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The use of caged dreissenid mussels to assess PCB contamination at Manistique River Area of Concern

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Introduction

NOAA Mussel Watch Program conducted a two-year caged mussel bioaccumulation study at Manistique River Area of Concern (AOC) as part of a larger multiagency investigation of the extent and magnitude of polychlorinated biphenyl (PCB) contamination in the river. Mussels harvested from the Manistique harbor breakwater were deployed in cages at 83 locations (both years together) grouped as Upper Channel, Upper Arm, Lower Arm, Harbor, Collection and Beach. The in situ mussels from an offshore site were chosen as the reference to compare and contrast the PCB concentration obtained from the caged mussels. Caged mussels were retrieved after 5 weeks and their soft tissue was analyzed for 80 PCB congeners. The Bayesian Analysis of Variance (BANOVA) showed that the Lower Arm had significantly higher concentrations than the rest of the strata and Upper Arm had significantly higher concentrations than Upper Channel and Harbor. We further examined the PCB homolog profile using Principal Component Analysis and the use of double ratio plot. The mussels from the Lower Arm and Upper Arm showed the dominance of tri and tetra homologs compared to the mussels from other strata and the offshore site. The consistent results obtained from this two-year study indicate the usefulness of caged mussels as a tool in identifying areas of PCB contamination and aiding managers in prioritizing areas for clean-up and restoration efforts.

Bivalves are used worldwide as indicator organisms to monitor and assess aquatic pollution. Several characteristics such as cosmopolitan distribution, ease of collection, ability to tolerate and bioaccumulate contaminants, limited mobility, and limited capacity to metabolize many contaminants make the filter feeding bivalves ideal candidates for environmental monitoring programs. Several studies have shown that the contaminant concentrations in bivalve tissue reflect contaminant concentrations in the water and in suspended particles filtered for food (Cunningham and Tripp, 1975; Pruell et al., 1987; Fisher and Teyssie, 1986; Roesijadi et al., 1984; Martin, 1985; Capuzzo et al., 1989; Sericano, 1993).

In the Great Lakes, dreissenid mussels are widely distributed (except in Lake Superior) and have been used by the NOAA Mussel Watch Program (MWP) as indicator organisms to monitor nearshore pollution since 1992. The dreissenid mussels are recognized for their ecological impacts on the Great Lakes ecosystem since their introduction into the Lakes in late 1980s (Vanderploeg, 2002). More importantly, these mussels have been suggested to play a major role in contaminant cycling and biomagnification of pollutants (indirectly via deposition of feces and psuedofeces, and directly via predation in the food chain) in the Great Lakes (Bruner et al., 1994a, b; Morrison et al., 1998; Marvin et al., 2000; Cho et al., 2004; Kwon et al., 2006). As such, elevated contaminant concentrations in dreissenid mussels that are the primary consumers at the base of the food chain suggest that higher consumers (fish, birds and humans) may be at risk of contamination. Therefore, dreissenid mussels can serve as a vital bioindicator in the Great Lakes.

MWP has documented the presence of heavy metals, organochlorine compounds and select contaminants of emerging concern in the Great Lakes based on *in situ* dreissenid mussel monitoring data (Kimbrough et al., 2013 and 2014). Under the Great Lakes Restoration Initiative (GLRI), the MWP conducted place-based contamination assessments and added

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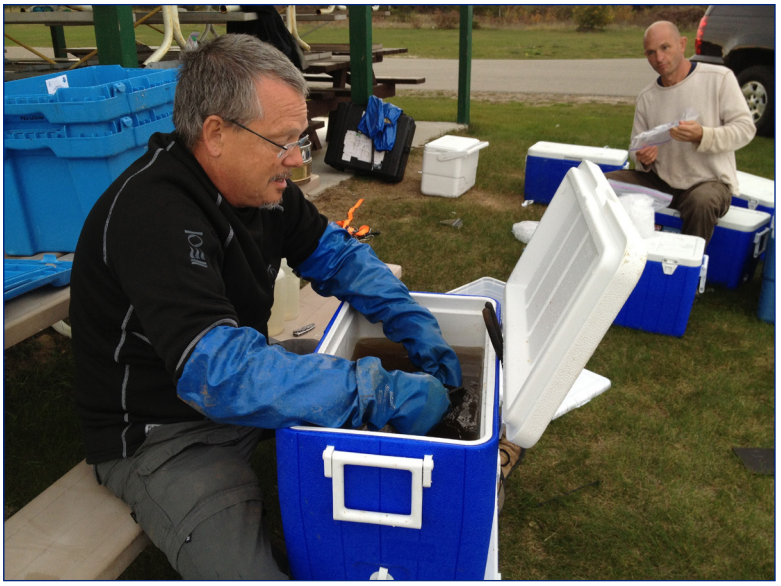
long-term monitoring sites in more impacted and urban areas in rivers and harbors. Further, the MWP also completed basinwide monitoring for baseline assessment of contaminant concentrations of *in situ* dreissenid mussels in the harbor areas of the Great Lakes (Kimbrough et al., 2014). This assessment identified harbors with elevated levels where additional studies and characterizations should be conducted.

To perform site based characterizations, MWP began to use caged dreissenid mussels in upstream areas where in situ mussels were either absent or their collection was logistically infeasible. Caging mussels offers an added advantage in which precise contaminant exposure period at specific sites can be assessed on temporal and spatial scales (Salazar and Salazar, 1997; deLafontaine, 2000; Bocchetti et al., 2008). There is also great potential for using caged mussels to identify areas with elevated contaminant levels as part of a weight of evidence approach, and bioeffects via the measurements of bivalve health through methods such as biomarkers, gene assays, and metabolomics.

Other studies have been conducted in the Great Lakes using caged native mussels as indicators of pollution (Kauss and Hamdy, 1985, 1991; Richman et al., 2011). We chose not to use caged native mussels mainly for two reasons: 1) many species of mussels are a scarce resource (threatened or endangered); 2) native mussels live in the sediment, which in some highly impacted areas may be uninhabitable due to anoxic conditions, high ammonia, or habitat disturbance from dredging or sediment capping activities. On the other hand, invasive dreissenid mussels can attach to hard substrates, unlike native mussels (Mills et al., 1996), and can be easily found on the breakwater boulders.

In this technical memorandum, we summarize the caged mussel results conducted as part of an extensive two-year study in collaboration with other agencies (US Environmental Protection Agency, NOAA, US Army Corps of Engineers, US Geological Survey, and the State of Michigan) to assess the spatial distribution of PCB contamination in lower Manistique River.



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Methods

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Study site and experimental design

The Manistique River study area comprises the last 1.7 miles (from the dam to the mouth of the harbor at Lake Michigan) of the Manistique River, which flows southwest through Schoolcraft County in Michigan's central Upper Peninsula. Also included in the study are an offshore site, the mussel Collection site, and nearby beach sites. A series of remedial actions to remove PCBs from sediments was conducted between 1993-2000.

The multi-agency technical team defined several unique strata in the study area based on its hydrographic characteristics as described in the conceptual site model development report (EA and Foth, 2013). Caged mussels were deployed following a stratified transect design in each of these zones. For the purpose of this manuscript, the caged mussel locations are grouped into Upper Channel, Upper Arm, Lower Arm, Harbor, Collection and Beach sites (Fig. 1). The transects were used to ensure a thorough spatial assessment of all relevant strata within the River and included targeted and random sites. In addition to transects, targeted stations were chosen based on prior knowledge of environmental condition, historical land use, and ongoing studies. To achieve a desired density of samples, additional stations were randomly selected using ArcGIS.

Dreissenid mussels harvested from established populations were collected from the stone breakwater of the outer harbor of Manistique River, placed in cages, and deployed throughout the study area. In year 1 and year 2 mussels were deployed on July 18 and June 6 respectively and retrieved after approximately 5 weeks. In one stratum the initial cages were lost, as a result additional mussels were deployed in October to characterize that stratum. In 2013, more caged mussel sites were added resulting in a total of 51 sites. Beach and Collection strata were added in 2013. We deployed three cages at the Collection sites but could successfully retrieve only two cages after 5 weeks. The mussel samples for caging were collected by scuba divers from breakwater boulders using a metal paint scraper. The mussels were placed into a separate nylon mesh dive bag and the bags were gently shaken underwater to remove debris. Upon surfacing, the harvested mussels in individual mesh bags were immediately placed in a 26 L cooler with site water (cooled by ice in sealed plastic bags to maintain near-ambient

Fig 1. Caged mussel locations in Manistique River Area of Concern (AOC) in 2012 and 2013. The upper inset map shows the location of the Manistique River AOC in the Great Lakes. The lower inset map shows the location of the offshore reference site sampled in both years .

temperatures) until deployment. Approximately 300-500 mussels were placed inside each cage, which consisted of torpedo shaped metal minnow traps that were tightly secured with cable ties. The cages were deployed approximately 0.5 m above the river bottom and secured to cinder blocks as anchors. A subsample of *in situ* mussels from the collection site and from an offshore site were sent to the laboratory for chemical analysis to provide baseline concentrations against which the caged mussel concentrations could be compared.

Chemical analysis

Protocols for analytical methods of organic contaminants in mussel tissue are detailed in Kimbrough et al. (2006). Briefly, a composite of 50-100 mussels from each station are shucked, homogenized, extracted with dichloromethane, purified and analyzed for PCB by gas chromatograph following a modified EPA Method 8082A. An extended list of PCB congeners (80 congeners) were selected for analysis in mussel tissue. The MWP uses a performance based quality assurance process to ensure data quality. This effort has been in operation since 1985 and is designed to document sampling protocols, analytical procedures, and laboratory performance.

Data analysis

Data Treatment: PCB concentration for a station was obtained by summing the concentration of 80 congeners. In both years, the concentrations were not correlated to lipid content. As such, we used PCB concentrations expressed as ng/g dry weight for all statistical analyses. For BANOVA analysis, we pooled both years of data because preliminary test showed that the year did not have an effect. As a result, samples from the same strata are replicates. For Principal Component Analysis (PCA), we used only 2013 data, which had more stations than the previous year.

BANOVA

BANOVA was performed to determine differences in total concentrations of PCBs among the 6 groups. The BANOVA sum to zero (STZ) method was used to determine the variance within and among groups by calculating the total amount of deflection of the mean from zero (Kruschke, 2010). The deflections provide a proxy method to the traditional ANOVA because the sum to zero measures how much a group deviates from the overall mean and mean deviations among groups. BANOVA tables include mean, median, mode, highest density interval (hdi)-mass, hdi-low, and hdi-high values for the posteriors of each group. The hdi-mass, hdi-low, and hdi-high were calculated based on Kruschke (2010) methods. The highest density interval, an alternative to the p-value, indicates differences among groups and is similar to the use of confidence intervals, in which low and high ranges that do not include zero are considered to indicate significance. Predictive posterior distributions for the sum to zero measures were simulated using the JAGS computer program via R computing software (R version 4.0.2; RCore Team, 2020). Twenty thousand simulations were performed for each of 6 groups with the retention of every other observation from the subsampling of a chain (i.e. thinning =1) and the discard of the first 5000 simulations (i.e. burn-in). The deterministic model, mu = a0 + a[xi], was used to compute the predictive posterior distributions, where mu is the predicted mean for a stratum, the a0 is the difference of the mean for all groups and each observation, and a[xi] is the individual observations within a group. Normal priors were assumed for a0 with a mean = 0 and standard deviation = 0.001.

PCA

PCA was performed to compare PCB congener composition among strata, using data normalized such that the sum of the homolog concentration was 100 ([Sum PCB by chlorination]/[Sum PCB]). Although preliminary analysis revealed similar PCB spatial distribution patterns for samples collected in 2012 and 2013, due to sampling inconsistency between years we only analyzed 2013 data, which had more stations than 2012 representing all strata. The non-detects and values below method detection limits were replaced with half of the detection limit for each congener. While PCA can be used to test statistical hypothesis about data, our use was primarily descriptive. A bi-plot of the scores and rotations for the first two components of the PCA illustrates congeners that have distinct associations with a station(s) within a stratum.

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Results and Discussion

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PCBs were detected in caged mussels at all stations in both years and the two years of data were pooled for statistical analysis. The total PCB tissue concentrations in caged mussels (expressed as the sum of 80 congeners) ranged from 50 - 7376 ng/g with a mean concentration of 981 ng/g, which was much higher than the mean PCB concentration from in situ mussels at the collection site (246 ng/g) and from the offshore site (109 ng/g) (Fig. 2, Table 1).

BANOVA results of the total PCB concentration among strata are given in Table 1. The absence of zero in the hdi-low and hdi-high ranges indicates there is a significant difference among strata. Of the six strata examined at the Manistique River study area, the Lower Arm exhibited the highest magnitude of PCB tissue concentrations, ranging from 2012 - 7376 ng/g (Fig. 2). All the stations within this Lower Arm stratum had higher concentrations than any other strata (Fig. 2). The results from multiple comparisons show that the mean PCB concentration in the Lower Arm was higher than those from the Collection, Beach, Harbor sites, Upper Arm and Upper Channel (Table 2).

The stratum with the second highest concentration was the Upper Arm, where the concentration ranged from 102 - 1774 ng/g. Multiple comparisons tests show that the mean concentration in the Upper Arm was significantly higher than Harbor and Upper Channel stations but lower than Lower Arm stations (Table 2). However, unlike the Lower Arm, the concentrations were more variable (Fig. 2). In fact, the mean PCB concentration in mussels in the Upper Arm was not different from those of Beach or Collection stations. The total PCB concentration in

caged mussels from the rest of the strata, Harbor, Beach, and Upper Channel, ranged from 50 – 595 ng/g and were comparable to concentrations from the Collection site and Offshore in situ mussels (Fig. 2). Further, the overall pattern depicted in the spatial distribution of total PCB concentrations in mussel tissue were similar in both years. However, due to the elevated levels of contamination found at the collection site, we primarily focused on increases in PCB concentrations as other comparisons may depend on the bivalves ability to depurate PCBs.

PCB concentrations in caged mussels specifically reflect the bioavailable fraction of total PCB that is available to aquatic organisms within the water column, as opposed to abiotic factors regulated by water or sediment (Gewurtz et al., 2003; Traina et al., 2021). Furthermore, the concentration in mussels also signals the potential for transfer to higher organisms directly through predation and indirectly through deposition of contaminated pseudofeces. The trophic transfer potential of PCB from mussels to round gobies and to small mouth bass was confirmed by the study conducted by Kwon *et al.* (2006). As these mussels have limited ability to move, both in situ and caged mussels can be used for contaminant source tracking and spatial characterizations of contaminants. Unlike fish, mussels bioaccumulate and integrate PCBs at the specific locations where they are found and have limited ability in metabolizing xenobiotics, including polycyclic aromatic hydrocarbons (PAHs) and PCBs (Farrington, 1983). Thus, contaminant concentrations in

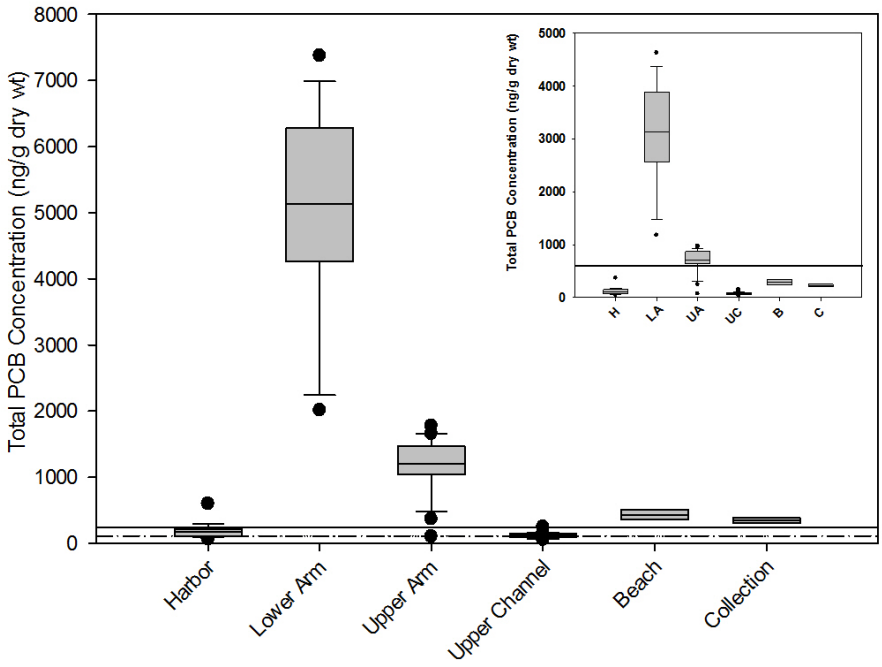


Fig 2. Total PCB concentrations (Sum of 80 congeners; ng/g dry wt) in caged mussels from the Manistique River AOC sampled in 2012 and 2013. The solid reference line indicates the mean pre-deployment body burden in mussels from the collection site and the dashed reference line indicates the body burden in mussels from an offshore site. The inset figure shows the total concentration using a reduced data set (sum of 39 congeners), making the data set comparable to the basin wide PCB measurements taken in 2009/2010. The solid line in the inset map is the mean PCB concentration found in the mussels taken from the harbor mouths of the AOCs throughout the basin during the 2009/2010 basinwide monitoring effort

Table 1. BANOVA table with mean, median, mode, highest density interval (hdi)-mass, hdi-low, and hdi-high values for the posteriors of each group. The hdi-mass, hdi-low, and hdi-high were calculated based on Kruschke (2010) methods. The hdi Mass = 0.95.

	mean	median	mode	hdiMass	hdiLow	hdiHigh
Upper Channel	-1090	-1091	-1116	0.95	-1395	-806
Lower Arm	3733	3733	3725	0.95	3322	4137
Upper Arm	-52	-53	-57	0.95	-384	273
Harbor	-1017	-1017	-1024	0.95	-1389	-671
Beach	-750	-749	-743	0.95	-1499	21
Collection	-825	-821	-823	0.95	-1604	-89

Table 2. Multiple comparison test of strata using BANOVA. The highest density interval (hdi), an alternative to the p-value, indicates differences among groups and is similar to the use of confidence intervals, in which low and high ranges that do not include zero (*) are considered to indicate "significance". The hdi Mass = 0.95.

	mean	median	mode	hdiMass	hdiLow	hdiHigh
Lower Arm vs. Upper Channel*	4823	4824	4821	0.95	4344	5266
Upper Arm vs. Upper Channel*	1039	1038	1038	0.95	680	1393
Harbor vs. Upper Channel	74	73	87	0.95	-313	474
Beach vs. Upper Channel	341	340	293	0.95	-599	1220
Collection vs. Upper Channel	266	271	301	0.95	-624	1188
Upper Arm vs. Lower Arm*	-3784	-3784	-3787	0.95	-4285	-3299
Harbor vs. Lower Arm*	-4749	-4747	-4720	0.95	-5285	-4227
Beach vs. Lower Arm*	-4483	-4481	-4464	0.95	-5430	-3492
Collection vs. Lower Arm*	-4558	-4556	-4588	0.95	-5559	-3610
Harbor vs. Upper Arm*	-965	-966	-972	0.95	-1392	-527
Beach vs. Upper Arm	-698	-699	-644	0.95	-1645	218
Collection vs. Upper Arm	-773	-764	-713	0.95	-1705	154
Beach vs. Harbor	267	264	239	0.95	-678	1204
Collection vs. Harbor	192	193	187	0.95	-730	1160
Collection vs. Beach	-75	-69	-44	0.95	-1295	1182

mussels can provide an additional line of biological evidence to support decision making with respect to contaminant concentrations burden in fish. Overall, elevated concentration levels were identified in the Lower Arm and the Upper Arm. However, elevated concentration levels detected at the collection site make additional comparisons of these compounds more subjective.

PCB Homolog profile

The homolog profile of normalized PCB concentration (normalized to the total PCB concentration) for each stratum was plotted to examine whether there were any differences among strata. Fig. 3 shows that the tri, tetra and penta homologs were predominant in all the strata and at the collection site. This pattern contrasted with the homolog profile in offshore in situ mussels, for which the penta homolog was the most dominant.

In order to compare PCB congener/homolog profile in mussel tissue among stations, we conducted PCA. Principal component 1 explained almost 65% of the variation in the data and the two components together (sites and homologs) explain 80% of the variation. The PCA biplot shows homolog association and a strata based clustering that is consistent with the BANOVA, in which the stations in the Lower Arm and Upper Arm form two distinct clusters adjacent to each other at the far left along the axis of component 1 and all other stations form a strata independent cluster to the right of the Upper Arm strata cluster along the axis of component 1 (Fig. 4). The homolog vectors radiate from the center of the plot axis and are ordered in a counter-clockwise direction. The close proximity of the strata clusterings and/or individual stations to the homolog vector indicates an association. The close proximity of the Lower Arm and the Upper Arm strata cluster in the quadrant between the tri and tetra homolog vectors indicates an association of these homologs in the Lower and Upper Arm. However, all the stations in the Lower Arm stations are tightly clustered compared to the stations in the Upper Arm as indicated by separation of stations into different quadrants and their nearest

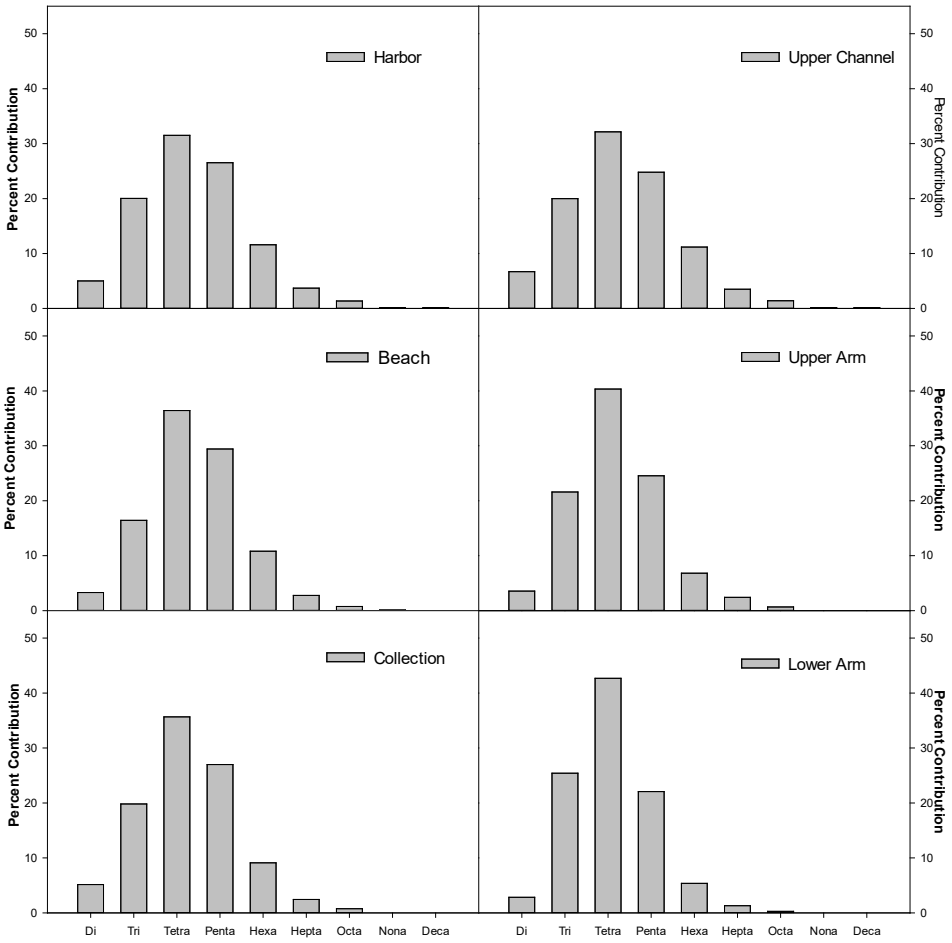
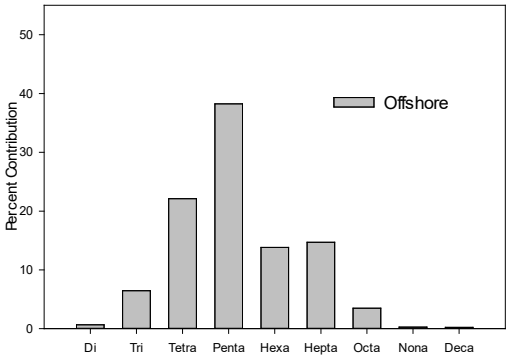


Fig 3: Comparison of homolog profile in caged mussels from the six strata (Upper Channel, Upper Arm, Lower Arm, Harbor, Collection and Beach) and *insitu* mussels from the offshore reference site. The bars represent the percent contribution of each homolog to the total PCB concentration.



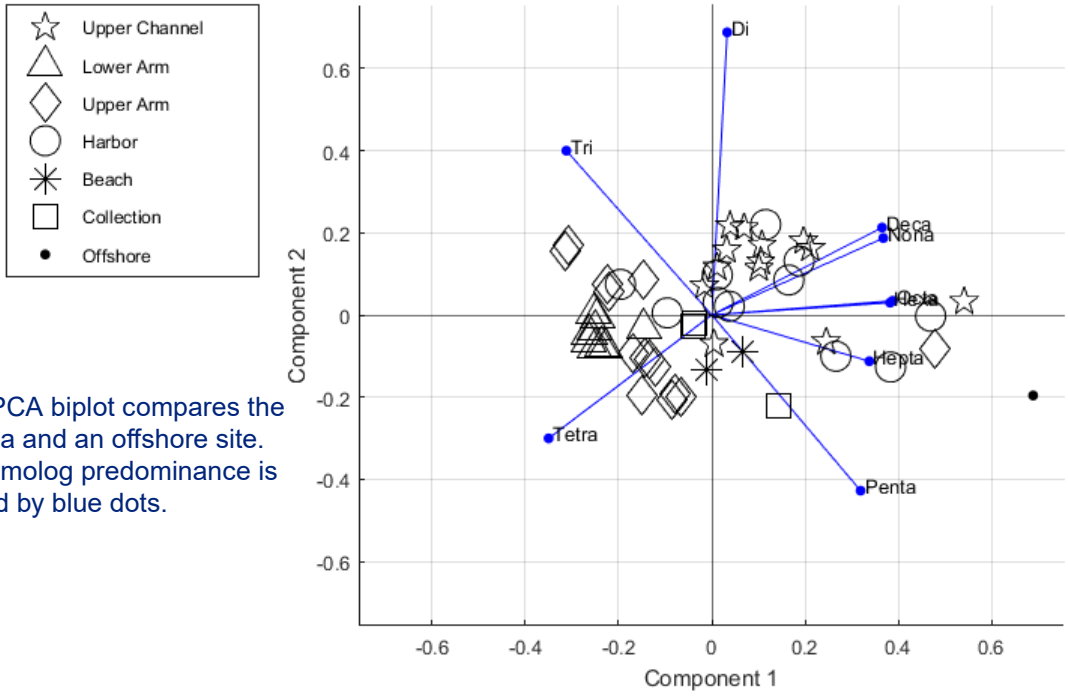


Fig 4: PCA biplot compares the six strata and an offshore site. PCB homolog predominance is depicted by blue dots.

homolog vector. Most of the other stations including the Upper Channel, Harbor, Beach, Collection and Offshore sites are in the same quadrant with the homolog vectors directed in the opposite direction of the tri homolog vector, indicating a large chemical composition difference among stations and strata clusters along opposing vectors.

Having found differences in the homolog profiles from PCA, we created a double ratio plot of the percent tri and tetra homologues, against penta and hexa homologues to zoom in on the key homologues. The double ratio plot shows the dominance of the tri and tetra homologues in all of the Lower Arm sites compared to other strata and particularly the offshore and collection sites (Fig. 5). The information given in the double ratio plot is an integration of the information in the PCA but clearly shows the key homologues responsible for the basis of separation of samples.

PCB congeners, once released to the environment, are subject to weathering from volatilization, solubilization, photolysis, chemical, and microbial degradation. The degree of weathering is site-specific and depends on the original PCB formulation. Lighter congeners are subject to volatilization and water washing effects more the heavier congeners. Larger proportion of heavier congeners may indicate greater PCB weathering (Samba and Boehm, 2011).
The pattern of PCBs found in an organism is influenced by many factors including the type of PCB mixtures to which the organisms were exposed, the extent of weathering of the PCB mixture before exposure, the uptake rates of each PCB congener, and metabolic capabilities of the organism. Further, mussels are exposed to both particulate and dissolved forms of contaminants, which can bioaccumulate via multiple pathways, including food, sediments and water (ASTM International, 2007) and hence are capable of integrating exposure from both the water column and benthic sources. In this study we were able to identify the dominance of certain homologs in the two strata that had the highest overall PCB concentration. However, we will not attempt to link the patterns in organisms back to specific Aroclors, rather we will simply recognize the pattern differences.

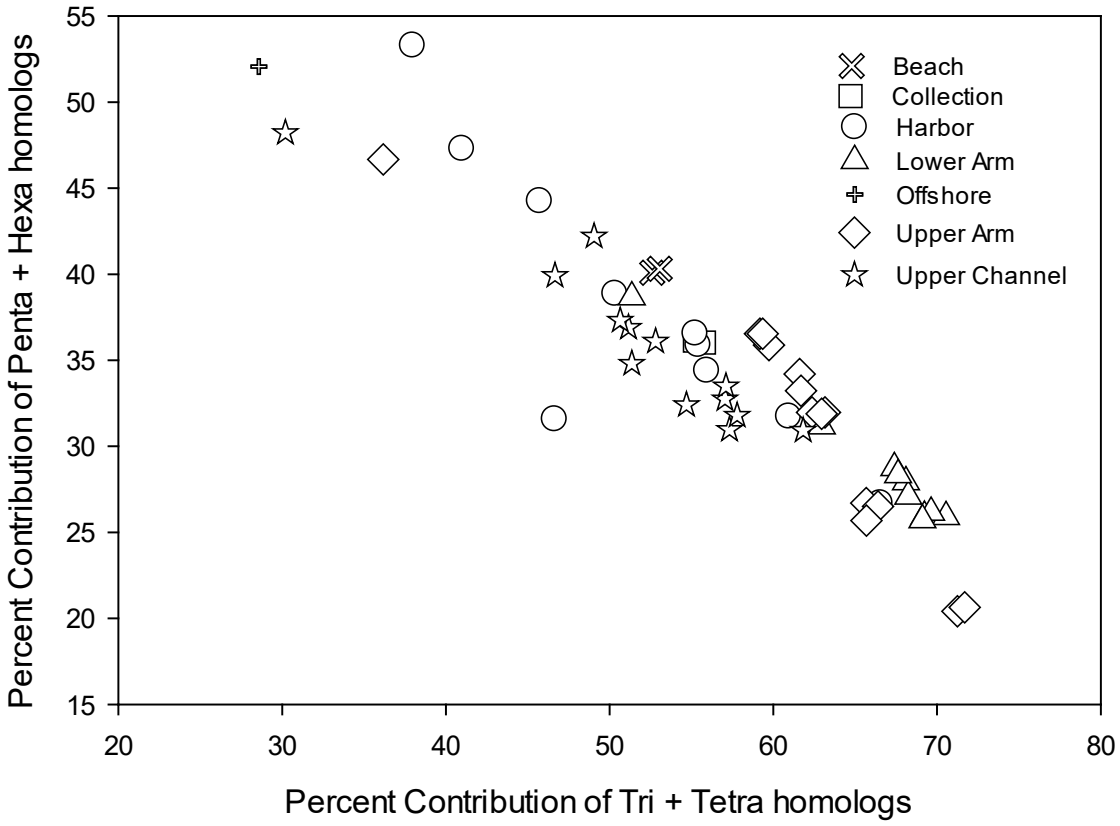


Fig 5: Double ratio plot of the percentage contribution of Penta + Hexa homolog versus Tri + Tetra homolog in mussel tissue from all stations within six strata and an offshore site collected in 2013.

Conclusion and Acknowledgements

Photographs provided by MAB photo Archives. Manistique River, MI.

This two-year bioaccumulation study using caged mussels highlights the usefulness of using dreissenid mussels in characterizing PCB contamination. Using PCB concentration in mussels, we were able to clearly identify strata with elevated concentration of bioavailable PCB in the lower Manistique River thus validating the use of mussel tissue concentrations for identifying areas with elevated PCB concentrations. Furthermore, the results from this study indicates that there is a clear relationship between patterns in the homolog profile of normalized PCB concentration for each strata and collection site. The contaminant concentration in caged mussels provides a valid biological line of evidence in the weight of evidence approach for assessing the extent and magnitude of PCB contamination in highly impacted locations. The next step would be incorporating bivalve health components in the future caged mussel studies, which can then link the contaminant exposure to bioeffects.

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