

Polychlorinated Biphenyls and Organochlorine Pesticide Concentrations in Whole Body Mummichog and Banded Killifish from the Anacostia River Watershed:

2018-2019

CBFO-C-20-01



*Left: Mummichog, female (L), male (R); Right: Banded killifish, left two are males, right two are females.
Photos: Fred Pinkney, USFWS*

U.S. Fish and Wildlife Service
Chesapeake Bay Field Office

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ABSTRACT

In 2018 and 2019, the U.S. Fish and Wildlife Service Chesapeake Bay Field Office (CBFO) monitored polychlorinated biphenyl (PCB) and organochlorine (OC) pesticide concentrations in whole body samples of forage fish. Fish were collected along the mainstem Anacostia River, Kingman Lake, five major tributaries, and (as a reference) a section of the Potomac River. Mummichog (*Fundulus heteroclitus*; referred to as MC in this report) and banded killifish (*F. diaphanus* BK) were chosen because of their high site fidelity and widespread presence in the watersheds. The objectives are to: 1) establish a pre-remedial baseline of these contaminants in fish from the Anacostia mainstem and major tributaries, Kingman Lake, and the Potomac River; 2) compare total PCB, total chlordane, and total DDT among sampling locations; and 3) interpret patterns in PCB homologs. Findings will be integrated with other analyses of the Anacostia River Sediment Project (ARSP) to develop a biota monitoring program.

Five Anacostia River tributaries were sampled: Northwest Branch (NW), Northeast Branch (NE), Lower Beaverdam Creek (two locations - LB1, LB2), Hickey Run (HR), and Watts Branch (WB). In the mainstem Anacostia and Kingman Lake, six locations were sampled: Bladensburg Waterfront Park (BL), Kingman Lake (KL), Pepco Cove (PC), north of the CSX Bridge (A2), between the 11th St. Bridge and Sousa Bridge (11A), and Poplar Point (A1). The three Potomac River locations were: along the shore of Theodore Roosevelt Island (P3), about 0.8 km upstream of Key Bridge (KB), and at Fletchers Cove (P4). For each species, composites of similar-sized fish were prepared with the goal of having at least 20 grams wet weight. The number of composite samples of a species at a location ranged from 1 to 13. At some locations only one species was obtained.

The primary finding was that PCB concentrations were elevated (719 to 1055 ppb wet weight) in both species collected from the two Lower Beaverdam Creek locations. The pattern of PCB homologs (enriched in di-, tr-, and tetra- congeners) in these fish suggested a different source of PCBs from that in all other locations. Intermediate concentrations of total PCBs (132 to 460 ppb) were detected in many locations in the tidal Anacostia River and several tributaries. The finding of low PCB concentrations in Potomac River BK (<100 ppb) supported the use of this collection area as a background location. Similar (<100 ppb) total PCB concentrations in the Northwest and Northeast Branch showed that these tributaries, which contribute large quantities of sediment to the tidal river, were not major sources of PCBs that accumulate in fish. A linear model, used to make statistical comparisons between locations, confirmed the elevated concentrations of total PCBs in Lower Beaverdam Creek fish. Total chlordane and total DDT had a very different pattern with smaller overall ranges and no single tributary consistently ranked highest.

Detection of the highest PCB concentrations in MC and BK from Lower Beaverdam Creek was consistent with 2017 loadings comparisons between these same tributaries, reported by the U.S. Geological Survey. The findings in fish were also consistent with those in deployed freshwater mussels and passive samplers.

We recommend monitoring PCB and OC pesticide concentrations in these species as a key component for evaluating the success of the remediation of the tidal Anacostia River. Such a strategy could be applied at Early Action Areas and Potential Environmental Cleanup Sites (PECS) locations as well as Lower Beaverdam Creek, identified as a continuing source of PCB loading. The Potomac River area is suitable as a reference location. In 2020, about 100 additional composite samples will be collected from a subset of these locations (including the Potomac at Key Bridge) to develop a 3-year pre-remedial database. We will use the same statistical models. Having an expanded data set may allow us to develop a combined species model with confidence limits. We will also test for differences in years, especially at Pepco Cove which has both 2018 and 2019 data. For the locations that can be pooled across years, we expect that the adjusted means for the single species models will have narrower confidence intervals. Future monitoring would be scheduled based on the timing of clean-up actions, perhaps on a 2- to 3-year cycle.

In addition to contaminant monitoring, we recommend using these species as indicators of the health of fish from specific locations and tributaries. The results could be applied to injury assessment for Natural Resources Damage Assessments and to supplement river-wide and PECS Ecological Risk Assessments. One approach would be to conduct necropsies on a subset of the fish collected for contaminant monitoring. Histopathology would be performed focusing on the gonads and liver. A second approach would be to measure the hatching success of MC and BK collected from locations with a wide range of PCB concentrations. A third would consist of laboratory exposures of fertilized eggs to sediments from these locations. All three approaches would directly assess adverse effects on fish, reducing the uncertainty in applying literature-based thresholds to interpret the contaminant data.

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INTRODUCTION

Background

The Anacostia River is one of three Chesapeake Bay Regions of Concern due to contamination of water, sediment, and biota. The major contaminants of concern (COCs) in the Anacostia are polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and organochlorine (OC) pesticides such as chlordane (Velinsky et al. 2011, Tetra Tech 2019). Within the District of Columbia, fish advisories warn the public to restrict their consumption, largely due to PCB contamination (https://doee.dc.gov/sites/default/files/dc/sites/ddoe/service_content/attachments/Fish%20Advisory%202-2-2016.pdf; Pinkney, 2014, 2018a). The elevated prevalence of liver tumors in brown bullhead catfish (*Ameiurus nebulosus*) has been linked with exposure to polycyclic aromatic hydrocarbons (PAHs) as initiators and possibly PCBs and DDT compounds as promoters (Pinkney et al. 2019).

As part of the Anacostia River Sediment Project (ARSP), the District of Columbia Department of Energy and Environment (DOEE) has conducted a Remedial Investigation/Feasibility Study (RI/FS) (Tetra Tech 2019). The purpose of the RI was to assess the nature and extent of contamination in the tidal river. The FS evaluated potential remedial actions (<https://www.anacostiasedimentproject.com/library>).

Additional DOEE-funded studies quantified contaminant loading from the watershed into the tidal river. Such data are critical to ensure that cleanup actions taken in the tidal river are long-lasting and not subject to re-contamination from tributary loadings. The U.S. Geological Survey (USGS) measured tributary loadings of contaminants during high and low flow periods over the course of 2017 (Wilson 2019). The sediment load was driven by the flow and size of the watersheds as follows: Northwest Branch (NW) 50 percent, Northeast Branch (NE) 33 percent, Lower Beaverdam (LB) 14 percent, Watts Branch (WB) 2 percent, and Hickey Run (1 percent). Total PCB loads (810 grams) had a very different pattern: an estimated 75 percent (610 grams) from Lower Beaverdam Creek, 12 percent (95 grams) from Northwest Branch, 7 percent (60 grams) from Northeast Branch, 3 percent (25 grams) from Watts Branch, and 2.5 percent (19 grams) from Hickey Run.

Ghosh et al. (2020) used passive samplers and freshwater mussels (*Elliptio complanata*) deployed in 2016 and 2017 to estimate the freely dissolved and bioavailable concentrations of PCBs, OC pesticides, and PAHs in these same five major Anacostia tributaries. The authors reported that Lower Beaverdam Creek contributed 285 grams of freely dissolved PCBs per year (72 percent of the total load of 395 grams/year) with the remainder coming primarily from the Northeast Branch (52 grams or 13 percent) and Northwest Branch (36 grams or 9 percent).

A measure of the success of remediation would be a decrease in PCB concentrations in tissues of fish that are caught and sometimes eaten (i.e., game fish), which may allow

consumption advisories to be relaxed. Unfortunately, the home ranges of many of the game species complicate this analysis. For example, blue catfish is the most commonly caught species by subsistence anglers in the District of Columbia (Fiske and Callaway 2019). In a Potomac River tagging study, the mean minimum recapture distance was 24.1 km with a range of 0 to 112.6 km (Tuckey et al. 2017). Thus, it is likely that fish move in and out of the Anacostia River into the Potomac River, both in the District of Columbia and downriver into Maryland. In past monitoring studies, PCB concentrations in many of the Anacostia and Potomac game fish samples have been similar and based on small numbers of composite samples (Pinkney 2014, 2018a).

For the current project, we collected mummichog (*Fundulus heteroclitus*, referred to as MC in this report, Figure 2a) and banded killifish (*F. diaphanus*, BK, Figure 2b) from 12 locations in the tidal Anacostia River, Kingman Lake, Anacostia tributaries, and 3 in the Potomac River and analyzed whole body PCB and OC pesticide concentrations (Table 1, Figure 1). Although PAHs are a contaminant of concern for the benthic community and the health of fish, these chemicals are not a driver of public health advisories (Pinkney 2018a) and therefore not investigated. Monitoring data from DOEE, Metropolitan Washington Council of Governments (MWCOC), and Maryland Department of Natural Resources (DNR) documented the presence of both species in these watersheds, although some locations only have one of the two species.

The primary consideration for the choice of species is site fidelity. Mummichog in a tidal creek in Delaware, had an estimated summer home range of 36 m, with a few fish moving 375 m (Lotrich 1975, Abraham 1985). Teo and Able (2003) estimated a home range of 15 ha in a tidal marsh in New Jersey monitored from April through November. No home range estimates for banded killifish were identified in the literature. Pinkney and McGowan (2006), however, compared concentrations of DDT isomers in whole body banded killifish collected from the tidal 78-ha Quantico embayment of the Potomac River and nearby creeks. They found a similar, elevated ratio of p,p'-DDD: p,p'-DDE concentrations in embayment sediments and fish; this ratio was not observed in nearby creeks. They concluded that there was site fidelity for banded killifish in the embayment. Thus, for the current study, we estimate that for both species there is site fidelity in a river segment with a length of 0.805 km (0.5 mi) and an area of 50 ha (124 ac).

Mummichogs have been used in fish health studies because of their propensity to develop liver tumors and pre-neoplastic lesions. Examples of their use include work in the Elizabeth River, Virginia (Vogelbein et al. 1990, Vogelbein and Unger 2006) and in Hershey Run, Delaware (Pinkney and Harshbarger 2006). Banded killifish were used to monitor contamination in the Quantico study described above (Pinkney and McGowan 2006). The life span of both species is 3 to 4 years (Abraham 1985, Phillips et al. 2007). This is advantageous in that new cohorts are representative of recent exposure, facilitating before and after monitoring studies.

Objectives

The objectives are to: 1) establish a pre-remedial baseline of PCB and OC pesticide concentrations in fish from the Anacostia River mainstem and major tributaries, Kingman Lake,

and the Potomac River; 2) compare total PCBs, total chlordane, and total DDT among sampling locations; and 3) interpret patterns in PCB homologs. The findings will be integrated with other analyses of the Anacostia River Sediment Project to develop a biota monitoring program. Monitoring is an essential component of the adaptive management approach of the ARSP.

METHODS

Site selection, schedule, and sampling methods

Sampling locations (Table 1, Figure 1) were chosen to establish baseline concentrations in tributaries, Kingman Lake, main stem areas near and remote from Potential Environmental Cleanup Sites (PECS), and the Potomac River from Key Bridge to Chain Bridge (used as background for sediment contamination in the RI (Tetra Tech 2019). Five tributaries were sampled near USGS gages: Northwest Branch (NW), Northeast Branch (NE), Lower Beaverdam Creek (LB1), Hickey Run (HR), and Watts Branch (WB). In Lower Beaverdam Creek, we sampled an upstream location (LB2) that extended downstream from the confluence with Cattail Branch just below Route 202. The Anacostia tributary locations match those used in Ghosh et al. (2020).

There were five mainstem sites in the tidal Anacostia River: Bladensburg Waterfront Park (BL), Pepco Cove (PC, a PECS), north of the CSX Bridge (A2), about halfway between the 11th Street Bridge and Sousa Bridge (11A), and Poplar Point (A1). Kingman Lake (KL) was sampled across an area from the East Capitol Bridge north to the footbridge. In the Potomac River, we collected from three locations: near the upstream edge of Theodore Roosevelt Island (P3), about 0.805 km (0.5 mi) upstream of Key Bridge (KB) along both shores, and at the outer edge of Fletcher's Cove (P4). Four of the locations were seining stations regularly sampled by DOEE—A1 and A2 in the Anacostia and P3 and P4 in the Potomac.

Fish from five locations (A1S, A2S, P3S, P4S, and KB) were collected with a 100-foot beach seine (Figure 2c) by DOEE. U.S. Fish and Wildlife Service, Chesapeake Bay Field Office (CBFO) biologists used 25- and 100-foot seines to collect additional fish from A1S and P4S, and from BL, 11A, KL, PC, and HR. All other locations were collected by CBFO biologists using minnow traps (Figure 2d) deployed overnight and, in the tributaries, with backpack electroshocking.

Permits were obtained from DOEE, Maryland DNR, and the National Park Service. We started field work in October 2018 and obtained samples from all Anacostia River mainstem, Kingman Lake, and tributary locations except 11A. We stopped sampling in mid-November because the colder water temperature made it difficult to collect fish. We resumed in May through November 2019, with the primary focus on the three Potomac River locations. Pepco Cove was sampled again in 2019 and 11A was added because we were unable to obtain additional samples from Poplar Point (A1).

Fish processing

Euthanasia was conducted by immersing the fish in a >1.0 mg/L solution of tricaine methanesulfonate (MS-222), as recommended by the American Veterinary Medical Association (AVMA 2013). Fish were double wrapped in aluminum foil and stored in plastic bags in a freezer. They were labeled with the species name and date.

At the CBFO laboratory, the fish from a single location were thawed, unwrapped, rinsed with deionized water, blotted dry, weighed to the nearest 0.1 gram, and measured for total length to the nearest millimeter. Species identifications were confirmed with field guides.

Composites were prepared within three sizes. For MC, the small (S) size class was 45 to 65 mm, the large (L) class was 66 to 84 mm, and the jumbo (J) class was ≥ 85 mm. The same length classes were used for banded killifish except the small class was from 50 to 65 mm. To the extent possible, fish lengths within a sample were such that the smallest fish was at least 75 percent of the largest fish, based on U.S. EPA (2000) guidance. The ratios [(smallest in range/largest in range) \times 100] were ≥ 69 percent for small MC, ≥ 77 percent for small BK, and ≥ 79 percent for both large MC and large BK, >95 percent for jumbo MC, and >90 percent for jumbo BK. If possible, composite samples had ≥ 20 grams of wet tissue to allow the laboratory to meet its detection limits. The composites consisted of 8–18 small, 3–9 large, and 2–5 jumbo fish.

In general, the longer fish are older although age-length relationships reported for these species have varied. For MC, Fritz and Garside (1975) reported the following ranges in Nova Scotia: age 1, 35 to 50 mm; age 2, 51 to 74 mm; age 3, 68 to 83 mm; and age 4, 78 to 95 mm. In a study of a Massachusetts marsh, Valiela et al. (1977) reported MC had mean lengths (mm) of 45 to 63 (male) and 48 to 66 (female) for age 1, 63 to 78 (male) and 68 to 85 (female) for age 2, and 78 to 90 (male) and 88 to 96 (female) for age 3. They did not report any age 4 fish and found no age 3 fish in samples in fall collections. For BK, Fritz and Garside (1975) reported length ranges of 24 to 48 mm for age 1, 49 to 68 mm for age 2, 67 to 80 mm for age 3, and 75 to 110 mm for age 4. In BK from the Presque Isle Bay area of Lake Erie, Phillips et al. (2007) reported mean lengths of 67 mm for age 1, 79 mm for age 2, and 93 mm for age 3.

Chemical analyses

Organic analyses were performed by SGS AXYS Analytical Services, Ltd. (Sidney, British Columbia, Canada), contracted by the Service's Analytical Control Facility (ACF). Samples were shipped on dry ice and documented with chain of custody forms. Fish samples were analyzed for lipid and moisture content, organochlorine pesticides, total PCBs, and 209 PCB congeners (see Table 2, for list of analytes and detection limits). PCB congeners, alpha BHC, alpha chlordane, beta BHC, cis-nonachlor, gamma BHC, gamma chlordane, HCB, mirex, o,p'-DDD, o,p'-DDE, o,p'-DDT, oxychlordane, p,p'-DDD, p,p'-DDE, p,p'-DDT, toxaphene, and trans-nonachlor were analyzed by Low Resolution Gas Chromatography/Mass Spectrometry (LRGC/MS) according to AXYS method MLA-007. Dieldrin, endrin, and heptachlor epoxide were analyzed by GC with an Electron Capture Detector according to AXYS method MLA-007.

See Appendix A for the Analytical Laboratory Report which includes the method description, results, and quality assurance review.

Quality Assurance

A Quality Assurance Project Plan (Pinkney 2018b) was prepared according to U.S. Environmental Protection Agency (USEPA 2001, 2002) guidance. Quality control/quality assurance procedures included the analysis of laboratory duplicates, internal standards, and matrix spikes. A QA review of the data was performed by ACF chemists, Brenda Montgomery and Steve Boateng (Appendix A). A full data validation (EPA Stage 4) was conducted by EcoChem, Inc. (Seattle, WA) (Appendix B) according to U.S. EPA (2017) guidance.

Data presentation and analysis

All data (including model-based estimates) were reported to three significant digits in wet weight parts per billion (ppb). PCBs were calculated as the sum of the congeners. Total DDT was determined as the sum of all six o,p'- and p,p'-DDD, DDE, and DDT isomers. Total chlordane was determined as the sum of alpha- and gamma-chlordane, cis- and trans-nonachlor, and oxychlordane (U.S. EPA 2000). For calculating these totals, non-detects were considered to be zeros. The mean, standard deviation, median, 25th and 75th percentile, and maximum concentrations of total PCBs, total DDT, and total chlordane were presented for each species–location pair (Table 3). For each composite sample, in addition to the COC results, we reported lipid content, sample weight, median length, and the number of individual fish. Results are displayed in bar graphs to show the patterns across locations and species. All analytical results are presented in Appendix A (report format) and Appendix C (spreadsheet).

We developed a linear model to take into consideration covariates for comparisons of the primary COC concentrations across locations. The lack of co-occurrence of both species at all locations led to an unbalanced two-way design for models that included both species and location. To avoid possible model biases associated with this unbalanced two-way model, the most rigorous comparisons among locations were obtained by analyzing the species separately using a one-way design. One location Pepco Cove (PC) was sampled in 2018 and 2019, and these collections were coded PC18 and PC19 to create an additional cell in the one-way design. The adjusted means were presented along with upper and lower 95th percentile confidence limits. The data were also analyzed using the two-way design in order to obtain a ranking of all locations that were sampled, but pairwise comparisons between locations based on the two-way analysis were considered less reliable. Therefore, we did not calculate confidence limits for the combined species models.

Two potentially confounding variables, median fish length and percent lipids were considered for inclusion as covariates in the model. Initial analysis produced negative coefficients for the median-length covariate which indicated an inverse relation to each COC. Because this finding is not consistent with our scientific understanding of bioaccumulation of PCBs (e.g., Sadraddini et al. 2011, Gewurtz et al. 2011), the median-length covariate was excluded from the analysis. In

their analysis, Gewurtz et al. (2011) stated that a positive association between PCBs and length was generally the case when there were both large size ranges and large sample sizes (neither of which were present in the current data set). Each COC showed a positive association with percent lipids and this variable was included in the model. Lipid content has been positively associated with PCB concentrations in some species (Gewurtz et al. 2011), although a simple normalization (dividing the contaminant by lipid content) to adjust for this relationship was not recommended (Hebert and Keenleyside 1995). Lipid content (as a percent) was centered by subtracting the mean by species for ease of interpretation of model parameters. Estimates of mean concentration by location were adjusted to the mean level of percent lipids for each species.

Linear models were fitted using the `lm()` function of the R statistical programming environment (R Core Team 2014). For each species, the linear model included location as a factor variable and percent lipids as a covariate. Following the linear model analysis, adjusted mean concentrations for pairs of locations were compared using a t-statistic computed as a linear function of the parameter vector (Rao 1973). The null hypothesis was that the difference between locations equals zero. Two methods were considered to adjust p-values for multiple comparisons: the method of controlling the false discovery rate (FDR) (Benjamini and Hochberg 1995) and Holm's method for controlling the family-wise false positive rate (Holm 1979). We chose to use Holm's method, because it is more conservative in assigning statistical groups and is analogous to a Tukey's test for one way ANOVA in that both Holm and Tukey procedures control the family-wise false positive rate (Westfall and Young 1993).

Location comparisons were presented using the compact letter display (CDL) tables, in which groups with different letters are significantly different at $p < 0.05$. The confidence intervals that are reported are for predicted values from the fitted linear model. These are not confidence intervals based on the mean and standard deviation from a sample population at one location. The standard error of the predicted value is a composite of all of the residuals from the model as accumulated in the mean squared error term and is justified by the homogeneous variance assumption. See Appendix D for statistical output.

RESULTS

Models

For the separate species models (Tables 4 a,b; 5 a,b; 6 a,b) location and lipid were the two predictor variables. Location was highly significant with all p values less than 0.0001. The p value was < 0.05 for lipid for all models except PCBs in MC with $p = 0.34$. We kept lipid in this model to be consistent with the PCB model for BK, although it likely had minor effects on the calculation of the adjusted mean for MC. Some of the confidence intervals went into negative numbers, which can be interpreted as zeros. The differences between the raw mean and adjusted means are shown on these tables and the lipid adjustment is shown in Appendix D.

The combined species model included both location and species as factor variables (no interaction term) and percent lipids as a covariate. The residuals from the combined species model were examined using normal probability plots, histograms, and density plots and were judged to be approximately normal. For the combined species models (Tables 4c, 4d, 5c, 5d, 6c, 6d), location was highly significant ($p < 0.0001$) for all COCs, species was highly significant ($p < 0.0001$) for total PCBs and total chlordane, but not for total DDT ($p = 0.68$). Lipid was significant for total PCBs ($p = 0.0037$), total chlordane ($p < 0.0001$), and total DDT ($p < 0.0001$).

Total PCBs

For total PCBs, there were considerable differences (as much as 19-fold) in median concentrations in the Anacostia tributaries (Table 3, Figure 3). By far, the highest concentrations (ppb wet weight) were in LB1 (BK 1055ppb, MC 719ppb) and LB2 (BK 923 ppb, MC 783 ppb). The next highest tributary was Watts Branch: MC 272 ppb and BK 273 ppb. The lowest median total PCB concentrations for BK were from Northwest Branch (54.6 ppb) and Northeast Branch (74.6 ppb). For MC, median total PCB concentrations in Northeast Branch were lowest at 48.0 ppb, followed by Hickey Run at 132 ppb.

In the mainstem Anacostia and Kingman Lake, many locations had fish with median total PCB concentrations in the 146 to 460 ppb range. For BK, these were A1 (460 ppb), KL (378 ppb), Pepco Cove 2019 (372 ppb), and A2 (347 ppb). BK were much lower at 11A (193 ppb) and Bladensburg (146 ppb). Concentrations in BK from the Potomac locations ranged from 29 ppb (P4) to 89 ppb (P4). In the mainstem and Kingman Lake, MC concentrations were as follows: Pepco Cove (444 ppb in 2018 and 223 ppb in 2019); A2 (206 ppb), Kingman Lake (241 ppb), and Bladensburg (77.0 ppb). No MC were collected from the Potomac River locations.

Model-based results for MC, yielded three statistical (A, B, and C) groups (Table 4b). The highest concentration group was for Lower Beaverdam Creek, in which both locations were significantly higher than all other locations. The adjusted mean concentrations were 741 ppb at LB1 and 728 ppb in LB2. The MC from Pepco Cove in 2018 had an adjusted mean concentration estimate of 466 ppb and were in the B group, statistically different from the LB1 and LB2 group and all other locations except WB (298 ppb, both B and C groups). The low concentration (C group) consisted of the Potomac locations and all mainstream, Kingman Lake, and tributaries (except LB). The pattern in BK was slightly different with LB1 (1099 ppb) and LB2 (909 ppb) significantly different from each other and all other locations (Table 4b).

Using the combined species model (Table 4c) the highest concentration group consists of the two Lower Beaverdam locations (Table 4d). Pepco Cove was placed in separate groups, based on collection year. The 2018 collection of MC was significantly elevated relative to nearly all of the mainstem Anacostia locations, Kingman Lake, the tributaries other than LB, and the Potomac locations. The Pepco 2019 collection was grouped with the other Anacostia mainstem and Kingman Lake locations and most of the tributaries.

PCB homologs

There were substantial differences in the PCB homolog patterns between the locations. For the collections from the Anacostia tributaries, the Lower Beaverdam Creek fish had primarily trichloro- and tetrachloro- homologs (Figure 4) whereas fish from the other tributaries had primarily penta- and hexa- homologs. The contributions of tri- and tetra- homologs accounted for 48 to 65 percent of total PCBs in the LB collections vs. 9 to 19 percent in the collections from the other tributaries. Di- homologs were 3 to 5 percent in LB fish compared with ≤ 1 percent at all other locations. Conversely, the penta- and hexa- homologs accounted for 27 to 40 percent of total PCBs in the LB collections compared with 57 to 79 percent in the other tributary collections. Penta- and hexa- homologs were dominant in the fish from the mainstem Anacostia/Kingman Lake (54 to 61 percent) and Potomac (62 to 68 percent) locations (Figure 5). There were lower contributions of the tri- and tetra- homologs in the Potomac (4 to 9 percent) vs. Anacostia mainstem/Kingman Lake (13 to 29 percent) fish.

Total chlordane

For total chlordane, median concentrations among the tributaries had only a 1.5 to 2-fold difference between the highest and lowest locations (Table 3, Figure 6). For BK, the range was from 102 ppb at Watts Branch to 156 at Northwest Branch. For MC, the range was from 51 ppb at LB1 to 102 ppb at Northeast Branch. In the mainstem Anacostia and Kingman Lake, the medians in MC ranged from 43.9 ppb in Pepco Cove in 2019 to 61.3 in A2. In BK, the median total chlordane concentrations ranged from 73.9 ppb (11A) to 218 ppb (A1). In Potomac River BK, median total chlordane concentrations ranged from 25.3 ppb at KB to 37.4 ppb at P4.

Model-based results (Table 5a) for MC resulted in only two statistical groups (Table 5b). Only the Northeast Branch location (adjusted mean estimate of 108 ppb) was in the highest (A) group. Four locations (LB2 (98 ppb), WB (79 ppb), PC18 (65 ppb), and BL (62 ppb) were placed in both statistical groups (A, B). The remaining locations had adjusted mean concentrations ranging from 50 to 60 ppb. For BK, there were four groups with A1 the only location in the highest (A) group with an adjusted mean concentration total chlordane estimate of 194 ppb. The following locations had estimates greater than 100 ppb: NW (162 ppb: group A, B), NE (147 ppb: A, B), LB2 (141 ppb: B), and BL (110 ppb: B, C). All other locations were in C and D groups and ranged from 31 ppb to 96 ppb.

Using the combined species model, there were four statistical groups (Table 5d) with only A1 (184 ppb) in the highest (A) group, NW (145 ppb) in the A, B groups, and NE (133 ppb: B). The Potomac locations were the three lowest: KB (11 ppb: D), P4 (16 ppb: D), and P3 (29 ppb: C, D).

Total DDT

For total DDT, there was a six to seven fold range in median concentrations in the tributaries (Table 3, Figure 7). In BK, total DDT ranged from 7.83 ppb in LB2 to 45.6 ppb in WB. In MC,

the range was from 6.81 ppb (LB2) to 53.7 ppb (WB). In the mainstem Anacostia and Kingman Lake, medians in MC ranged from 17.4 (Bladensburg) to 46.3 (A2). In BK, medians ranged from 26.7 (Bladensburg) to 74.6 (A1). In Potomac River BK, median total DDT concentrations ranged from 13.9 (KB) to 28.0 in P4.

Model-based results (Table 6a) for MC, resulted in three statistical groups (Table 6b). Only the Watts Branch location (adjusted mean total DDT estimate of 49 ppb) was in the highest (A) group. Four locations (A2 (44 ppb), KL (36 ppb), PC18 (34 ppb), and PC19 (27.4 ppb) were placed in statistical groups A and B. Two locations were in only the B group: HR (29.9 ppb) and LB1 (26 ppb). The remaining locations were in group B and C or group C with adjusted mean concentrations ranging from 9 ppb to 15 ppb. For BK, there were five groups with A1 the only location in the highest (A) group with an adjusted mean total DDT concentration of 69 ppb. Three locations were in the next highest group either alone (A2, 54 ppb: B) or with other groups (KL (52 ppb: A, B, C) and WB (44 ppb: B, C). All other locations were in C, D, and E groups and ranged from 7 ppb to 37 ppb.

Using the combined species model (Table 6c), there were five statistical groups (Table 6d) with only A1 (67 ppb) in the highest (A) group. The groupings were similar to those for the single species models. The lowest (C, D, E) groups ranged from 8 ppb in LB2 to 11A (36 ppb). The Potomac locations were KB (13 ppb: D, E), P4 (22 ppb: D, E), and P3 (23: C, D, E).

DISCUSSION

PCBs

For the five major tributaries, we found consistent rankings in total PCB concentrations in MC and BK (this study) and the mussels and passive samplers deployed in 2016 and 2017 by Ghosh et al. (2020; Table 7). Lower Beaverdam Creek was ranked highest for all three media. This tributary was also ranked first in percent of 2017 total tributary loading both by Wilson (2019; 75 percent) and Ghosh et al. (2020; 72 percent). This high contribution to loadings to the tidal river, reflects higher concentrations in both suspended sediments and the freely dissolved phase. The large contribution occurred despite the finding that Lower Beaverdam Creek only contributed 14 percent of the sediment load to the tidal river, compared with 50 percent for Northwest Branch and 33 percent for Northeast Branch (Wilson 2019).

The same pattern of enrichment of the lower homolog congeners in 2018 Lower Beaverdam Creek MC and BK (Figure 4) was also found in the 2016 and 2017 passive samplers (Ghosh et al. 2020). Combined tri- and tetra- homologs comprised 58 to 79 percent of total PCBs in the Lower Beaverdam passive samplers. They comprised 35 to 62 percent of total PCBs from passive samplers from the other four tributaries. In addition, di-homologs comprised 14 percent at the Lower Beaverdam 1 location in 2016 and 12 percent in 2017. Passive samplers deployed at our Lower Beaverdam 2 location in 2017 (termed Lower Beaverdam 3 in Ghosh et al. 2020) had 38 percent dichlorobiphenyls. The dichlorobiphenyl contribution in passive samplers deployed in 2016 and 2017 in the four other tributaries ranged from 0 to 3 percent of total homologs. Wilson

(2019) did not present calculations of homolog percentages. They stated, however, that, “The makeup of PCB congeners in samples from the LBDC differed from that in samples from the other tributaries, as high percentages of congeners from the mono- through tetra-homolog groups were present.”

Total chlordane and total DDT

In the tributaries, the concentrations in the fish samples were largely consistent with the findings of Wilson (2019) and Ghosh et al. (2020). For total chlordane, the concentrations in fish, mussels, and passive samplers were all highest in samples from the Northwest Branch (Table 7). This tributary was ranked first in total loading by Ghosh et al. (2020; 44% of total load) and second by Wilson (2019; 28 percent of total load). For total DDT, the concentrations in fish were highest in Watts Branch, which ranked first or second in mussel and passive sampler concentrations. Watts Branch was estimated to have the highest loading (46 percent of total loads) by Wilson (2019) and the third highest (20 percent of total loads) by Ghosh et al. (2020).

In the mainstem Anacostia and Kingman Lake, by far the highest median total chlordane concentration (218 ppb) was in BK from Poplar Point (A1). This concentration may reflect the high surface sediment chlordane concentrations found nearby. In the RI, Tetra Tech (2019) reported that 10 samples between the 11th St. and South Capitol Street Bridges had chlordane concentrations ranging from 110 ppb to 320 ppb. In the entire RI data base of 368 samples, only two samples were higher than 320 ppb (340 and 1300 ppb). No samples had chlordane concentrations between 200 and 320 ppb; only 23 samples had concentrations of 110 to 190 ppb.

Background location for future monitoring

Tetra Tech (2019) used the area of the Potomac River between Key Bridge and Chain Bridge as background for the ARSP RI because of low sediment concentrations of PCBs and OC pesticides. The low concentrations of total PCBs, total chlordane, and total DDT we report in Potomac River BK (no MC were collected) are another line of evidence for the use of this area as background for sediment and for forage fish. Furthermore, the depth and width of this section of the Potomac is more similar to the tidal Anacostia than the narrower and shallower Northwest and Northeast Branches, used by Tetra Tech (2019) as background for fish tissue in the Human Health Risk Assessment. Although we detected low total PCB and total DDT concentrations in MC and BK from the Northwest and Northeast Branches, total chlordane was about 5 times higher than in those from the Potomac (Figure 6). Median total chlordane concentrations in the Northwest and Northeast Branch fish were higher than in fish from all the other Anacostia tributaries and most of the mainstem locations. Thus, we recommend the Potomac area as background for sediment and forage fish in pre-and post-remedial monitoring of clean-up actions conducted through the ARSP.

Possible adverse effects of PCB residues in mummichogs and banded killifish

Recently Berninger and Tillitt (2019) performed a meta-analysis of PCB-induced adverse effects data in many fish species to develop tissue concentration-based effects thresholds. The endpoints were increased mortality, decreased reproduction, and impaired growth, all of which can result in deleterious effects on fish populations. The authors used 31 studies to perform regression analyses. Two of the studies in the database used MC whereas none used BK. Both MC studies estimated rather than measured whole body residues. In one study fish were dosed by injection. Because of these uncertainties, we relied on the regression equations developed for all 31 species rather than these two studies.

The regressions predicted that a whole body total PCB concentration of 100 ppb would not result in increased mortality, but was associated with a 6 percent decrease in growth, and a 27 percent inhibition of reproduction. Total PCBs at 500 ppb would result in a 10 percent increase in mortality, a 12 percent inhibition of growth, and 35 percent inhibition of reproduction. A whole body residue of 1000 ppb would result in a 17 percent increase in mortality, a 15 percent inhibition of growth, and a 39 percent inhibition of reproduction. This concentration range (100 to 1000 ppb) overlaps with the PCB concentrations we detected in nearly all of our sampling locations. Based on the tissue-effects database, our greatest likelihood of adverse effects would be in Lower Beaverdam Creek fish. Of the three endpoints, the greatest concern is for inhibition of reproduction.

Uncertainty

Uncertainties in the concentration data arise from several factors. The number of composite samples varied per location/species combination from 1 to 13. Clearly, as the number of samples decreases, the confidence interval in the model-based estimates increases. For example, the confidence interval for BK from 11A (n=13, adjusted mean: 214 ppb, confidence interval: 173 to 254 ppb) was much less than that for BK from P3 (n=1, adjusted mean: 134, confidence interval: -6.2 to 273 ppb). We are unable to provide confidence intervals for the combined species model because 1) only seven locations had both species and 2) there were few composite samples at several of these locations.

There is uncertainty regarding the possible effects of these whole body residues on the health of the fish. We do not know whether these literature-based thresholds are overestimates or underestimates of the thresholds for MC and BK. We could not identify laboratory toxicity studies with PCBs and BK, which in general are used less frequently in field studies.

Recommendations for monitoring and applied research

We recommend monitoring PCB and OC pesticide concentrations in these species as a key component for evaluating the success of remediation of the tidal Anacostia River. Such a strategy could be applied at Early Action Areas and PECS locations as well as Lower Beaverdam Creek, identified as a continuing source of PCB loading. The Potomac River area is suitable as a

reference location. In 2020, about 100 additional composite samples will be collected from a subset of these locations (including the Potomac at Key Bridge) to develop a 3-year pre-remedial database. We will use the same statistical models. Having an expanded data set may allow us to develop a combined species model with confidence limits. We will also test for differences in years, especially at Pepco Cove which has both 2018 and 2019 data. For the locations that can be pooled across years, we expect that the adjusted means for the single species models will have narrower confidence intervals. Future monitoring would be scheduled based on the timing of clean-up actions, perhaps on a 2- to 3-year cycle.

In addition to contaminant monitoring, we recommend using these species as indicators of the health of fish from specific locations and tributaries. The results could be applied to injury assessment for Natural Resources Damage Assessments and to supplement river-wide and PECS Ecological Risk Assessments. One approach would be to conduct necropsies on a subset of the fish collected for contaminant monitoring. Histopathology would be performed focusing on the gonads and livers. A second approach would be to measure the hatching success of MC and BK collected from locations with a wide range of PCB concentrations. A third would consist of laboratory exposures of fertilized eggs to sediments from these locations. All three approaches would directly assess adverse effects on fish, reducing the uncertainty in applying literature-based thresholds to interpret the contaminant data.

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TABLES

Table 1. Fish sampling locations and information.

Waterbody, Location	ID	Description	Methods	Range description					
					Latitude	Longitude		Latitude	Longitude
2018 Collections									
Anacostia R., Bladensburg, MD	BL	Gravel bar north of Boat Ramp + inlet	Seine, traps	gravel bar (seining)	38.93630	-76.94038	inlet (traps)	38.93734	76.94042
Anacostia trib, Northwest Branch, MD	NW	Downstream of USGS gage	Shock	Start east (downriver)	38.95095	-76.96091	End west (upriver)	38.95341	-76.96163
Anacostia trib, Northeast Branch, MD	NE	Downstream of USGS gage	Shock	Start south (downriver)	38.95541	-76.9287	End north (upriver)	38.95694	-76.92790
Anacostia trib, Watts Branch, DC	WB	Upstream of USGS gage	Shock	Start east (upriver)	38.89886	-76.9403	End west (downriver)	38.89919	-76.94049
Anacostia trib, Hickey Run, DC	HR	Downstream of USGS gage	Shock, traps	Start east (downriver)	38.91148	-76.9639	End west (upriver)	38.91222	-76.96530
Anacostia trib, Lower Beaverdam Creek, MD	LB1	Near USGS gage; Route 201	Shock, traps	Start east (upriver)	38.91612	-76.93181	End west (downriver)	38.91628	-76.93389
Anacostia trib, Lower Beaverdam Creek, MD	LB2	Downstream of Route 202 and Cabin Branch	Shock, traps	Start east (upriver)	38.928424	-76.894427	End west (downriver)	38.9271	-76.89760
Kingman Lake, DC	KL	E. Capitol Br. north to footbridge	Seine, traps	Start north (upriver) seining	38.89403	-76.9659	End south (downriver) traps	38.89019	-76.96567
Anacostia R., DC	A2	Above CSX Bridge	Seine	Single area-- seining	38.88432	-76.96668			
Anacostia R., DC	PC	Pepco Cove (above Benning Road)	Seine	Single area-- seining	38.90124	-76.96044			
Anacostia R., DC	A1	Near Poplar Point	Seine	Single area-- seining	38.86929	-76.997			

Table 1 (continued)									
Waterbody, Location	ID	Description	Methods	Range description					
					Latitude	Longitude		Latitude	Longitude
2019 Collections									
Anacostia R., DC	11A	Between 11th St. and Sousa Br. (11A)	Seine	Single area--seining	38.87324	-76.98543			
Anacostia R., DC	PC	Pepco Cove (above Benning Road)	Seine	Single area--seining	38.90124	-76.96044			
Potomac R., DC	P3	Theo Roosevelt I. (Potomac)	Seine	Single area--seining	38.89985	-77.0662			
Potomac R., DC	P4	Fletcher's Cove (Potomac)	Seine	Single area--seining	38.91847	-77.1031			
Potomac R., DC	K	Key Bridge (Potomac)	Seine	VA side of river	38.90238	-77.0794	DC side of river	38.90488	-77.07908

^^

Table 2. Analytes and detection limits.

PCBs: Individual and Co-Eluting Congeners						
1	30	58	95/93	129	156	184
2	31	74/61	94	130	157	185
3	35	65/62	96	131/142	158/160	186
4/10	36	63	98/102	132/168	159	188
8/5	37	66/80	99	133	161	189
6	38	67	100	134/143	162	191
7/9	39	69	103	144/135	165	193
11	40	70/76	104	136	166	194
12/13	41/71/64/68	72	105/127	137	167	195
14	42/59	77	118/106	138/163/164	169	196/203
15	49/43	78	107/109	149/139	170/190	197
16/32	44	79	110	140	171	198
17	45	81	111/117	141	172/192	199
18	46	82	112	145	173	200
19	47/48/75	83/108	113	146	174/181	201
33/20/21	50	84	114	147	175	202
22	51	85/120	119	148	176	204
34/23	52/73	97/86	122	150	177	205
24/27	53	87/115/116	123 ¹	151	178	206
25	54	88/121	124	152	179	207
26	55	90/101/89	125	153	180	208
28	56/60	91	126	154	187/182	209
29	57	92	128	155	183	
<i>E1 Pesticides</i>				<i>E2 Pesticides</i>		
Hexachlorobenzene		Nonachlor, cis-		HCH, delta		
HCH, alpha		2,4'-DDD		Heptachlor Epoxide		
HCH, beta		4,4'-DDD		alpha-Endosulphan		
HCH, gamma		2,4'-DDE		Dieldrin		
Heptachlor		4,4'-DDE		Endrin		
Aldrin		2,4'-DDT		beta-Endosulphan		
Chlordane, oxy-		4,4'-DDT		Endosulphan Sulphate		
Chlordane, gamma (tr)		Mirex		Endrin Aldehyde		
Chlordane, alpha (cis)		Technical Toxaphene		Endrin Ketone		
Nonachlor, trans-				Methoxychlor		

Detection limits: Approximately 0.00001–0.0002 wet weight ppm (0.01–0.2 wet weight ppb) for PCB congeners and OC pesticides

Table 3. Composite sample list, sample details, lipid data, and summary concentration data (parts per billion wet weight). BK: Banded killifish, MC: Mummichog, S: Small, L: Large, J: Jumbo. See Table 1 for location (loc) descriptions and abbreviations.

Loc	Species	Sample ID	Date	N	% - lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
A1	BK	A1BKL01	9/18/2018	2	3.32	475	227	75.9	69.0	6	19.77
A1	BK	A1BKS01	9/18/2018		3.65	444	210	73.2	58.5	8	14.8
			mean		3.49	460	218	74.6	63.8		
			stdev		0.23	21	12	1.9	7.4		
			median		3.49	460	218	74.6	63.8		
			25th		3.40	452	214	73.9	61.1		
			75th		3.57	467	223	75.2	66.4		
			max		3.65	475	227	75.9	69.0		
A2	BK	A2BKL01	9/18/2018	4	2.40	223	84.1	40.6	69.0	9	30.28
A2	BK	A2BKS01	9/18/2018		3.93	366	132	69.7	62.0	13	26.72
A2	BK	A2BKS02	9/18/2018		3.65	334	121	62.4	55.0	18	28.9
A2	BK	A2BKS03	9/18/2018		4.23	360	141	65.2	60.0	18	32.46
			mean		3.55	321	120	59.5	61.5		
			stdev		0.80	66	25	12.9	5.8		
			median		3.79	347	127	63.8	61.0		
			25th		3.34	306	112	57.0	58.8		
			75th		4.01	362	135	66.3	63.8		
			max		4.23	366	141	69.7	69.0		
BL	BK	BLBKJ01	10/18/2018		2.22	65.7	95.3	17.1	88.0	4	23.65
BL	BK	BLBKJ02	10/18/2018		1.76	47.7	55.4	13.1	87.5	4	24.74
BL	BK	BLBKL01	10/18/2018		1.81	140	91.4	26.4	73.0	7	25.8
BL	BK	BLBKL02	10/18/2018		1.69	152	88.2	27.0	75.0	7	26.63
BL	BK	BLBKS01	10/18/2018		3.66	161	134	30.6	55.5	18	29.41

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
BL	BK	BLBKS02	10/18/2018	6	3.43	155	132	33.9	57.0	18	29.53
			mean		2.43	120	99.4	24.7	72.7		
			stdev		0.89	50	29.7	8.0	14.1		
			median		2.02	146	93.3	26.7	74.0		
			25th		1.77	84	89.0	19.4	61.0		
			75th		3.13	154	123	29.7	84.4		
			max		3.66	161	134	33.9	88.0		
KL	BK	KLBKS01	9/18/2018	1	3.98	378	116	60.5	58.0	9	14.95
LB1	BK	LB1BKL01	10/18/2018	3	2.32	1055	81.5	22.8	69.0	7	24.2
LB1	BK	LB1BKL02	10/18/2018		3.48	1019	122	27.3	71.5	4	14.25
LB1	BK	LB1BKS01	10/18/2018		3.13	1263	110	21.4	55.0	10	16.05
			mean		2.98	1112	105	23.8	65.2		
			stdev		0.60	132	21	3.1	8.9		
			median		3.13	1055	110	22.8	69.0		
			25th		2.73	1037	95.9	22.1	62.0		
			75th		3.31	1159	116	25.1	70.3		
			max		3.48	1263	122	27.3	71.5		
LB2	BK	LB2BKL01	10/18/2018	5	3.19	902	161	11.2	68.0	9	25.14
LB2	BK	LB2BKL02	10/18/2018		2.57	924	130	6.68	70.0	9	24.52
LB2	BK	LB2BKL03	10/18/2018		3.23	923	138	7.83	70.0	9	26.98
LB2	BK	LB2BKL04	10/18/2018		2.03	716	94.3	4.78	79.0	7	24.31
LB2	BK	LB2BKS01	10/18/2018		3.38	1121	206	11.3	60.0	16	26.31
			mean		2.88	917	146	8.37	69.4		
			stdev		0.57	144	41	2.87	6.8		

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
			median		3.19	923	138	7.83	70.0		
			25th		2.57	902	130	6.68	68.0		
			75th		3.23	924	161	11.2	70.0		
			max		3.38	1121	206	11.3	79.0		
NE	BK	NEBKJ01	10/18/2018	3	4.12	87.6	198	17.5	88.0	5	30.49
NE	BK	NEBKJ02	10/18/2018		2.68	74.6	130	13.5	87.0	5	28.81
NE	BK	NEBKJ03	10/18/2018		2.59	69.1	152	12.3	90.0	4	26.45
			mean		3.13	77.1	160	14.4	88.3		
			stdev		0.86	9.5	35	2.7	1.5		
			median		2.68	74.6	152	13.5	88.0		
			25th		2.64	71.8	141	12.9	87.5		
			75th		3.40	81.1	175	15.5	89.0		
			max		4.12	87.6	198	17.5	90.0		
NW	BK	NWBKL01	9/18/2018	4	2.46	48.8	127	23.2	79.0	7	26.49
NW	BK	NWBKS01	9/18/2018		2.78	55.6	155	22.0	55.0	15	19.83
NW	BK	NWBKS02	9/18/2018		2.57	59.3	156	22.0	55.0	17	21.83
NW	BK	NWBKS03	9/18/2018		3.38	53.6	220	21.9	56.0	17	23.59
			mean		2.80	54.3	165	22.3	61.3		
			stdev		0.41	4.4	39	0.6	11.8		
			median		2.68	54.6	156	22.0	55.5		
			25th		2.54	52.4	148	21.9	55.0		
			75th		2.93	56.5	172	22.3	61.8		
			max		3.38	59.3	220	23.2	79.0		
WB*	BK	WCBKL01	10/18/2018	2	3.48	300	117	54.5	82.0	4	17.34

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
WB	BK	WCBKL02	10/18/2018		2.49	246	86.2	36.8	83.0	3	16.57
			mean		2.99	273	102	45.6	82.5		
			stdev		0.70	39	22	12.5	0.7		
			median		2.99	273	102	45.6	82.5		
			25th		2.74	259	93.9	41.2	82.3		
			75th		3.23	287	109	50.0	82.8		
			max		3.48	300	117	54.5	83.0		
A2	MC	A2MCL01	9/7/2018	4	2.83	179	51.3	40.1	71.0	5	22.92
A2	MC	A2MCL02	9/7/2018		3.46	180	58.2	40.6	70.0	5	22.35
A2	MC	A2MCS01	9/7/2018		3.57	232	69.4	52.5	56.0	10	23.97
A2	MC	A2MCS02	9/7/2018		3.62	234	64.5	51.9	57.0	14	28.73
			mean		3.37	206	60.8	46.3	63.5		
			stdev		0.37	31	7.8	6.9	8.1		
			median		3.52	206	61.3	46.3	63.5		
			25th		3.30	180	56.5	40.5	56.8		
			75th		3.58	232	65.7	52.1	70.3		
			max		3.62	234	69.4	52.5	71.0		
BL	MC	BLMCL01	10/15/2018	4	3.15	68.8	49.6	13.9	69.0	5	25.24
BL	MC	BLMCL02	10/15/2018		3.24	59.3	45.7	13.7	82.0	3	22.22
BL	MC	BLMCS01	10/15/2018		3.75	100	95.1	26.6	57.0	9	23.42
BL	MC	BLMCS02	10/15/2018		3.95	85.1	73.6	20.9	58.0	11	24.46
			mean		3.52	78.2	66.0	18.7	66.5		
			stdev		0.39	17.8	23.0	6.2	11.7		
			median		3.50	77.0	61.6	17.4	63.5		
			25th		3.22	66.4	48.6	13.8	57.8		

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
			75th		3.80	88.8	79.0	22.3	72.3		
			max		3.95	100	95.1	26.6	82.0		
HR	MC	HRMCJ01	10/18/2018	5	5.11	115	58.2	41.2	85	3	29.14
HR	MC	HRMCJ02	10/18/2018		4.73	173	77.0	50.2	88.5	2	21.56
HR	MC	HRMCL01	10/18/2018		4.47	132	60.4	37.6	73.0	6	39.58
HR	MC	HRMCL02	10/18/2018		3.94	98.6	53.2	46.1	72.5	6	34.23
HR	MC	HRMCS01	10/18/2018		3.04	164	64.6	32.1	59.5	10	25.88
			mean		4.26	137	62.7	41.4	75.7		
			stdev		0.80	32	9.0	7.1	11.5		
			median		4.47	132	60.4	41.2	73.0		
			25th		3.94	115	58.2	37.6	72.5		
			75th		4.73	164	64.6	46.1	85.0		
			max		5.11	173	77.0	50.2	88.5		
KL	MC	KLMCL01	10/15/2018	3	2.36	197	40.3	29.8	69.0	4	16.54
KL	MC	KLMCS01	10/15/2018		2.64	263	57.2	31.9	55.5	10	20.09
KL	MC	KLMCS02	10/15/2018		2.56	241	63.4	28.8	54.0	10	19.58
			mean		2.52	234	53.7	30.2	59.5		
			stdev		0.14	33	12.0	1.6	8.3		
			median		2.56	241	57.2	29.8	55.5		
			25th		2.46	219	48.8	29.3	54.8		
			75th		2.60	252	60.3	30.9	62.3		
			max		2.64	263	63.4	31.9	69.0		
LB1	MC	LB1MCL01	10/18/2018	5	2.57	759	51.0	20.9	72.0	5	24.79
LB1	MC	LB1MCS01	10/18/2018		2.29	677	29.2	10.2	60.5	6	16.83
LB1	MC	LB1MCS02	10/18/2018		3.45	846	86.4	32.6	51.0	11	17.42

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
LB1	MC	LB1MCS03	10/18/2018		2.51	719	54.5	21.8	47.0	9	15.96
LB1	MC	LB1MCS04	10/18/2018		2.57	643	49.7	19.8	51.5	8	15.35
			mean		2.68	729	54.1	21.0	56.4		
			stdev		0.45	79	20.6	7.9	10.0		
			median		2.57	719	51.0	20.9	51.5		
			25th		2.51	677	49.7	19.8	51.0		
			75th		2.57	759	54.5	21.8	60.5		
			max		3.45	846	86.4	32.6	72.0		
LB2	MC	LB2MCL01	10/18/2018	5	3.18	891	99.0	5.85	68.0	7	27.51
LB2	MC	LB2MCL02	10/18/2018		2.72	577	90.6	7.80	71.5	6	25.66
LB2	MC	LB2MCL03	10/18/2018		2.68	784	86.5	6.50	70.0	7	28.66
LB2	MC	LB2MCL04	10/18/2018		3.31	783	112	14.5	69.0	7	27.3
LB2	MC	LB2MCS01	10/18/2018		3.53	599	98.7	6.81	58.5	10	20.46
			mean		3.08	727	97.3	8.29	67.4		
			stdev		0.37	134	9.76	3.53	5.1		
			median		3.18	783	98.7	6.81	69.0		
			25th		2.72	599	90.6	6.50	68.0		
			75th		3.31	784	99.0	7.80	70.0		
			max		3.53	891	112	14.5	71.5		
NE	MC	NEMCL01	10/18/2018	2	2.61	53.3	80.4	9.65	77.0	5	30.75
NE	MC	NEMCL02	10/18/2018		2.54	43.4	124	8.34	77.0	5	31.37
			mean		2.58	48.4	102	9.00	77.0		
			stdev		0.05	7.0	31	0.93	0.0		
			median		2.58	48.4	102	9.00	77.0		
			25th		2.56	45.9	91.2	8.67	77.0		

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
			75th		2.59	50.8	113	9.32	77.0		
			max		2.61	53.3	124	9.65	77.0		
PC	MC	PCMCS01	10/15/2018	4	2.99	417	58.8	26.8	57.0	9	18.9
PC	MC	PCMCS02	10/15/2018		2.76	375	53.4	26.0	58.0	8	21.38
PC	MC	PCMCS03	10/15/2018		2.20	472	45.9	21.9	62.0	6	17.01
PC	MC	PCMCS04	10/15/2018		2.22	618	78.0	38.9	64.5	6	15.61
			mean		2.54	470	59.0	28.4	60.4		
			stdev		0.40	106	13.7	7.3	3.5		
			median		2.49	444	56.1	26.4	60.0		
			25th		2.22	406	51.5	25.0	57.8		
			75th		2.82	508	63.6	29.8	62.6		
			max		2.99	618	78.0	38.9	64.5		
WB	MC	WCMCJ01	10/18/2018	3	4.16	272	75.7	53.7	90.5	2	20.93
WB	MC	WCMCL01	10/18/2018		4.44	407	94.1	78.2	80.0	2	16.21
WB	MC	WCMCL02	10/18/2018		2.67	268	88.0	35.6	73.0	3	17.84
			mean		3.76	315	85.9	55.8	81.2		
			stdev		0.95	79	9.4	21.4	8.8		
			median		4.16	272	88.0	53.7	80.0		
			25th		3.42	270	81.8	44.6	76.5		
			75th		4.30	339	91.0	65.9	85.3		
			max		4.44	407	94.1	78.2	90.5		
11A	BK	11ABKJ01	6/27/2019	13	2.23	166	58.0	31.1	86.5	4	27.13
11A	BK	11ABKJ02	6/27/2019		2.38	158	57.8	33.1	85.5	4	29.04
11A	BK	11ABKL01	6/27/2019		2.63	204	78.6	36.3	74.0	6	27.71
11A	BK	11ABKL02	6/27/2019		2.00	193	70.7	37.1	72.5	6	26.67

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
11A	BK	11ABKL03A	6/27/2019		2.35	178	77.1	37.2	78.0	6	30.06
11A	BK	11ABKL03B	6/27/2019		2.32	171	73.6	35.5	dup	dup	30.06
		dup mean			2.34	175	75.4	36.3	78.0	6	30.06
11A	BK	11ABKL04	6/27/2019		2.50	211	80.4	33.7	71.0	6	25.25
11A	BK	11ABKL05	6/27/2019		2.18	146	55.8	23.8	74.0	6	28.82
11A	BK	11ABKL06	6/27/2019		2.44	181	70.4	29.1	71.5	6	26.1
11A	BK	11ABKL07	6/27/2019		1.91	127	51.3	24.6	73.0	6	26.41
11A	BK	11ABKL08	6/27/2019		2.31	250	85.5	37.9	73.5	6	27.14
11A	BK	11ABKL09	6/27/2019		2.62	196	79.7	34.4	75.0	6	28.75
11A	BK	11ABKL10	6/27/2019		2.37	244	96.0	48.0	73.5	6	29.34
11A	BK	11ABKS01	6/27/2019		2.41	255	93.6	36.6	65.0	10	26.93
			mean		2.33	193	73.3	34.0	74.8		
			stdev		0.20	38	13.3	5.8	5.6		
			median		2.37	193	75.4	34.4	73.5		
			25th		2.27	169	64.2	32.1	72.6		
			75th		2.43	208	80.1	36.9	77.3		
			max		2.63	255	96.0	48.0	86.5		
KEY BR	BK	K19BKL01	8/6/2019	8	1.65	36.8	19.3	12.9	67.0	6	20.17
KEY BR	BK	K19BKL02	8/6/2019		1.63	32.7	11.9	8.27	72.0	4	14.5
KEY BR	BK	K19BKS01	8/6/2019		2.48	39.1	26.8	14.2	60.0	10	21.42
KEY BR	BK	K19BKS02	8/6/2019		2.76	40.1	30.9	13.2	60.5	10	20.9
KEY BR	BK	K19BKS03	8/6/2019		2.81	98.7	24.5	15.8	59.0	10	20.07
KEY BR	BK	K19BKS04	8/6/2019		3.01	37.1	29.9	12.2	56.0	12	20.43
KEY BR	BK	K19BKS05	8/6/2019		3.02	66.0	26.4	14.8	57.0	12	22.58
KEY BR	BK	K19BKS06	8/6/2019		2.80	39.5	22.8	12.4	58.0	13	24.96

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
			mean		2.52	48.8	24.1	13.0	61.2		
			stdev		0.53	21.2	5.8	2.1	5.15		
			median		2.78	39.3	25.5	13.0	59.5		
			25th		2.76	39.1	24.5	12.9	59.0		
			75th		2.86	46.6	27.6	14.3	62.1		
			max		3.02	98.7	30.9	15.8	72.0		
P3	BK	P319BKL01	8/8/2019	1	1.89	89.5	28.7	17.9	71.5	4	14.32
P4	BK	P419BKL01	8/8/2019	3	1.88	13.5	13.7	11.2	76.0	3	14.94
P4	BK	P419BKS01	8/8/2019		2.84	45.6	42.1	28.0	61.0	10	22.8
P4	BK	P419BKS02	8/8/2019		3.16	28.9	37.4	28.1	57.0	10	21.15
			mean		2.63	29.3	31.1	22.4	64.7		
			stdev		0.67	16.1	15.2	9.7	10.0		
			median		2.84	28.9	37.4	28.0	61.0		
			25th		2.36	21.2	25.5	19.6	59.0		
			75th		3.00	37.3	39.7	28.0	68.5		
			max		3.16	45.6	42.1	28.1	76.0		
PEPCO	BK	PC19BKL01	7/19/2019	4	2.58	345	80.5	30.0	70.0	5	17.01
PEPCO	BK	PC19BKL02	7/19/2019		3.04	422	93.2	33.5	70.5	4	20.2
PEPCO	BK	PC19BKS01	7/19/2019		2.90	399	63.9	25.4	53.5	10	20.2
PEPCO	BK	PC19BKS02	8/6/2019		2.66	84.7	40.8	20.4	63.0	10	17.79
			mean		2.80	313	69.6	27.3	64.3		
			stdev		0.21	155	22.6	5.7	7.9		
			median		2.78	372	72.2	27.7	66.5		
			25th		2.64	280	58.1	24.1	60.6		

Table 3 (continued)											
Loc	Species	Sample ID	Date	N	% lipid	Total PCBs (sum congeners)	tChlordane	tDDT	Median length (mm)	# in composite	sample wet wt (g)
			75th		2.94	405	83.7	30.9	70.1		
			max		3.04	422	93.2	33.5	70.5		
PEPCO	MC	PC19MCL01	7/19/2019	3	1.78	205	40.5	15.7	72.5	4	21.3
PEPCO	MC	PC19MCL02	7/19/2019		2.19	223	43.9	17.6	70.0	3	16.88
PEPCO	MC	PC19MCS01	7/19/2019		2.58	329	51.9	20.6	48.0	11	17.05
			mean		2.18	252	45.4	18.0	63.5		
			stdev		0.40	67	5.9	2.4	13.5		
			median		2.19	223	43.9	17.6	70.0		
			25th		1.99	214	42.2	16.7	59.0		
			75th		2.39	276	47.9	19.1	71.3		
			max		2.58	329	51.9	20.6	72.5		

*Watts Branch (WB) sample IDs were labeled WC in the analytical catalog.

Table 4a Linear models for total PCBs; model: totPCB ~ as.factor(LocCod) + cLipid
df: degrees of freedom AIC: Aikake Information Criterion.

source	sum of squares	df	mean square	F-stat	p-value
Mummichog					
as.factor(LocCod)	2345090	9	260565	44.9	<0.0001
cLipid	5489	1	5489	0.95	0.3397
Residuals	156853	27	5809	NA	
total	2507432	37	NA	NA	
Slope estimate: 27.3; Model AIC 448; Model Root Mean Square Error: 76.2					
Banded killifish					
as.factor(LocCod)	5425488	13	417345	91.1	<0.0001
cLipid	39168	1	39168	8.6	0.0054
Residuals	201529	44	4580	NA	
total	5666185	58	NA	NA	
Slope estimate: 52.5; Model AIC 679; Model Root Mean Square Error: 67.7					

Table 4b. Comparison of model based mean concentrations of total PCBs (ppb wet weight); Holm multiple comparison test with experiment-wise error rate of $p=0.05$. Lower Estimate and Upper Estimate: 95th percentile confidence intervals for the mean.

Location	N	Mean \pm SD	Adjusted Mean	Lower Est.	Upper Est.	Group ^a
Mummichogs						
LB1	5	729 \pm 79	741	666	815	A
LB2	5	727 \pm 134	728	658	798	A
PC18	4	470 \pm 106	486	401	571	B
WB	3	315 \pm 79	298	201	396	B,C
PC19	3	252 \pm 67	278	173	383	C
KL	3	234 \pm 33	250	153	346	C
A2	4	206 \pm 31	199	120	279	C
HR	5	137 \pm 32	105	10	201	C
BL	4	78.2 \pm 17.8	67	-14	149	C
NE	2	48.4 \pm 7.0	63	-52	178	C
Banded killifish						
LB1	3	1112 \pm 132	1099	1020	1179	A
LB2	5	917 \pm 144	909	848	971	B
A1	2	460 \pm 21	420	320	520	C
KL	1	378	312	169	456	C,D,E
PC19	4	313 \pm 155	309	241	378	C,D
A2	4	321 \pm 66	278	203	352	C,D
WB	2	273 \pm 39	260	163	357	C,D
11A	13	193 \pm 38	214	173	254	D
BL	6	120 \pm 50	136	79	193	D,E
P3	1	89.5	134	-6	273	C,D,E
KB	8	48.8 \pm 21.2	60	11	109	E
NE	3	77.1 \pm 9.5	56	-24	136	D,E
NW	4	54.3 \pm 4.4	51	-18	119	E
P4	3	29.3 \pm 16.	35	-44	114	E

^aGroups with different letters are significantly different at $p<0.05$.

Table 4c. Linear model analysis for combined species for total PCBs. Model: totPCB ~ as.factor(SpeCod) + as.factor(LocCod) + cLipid. Slope estimate: 46.8

source	sum of squares	df	mean square	F-stat	p-value
Species	128003	1	128003	18.5	<0.0001
Location	7565497	15	504366	72.9	<0.0001
Lipid	61731	1	61731	8.9	0.0037
Residuals	546389	79	6916		
Total	8301620	96			

Table 4d. Comparison of model based mean concentrations of total PCBs (ppb wet weight) for combined species; Holm multiple comparison test with experiment-wise error rate of $p=0.05$. Number of samples per species; BK: banded killifish; MC: mummichog.

location	Adjusted Mean	BK n=	MC n=	Group ^a	Ranking of Tribs ^b
LB1	895	3	5	A	1
LB2	819	5	5	A	1
PC18	552	0	4	B	
A1	370	2	0	B,C	
KL	303	1	3	C	
PC19	296	4	3	C	
WB	287	2	3	C	2
A2	238	4	4	C	
11A	157	13	0	C,D	
HR	138	0	5	C,D	3
BL	93	6	4	D	
P3	74	1	0	C,D	
NE	54	3	2	D	4
KB	4	8	0	D	
NW	-3	4	0	D	5
P4	-20	3	0	D	

^a Groups with different letters are significantly different at $p<0.05$.

^b Average of LB1 and LB2 adjusted mean concentrations used for tributary ranking.

Table 5a Model results for total chlordane; model: $tChlordane \sim as.factor(LocCod) + cLipid$. df: degrees of freedom AIC: Aikake Information Criterion.

source	sum of squares	df	mean square	F-stat	p-value
Mummichog					
as.factor(LocCod)	11403	9	1267	6.4	<0.0001
cLipid	880	1	880	4.5	0.0442
Residuals	5333	27	198	NA	
total	17617	37	NA	NA	
Slope estimate: 10.9; Model AIC:319.72; Model Root Mean Square Error:14.05					
Banded killifish					
as.factor(LocCod)	144325	13	11102	43.8	<0.0001
cLipid	15220	1	15220	60.0	<0.0001
Residuals	11158	44	254	NA	
total	170703	58	NA	NA	
Slope estimate: 32.7; Model AIC: 508.7; Model Root Mean Square Error: 15.9					

Table 5b. Comparison of model based mean concentrations of total chlordane (ppb wet weight); Holm multiple comparison test with experiment-wise error rate of $p=0.05$. Adj: adjusted mean; Lower Estimate and Upper Estimate are the 95th percentile confidence intervals for the mean.

location	N	Mean \pm SD	Adjusted Mean	Lower Est.	Upper Est.	Group ^a
Mummichogs						
NE	2	102 \pm 31	108	87	129	A
LB2	5	97.3 \pm 9.8	98	85	111	A, B
WB	3	85.9 \pm 9.4	79	61	97	A, B
PC18	4	59.0 \pm 13.7	65	50	81	A, B
BL	4	66.0 \pm 23.0	62	47	77	A, B
KL	3	53.7 \pm 12.0	60	42	78	B
LB1	5	54.1 \pm 20.6	59	45	73	B
A2	4	60.8 \pm 7.8	58	43	73	B
PC19	3	45.4 \pm 5.9	56	36	75	B
HR	5	62.7 \pm 9.0	50	33	68	B
Banded killifish						
A1	2	218 \pm 12	194	170	217	A
NW	4	165 \pm 39	162	146	178	A,B
NE	3	160 \pm 35	147	128	166	A,B
LB2	5	146 \pm 41	141	127	155	B
BL	6	99.4 \pm 29.7	109	96	123	B,C
LB1	3	105 \pm 21	96	78	115	C
WB	2	102 \pm 22	93	71	116	C
A2	4	120 \pm 25	93	75	110	C
11A	13	73.3 \pm 13.3	86	77	96	C
KL	1	116	75	41	109	C,D
PC19	4	69.6 \pm 22.6	67	51	84	C,D
P3	1	28.7	56	23	89	C,D
P4	3	31.1 \pm 15.2	34	16	53	D
KB	8	24.1 \pm 5.8	31	19	42	D

^aGroups with different letters are statistically different at $p<0.05$.

Table 5c. Linear models analysis for combined species^a for total chlordanes. Model: tot chlordanes ~ as.factor(SpeCod) + as.factor(LocCod) + cLipid. Slope estimate: 21.8.

source	sum of squares	df	mean square	F-stat	p-value
Species	16182	1	16182	57.8	<0.0001
Location	152792	15	10186	36.4	<0.0001
Lipid	13428	1	13428	48.0	<0.0001
Residuals	22101	79	279.8		
total	204503	96			

Table 5d. Comparison of model based mean concentrations of total chlordane (ppb wet weight) for combined species; Holm multiple comparison test with experiment-wise error rate of $p=0.05$. Number of samples per species; BK: banded killifish; MC: mummichog.

location	Adjusted Mean	BK n=	MC n=	Group ^a	Ranking of Tribs ^b
A1	184	2	0	A	
NW	145	4	0	A,B	1
NE	133	3	2	B	2
LB2	120	5	5	B,C	3
PC18	89	0	4	C	
WB	85	2	3	C	4
BL	83	6	4	C	
LB1	81	3	5	C	3
KL	81	1	3	C	
A2	78	4	4	C	
PC19	65	4	3	C	
11A	64	13	0	C	
HR	55	0	5	C,D	5
P3	29	1	0	C,D	
P4	16	3	0	D	
KB	11	8	0	D	

^a Groups with different letters are significantly different at $p<0.05$.

^b Average of LB1 and LB2 adjusted mean concentrations used for tributary ranking.

Table 6a Model results for total DDT. model: totDDT ~ as.factor(LocCod) + cLipid.
df: degrees of freedom AIC: Aikake Information Criterion.

source	sum of squares	df	mean square	F-stat	p-value
Mummichogs					
as.factor(LocCod)	8124	9	903	22.3	<0.0001
cLipid	761	1	761	18.8	0.0002
Residuals	1092	27	40.4	NA	
Total	9977	37	NA	NA	
Slope estimate: 10.1, Model AIC: 259.4; Model Root Mean Square Error: 6.4					
Banded killifish					
as.factor(LocCod)	15172	13	1167	44.3	<0.0001
cLipid	681	1	681	25.0	<0.0001
Residuals	1158	44	26.3	NA	
Total	17012	58	NA	NA	
Slope estimate: 6.9, Model AIC: 375.1; Model Root Mean Square Error: 5.1					

Table 6b. Comparison of model based mean concentrations of total DDT (ppb wet weight); Holm multiple comparison test with experiment-wise error rate of $p=0.05$. Lower Estimate and Upper Estimate are the 95th percentile confidence intervals for the adjusted mean.

location	N	Mean \pm SD	Adjusted Mean	Lower Est.	Upper Est.	Group ^a
Mummichogs						
WB	3	55.8 \pm 21.4	49	41	58	A
A2	4	46.3 \pm 6.9	44	37	50	A,B
KL	3	30.2 \pm 1.6	36	28	44	A,B
PC18	4	28.4 \pm 7.3	34	27	41	A,B
HR	5	46.4 \pm 7.1	30	22	38	B
PC19	3	18.0 \pm 2.4	27	19	36	A,B
LB1	5	21.0 \pm 7.9	26	19	32	B
BL	4	18.7 \pm 6.2	15	8	22	B,C
NE	2	9.00 \pm 0.93	15	5	24	B,C
LB2	5	8.29 \pm 3.53	9	3	14	C
Banded killifish						
A1	2	74.6 \pm 1.9	69	62	77	A
A2	4	59.5 \pm 12.9	54	48	59	B
KL	1	60.5	52	41	63	A,B,C
WB	2	45.6 \pm 12.5	44	37	51	B,C
11A	13	34.0 \pm 5.8	37	34	40	C
PC19	4	27.3 \pm 5.7	27	22	32	C,D
BL	6	24.7 \pm 8.0	27	23	31	D
P3	1	17.9	24	13	34	C,D,E
P4	3	22.4 \pm 9.7	23	17	29	D,E
LB1	3	23.8 \pm 3.1	22	16	28	D,E
NW	4	22.3 \pm 0.6	22	17	27	D,E
KB	8	13.0 \pm 2.1	14	11	18	E
NE	3	14.4 \pm 2.7	12	6	18	E
LB2	5	8.37 \pm 2.87	7	3	12	E

^a Groups with different letters are significantly different at $p<0.05$.

Table 6c. Linear models analysis for combined species^a for total DDT. Model: totDDT ~ as.factor(SpeCod) + as.factor(LocCod) + cLipid. Slope estimate: 7.89

source	sum of squares	df	mean square	F-stat	p-value
as.factor(SpeCod)	6	1	6	0.17	0.68
as.factor(LocCod)	22308	15	1487	40.2	<0.0001
cLipid	1756	1	1756	47.4	<0.0001
Residuals	2925	79	37.0		
total	26995	96			

Table 6d. Comparison of model based mean concentrations of total DDT (ppb wet weight) for combined species; Holm multiple comparison test with experiment-wise error rate of $p=0.05$. Number of samples per species; BK: banded killifish; MC: mummichog.

location	Adjusted Mean	BK n=	MC n=	Group ^a	Ranking of Tribs ^b
A1	67	2	0	A	
A2	49	4	4	B	
WB	48	2	3	B	1
KL	40	1	3	B,C	
11A	36	13	0	C	
PC18	34	0	4	C,D	
HR	34	0	5	C,D	2
PC19	26	4	3	C,D	
LB1	24	3	5	D	3,5
P3	23	1	0	C,D,E	
BL	22	6	4	D,E	
P4	22	3	0	D,E	
NW	20	4	0	D,E	4
KB	13	8	0	D,E	
NE	12	3	2	E	5
LB2	8	5	5	E	3,5

^a Groups with different letters are significantly different at $p<0.05$.

^b Average of LB1 and LB2 adjusted mean concentrations used for tributary ranking.

Table 7. Ranking (in bold) of loading estimates and concentrations in suspended sediments, fish, mussels, and passive samplers from five tributaries. PS: passive samplers.

	LB	WB	HR	NE	NW
Total PCBs					
Load (g, %) ^a	1 (590, 75%)	4 (25, 3.2%)	5 (19, 2.4%)	3 (60, 7.6%)	2 (95, 12%)
Load (g, %) ^b	1 (285, 72%)	4 (15, 3.8%)	5 (7.4, 1.9%)	2 (52, 13%)	3 (36, 9.1%)
Susp. sed. ppb ^a	1	3	2	5	4
Fish ppb (2018)	1	2	3	4	5
Mussels ppb (2016) ^b	1	2	5	4	3
Mussels ppb. (2017) ^b	1	2	3	4	5
PS ppb (2016) ^b	1	2	5	4	3
PS ppb (2017) ^b	1	2	4.5	4.5	3
Total Chlordane					
Load (g, %) ^a	3 (310, 28%)	4 (56, 5%)	5 (11, 1%)	1 (430, 39%)	2 (320, 28%)
Load (g, %) ^b	4 (16.5, 3.4%)	3 (79, 17%)	5 (6.2, 1.3%)	2 (166, 35%)	1 (211, 44%)
Susp. sed. ppb ^a	2	1	4	3	5
Fish ppb (2018)	3	4	5	2	1
Mussels ppb (2016) ^b	4	2	5	3	1
Mussels ppb (2017) ^b	2.5	2.5	5	4	1
PS ppb (2016) ^b	3	2	5	4	1
PS ppb (2017) ^b	3	2	4	5	1
Total DDT					
Load (g, %) ^a	3 (25.4, 13%)	1 (90.5, 46%)	5 (9.4, 4.7%)	4 (10.7, 5.4%)	2 (70.1, 35%)
Load (g, %) ^b	5 (3.9, 2.0%)	3 (38, 20%)	4 (5, 2.6%)	2 (69, 36%)	1 (76, 40%)
Susp. sed. ppb ^a	NA	NA	NA	NA	NA
Fish ppb (2018)	3	1	2	5	4
Mussels ppb (2016) ^b	5	1	2	3	4
Mussels ppb (2017) ^b	3.5	2	1	5	3.5
PS ppb (2016) ^b	3	1	2	4	5
PS ppb (2017) ^b	4	2	1	5	3

^a Suspended sediment and loading data from 2017 by Wilson (2019)

^b Passive sampler and mussel data from Ghosh et al. (2020)

FIGURES

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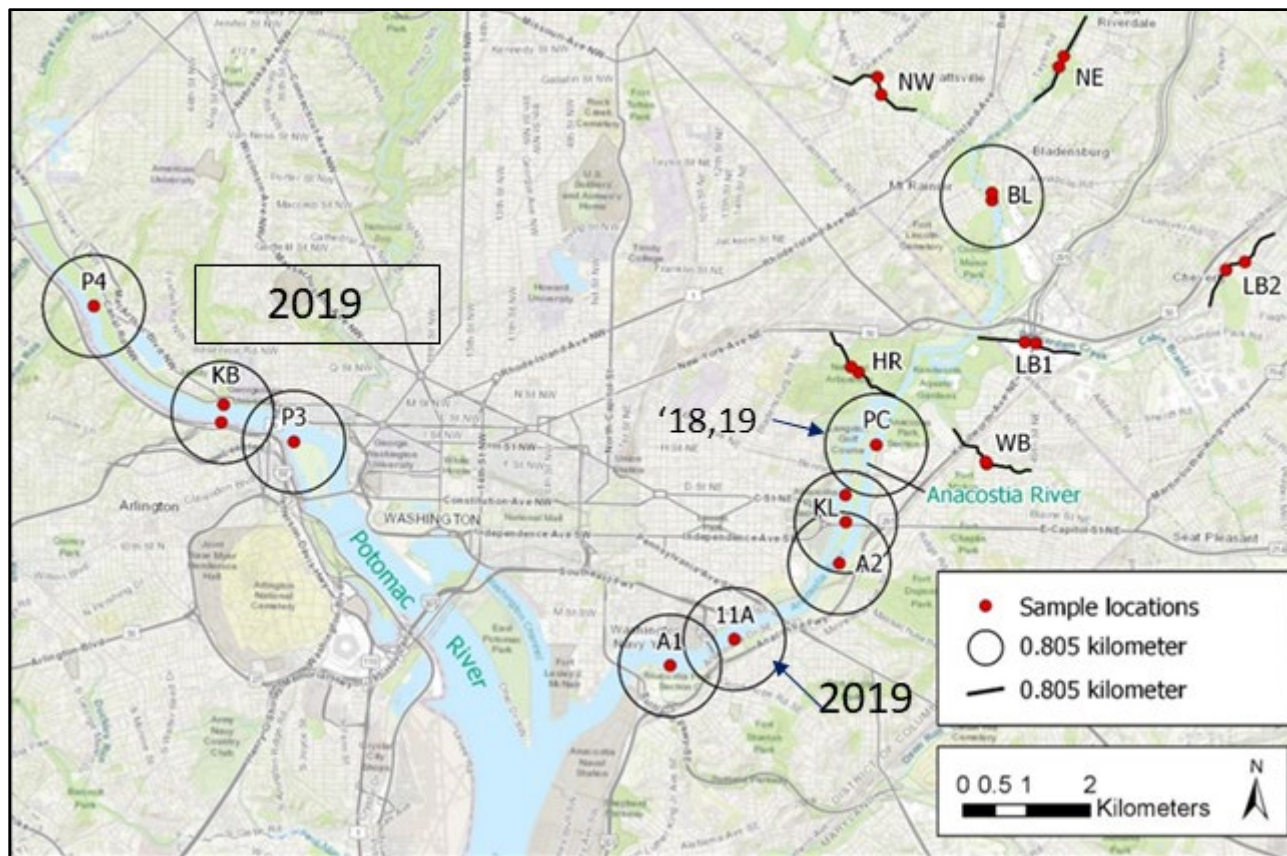


Figure 1. Locations for the 2018 (all Anacostia mainstem and tributaries except 11A) and 2019 (all Potomac locations, 11A, additional PC samples) collections. See Table 1 for abbreviations and descriptions.



Figure 2. Species and sampling photos. a) Mummichog (MC): *Fundulus heteroclitus*: left–female, right–male; b) Banded killifish (BK) *F. diaphanus*: left two —males, right two —females; c) Beach seining; d) Setting a minnow trap

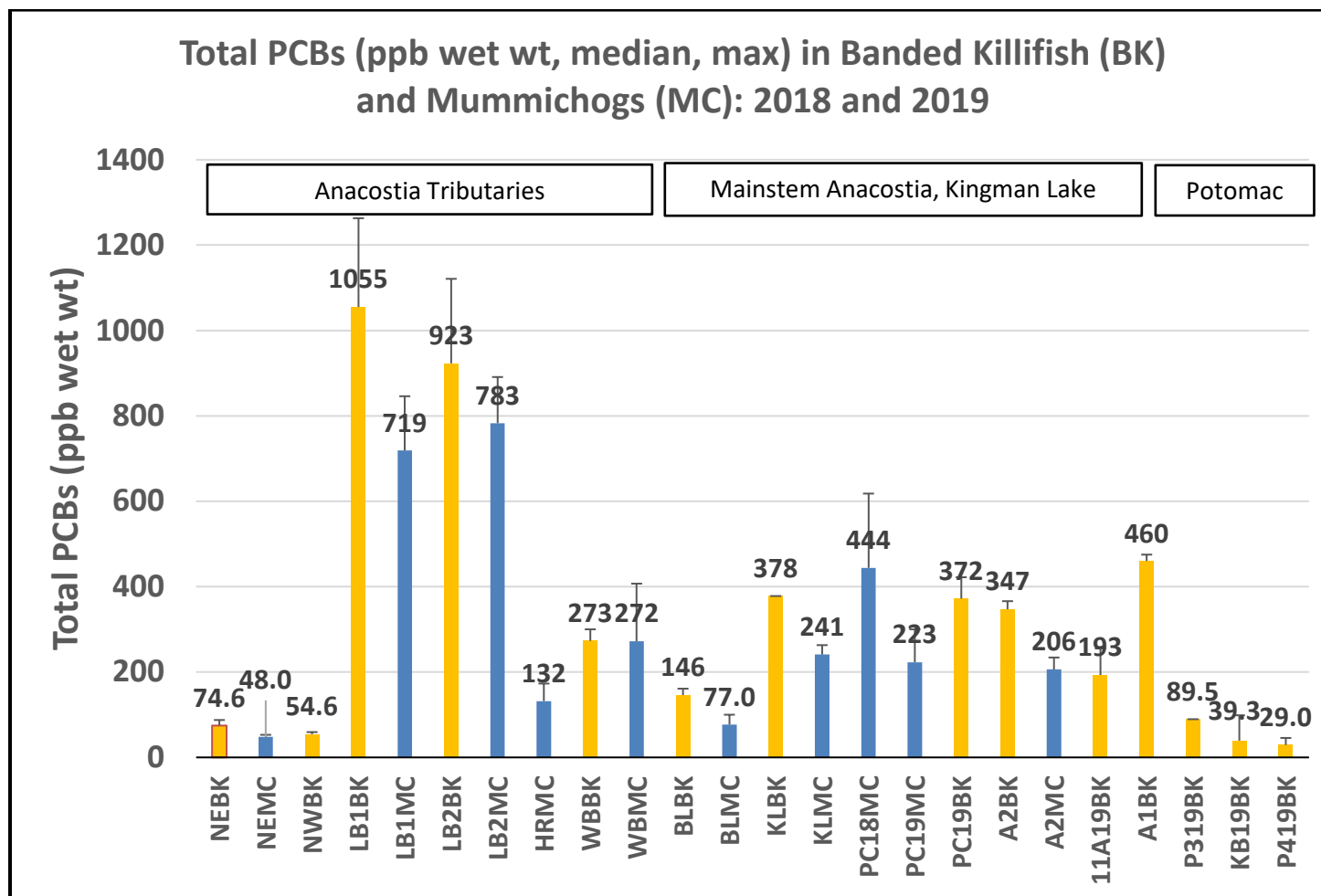


Figure 3. Total PCBs (ppb wet weight; median, max) in banded killifish (BK) and mummichogs (MC) from the mainstem Anacostia, tributaries, Kingman Lake, and Potomac River: 2018 and 2019. See Table 1 for abbreviations and descriptions, Table 3 for data.

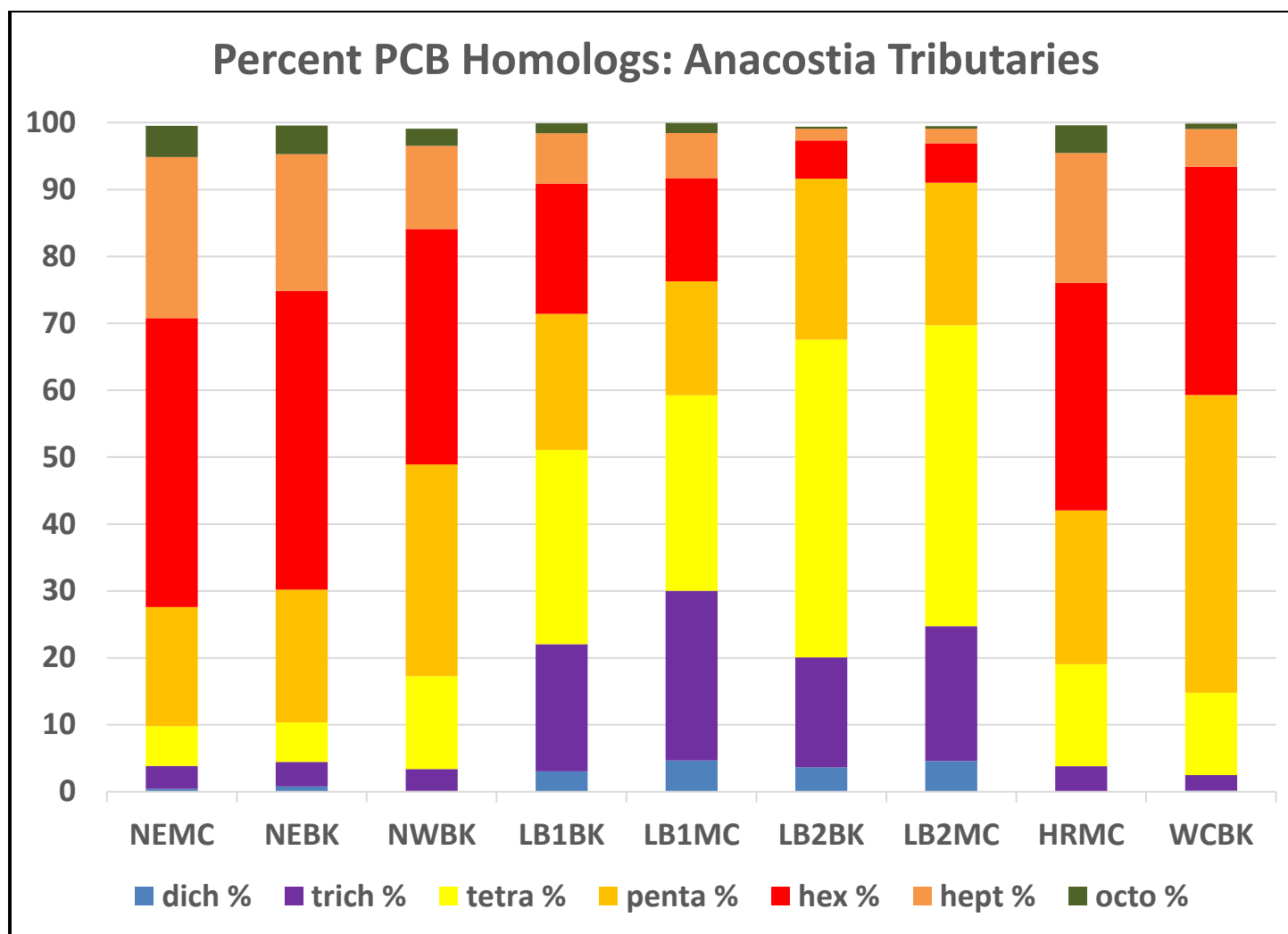


Figure 4. Median percent PCB homologs in banded killifish (BK) and mummichogs (MC) from the Anacostia River tributaries:2018 and 2019. Mono-, nano-, and deca- were minimal. See Table 1 for abbreviations and descriptions.

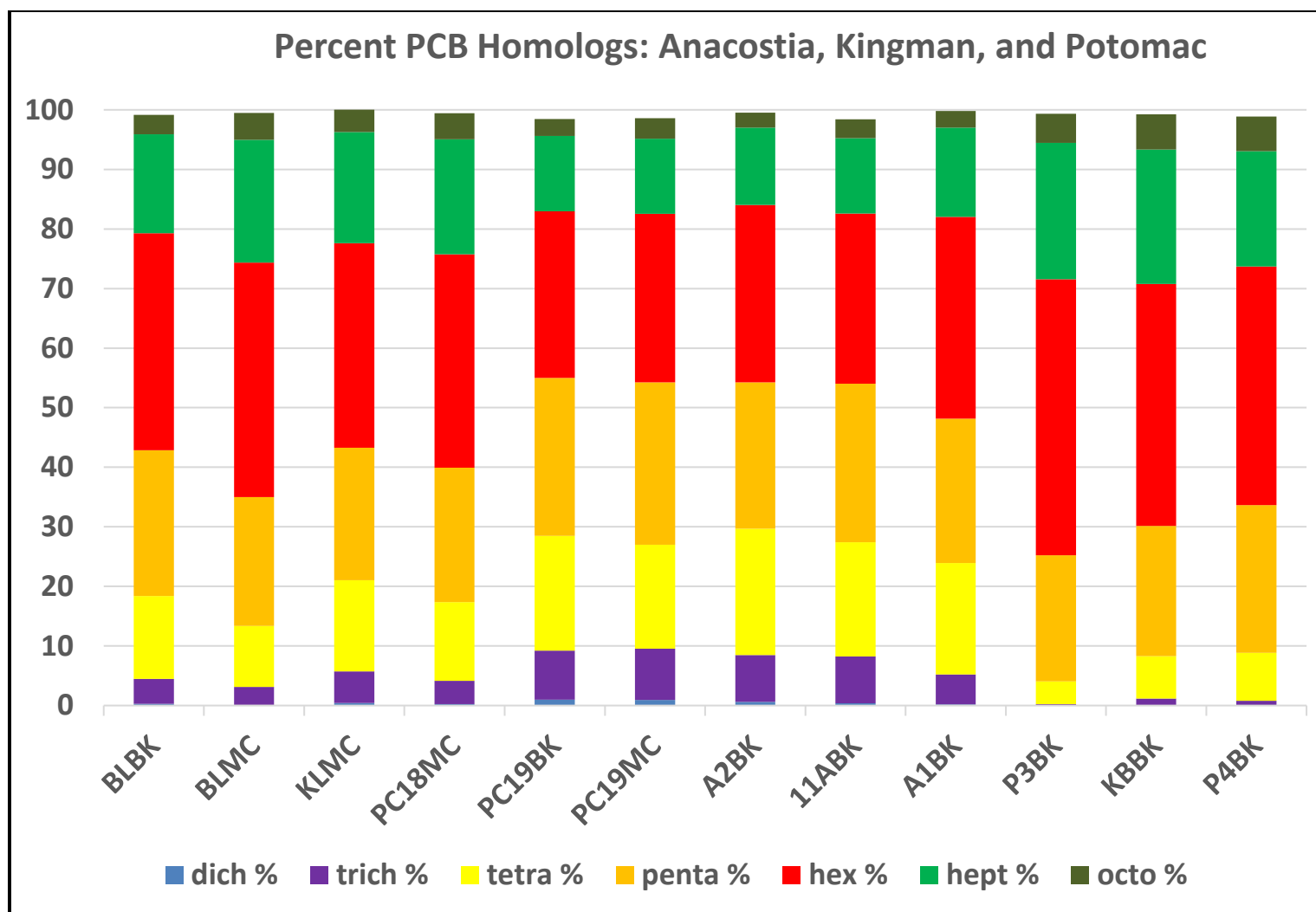


Figure 5. Median percent PCB homologs in banded killifish (BK) and mummichogs (MC) from mainstem Anacostia River, Kingman Lake, and Potomac River: 2018 and 2019. Mono-, nano-, and deca- were minimal. See Table 1 for abbreviations and descriptions.

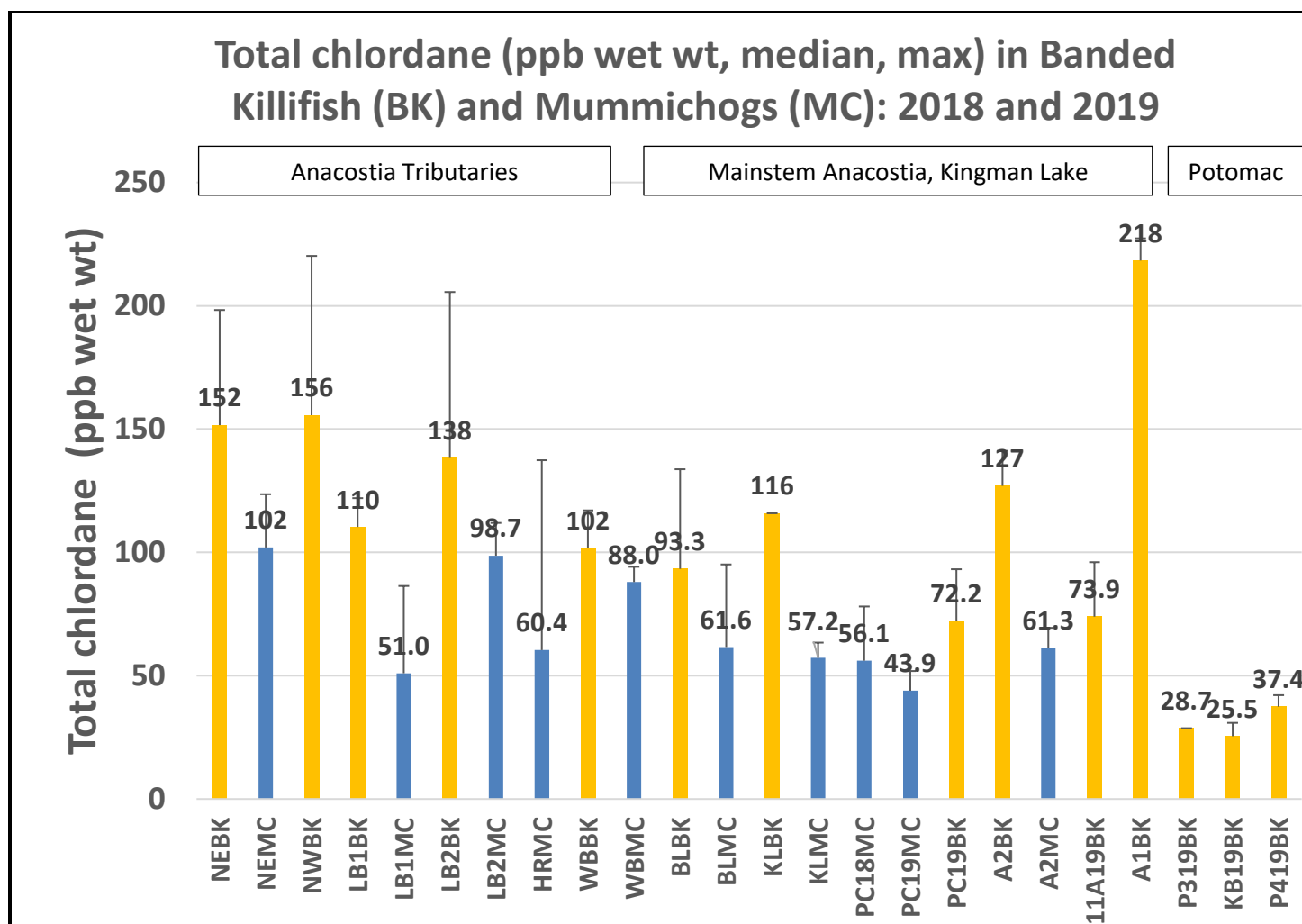


Figure 6. Total chlordane (ppb wet weight; median, max) in banded killifish (BK) and mummichogs (MC) from the mainstem Anacostia, tributaries, Kingman Lake, and Potomac River: 2018 and 2019. See Table 1 for abbreviations and descriptions; Table 3 for data.

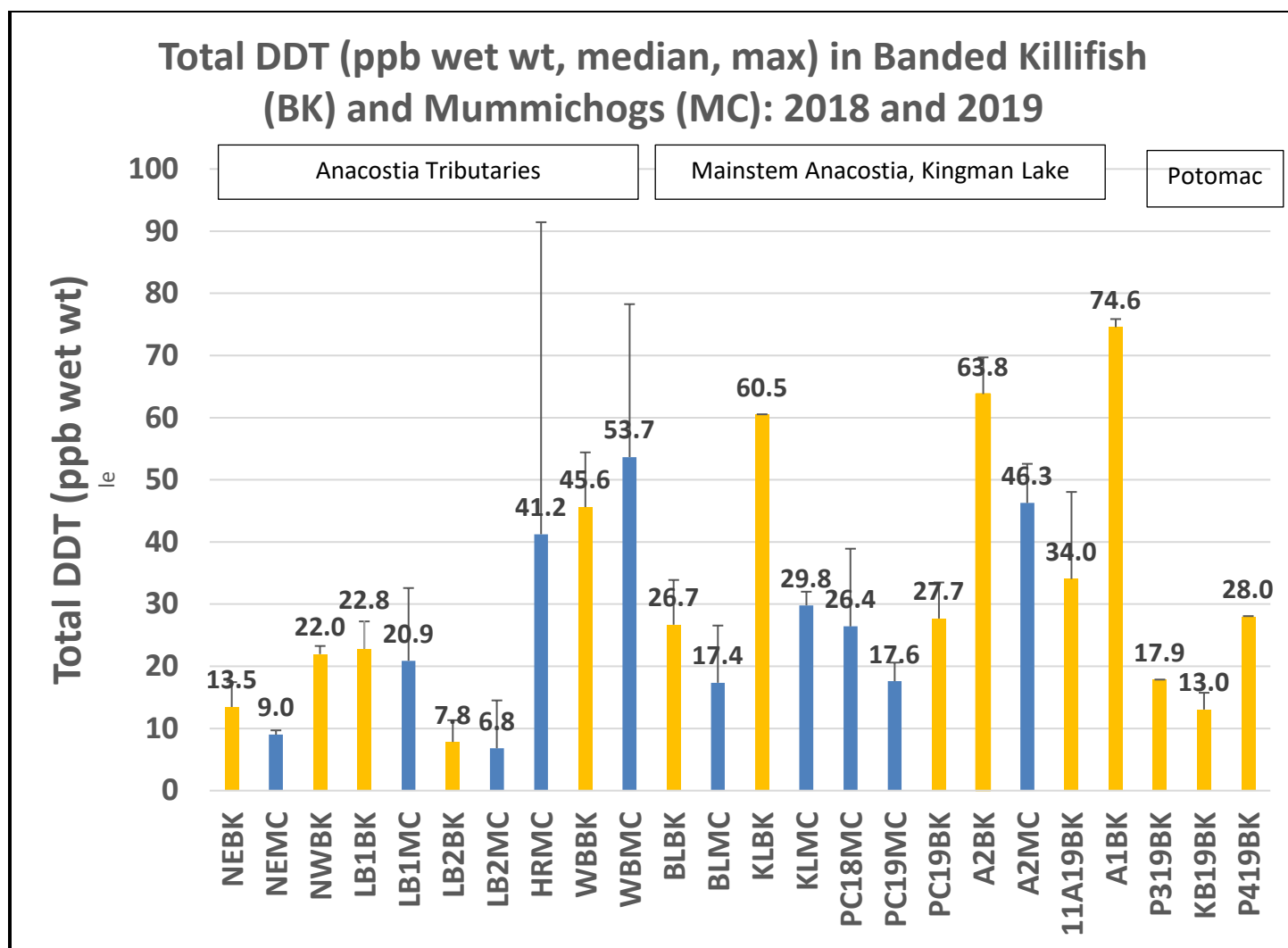


Figure 7. Total DDT (ppb wet weight; median, max) in banded killifish (BK) and mummichogs (MC) from the mainstem Anacostia, tributaries, Kingman Lake, and Potomac River: 2018 and 2019. See Table 1 for abbreviations and descriptions, Table 3 for data.