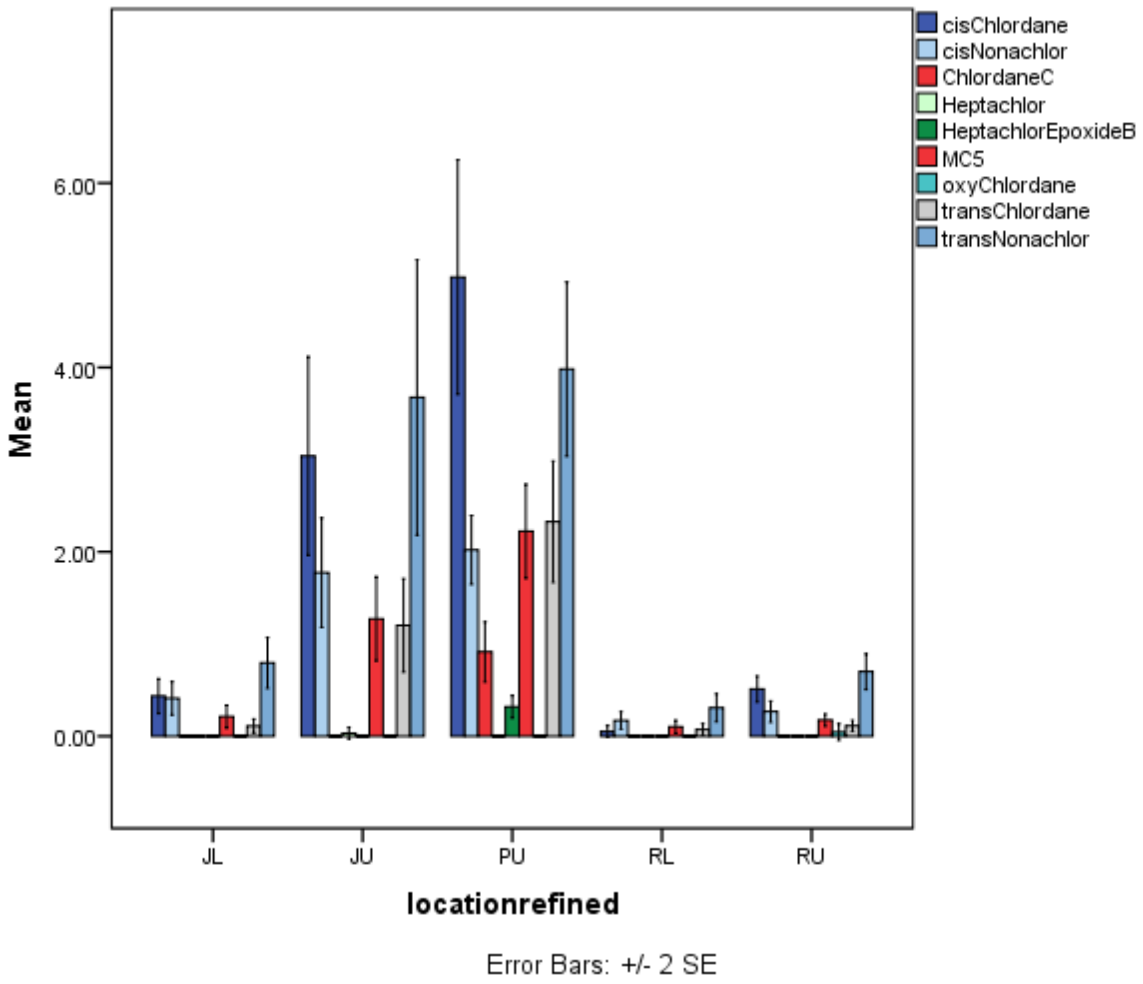
a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Sidak.

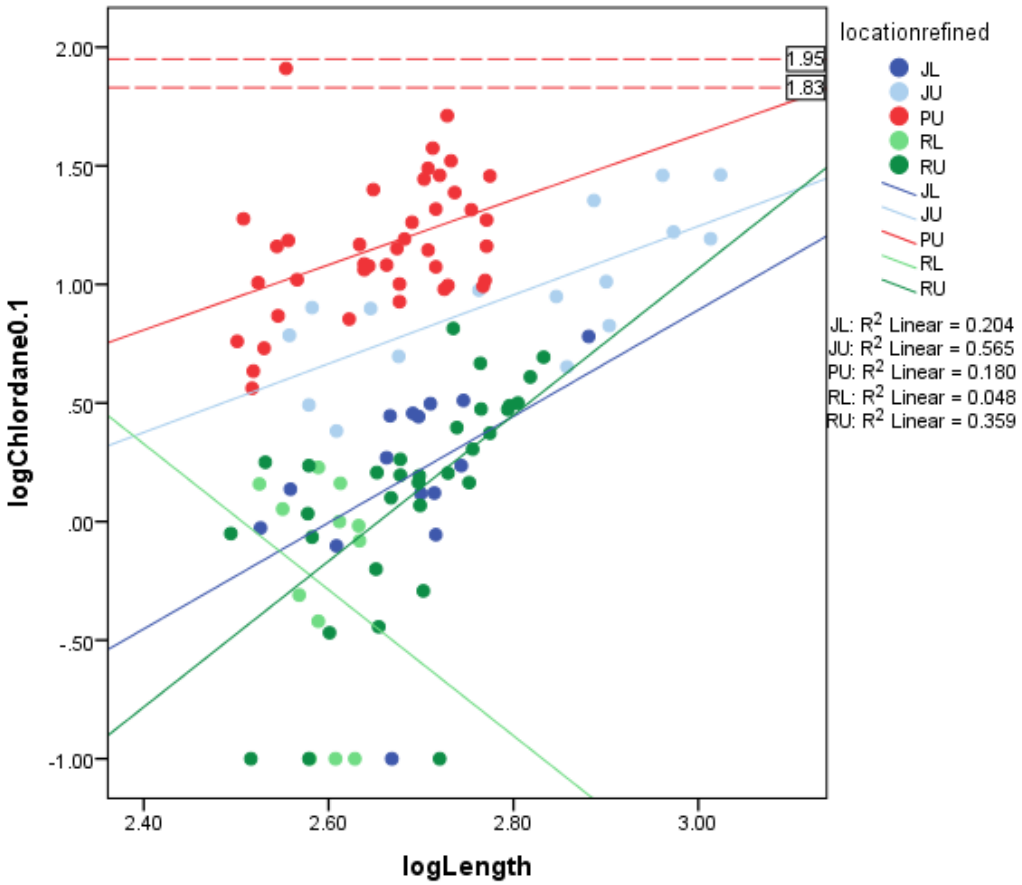
For blue catfish from the James River, a 10% increase in length resulted in a 23% increase in summed DDT levels, whereas in the Potomac and Rappahannock river fish, the same increase in length resulted in an increase of 14% and 21%, respectively. Summed DDT levels in Potomac River blue catfish were generally higher than comparably-sized fish from the James and Rappahannock rivers, with most fish less than 600 mm FL in the Potomac River exceeding the EPA consumption limit (8.6 ng/g; <16 meals/month). Note that 16 meals per month is a far greater number of meals than is generally delineated in advisories in MD and VA (i.e. normally 2-3 meals; with attendant higher allowable concentration limits). Similarly, most blue catfish from the James River exceeded EPA limits (8.6 ng/g; <16 meals/month). Blue catfish >550 mm FL collected in the Rappahannock River exceeded EPA limits (8.6 ng/g; <16 meals/month), but Rappahannock River fish smaller than 550 mm FL were below the advisory threshold for unlimited consumption. Two large fish from the upper James River exceeded the EPA three meals/month advisory limit (see figure above).

Chlordanes: Chlordane was primarily used to control termites and its use was banned in 1988. However, like most of the other contaminants we examined, it is environmentally persistent. Total chlordane as defined here consisted of the sum of 15 components, the most abundant of which are shown in the below figure. Cis-chlordane and trans-nonachlor were the dominant components in fish fillets from the three rivers sampled. Concentrations (expressed in ng/g wet weight) were generally highest in fillets from the Potomac and upper James rivers. Component profiles were similar in fish from the three rivers.



Contributions (ng/g wet weight) of the dominant chlordane components detected in blue catfish fillets from tributaries of Chesapeake Bay (location abbreviations as defined previously).

Chlordane levels in blue catfish increased significantly with fish length in the James and upper Rappahannock rivers. Chlordanes were not detected in all samples, particularly in those from Rappahannock River fish. In general, a 10% change in fish length was associated with an increase in total chlordane levels of 15.7% in blue catfish from the James River and 18% in fish from the Rappahannock River.



Log of summed chlordane +0.1 ng/g (to allow calculation of log concentrations for non-detected values) versus log fish length (mm) for blue catfish from Chesapeake Bay tributaries (location abbreviations as defined previously). The EPA two and three meals/month cancer advisory limits are 89 and 67 ng/g (log equivalent 1.95 and 1.83, respectively). The high outlier (red dot) is F021. The EPA unrestricted consumption limit is 8.4 ng/g (log=0.92).

As was the case for the organic contaminants previously discussed, sampling location, fish length, and fillet %lipid were good predictors (all $p<0.001$). $\delta^{15}N$ and sex were poorer predictors ($p=0.062$ and 0.066, respectively). The GLM using these factors accounted for 80% of the total variation in log summed chlordane (0.1 was added to all values to account for concentrations not detected) (see table below). Summed chlordanes in Potomac River fish fillets exceeded those in fish from all other locations. Total chlordane levels in lower and upper Rappahannock fish fillets were similar; but lower than upper James and Potomac fish. Summed chlordane was lower in fish from the lower James River than in fish from the Potomac River, but greater than those from the lower Rappahannock River.

Tests of Between-Subjects Effects

Dependent Variable: logChlordane0.1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	41.886[a]	14	2.992	33.763	.000
Intercept	.174	1	.174	1.968	.164
loglipidwet	2.463	1	2.463	27.796	.000
log15N	.316	1	.316	3.561	.062
logLength	3.038	1	3.038	34.281	.000
locationrefined	13.791	4	3.448	38.908	.000
sex	.496	2	.248	2.799	.066
locationrefined * sex	.343	5	.069	.773	.571
Error	8.950	101	.089		
Total	88.268	116			
Corrected Total	50.836	115			

a. R Squared = .824 (Adjusted R Squared = .800)

Pairwise Comparisons

Dependent Variable: logChlordane0.1

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
JL	JU	-.278[a,b]	.125	.251	-.637	.080
	PU	-.896[a,*]	.155	.000	-1.341	-.451
	RL	.439[a,*]	.130	.010	.068	.810
	RU	.244[a,b]	.103	.177	-.050	.538
JU	JL	.278[a,b]	.125	.251	-.080	.637
	PU	-.618[a,*]	.179	.008	-1.131	-.105
	RL	.717[a,*]	.148	.000	.292	1.142
	RU	.523[a,b,*]	.110	.000	.209	.837
PU	JL	.896[b,*]	.155	.000	.451	1.341
	JU	.618[b,*]	.179	.008	.105	1.131
	RL	1.335[*]	.133	.000	.955	1.715
	RU	1.140[b,*]	.128	.000	.775	1.506
RL	JL	-.439[b,*]	.130	.010	-.810	-.068
	JU	-.717[b,*]	.148	.000	-1.142	-.292
	PU	-1.335[*]	.133	.000	-1.715	-.955
	RU	-.195[b]	.110	.566	-.510	.120
RU	JL	-.244[a,b]	.103	.177	-.538	.050
	JU	-.523[a,b,*]	.110	.000	-.837	-.209
	PU	-1.140[a,*]	.128	.000	-1.506	-.775
	RL	.195[a]	.110	.566	-.120	.510

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

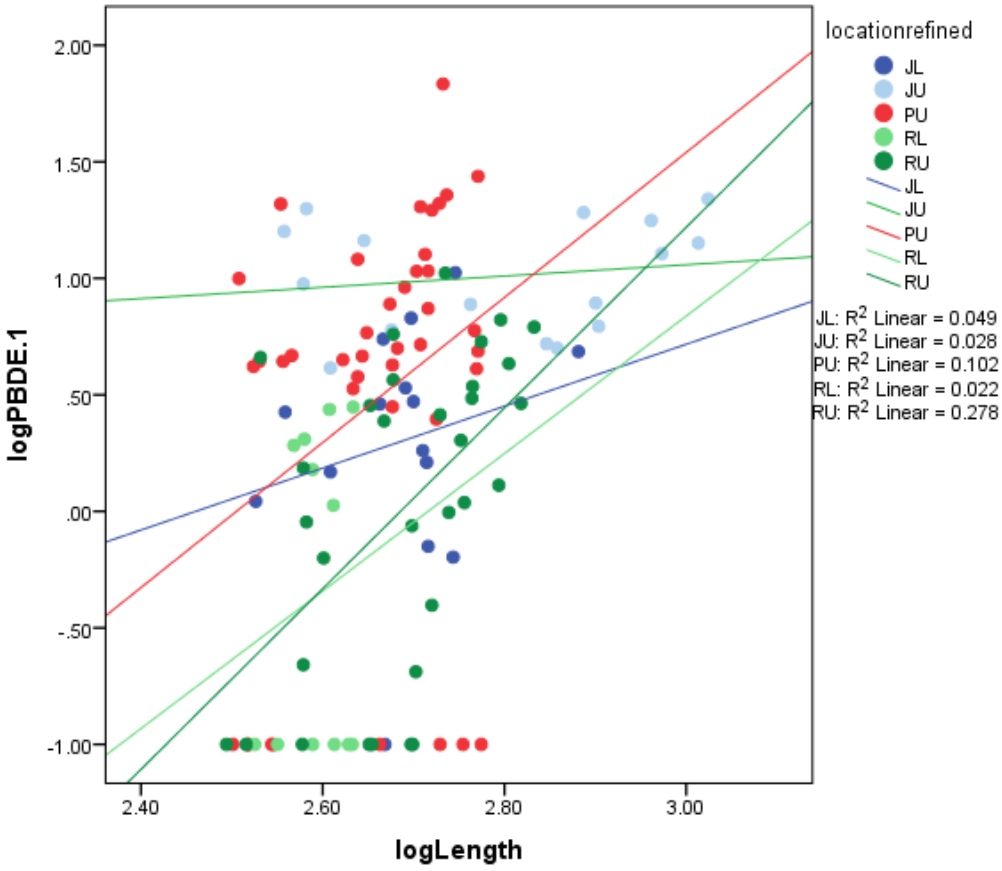
a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Sidak.

Most catfish sampled from the Potomac River exceeded the EPA chlordane advisory limit for unrestricted consumption (8.4 ng/g; <16 meals/month). The highest total chlordane level was found in the fillet of a 358 mm FL blue catfish from the Potomac River (F021; also a high concentration outlier for summed DDT and % lipid): this fish (F021) contained summed chlordanes of 81.2 ng/g, exceeding the EPA three meals/month advisory limit (67 ng/g). Fillets from blue catfish in the James River larger than 600 mm FL exceeded advisory limits for chlordane, while smaller fish approached the 16 meals/month consumption threshold. No blue catfish from the Rappahannock River exceeded EPA advisory limits for chlordane at the sizes analyzed in this study.

PBDEs: PBDEs were also detected in some fish fillets. These compounds were used extensively as flame retardants in polymers, but their manufacture for use in new products was discontinued in December 2004. However, materials with PBDEs as additives remain in wide use today. The analysis scheme employed here focused on the tri- to octabrominated congeners, which are the most bioaccumulative. As was the case for chlordanes, PBDEs were not detected in some fish, so to permit statistical analyses of log transformed data, 0.1 ng/g was added to all measured concentrations. In general, PBDE concentrations increased with fish length and were highest for fish from the Potomac and upper James rivers, and lowest for Rappahannock River fish. VDH previously developed a two meal per month PBDE advisory limit of 500 ng/g for fish (http://leg2.state.va.us/dls/h&sdocs.nsf/fc86c2b17a1cf388852570f9006f1299/6f3e559a07dacacd85257814006ee68d/$FILE/RD423.pdf). None of these blue catfish fillet concentrations approached the 500 ng/g limit. However, this limit was not based on potential neurodevelopmental effects in prenatally exposed humans, likely the most sensitive endpoint (Herbstman et al 2010). Hence, it is not toxicologically conservative.



Summed PBDE concentrations +0.1 ng/g (to allow calculation of log values) versus fish length for blue catfish sampled from Chesapeake Bay tributaries in 2011 and 2012 (location abbreviations as previously defined).

Consistent with the other organic contaminants discussed above, GLM analysis suggested that fish length, fillet %lipid and sampling location predicted PBDE concentration ($p<0.05$). Fish sex and fillet $\delta^{15}N$ were not significant predictors ($p=0.595$ and 0.098, respectively; see table below). However, this model accounted for only 32% of the variation in PBDE concentrations.

Tests of Between-Subjects Effects

Dependent Variable: logPBDE.1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	28.196[a]	14	2.014	4.921	.000
Intercept	8.544E-005	1	8.544E-005	.000	.989
loglipidwet	1.949	1	1.949	4.763	.031
log15N	1.142	1	1.142	2.791	.098
logLength	3.435	1	3.435	8.395	.005
locationrefined	10.652	4	2.663	6.508	.000
sex	.427	2	.213	.521	.595
locationrefined * sex	3.048	5	.610	1.489	.200
Error	41.332	101	.409		
Total	80.826	116			
Corrected Total	69.527	115			

a. R Squared = .406 (Adjusted R Squared = .323)

Pairwise Comparisons

Dependent Variable: logPBDE.1

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
JL	JU	-.143[a,b]	.269	1.000	-.914	.627
	PU	-.265[a]	.334	.996	-1.221	.691
	RL	.626[a]	.279	.239	-.172	1.424
	RU	.533[a,b]	.221	.161	-.098	1.165
JU	JL	.143[a,b]	.269	1.000	-.627	.914
	PU	-.122[a]	.385	1.000	-1.224	.980
	RL	.769[a]	.319	.163	-.144	1.682
	RU	.677[a,b,*]	.236	.049	.002	1.352
PU	JL	.265[b]	.334	.996	-.691	1.221
	JU	.122[b]	.385	1.000	-.980	1.224
	RL	.891[*]	.285	.023	.074	1.708
	RU	.799[b,*]	.274	.043	.013	1.584
RL	JL	-.626[b]	.279	.239	-1.424	.172
	JU	-.769[b]	.319	.163	-1.682	.144
	PU	-.891[*]	.285	.023	-1.708	-.074
	RU	-.092[b]	.237	1.000	-.770	.585
RU	JL	-.533[a,b]	.221	.161	-1.165	.098
	JU	-.677[a,b,*]	.236	.049	-1.352	-.002
	PU	-.799[a,*]	.274	.043	-1.584	-.013
	RL	.092[a]	.237	1.000	-.585	.770

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (I).

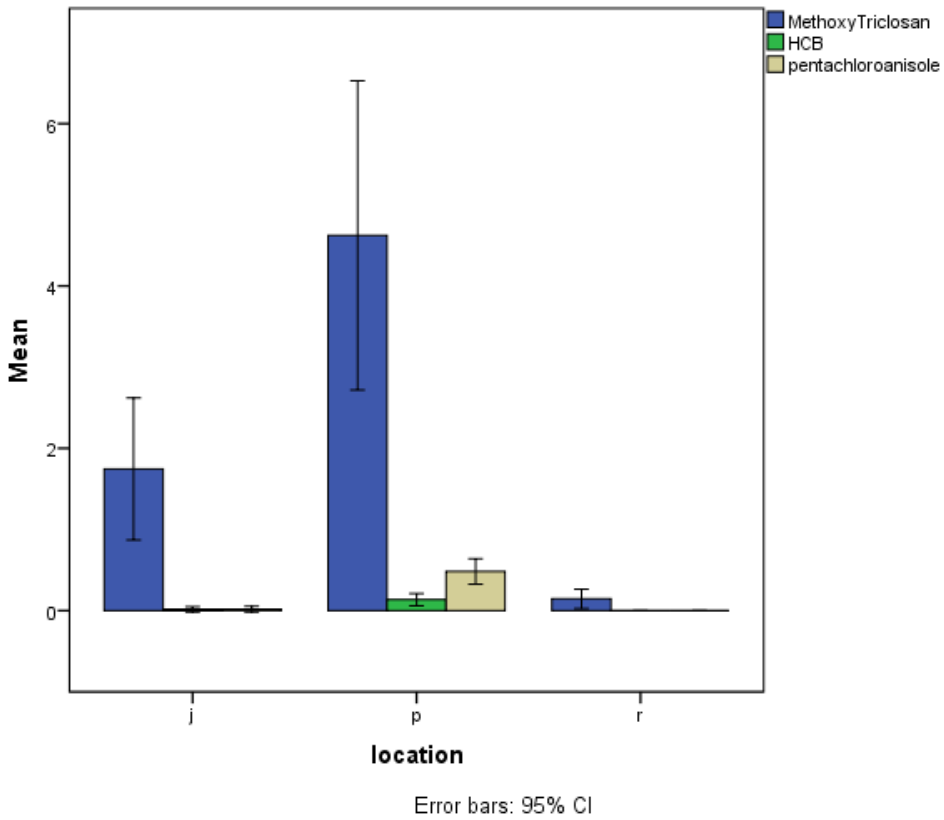
b. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Sidak.

Fillets from fish from the upper James River exhibited significantly higher PBDE concentrations than those from the upper Rappahannock River, based on Sidak's *post-hoc* test. Potomac River fish fillet levels were higher than those from either Rappahannock River segment.

Other emerging contaminants

Among these, methoxytriclosan was the most abundant, followed by pentachloroanisole and hexachlorobenzene (HCB). Concentrations of all three were highest in the Potomac River fish. Methoxytriclosan is a moderately lipophilic environmental transformation product of the antibacterial agent, triclosan. HCB was used as a fungicide. Pentachloroanisole is believed to typically be a degradate of the preservative pentachlorophenol.

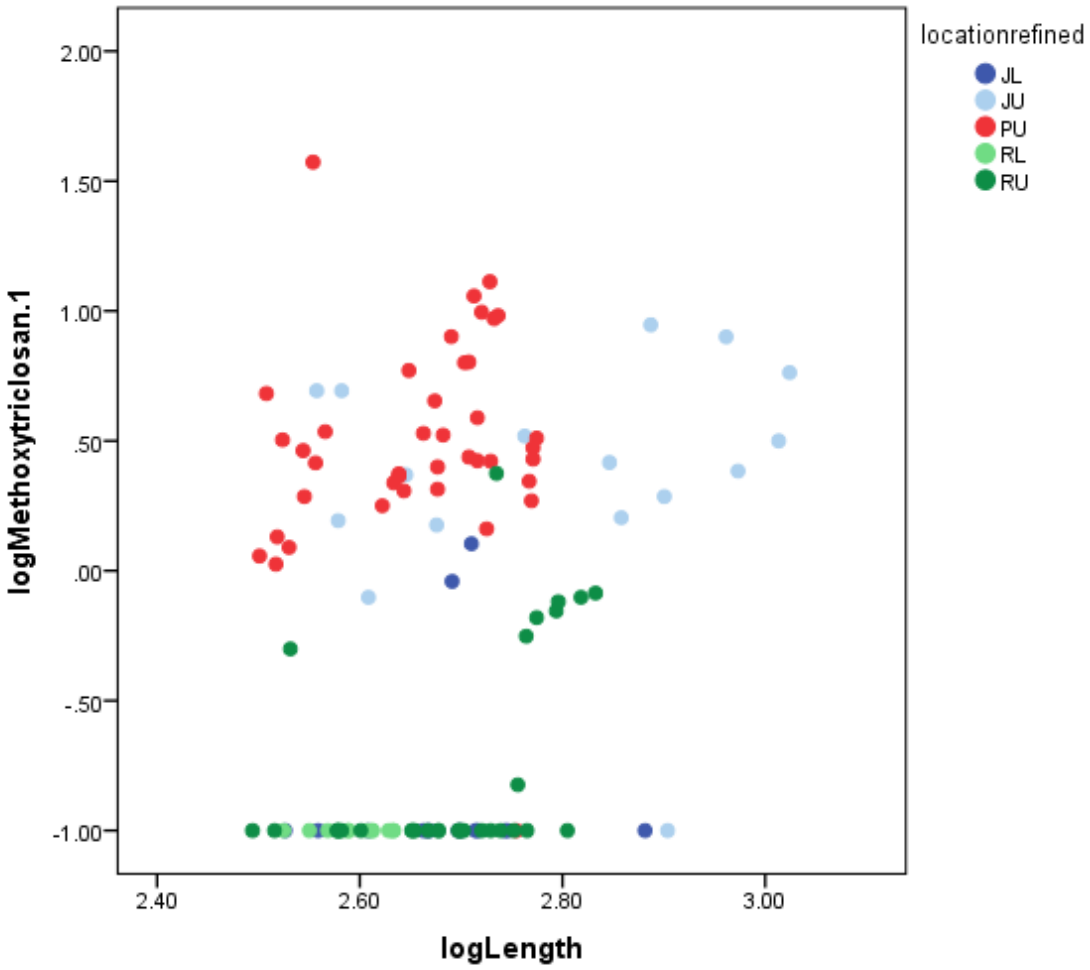


Mean (SE) Methoxytriclosan, Hexachlorobenzene, and Pentachloranisole levels in the James (J), Potomac (P), and Rappahannock (R) rivers in 2011 and 2012.

Pentachloroanisole: Concentrations in fillets were low in the blue catfish analyzed in this study. Pentachloroanisole levels were significantly correlated with hexachlorobenzene concentrations (Pearson's rho=0.78, $p < 0.001$), suggesting a similar source for these two contaminants.

Hexachlorobenzene: Detected levels were low in fillets, with the highest (1.18 ng/g) observed in the blue catfish from the Potomac River (F021) that exhibited the highest concentration of summed chlordanes. Hexachlorobenzene levels in blue catfish fillets were greatest in Potomac River fish. However, values were well below advisory limits for consumption at all sizes observed. Only one other fish (from the James River) had levels of hexachlorobenzene above the analytical detection limit.

Methoxytriclosan: This triclosan degradate (also known as methyl triclosan) was detected at highest concentrations in blue catfish fillets from the Potomac and upper James Rivers. Fish F021 from the Potomac River contained distinctly higher methoxytriclosan than the other fish fillets. Concentrations were lowest in fish from the Rappahannock and lower James rivers. Methoxytriclosan levels were not significantly related to fish length, which is consistent with opportunistic and recent exposures.



Log methoxytriclosan concentrations + 0.1 ng/g versus fish length for blue catfish sampled in 2011 and 2012 from Chesapeake Bay tributaries (location abbreviations as previously defined).

Fillet lipid content, fish length and sampling location were significant ($p<0.001$, $p=0.042$, $p<0.001$, respectively) factors accounting for 82% of the total variation in log methoxytriclosan concentrations in blue catfish (0.1 was added to the methoxytriclosan values to permit log transformation prior to analysis). Sex and $\delta^{15}N$ were not significant ($p=0.574$ and 0.105, respectively) factors in this model.

Tests of Between-Subjects Effects

Dependent Variable: logMethoxytriclosan.1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	55.908[a]	14	3.993	38.906	.000
Intercept	.001	1	.001	.012	.912
logLength	.436	1	.436	4.246	.042
loglipidwet	3.554	1	3.554	34.627	.000
log15N	.275	1	.275	2.679	.105
locationrefined	22.706	4	5.677	55.304	.000
sex	.114	2	.057	.557	.574
locationrefined * sex	.797	5	.159	1.552	.180
Error	10.367	101	.103		
Total	70.986	116			
Corrected Total	66.275	115			

a. R Squared = .844 (Adjusted R Squared = .822)

Pairwise Comparisons

Dependent Variable: logMethoxytriclosan.1

(I) locationrefined	(J) locationrefined	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
JL	JU	-.925[*,b,c]	.135	.000	-1.311	-.539
	PU	-1.243[*,b]	.167	.000	-1.722	-.764
	RL	.189[b]	.140	.859	-.210	.589
	RU	.043[b,c]	.110	1.000	-.274	.359
JU	JL	.925[*,b,c]	.135	.000	.539	1.311
	PU	-.319[b]	.193	.658	-.870	.233
	RL	1.114[*,b]	.160	.000	.657	1.571
	RU	.967[*,b,c]	.118	.000	.629	1.305
PU	JL	1.243[*,c]	.167	.000	.764	1.722
	JU	.319[c]	.193	.658	-.233	.870
	RL	1.433[*]	.143	.000	1.024	1.842
	RU	1.286[*,c]	.137	.000	.893	1.679
RL	JL	-.189[c]	.140	.859	-.589	.210
	JU	-1.114[*,c]	.160	.000	-1.571	-.657
	PU	-1.433[*]	.143	.000	-1.842	-1.024
	RU	-.147[c]	.119	.915	-.486	.192
RU	JL	-.043[b,c]	.110	1.000	-.359	.274
	JU	-.967[*,b,c]	.118	.000	-1.305	-.629
	PU	-1.286[*,b]	.137	.000	-1.679	-.893
	RL	.147[b]	.119	.915	-.192	.486

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. An estimate of the modified population marginal mean (I).

c. An estimate of the modified population marginal mean (J).

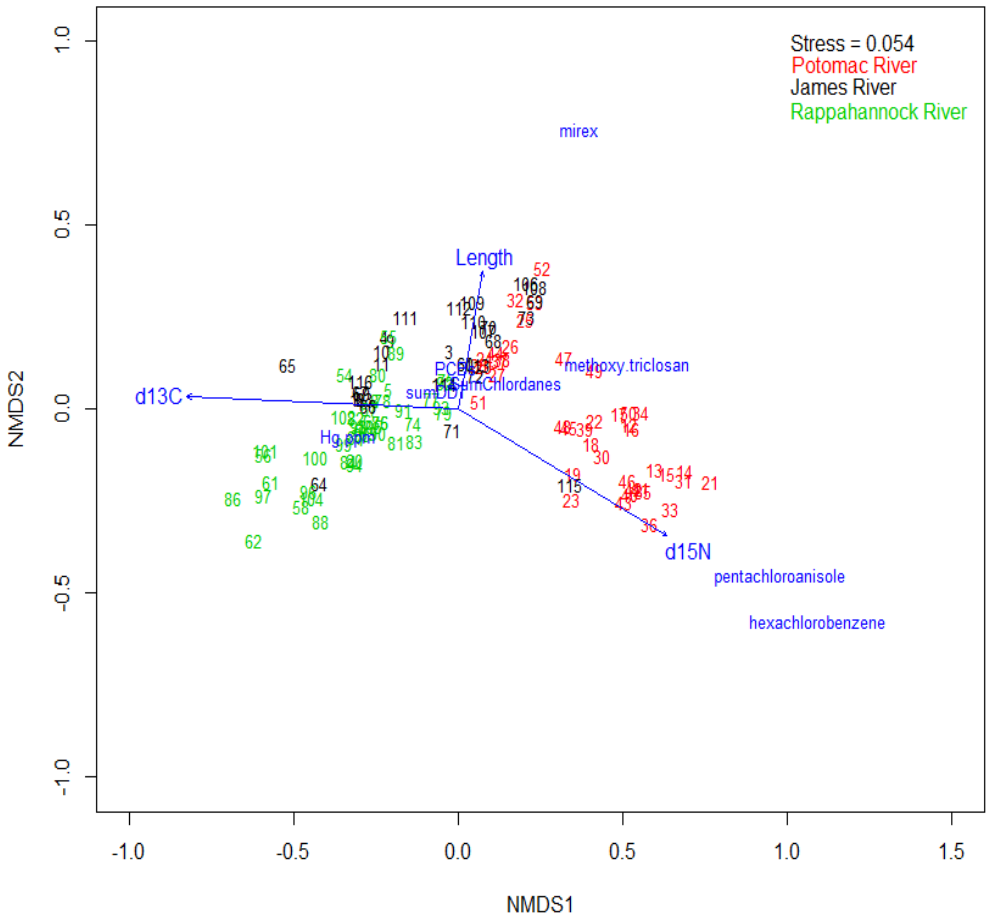
d. Adjustment for multiple comparisons: Sidak.

Based on Sidak's *post-hoc* test, blue catfish fillets from the Potomac contained higher methoxytriclosan concentrations than all but the upper James. The upper James River contained significantly higher methoxytriclosan levels than fish from the Rappahannock and the lower James. Fish from the lower James River exhibited concentrations of methoxytriclosan similar to those observed in fish from the Rappahannock River.

Mirex is an insecticide that was used in the southeast US primarily for fire ant control. It was banned in 1976. Mirex was detected only in blue catfish greater than 700 mm FL from the James and Potomac rivers. Observed Mirex levels were well below advisory limits for all fish analyzed in this study.

Multivariate analysis

Multivariate analysis (using NMDS) of contaminants found in blue catfish fillets demonstrated river-specific contaminant signatures in the James, Rappahannock, and Potomac rivers as indicated by the clustering of samples from each river (NMDS figure below). Fish from all three rivers contained relatively similar levels of PCBs, chlordanes, Hg, and DDTs as indicated by the grouping of these contaminants in the center of the ordination. Other contaminants were more river-specific, resulting in the spread of the observations in the ordination plane. For example, fish from the James and Potomac rivers were characterized by higher Mirex concentrations, whereas fish from the Potomac River exhibited higher hexachlorobenzene and pentachloroanisole levels. Potomac River fish were also characterized by higher levels of methoxytriclosan. Trophic position was significantly correlated with NMDS axes, with the Potomac River fish exhibiting significantly higher $\delta^{15}N$ values. Length was significantly correlated with James River blue catfish, however the largest fish were from the James River. The low stress level (stress = 0.054) indicated that the two-dimensional plot of the multivariate data cloud was representative of the relationship between rivers and contaminants and was a good fit to the data. We plan to pursue additional model fitting with these data.



Non-metric multidimensional analysis of individual blue catfish contaminant loads (chlorinated pesticides, PCBs, Hg, and emerging chemicals of concern) from the James (black), Rappahannock (green), and Potomac (red) rivers collected in 2011 and 2012. Blue arrows indicate significant correlations between NMDS axes and $\delta^{13}C$, $\delta^{15}N$, and fish length with length of the arrow indicative of the strength of the correlation. Relative contaminant loads are shown in blue.

Discussion

Pollutant concentration thresholds for issuance of fish consumption advisories are a function of the amount of fish consumed (typically tracked by the number of 8 oz meals consumed per month). MD and VA typically issue advisories based on an anticipated

consumption of a 2-3 meals/month. However, the US EPA provides advisory guidance for up to 16 meals/month (see tables in the Appendix), with decreasing concentration limits as the number of monthly meals increases. "No restrictions" by the EPA refers to the consumption of more than 16 meals/month. Hence, the absence of a publicized (2-3 meal/month) state/commonwealth consumption advisory does not necessarily mean it is safe to consume an unlimited amount of fish. Typically, EPA and state health departments suggest further limiting consumption of contaminated fish by particularly vulnerable members of the population, e.g. pregnant women, women of child-bearing age and children.

Contaminant concentrations in many blue catfish fillets we sampled exceeded EPA limits for unrestricted consumption and, in some cases, exceeded existing VA or MD limits based on two or fewer meals per month. The latter findings were consistent with pre-existing state advisories for the locale and similar species. However, contaminants in blue catfish in many smaller Bay watershed tributaries have not been evaluated. Our work focused on the main stems of the James, Rappahannock and Potomac rivers. Assuming the absence of organic contaminant point sources, we anticipate pollutant concentrations (with the possible exception of chlordanes) in fish from smaller tributaries will be similar or lower than concentrations reported here from the major tributaries. However, mercury may be higher due to greater *in-situ* methylation in smaller tributaries and wetlands (St Louis et al, 1994). Therefore, additional sampling to augment our study appears warranted.

The relationship between fish length and contaminant concentration varied as a function of pollutant identity and location. The pollutant burdens accumulated in individual fish are a product of the degree of contaminant exposure (e.g. the presence of local point sources or biotransformation potential in the case of methylmercury) and species-specific characteristics such as diet and physiological condition. Hence, simple length-based advisories for blue catfish encompassing wide geographical areas, while useful, cannot ensure consumers will not be exposed to unacceptable contaminant burdens. Individual fish may also exhibit pollutant contaminant concentrations well in excess of the mean observed for a given tributary (e.g. F021 in our study). However, other fish from the same location may exhibit

proportionately lower levels. The approach taken in this study -- to examine individual specimens instead of compositing multiple individuals, as is often done in state monitoring programs due to budgetary limitations -- allowed for the detection of such outliers. Furthermore, such analyses permitted a more accurate characterization of the distribution of contaminants among wild populations by accounting for individual variation.

We observed that PCB levels in blue catfish surpassed more existing advisory thresholds than other contaminants examined here, including mercury. The highest PCB concentrations were detected in blue catfish from the (upper) Potomac River and the upper James River. This pattern was likely related to substantial urbanization of these watersheds (e.g. Washington DC and Richmond VA). Accordingly, one might anticipate elevated PCB levels in lower James River fish near Hampton Roads, but this was not the case. This may reflect historically lower input of PCBs or tidal flushing of the lower James River.

Blue catfish from the Potomac River exhibited organic contaminant levels similar to, or in excess of those observed in fish from the other river systems sampled. High levels of fillet lipid may have influenced the concentrations of lipophilic contaminants in these fish (e.g. chlordanes, DDTs and PCBs in F021). Indeed, lipid content and PCBs, DDTs and chlordane concentrations were significantly correlated ($p<0.001$; two-tailed Pearson Correlation), but concentrations of non-lipophilic mercury were not correlated with lipid content. Methylmercury, the common form bioaccumulated in fish, associates with proteins (Kutscher et al, 2012).

Methoxytriclosan appears to be a contaminant of emerging concern, especially as domestic wastewater volumes increase in the Chesapeake Bay watershed. The parent compound, triclosan, is an antibacterial agent used in many household products. It is fairly polar and thus exhibits a modest bioaccumulation potential. Methoxytriclosan is more bioaccumulative and its distribution in fish likely relates to recent releases from urban wastewater treatment plants (Balmer et al 2004). Large treatment plants currently exist near Richmond, VA and Washington DC.

Blue catfish from the James and Rappahannock rivers exhibited $\delta^{15}N$ and $\delta^{13}C$ signatures expected for a predator in an estuarine environment. However, signatures from the Potomac River fish fillets differed substantially from those observed for other blue catfish from the Chesapeake Bay region. Generally, $\delta^{15}N$ increases by 3 per mil per trophic level. The higher $\delta^{15}N$ values of Potomac River blue catfish thus suggest that such fish are feeding at a higher trophic level compared with those from the James and Rappahannock rivers. If so, then the higher contaminant levels may reflect both enhanced biomagnification from prey and proximity to contaminant source(s). Hg concentrations are expected to increase with trophic level, i.e. rise with increasing $\delta^{15}N$ (Power et al 2002). This was not observed here. For example, Potomac River fillet samples exhibited the highest $\delta^{15}N$ values, but lowest Hg concentrations. Previous diet studies of Potomac River blue catfish, however, do not support the inference of higher-trophic-level feeding suggested by $\delta^{15}N$ values for these fish (Schloesser et al. 2011). Thus, further research on the $\delta^{15}N$ values of blue catfish prey from the upper Potomac River is warranted.

The lower $\delta^{13}C$ values observed for Potomac River fish fillets may relate to the higher lipid content of these samples. However, the mean lipid content of lower Rappahannock fish was similar, but $\delta^{13}C$ values in lower Rappahannock River fish were higher than those observed in Potomac River fish. Sweeting et al. (2006) reported $\delta^{13}C$ was depleted in lipids relative to proteins and recommended that lipid-rich fish tissues be pre-extracted prior to isotope analyses. Lipid-content adjustments of $\delta^{13}C$ values are possible using published mathematical functions and their influence will be evaluated prior to publication of these data. As with $\delta^{15}N$, knowledge of isotopic signatures of prey items from the Potomac River food web supporting blue catfish would aid in interpreting these results.

Neither sex nor $\delta^{15}N$ values of blue catfish were statistically significant factors accounting for variation in contaminant concentrations. The lipid content of fillets, however, was significant for many of the lipophilic contaminants. However, this parameter is not convenient for fishers and consumers to measure and therefore, cannot be readily used to formulate consumption advisories.

As noted above, blue catfish frequenting areas impacted by urban or industrial pollution typically have the highest contaminant levels. Hence, sub-sections of the rivers were examined to better characterize contaminant profiles. Unfortunately, higher resolution sampling increases monitoring costs and its value may be compromised by fish relocation. Currently, the extent of individual blue catfish movements in the Chesapeake watershed is uncertain. However, several blue catfish tracking projects are now underway (e.g. http://www.serc.si.edu/labs/fish_invert_ecology/invasives/overview.aspx and a mark-recapture study in the Potomac River jointly conducted by MD DNR and VIMS). Previous research in the Missouri River indicates seasonal blue catfish movement, with some fish maintaining a limited home range, and others traveling substantial distance (Garrett and Rabeni, 2011). One aspect meriting investigation is whether the higher salinity waters at the mouths of the Chesapeake Bay tributaries deter blue catfish movement.

Recreational anglers know the capture point of their fish (although past movements may cloud certainty of contaminant exposure history), and often possess additional information on the quality of the local environment. Hence, anglers are in a position to reduce their contaminant exposure. In contrast, consumers purchasing fish at commercial markets generally have limited information regarding fish origin and contaminant load. In addition, limits on contaminants in commercial fish are typically governed by more lenient standards. For example, the commercial PCB limit in edible tissue of fish (2000 ng/g) is 40-fold higher than the recreational advisory level (US FDA 2013). However, an underlying assumption for commercial fish is that contaminant burdens will be effectively diluted by fish exhibiting low contamination. This may or may not be true, depending upon an individual consumer's shopping habits.

Acknowledgments

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synthesis. Gary Rice (W&M Chemistry Department) and associates performed the mercury determinations.

Literature Cited

Balmer, M.E., Poiger T., Droz, C., Romanin, K., Bergqvist, P., Müller, M.D., Buser, H. 2004. Occurrence of methyl triclosan, a transformation product of the pactericide triclosan, in fish from various lakes in Switzerland. Environ. Sci. Technol., 38 (2):390–5.

Garrett, D., Rabeni, C.F. 2011. Intra-annual movement and migration of flathead catfish and blue catfish in the Lower Missouri River and tributaries. American Fisheries Society Symposium 77:000–000.

Greenlee, R.S. and C. N. Lim. 2011. Searching for equilibrium: Population parameters and variable recruitment in introduced blue catfish populations in four Virginia tidal river systems. In P. Michaletz and V. Travnichek, eds., Conservation, Ecology, and Management of Worldwide Catfish Populations and Habitats. American Fisheries Society, Symposium 77, Bethesda, MD.

Herbstman J.B., Sjödin A., Kurzon M., Lederman S.A., Jones R.S., Rauh V., Needham L.L., Tang D., Niedzwiecki M., Wang R.Y., Perera F. 2010. Prenatal exposure to PBDEs and neurodevelopment. Environ Health Perspect. 118(5):712-9.

Kutscher, D.J., Sanz-Medel, A., Bettmer, J. 2012. Metallomics investigations on potential binding partners of methylmercury in tuna fish muscle tissue using complementary mass spectrometric techniques. Metallomics 4(8):807-13.

La Guardia, M.J., Hale, R.C., Harvey, Mainor, T.M., Ciparis, S. 2012. In-situ accumulation of HBCD, PBDEs and several alternative flame-retardants in the bivalve (Corbicula fluminea) and gastropod (Elimia proxima). Environ. Sci. Technol. 46(11):5798-805.

MacAvoy, S., Macko, S., McInninch, S., and Garman, G.. 2000. Marine nutrient contributions to freshwater apex predators. Oecologia, 122: 568–573.

MacAvoy, S., Garman, G., and Macko S. 2009. Anadromous fish as marine nutrient vectors. Fishery Bulletin 107:165-174.

Power, M., Klein G.M., Guiguer, K.R.R.A., Kwan, M.K.H. 2002. Mercury accumulation in the fish community of a sub-Arctic lake in relation to trophic position and carbon sources. J. Applied Ecol. 39(5), 819–830.

R Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/.

Rypel, A.L., Findlay, R. H., Mitchell, J.B., Bayne, D.R. 2007. Variations in PCB concentrations between genders of six warmwater fish species in Lake Logan Martin, Alabama, USA. Chemosphere 68, 1707–1715.

Schloesser, R.W., M.C. Fabrizio, R.J. Latour, G.C. Garman, R. Greenlee, M. Groves, J. Gartland. 2011. Ecological role of blue catfish (Ictalurus furcatus) in Chesapeake Bay communities and implications for management. Pages 369-382 in P. Michaletz and V. Travnichek, eds., Conservation, ecology, and management of worldwide catfish populations and habitats. American Fisheries Society, Symposium 77, Bethesda, MD.

St. Louis, V.L. , Rudd, J.W.M., Kelly, C.A., Beaty, K.G., Bloom, N.S., Flett, R.J. 1994. Importance of wetlands as sources of methyl mercury to boreal forest ecosystems. Can. J. Fish. Aquat. Sci. 51(5): 1065-1076.

Sweeting C.J., Polunin N.V., Jennings S. 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. Rapid Commun Mass Spectrom. 20(4):595-601.

Thomas C.J., Cahoon L.B. (1993) Stable isotope analyses differentiate between different trophic pathways supporting rocky-reef fishes. Mar Ecol Prog Ser 95:19–24.

U.S. Food and Drug Administration. 2013. Title 21-Food and drugs. Chapter 1-Food and Drug Administration, Dept. of Health and Human Services, Subchapter B-Food and Human Consumption. Part 109: Unavoidable contaminants in human consumption and food-packaging material. Subpart B-Tolerances for unavoidable poisonous or deleterious substances. 21CFR109.30

Xu, X., Newman M.C., Fabrizio, M.C., Liang, L. 2013. Xu et al. 2013. An ecologically framed mercury survey of finfish of the lower Chesapeake Bay. Arch Environ Contam Toxicol 65:510–520.

Appendices

Non-PCB organochlorine compounds analyzed.

ID	compound	CASNum	numchlorines
1	p-terphenyl (ISTD)	92-94-4	x
2	1,2,4,5-tetrachlorobenzene	95-94-3	tri
3	1,2,3,4-tetrachlorobenzene	634-66-2	tri
4	alpha-BHC	319-84-6	tri
5	13C-diPCB-8 (SSTD)	N/A	tri
6	hexachlorobenzene	118-74-1	tri
7	pentachloroanisole	1825-21-4	tri
8	beta-BHC	319-85-7	tri
9	gamma-BHC	58-89-9	tri
10	delta-BHC	319-89-9	tri
11	compound "C" (chlordane)	63767-51-1	tri
12	13C-triPCB-28 (SSTD)	208263-76-7	tri
13	heptachlor (chlordane)	76-44-8	tetra
14	13C-tetraPCB-52 (SSTD)	208263-80-3	tetra
15	aldrin	309-00-2	penta
16	chlorpyrifos	2921-88-2	penta
17	isodrin	465-73-6	penta

18	**MC 1 (chlordane)**	**98318-98-0**	**penta**
19	**heptachlor epoxide isomer "B" (chlordane)**	**1024-57-3**	**penta**
20	**oxychlordane**	**27304-13-8**	**penta**
21	**MC 3 (chlordane)**	**68961-33-1**	**penta**
22	**MC 2 (chlordane)**	**98318-99-1**	**penta**
23	**trans-chlordane**	**5103-74-2**	**penta**
24	**p,p'-DDMU**	**1022-22-6**	**penta**
25	**o,p'-DDE**	**3424-82-6**	**penta**
26	**MC 5 (chlordane)**	**31503-68-1**	**penta**
27	**methoxy-triclosan**	**4640-01-1**	**penta**
28	**endosulfan I**	**959-98-8**	**penta**
29	**MC 6 (chlordane)**	**98318-97-9**	**penta**
30	**cis-chlordane**	**5103-71-9**	**penta**
31	**trans-nonachlor**	**39765-80-5**	**penta**
32	**p,p'-DDMS**	**2642-80-0**	**penta**
33	**MC 7 (chlordane)**	**142291-67-6**	**penta**
34	**p,p'-DDE**	**72-55-9**	**penta**
35	**dieldrin**	**60-57-1**	**penta**
36	**o,p'-DDD**	**53-19-0**	**penta**
37	**endrin**	**72-20-8**	**penta**
38	**perthane**	**72-56-0**	**penta**
39	**endosulfan II**	**33213-65-9**	**penta**
40	**13C-pentaPCB-118**	**104130-40-7**	**penta**

	(SSTD)		
41	**MC 8 (chlordane)**	**98523-46-7**	**hexa**
42	**p,p'-DDD**	**72-54-8**	**hexa**
43	**o,p'-DDT**	**789-02-6**	**hexa**
44	**cis-nonachlor**	**5103-73-1**	**hexa**
45	**13C-hexaPCB-153 (SSTD)**	**185376-58-3**	**hexa**
46	**p,p'-DDT**	**50-29-3**	**octa**
47	**13C-octaPCB-202 (SSTD)**	**105600-26-8**	**octa**
48	**methoxychlor**	**72-43-5**	**hepta**
49	**dicofol**	**115-32-2**	**hepta**
50	**13C-heptaPCB-180 (SSTD)**	**160901-82-6**	**hepta**
51	**mirex**	**2385-85-5**	**nona**
52	**13C-nonaPCB-206 (SSTD)**	**208263-75-6**	**nona**
53	**13C-decaPCB-209 (SSTD)**	**105600-27-9**	**deca**

VDH Mercury Advisory

VIRGINIA DEPARTMENT OF HEALTH (VDH) GUIDELINE FOR ISSUANCE OF FISH-EATING ADVISORY DUE TO CONTAMINATION OF FISH WITH MERCURY

Mercury is a naturally occurring metal which is widespread and persistent in the environment. It exists in three forms: elemental or metallic mercury, inorganic mercury, and organic mercury. Elemental mercury is a silver-white liquid at room temperature that vaporizes readily when heated. Inorganic mercury compounds occur when mercury combines with elements such as chlorine, sulfur, or oxygen.

Most inorganic compounds are powders or crystals. Organic mercury compounds occur when mercury combines with carbon. One organic form of mercury, methylmercury, is produced when a carbon and three hydrogen molecules are attached to the elemental mercury. Methylmercury is of particular concern because it can accumulate up the food chain in aquatic systems and can lead to high concentrations in predatory fish.

Uses of Mercury

Elemental mercury is used in thermometers, thermostats, switches, barometers, batteries, dental amalgam, and other products. Inorganic mercury compounds are commonly used in electrical equipment (e.g., switches and lamps), and in medicinal and skin care products, such as antiseptic creams and ointments. Organic mercury compounds are used in industry as pigments in paints and as fungicides.

Sources of Mercury in the Environment

Mercury is released to the environment by both natural sources and human activities. Most of the mercury in air, water, and soil is inorganic mercury. This inorganic mercury can enter the air from deposits of ore that contain mercury, from burning fuels or garbage, and from emissions by factories that use mercury. Inorganic mercury may also enter water or soil from rocks that contain mercury, releases of water containing mercury from factories or water treatment facilities, and the disposal of wastes. Organic compounds of mercury may be released in the soil through the use of mercury-containing fungicides. Metallic mercury can evaporate easily into the air and be carried a long distance before returning to water or soil in rain or snow. Once mercury enters lakes, rivers, or oceans in any form, it is converted to methylmercury by microorganisms (bacteria and fungi) or by chemical reactions.

Fish absorb methylmercury directly from water and from eating smaller aquatic organisms that contain methylmercury. Although virtually all fish species contain at least trace amounts of methylmercury, larger predatory fish species have the highest concentrations. Methylmercury is tightly bound to proteins in all fish tissue, including muscle. There is no method of cooking or cleaning fish which will reduce the amount of mercury in a meal. Since almost all of the mercury in fish is in the form of methylmercury, the fish-eating advisory guideline is based on methylmercury.

Toxicity of Mercury

Evidence from human and animal studies indicates that the nervous system is sensitive to all forms of mercury. Exposure to high levels of all forms of mercury can permanently damage the brain, kidney, and developing fetus. Methylmercury is more harmful because more mercury in this form reaches the brain. Methylmercury is rapidly absorbed from the gastrointestinal tract (about 95%) and readily enters the adult and fetal brain where it accumulates and slowly converts to inorganic mercury. The exact mechanism by which mercury causes neurotoxic effects is not known, and data are not avail-available on how exposure to other forms of mercury affects methylmercury toxicity.

Acute high-dose exposure to methylmercury can result in adverse effects in several organ systems throughout the life span of humans and animals. Extensive data exist on the effects of methylmercury on the development of the brain in humans and animals. The most severe effects reported in humans were seen following high-dose acute poisoning episodes in Japan and Iraq. The outbreak of neurological dis-orders in Japan in the 1950s was attributed to the consumption of fish contaminated with methylmercury. Industrial waste containing inorganic mercury had been discharged into Minimata Bay and was converted

by microorganisms into methylmercury. This resulted in contamination of fish, a major food source to the surrounding population. In this incident 700 people died and approximately 9,000 suffered severe health effects. A similar epidemic of neurological disorders occurred in Iraq in 1971 as a result of consumption of contaminated food. In this case flour was ground from grain treated with methylmercury fungicide. This incident affected more than 6,000 people. The health effects on brain functioning included irritability, mental retardation, shyness, tremors, cerebral palsy, deafness, and blindness in individuals who were exposed *in utero,* and sensory and motor impairment in exposed adults.

Chronic, low-dose prenatal methylmercury exposure from maternal consumption of fish has been associated with more subtle endpoints of neurotoxicity in children. Results from the three large epidemiological studies (the Faroe Islands, Seychelles Islands, and New Zealand studies) have added substantially to the body of knowledge on brain development following long-term exposure to small amounts of methylmercury. The Faroe Islands study reported associations between low-dose prenatal methylmercury exposure and children's performance on standardized neurobehavioral tests, particularly on the tests of attention, fine motor functions, confrontational naming, visual-spatial abilities (*e.g.,* drawing), and verbal memory. The Seychelles Islands study did not report such associations. The New Zealand study also observed associations, as did the large pilot study conducted in the Faroe Islands.

There is evidence in humans and animals that exposure to methylmercury can have adverse effects on the developing and adult cardiovascular system (blood pressure regulation, heart rate variability, and heart disease). There is also evidence in animals that the immune and reproductive systems are sensitive targets for methylmercury.

VDH had historically used the Food and Drug Administration's (FDA) action level of one part per million (ppm) for issuing fish consumption advisories for mercury. However, recently, the National Academy of Sciences (NAS) has produced a review of the toxicity of methylmercury and has recommended a reference dose (RfD) of 0.0001 milligrams per kilograms per day (mg/kg/day) for sensitive and nonsensitive populations. The RfD is an estimate of a daily exposure to the human population (including sensitive subpopulations) that is likely to be without appreciable risk of deleterious effects during a lifetime. In view of the NAS recommendation, VDH has revised its existing guidelines for issuing fish consumption advisories due to mercury contamination.

Derivation of Acceptable Concentration of Methylmercury in Fish

The formula for calculating an acceptable concentration, corresponding to a recommended two meals per month of methylmercury in edible fish tissue, for protecting fish consumers from noncancer health effects is as follows:

$$C = \frac{RfD \times BW \times T}{MS \times NM}$$

Where:

C = acceptable concentration of methylmercury in edible portions of fish in milligrams per kilograms (mg/kg).

RfD = reference dose (RfD) for methylmercury in milligrams per kilogram per day (0.0001 mg/kg/day).

BW = consumer adult body weight in kilograms (70 kg). A body weight of 70 kilograms for the average adult male is widely accepted by many regulatory agencies for risk assessment and establishing guidelines and standards for chemical exposure.

T = time period 30 days (days/month). Time period of 30 days/month was used to calculate fish meal consumption limits, in a 30-day period as a function of meal size.

MS = average fish meal size of 8 ounces (oz) or 0.227 kg. Meal size is defined as the amount of fish (in kilograms) consumed at one meal. An 8-oz or 0.227 kg meal size was assumed.

NM = number of allowable meals per month (2 meals/month). Number of meals consumption limit is expressed as the maximum allowable fish meals in a 30-day time period. These are based on the total dose allowable over a 1-month period (based on the the RfD).

Substituting for assumptions in the above equation, an acceptable methylmercury concentration of 0.5 mg/kg in edible fish tissue was derived.

$$C = \frac{0.0001 \text{ mg/kg/day} \times 70 \text{ kg} \times 30 \text{ day/month}}{0.227 \text{ kg/meal} \times 2 \text{ meals/month}}$$

$$= 0.4625 \text{ mg/kg} \approx 0.5 \text{ mg/kg}$$

Conclusion

Based on the above calculation, VDH would use 0.5 mg/kg or 0.5 ppm of methylmercury in fish as the trigger level for the issuance of a fish-eating advisory. When individual fish data are available, if 50% of fish samples exceed the guidance levels, this would trigger an advisory. VDH will use a four-tiered approach when issuing a fish- eating advisory.

- Average fish tissue concentrations ranging from non-detectable to below 0.5 ppm will not warrant issuance of a fish-eating advisory.

- When the average concentrations in fish range from 0.5 ppm to below 1 ppm, VDH will recommend limiting consumption of contaminated species to two, 8-oz meals per month.

- When the average concentration in fish range from 1 ppm to below 2.0 ppm, VDH will recommend limiting consumption of contaminated species to one, 8-oz meal per month.

- When the average concentrations in fish exceed 2.0 ppm, VDH will recommend that contaminated fish should not be consumed.

VDH would also recommend that pregnant women, nursing mothers, and young children should not consume fish contaminated with methylmercury at concentrations above 0.5 ppm.

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October 25, 2000

VDH PCB Advisory notification

http://www.vdh.state.va.us/epidemiology/dee/publichealthtoxicology/documents/pdf/new%20pcb%20advisories%2012.13.pdf

News Release

109 Governor Street, Virginia 23219 ● www.vdh.virginia.gov

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VIRGINIA DEPARTMENT OF HEALTH STRENGTHENS GUIDELINES FOR FISH CONSUMPTION ADVISORIES IN STATE WATERS

(Richmond, Va.)—The Virginia Department of Health (VDH) has revised guidelines for issuing fish consumption advisories due to contamination of fish with polychlorinated biphenyls (PCBs). The changes lower the levels of PCBs required for VDH to issue a fish consumption advisory.

Eleven old advisories have been modified and 27 new advisories have been issued as a result of the changes. “The number of fish consumption advisories will increase as a result of these more stringent guidelines,” said State Health Commissioner Robert B. Stroube, M.D., M.P.H. “The levels of PCBs have not increased, but our guidelines for determining what is acceptable for human consumption have become more protective.”

The guidelines divide PCB levels into the following:

• Fish with less than 50 parts per billion (ppb) have no restrictions on consumption

• Fish with 50-500 ppb are limited to no more than two meals per month

• Fish with greater than 500 ppb should not be consumed

Women who are pregnant or may become pregnant, nursing mothers and young children should not eat any fish from the advisory areas.

Historically, Virginia has steadily lowered the levels of PCBs considered acceptable in fish. Prior to 1980, Virginia followed guidelines developed by the U.S. Food and Drug Administration (FDA) that considered PCB levels higher than 5,000 ppb in exceedance of safety guidelines. In 1984, the FDA reduced the level to 2,000 ppb. In 1998, VDH developed its own fish consumption advisory guidelines and set its levels of concern for PCBs in fish to no more than 600 ppb. The new guidelines announced today further reduce that level to 50 ppb.

The levels of concern for PCBs are calculated in part based on how long an individual may consume fish from the same water source. Previously those calculations were based on the assumption that an individual may consume fish, at most, between 9 to 12 years from the same source. The new guidelines take into consideration the possibility that an individual may consume fish for as many as 30 years from the same location.

Both Maryland and North Carolina have made similar adjustments in recent years. Virginia shares water bodies with both states and the new guidelines provide more consistent advice to regional fishers.

An interactive online map detailing the complete list of fish consumption advisories is available on the Virginia Department of Health Web site at www.vdh.virginia.gov/HHControl/fishingadvisories.asp. A fact sheet with answers to frequently asked questions on fish consumption advisories is available at www.vdh.virginia.gov/HHControl/advisoriesq&a.pdf.

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EPA fish consumption advisory tables for various contaminants

United States Environmental Protection Agency | Office of Water (4305) | EPA 823-B-00-008 November 2000

Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories

Volume 2
Risk Assessment and Fish Consumption Limits
Third Edition

Table 4-6. Monthly Fish Consumption Limits for Carcinogenic and Noncarcinogenic Health Endpoints - Chlordane

Risk Based Consumption Limit[a]	Noncancer Health Endpoints[b]	Cancer Health Endpoints[c]
Fish Meals/Month	Fish Tissue Concentrations (*ppm, wet weight*)	Fish Tissue Concentrations (*ppm, wet weight*)
Unrestricted (>16)	0 - 0.15	0 - 0.0084
16	>0.15 - 0.29	>0.0084 - 0.017
12	>0.29 - 0.39	>0.017 - 0.022
8	>0.39 - 0.59	>0.022 - 0.034
4	>0.59 - 1.2	>0.034 - 0.067
3	>1.2 - 1.6	>0.067 - 0.089
2	>1.6 - 2.3	>0.089 - 0.13
1	>2.3 - 4.7	>0.13 - 0.27
0.5	>4.7 - 9.4	>0.27 - 0.54
None (<0.5)	>9.4	>0.54

* The assumed meal size is 8 oz (0.227 kg). The ranges of chemical concentrations presented are conservative, e.g., the 12-meal-per-month levels represent the concentrations associated with 12 to 15.9 meals.

[b] Chronic, systemic effects.

[c] Cancer values represent tissue concentrations at a 1 in 100,000 risk level.

Notes:

1. Consumption limits are based on an adult body weight of 70 kg, an RfD of 5×10^{-4} mg/kg-d, and a cancer slope factor (CSF) of 0.35 $(mg/kg\text{-}d)^{-1}$
2. None = No consumption recommended.
3. In cases where >16 meals per month are consumed, refer to Equations 3-1 and 3-2, Section 3.2.1.2, for methods to determine safe consumption limits.
4. The detection limit for chlordane is 1×10^{-3} mg/kg.
5. Instructions for modifying the variables in this table are found in Section 3.3.
6. Monthly limits are based on the total dose allowable over a 1-month period (based on the RfD). When the monthly limit is consumed in less than 1 month (e.g., in a few large meals), the daily dose may exceed the RfD (see Section 2.3).

Table 4-7. Monthly Fish Consumption Limits for Carcinogenic and Noncarcinogenic Health Endpoints - DDT

Risk Based Consumption Limit[a]	Noncancer Health Endpoints[b]	Cancer Health Endpoints[c]
Fish Meals/Month	Fish Tissue Concentrations (*ppm, wet weight*)	Fish Tissue Concentrations (*ppm, wet weight*)
Unrestricted (>16)	0 - 0.015	0 - 0.0086
16	>0.015 - 0.029	>0.0086 - 0.017
12	>0.029 - 0.039	>0.017 - 0.023
8	>0.039 - 0.059	>0.023 - 0.035
4	>0.059 - 0.12	>0.035 - 0.069
3	>0.12 - 0.16	>0.069 - 0.092
2	>0.16 - 0.23	>0.092 - 0.14
1	>0.23 - 0.47	>0.14 - 0.28
0.5	>0.47 - 0.94	>0.28 - 0.55
None (<0.5)	>0.94	>0.55

* The assumed meal size is 8 oz (0.227 kg). The ranges of chemical concentrations presented are conservative, e.g., the 12-meal-per-month levels represent the concentrations associated with 12 to 15.9 meals.

[b] Chronic, systemic effects.

[c] Cancer values represent tissue concentrations at a 1 in 100,000 risk level.

Notes:

1. Consumption limits are based on an adult body weight of 70 kg, an RfD of 5×10^{-4} mg/kg-d, and a cancer slope factor (CSF) of 0.34 $(mg/kg\text{-}d)^{-1}$
2. None = No consumption recommended.
3. In cases where >16 meals per month are consumed, refer to Equations 3-1 and 3-2, Section 3.2.1.2, for methods to determine safe consumption limits.
4. The detection limit for DDT is 1×10^{-4} mg/kg.
5. Instructions for modifying the variables in this table are found in Section 3.3.
6. Monthly limits are based on the total dose allowable over a 1-month period (based on the RfD). When the monthly limit is consumed in less than 1 month (e.g., in a few large meals), the daily dose may exceed the RfD (see Section 2.3).

Table 4-13. Monthly Fish Consumption Limits for Carcinogenic and Noncarcinogenic Health Endpoints - Hexachlorobenzene

Risk Based Consumption Limit[a]	Noncancer Health Endpoints[b]	Cancer Health Endpoints[c]
Fish Meals/Month	Fish Tissue Concentrations (*ppm, wet weight*)	Fish Tissue Concentrations (*ppm, wet weight*)
Unrestricted (>16)	0 - 0.23	0 - 0.0018
16	>0.23 - 0.47	>0.0018 - 0.0037
12	>0.47 - 0.63	>0.0037 - 0.0049
8	>0.63 - 0.94	>0.0049 - 0.0073
4	>0.94 - 1.9	>0.0073 - 0.015
3	>1.9 - 2.5	>0.015 - 0.02
2	>2.5 - 3.8	>0.02 - 0.029
1	>3.8 - 7.5	>0.029 - 0.059
0.5	>7.5 - 15	>0.059 - 0.12
None (<0.5)	>15	>0.12

[a] The assumed meal size is 8 oz (0.227 kg). The ranges of chemical concentrations presented are conservative, e.g., the 12-meal-per-month levels represent the concentrations associated with 12 to 15.9 meals.
[b] Chronic, systemic effects.
[c] Cancer values represent tissue concentrations at a 1 in 100,000 risk level.

Note:
1. Consumption limits are based on an adult body weight of 70 kg, an RfD of 8×10^{-4} mg/kg-d, and a cancer slope factor (CSF) of 1.6 $(mg/kg\text{-}d)^{-1}$
2. None = No consumption recommended.
3. In cases where >16 meals per month are consumed, refer to Equations 3-1 and 3-2, Section 3.2.1.2, for methods to determine safe consumption limits.
4. The detection limit for hexachlorobenzene is 1×10^{-4} mg/kg.
5. Instructions for modifying the variables in this table are found in Section 3.3.
6. Monthly limits are based on the total dose allowable over a 1-month period (based on the RfD). When the monthly limit is consumed in less than 1 month (e.g., in a few large meals), the daily dose may exceed the RfD (see Section 2.3).

Table 4-3. Monthly Fish Consumption Limits for Noncarcinogenic Health Endpoint - Methylmercury

Risk Based Consumption Limit[a]	Noncancer Health Endpoints[b]
Fish Meals/Month	Fish Tissue Concentrations (*ppm, wet weight*)
Unrestricted (>16)	0 - 0.029
16	>0.029 - 0.059
12	>0.059 - 0.078
8	>0.078 - 0.12
4	>0.12 - 0.23
3	>0.23 - 0.31
2	>0.31 - 0.47
1	>0.47 - 0.94
0.5	>0.94 - 1.9
None (<0.5)	>1.9

[a] The assumed meal size is 8 oz (0.227 kg). The ranges of chemical concentrations presented are conservative, e.g., the 12-meal-per-month levels represent the concentrations associated with 12 to 15.9 meals.

[b] Chronic, systemic effects.

Notes:

1. Consumption limits are based on an adult body weight of 70 kg and an interim RfD of 1×10^{-4} mg/kg-d.
2. None = No consumption recommended.
3. In cases where >16 meals per month are consumed, refer to Equations 3-1 and 3-2, Section 3.2.1.2, for methods to determine safe consumption limits.
4. The detection limit for methylmercury is 1×10^{-3} mg/kg.
5. Instructions for modifying the variables in this table are found in Section 3.3.
6. Monthly limits are based on the total dose allowable over a 1-month period (based on the RfD). When the monthly limit is consumed in less than 1 month (e.g., in a few large meals), the daily dose may exceed the RfD (see Section 2.3).

Table 4-24. Monthly Fish Consumption Limits for Carcinogenic and Noncarcinogenic Health Endpoints - PCBs

Risk Based Consumption Limit[a]	Noncancer Health Endpoints[b]	Cancer Health Endpoints[c]
Fish Meals/Month	Fish Tissue Concentrations (*ppm, wet weight*)	Fish Tissue Concentrations (*ppm, wet weight*)
Unrestricted (>16)	0 - 0.0059	0 - 0.0015
16	>0.0059 - 0.012	>0.0015 - 0.0029
12	>0.012 - 0.016	>0.0029 - 0.0039
8	>0.016 - 0.023	>0.0039 - 0.0059
4	>0.023 - 0.047	>0.0059 - 0.012
3	>0.047 - 0.063	>0.012 - 0.016
2	>0.063 - 0.094	>0.016 - 0.023
1	>0.094 - 0.19	>0.023 - 0.047
0.5	>0.19 - 0.38	>0.047 - 0.094
None (<0.5)	>0.38	>0.094

* The assumed meal size is 8 oz (0.227 kg). The ranges of chemical concentrations presented are conservative, e.g., the 12-meal-per-month levels represent the concentrations associated with 12 to 15.9 meals.

[b] Chronic, systemic effects

[c] Cancer values represent tissue concentrations at a 1 in 100,000 risk level.

* Concentration reported in parts per quadrillion (nanogram per kg or 10-9 g/kg.

Notes:

1. Consumption limits are based on an adult body weight of 70 kg, and RfD of 2×10^{-5}, and a cancer slope factor (CSF) of 2 $(mg/kg\text{-}d)^{-1}$.
2. NONE = No consumption recommended.
3. In cases where >16 meals per month are consumed, refer to Equations 3-1 and 3-2, Section 3.2.1.2, for methods to determine safe consumption limits.
4. The detection limit for PCBs (sum of Aroclors) is 2×10^{-2} mg/kg.
5. Instructions for modifying the variables in this table are found in Section 3.3.
6. Monthly limits are based on the total dose allowable over a 1-month period (based on the RfD). When the monthly limit is consumed in less than 1 month (e.g., in a few large meals), the daily dose may exceed the RfD (see Section 2.3).