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An Investigation into the Trophic Magnification of Polychlorinated Biphenyls in the Lake Michigan Food Web

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AN INVESTIGATION INTO THE TROPHIC MAGNIFICATION OF POLYCHLORINATED BIPHENYLS IN THE LAKE MICHIGAN FOOD WEB

by

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ABSTRACT

AN INVESTIGATION INTO THE TROPHIC MAGNIFICATION OF POLYCHLORINATED BIPHENYLS IN THE LAKE MICHIGAN FOOD WEB

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Under the Supervision of Professor Harvey A. Bootsma

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants known to contribute to several adverse health conditions in humans including cancers and a suite of liver diseases. While there exist 209 unique PCB congeners, the World Health Organization has identified 12 that pose the greatest health risk to humans due to these congeners' functional similarity to dioxins, another notoriously toxic class of contaminants. Along with methylmercury, PCBs are the primary drivers behind fish consumption advisories in the Great Lakes. These guidelines are informed primarily by surveys of contaminants in freshwater biota. However, the proliferation of invasive species, such as dreissenid mussels and round gobies, has dramatically restructured the food web and potentially the flow of contaminants in Lake Michigan.

This research examined the relationship between food web structure and PCB concentrations in Lake Michigan biota. A second objective was to determine how invasive species have shifted trophic structure and what implications these alterations might have on contaminant transfer. Aquatic biota were sampled throughout the southern basin of

Lake Michigan and analyzed for lipid content, WHO PCB congeners, and stable isotopes of nitrogen and carbon. Nitrogen isotopes were used to calculate trophic level, while carbon isotopes indicated the primary energy source of an organism. PCBs were extracted from lyophilized tissue using a microwave-assisted extraction technique and a hexane:acetone extraction solution. Crude extracts underwent silica and Florisil® clean-up protocols to remove interferences prior to gas chromatography – mass spectrometric determination.

Stable isotope analysis revealed substantial shifts in trophic structure between 2002-2003 and 2019. Lake trout have significantly dropped in mean trophic level while slimy sculpin are relying more heavily on nearshore carbon relative to the 2002-2003 results. The PCB congeners observed in the highest concentrations in Lake Michigan biota in 2002-2003 closely paralleled those manufactured most commonly. Lipid content and PCB concentration showed a strong significant relationship. Following lipid correction, ΣWHO congeners were shown to increase by a factor of 2.8 per trophic level. In addition, specific congeners appeared to magnify at different rates. Multiple regression analyses revealed that trophic level and lipid content were the best predictors for concentration of total WHO congeners as well as for congeners 105 and 118 specifically. Lake trout, deepwater sculpin, slimy sculpin, bloater chub, and lake whitefish all displayed elevated concentrations of WHO congeners relative to other species. Lake trout and yellow perch displayed an exponential relationship between total length and concentration of WHO congeners.

Lake Michigan's PCB trophic magnification effect in 2002-2003 was within range of that observed in other ecological systems. Possible explanations for the disparities between lakes are seasonal sampling biases, differences in primary productivity, and differential rates of PCB metabolism by fauna. For lake trout and yellow perch, changes in feeding behavior as they grow in size results in an increase of trophic level, thereby increasing PCB loads for larger members of these species. The trophic magnification factor and species-specific PCB results reported in this study enhance the scientific understanding of contaminant transfer in freshwater systems.

Dedication: to my niece, Ruth

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Chapter 1: Introduction and Background

1.1: A Brief History of PCBs

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants known to have adverse effects on environmental and human health. Although PCBs were first synthesized in 1881, it was not until nearly fifty years later that they were first produced on an industrial scale. The Swann Chemical Company briefly synthesized PCBs for a few years during the Great Depression before being absorbed by the Monsanto Corporation in 1935 (Monsanto 1935). PCBs have a variety of desirable characteristics that make them an attractive option for industry; they are chemically stable, strong insulators, and hydrophobic (Van den Berg et al. 1998). Upon monopolizing PCB synthesis, Monsanto utilized these chemicals primarily as insulators in electrical transformers and capacitors (Cairns and Siegmund 1981). Additionally, PCBs were incorporated into paint, pesticides, hydraulic fluids, and a variety of other products (Swann Use Codes 1935).

Even early in their usage, there was a growing body of evidence warning of the hazards of PCBs. A Swann briefing on worker safety indicated that some technicians experienced dermatitis after skin exposure to PCBs, while others inhaled chlorine gas that was emitted as a byproduct of a reaction that dechlorinated PCBs. The document emphasized use of protective equipment and suggested working in well-ventilated areas (Diphenyl and Chlorinated Diphenyl Derivatives 1935). The president of a Monsanto subsidiary argued in 1937 that executing toxicology studies on all chemicals utilized by the company would be unnecessary and financially burdensome (Francis 1998).

Regardless, researchers unaffiliated with Monsanto began investigating the health effects of PCBs. By the late 1930s, evidence was starting to emerge that the effects of PCBs were more than skin-deep; a team of pathologists found that even low concentrations can cause liver damage, though the mechanism of action was unknown at that time (Drinker et al. 1937). Monsanto officials dismissed the findings of this study and insisted that bathing after exposure to PCBs would be sufficient to protect workers from adverse health effects (Monsanto 1944).

Following World War II, Monsanto's approach shifted from denial to downplaying the hazards of PCBs. A 1953 Monsanto briefing indicated that PCBs "cannot be considered nontoxic" but are harmless at typical exposure levels for workers. Nevertheless, many consumers were becoming increasingly skeptical; the United States Navy refused to utilize products containing PCBs due to toxicity concerns (Monsanto 1956). Meanwhile, a growing body of literature continued to underscore the adverse health effects of PCBs, including elevated rates of heart disease (Treon et al. 1955).

The budding environmental movement of the 1960s and breakthroughs in analytical technology helped unearth the negative environmental effects of PCBs. One researcher utilized gas chromatography with electron capture detection, a revolutionary technique for that era, to investigate dichlorodiphenyltrichloroethane (DDT) in several fish and bird species. In addition to finding DDT, he also recognized several structurally similar chlorinated molecules that he identified as PCBs (Jensen 1966). This landmark study was the first to characterize PCB accumulation in nature, and it prompted a cascade of other research on the subject (Gustafson 1970, Vos and Koeman 1970). Risebrough et

al. (1968) were one of the first researchers to document PCB contamination in areas geographically distant from any industry. Over the ensuing decades, this finding has been consistently reproduced, with PCBs having been found in the most remote areas of the planet including the Arctic (Muir et al. 1992, Corsolini et al. 2002), Antarctic (Corsolini et al. 2006, Negri et al. 2006, Corsolini et al. 2019), Mount Everest (Guzzella 2016), and the Mariana Trench (Dasgupta et al. 2018). Atmospheric deposition is the primary mechanism through which PCBs are distributed to remote locations (Lohmann et al. 2007).

In the face of growing evidence of the hazards of PCBs, Monsanto continued to defend their products in the early 1970s. Monsanto publicists split hairs between classifying PCBs being "carcinogenic" or "tumorigenic" (Calandra 1975). In addition, one Monsanto official touted the benefits of incineration instead of "dump[ing] scrap [PCBs] down the sewer" (Graham 1970). In a letter to customers, Monsanto disputed the evidence that PCBs can migrate beyond the location of disposal (Papageorge 1970). Monsanto also began shifting responsibility by requiring purchasers of PCBs to sign waivers that freed Monsanto of legal culpability related to worker illness or environmental harm (Monsanto 1972).

Scientific and public outcry spurred the newly formed United States Environmental Protection Agency (EPA) to explore options for curtailing PCBs. A 1972 EPA report aiming to curb industrial discharges of PCBs suggested restricting these chemicals to only essential and non-replaceable functions (Mead 1972). The Toxic Substances Control Act (TSCA) that was working through Congress was an ominous sign for Monsanto; shortly before this bill was passed, the company announced that it would voluntarily cease PCB

production in the upcoming year (Monsanto 1976). This somewhat proactive approach was inspired by a mindset outlined in a company memo that "Monsanto must not be viewed as being forced into a decision to withdraw from PCB manufacture by either government action or public pressure. Rather, key audiences must perceive Monsanto as having initiated responsible action" (Monsanto 1975). The EPA officially banned PCB manufacture in 1979 to enforce the TSCA (EPA 1979), and almost all industrialized nations had similar bans in place by the 1990s (Stockholm Convention 2016). Although active PCB production is no longer a concern, these chemicals persist in the environment due to legacy contamination from previously released PCBs and from old equipment still in operation. While PCBs are distributed around the planet by precipitation, their concentration is highest in the regions where they were originally manufactured and utilized (Figure 1).

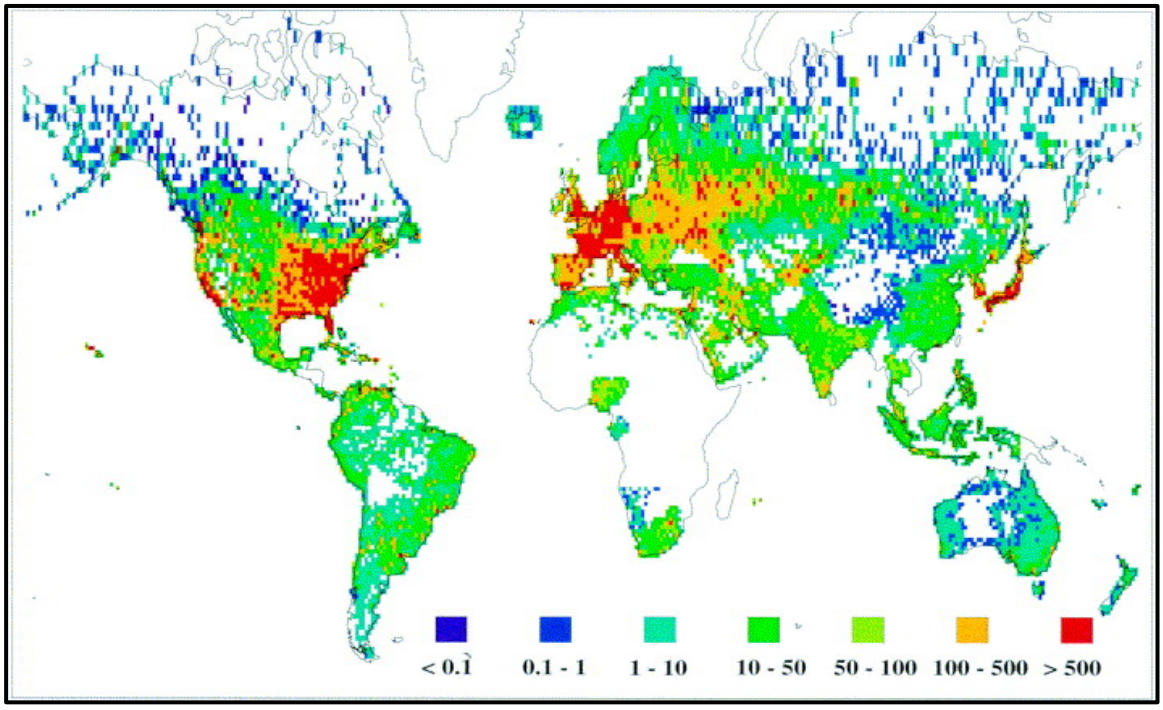


Figure 1. Estimated cumulative global accumulation of PCBs in tonnes (Breivik et al. 2002).

1.2 PCB Pathology

PCB exposure can cause an array of adverse health effects in humans. The co-planar orientation of WHO congeners allows these molecules to navigate through the plasma membrane (Totland et al. 2016). Once inside the cell, they can interfere with a number of cellular mechanisms. For instance, PCBs function as an agonist on the aryl hydrocarbon receptor (AhR), a prominent transcription factor. This protein complex consists of 848 amino acid residues and 3 functional domains (Schulte et al. 2017). The ligand-binding domain evolved to bind with endogenous compounds such as heme metabolites and tryptophan derivatives but can also interact with synthetic hydrocarbons such as PCBs, PAHs, and dioxins (Stejskalova et al. 2011). Interactions resulting in ligand biding with AhR are still an active area of research, but it is probable that an abundance of histidine residues facilitates pi-pi interactions with the conjugated system of PCBs (Pandini et al. 2007). Once activated, AhR travels from the cytoplasm to the nucleus, where it binds to a promoter sequence called the xenobiotic responsive element. This interaction recruits RNA polymerase and therefore induces transcription of several downstream genes with various functions (Puga et al. 2000). Excessive activation of this AhR pathway due to PCB exposure can induce several alarming cardiovascular issues. For instance, activation of AhR disrupts the differentiation of cardiac stem cells, leading to heart atrophy (Zhu et al. 2012). Although the cellular mechanism is not yet clear, excessive AhR activation also contributes to erratic swings in blood pressure (Yi et al. 2018). By interacting with the aryl hydrocarbon receptor, PCBs artificially trigger a cellular pathway that can ultimately lead to numerous adverse health effects.

A number of studies have also investigated the carcinogenicity of PCBs. Although the cellular evidence is not yet as clear as the link to cardiovascular disease, substantial research has made a correlational link between PCBs and various cancers. A pair of studies investigating workers exposed to high levels of PCBs in a capacitor manufacturing plant in Indiana observed increased incidence of brain cancer (Sinks et al. 1992, Ruder et al. 2006). Other forms of cancer associated with PCBs include melanomas (Bahn et al. 1976), non-Hodgkin lymphoma (Engel et al. 2007), and liver cancer (Kuratsune 1987). Tharappel et al. (2002) suggested that co-planar PCBs can set off a molecular pathway in hepatic cells that downregulates tumor suppressor genes, ultimately resulting in either apoptosis or unchecked cell proliferation. Collectively, this body of research was sufficient for the WHO to officially designate these chemicals as "human carcinogens" (IARC 2013) and the EPA to classify them as "probable human carcinogens" (IRIS 2012).

There is also substantial evidence that PCBs interfere with endocrine function in humans. The presence of WHO congeners has been shown not only to interfere with testosterone production in Leydig cells (Kovacevic et al. 1995) but also to compete for testosterone binding sites in receptors throughout the male reproductive tract (Portigal 2002). In women, coplanar PCBs downregulate the *Wnt7a* gene in embryos, leading to abnormal uterine development (Ma and Sassoon 2006). When hydroxylated, these molecules can also act as weak structural analogues of estradiol, further disrupting female reproductive function (McKinney and Waller 1994).

1.3 Routes of Human Exposure to PCBs

Given the high toxicity of PCBs, it is a public health priority to limit human exposure to these hazardous chemicals. Drinking water, air, and food are the primary means by which humans could conceivably be exposed to environmental contaminants. Fortunately, volatilized PCBs have become far less of a concern since their production halted (Frederiksen et al. 2012). Furthermore, the hydrophobicity of PCBs means that these molecules are insoluble in water. However, that same physical property also attracts PCBs to fat tissue of aquatic organisms. Ingestion of contaminated fish is therefore the main route of human exposure to PCBs (Carpenter 2006).

Wild-caught fish are a crucial component to the diets of many individuals. Therefore, the community must be aware of the potential contaminants to which they are exposed through their diets. Since lower-income populations consume seafood at higher rates (Jahns et al. 2014), fish contamination could even be considered a social justice issue. Lake Michigan is a major source of food for residents of the Upper Midwest, and consumers must be cautious of contaminants they are ingesting. Cooking methods such as broiling or grilling allow fat to drip away, thereby reducing the amount of PCBs ingested.

Due to concerns related to contaminants such as PCBs and methylmercury, the Wisconsin Department of Natural Resources has instituted fish consumption advisories throughout the state. Table 1 outlines the guidelines specific to Lake Michigan. Advisories are often stricter for children and pregnant women. These guidelines are affected by the size, age, fat content, and life history of particular species. Internal sampling by the Wisconsin DNR and external studies help inform fish consumption guidelines.

Table 1. Consumption guidelines for common Lake Michigan fish (Wisconsin DNR 2016).

Lake Michigan and its tributaries

See also advice for the Ahnapee, Kewaunee, Manitowoc, Milwaukee, Pike, Root, Twin, and Sheboygan Rivers.

Guidelines for everyone (PCBs)

Species	Unrestricted	Up to 1 meal per week	Up to 1 meal per month	Up to 6 meals per year	DO NOT EAT
Brown trout			All sizes		
Chinook salmon			All sizes		
Chubs			All sizes		
Coho salmon		Under 24"	Over 24"		
Lake trout			Under 30"		Over 30"
Lake whitefish			All sizes		
Rainbow trout		Under 28"	Over 28"		
Smelt		All sizes			
Yellow perch		Under 11"	Over 11"		

1.4 Classification, Properties, and Production of PCBs

Toxicity of PCBs is driven primarily by their chemical structure. PCBs consist of two phenyl rings with multiple chlorines (Figure 2). This structure follows the chemical formula $C_{12}H_{10-x}Cl_x$ and allows for 209 unique molecules or *congeners*. PCBs can be classified as *homologs*, groupings based upon the number of chlorines bonded to the phenyl rings. For instance, the heptachlorobiphenyl homolog is the group of PCBs containing seven chlorine atoms. Since it is quite challenging to selectively produce individual congeners, Monsanto manufactured PCBs in mixtures sold under the brand name "Aroclors." These mixtures were assigned a four-digit identification number. The

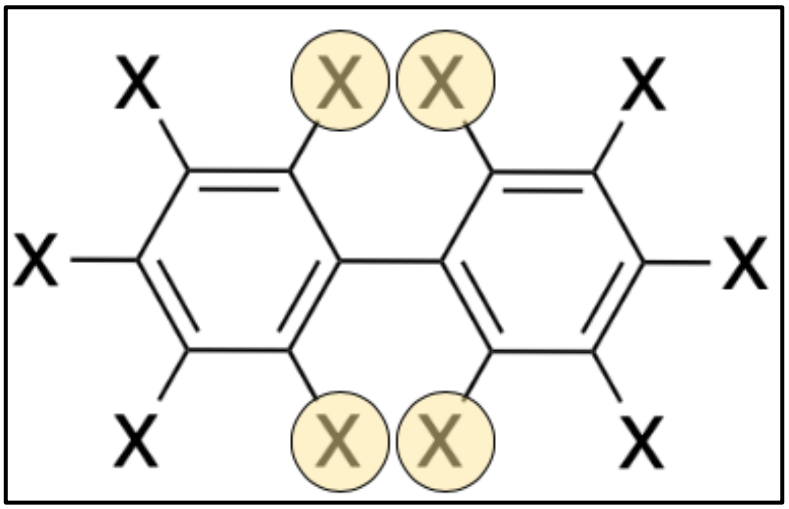


Figure 2. Generic structure of polychlorinated biphenyls with ortho positions highlighted. "X" can represent either hydrogen or chlorine.

first two digits referred to the number of carbons present in the ring complex (always 12 for PCBs), and the final two represented the percent chlorine by mass. For example, Aroclor 1248 is a biphenyl mixture that is 48% chlorine by mass. The degree of chlorination is controlled by the amount of time pure biphenyl is exposed to chlorine gas during PCB synthesis (US Department of Health and Human Services 2000). This halogenation reaction is catalyzed by iron and follows a conventional mechanism for electrophilic aromatic substitution (Figure 3). This reaction is repeated for each subsequent chlorine added to the phenyl rings. However, subsequent chlorines can only be added in particular locations due to interactions with preexisting substituent groups attached to the rings.

With log K_{ow} values of many congeners reaching above 7, PCBs are quite hydrophobic (Hawker and Connell 1988). As a result, they typically associate with other hydrophobic molecules such as lipids. Depending on the lipid content of the organism, lipophilic molecules are often not excreted and instead accumulate in body tissue. Therefore, predators simultaneously consume not only the energy-dense fat of their prey, but also the lipophilic contaminants found in that prey tissue. As a result, the

concentration of lipophilic molecules such as PCBs tends to increase substantially with each trophic level.

While there are 209 PCB congeners, not all are equally toxic. The World Health Organization (WHO) identified 12 particular congeners (henceforth "WHO congeners") that are suspected to be most hazardous to humans (Table 2). Like dioxins, these WHO congeners have a planar geometry. That orientation is possible because they lack the steric hindrance provided by bulky chlorine atoms in the ortho position. This abundance of space allows rotation of the bond between the phenyl rings, creating a planar molecule. Thus, the WHO congeners are alternatively referred to as "dioxin-like PCBs," "co-planar PCBs," and "non-ortho/mono-ortho PCBs."

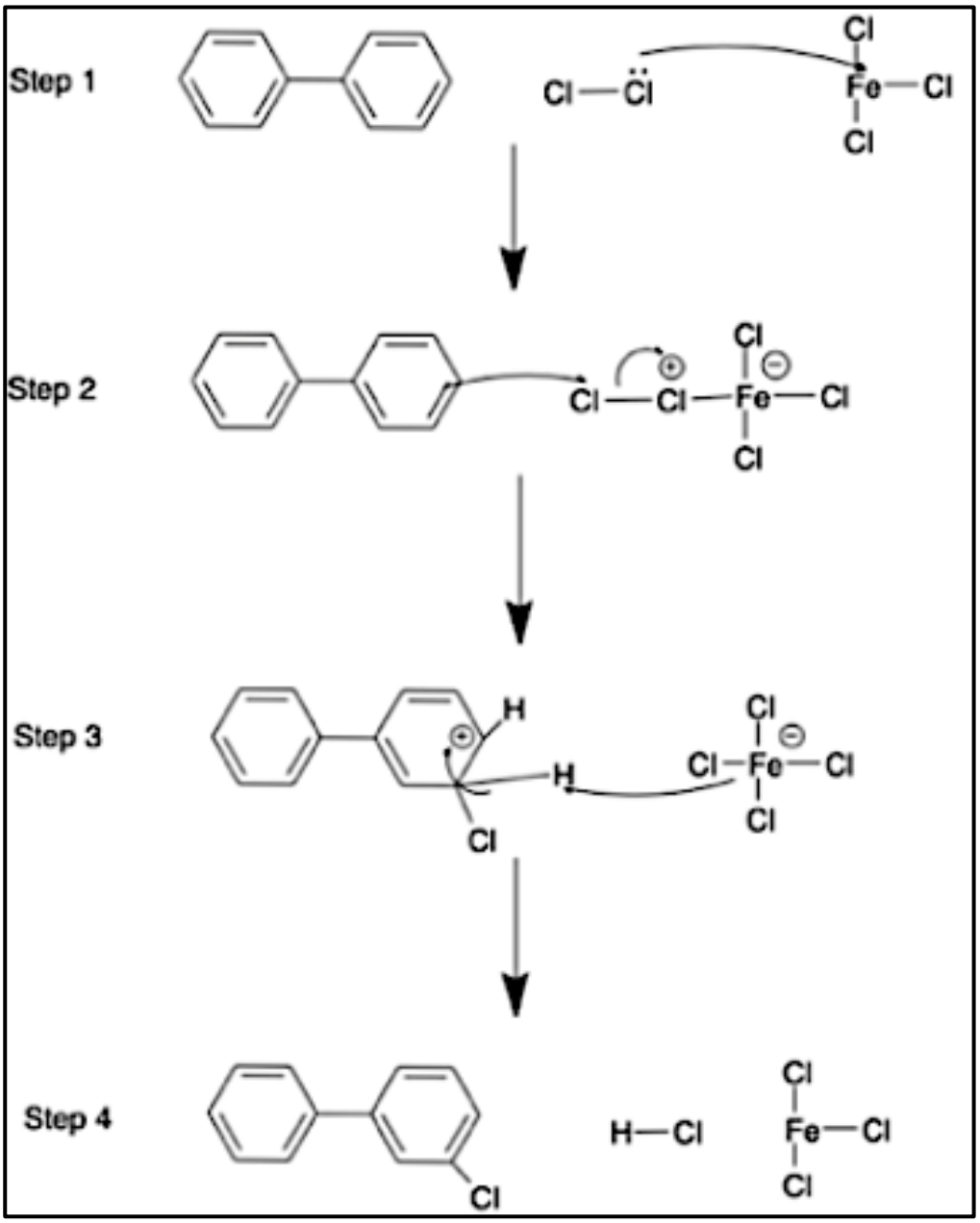
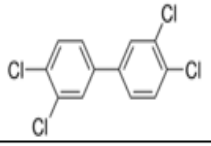
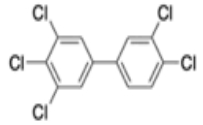
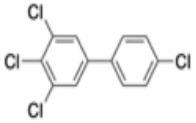
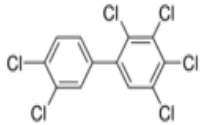
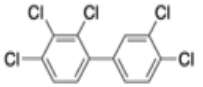
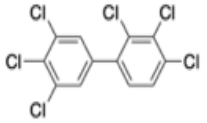
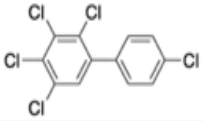
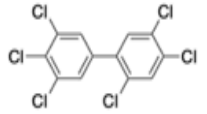
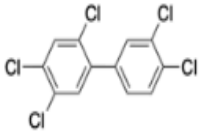
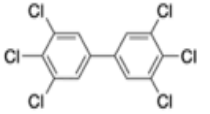
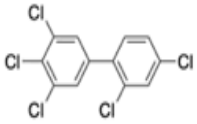
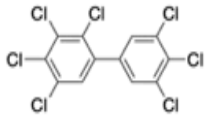


Figure 3. Mechanism for the iron-catalyzed chlorination of biphenyl. Step 1: Cl_2 reacts with an $FeCl_3$ catalyst to create an intermediate complex. Step 2: A high electron density region of biphenyl performs a nucleophilic attach on the terminal chlorine; simultaneously, an electron from the neighboring bond fills the octet of the electron-deficient chlorine. Step 3: An electron from the $FeCl_4^-$ anion attacks the alpha hydrogen to form HCl. Step 4: The final products have formed, and the catalyst has been regenerated

Table 2. List of WHO PCB congeners. These molecules contain either 0 or 1 chlorine atoms in the ortho positions.

Congener #	IUPAC Name	CAS #	Structure	Congener #	IUPAC Name	CAS #	Structure
77	3,3',4,4'-Tetrachlorobiphenyl	32598-13-3		**126**	3,3',4,4',5-Pentachlorobiphenyl	57465-28-8	
81	3,4,4',5-Tetrachlorobiphenyl	70362-50-4		**156**	2,3,3',4,4',5-Hexachlorobiphenyl	38380-08-4	
105	2,3,3',4,4'-Pentachlorobiphenyl	32598-14-4		**157**	2,3,3',4,4',5'-Hexachlorobiphenyl	69782-90-7	
114	2,3,4,4',5-Pentachlorobiphenyl	74472-37-0		**167**	2,3',4,4',5,5'-Hexachlorobiphenyl	52663-72-6	
118	2,3',4,4',5-Pentachlorobiphenyl	31508-00-6		**169**	3,3',4,4',5,5'-Hexachlorobiphenyl	32774-16-6	
123	2,3'4,4',5'-Pentachlorobiphenyl	65510-44-3		**189**	2,3,3',4,4',5,5'-Heptachlorobiphenyl	39635-31-9	

1.5 Analytical Chemistry of PCBs

Determining 209 analytes at trace levels in complex matrices makes the analysis of PCBs one of the most challenging methods in environmental chemistry (Erickson 1997). There exist several methods for PCB extraction from sediment or biological tissue. The traditional Soxhlet extraction is described in EPA Method 3540 but is slow, requires high levels of technical expertise, and utilizes substantial volumes of hazardous solvents such as methylene chloride (EPA 1996A). Alternatively, Method 3546 describes a microwave-assisted extraction (MAE) protocol that overcomes these issues (EPA 2007). In addition, other methods such as pressurized liquid extraction and supercritical fluid extraction utilize elevated temperatures and pressures to drastically improve the speed of extraction over the Soxhlet method (Eskilsson and Bjorklund 2000; Szostek et al. 1999). Regardless of the method chosen, extraction is typically followed by at least one round of solid-phase extraction (SPE) clean-up to remove interferences. Common techniques include silica gel cleanup, sulfuric acid/permanganate clean-up, and Florisil® clean-up (EPA 1996B).

Analytical determination of PCBs within the extract is most often achieved by gas chromatography (GC). The advent of capillary columns in the 1980s generated the efficiency necessary for PCB separation (Rahman et al. 2015). However, no single column and set of chromatographic parameters have been able to separate all 209 congeners (Ruddy et. al 2008). To overcome this challenge, researchers can perform multiple injections into the same GC using different temperature programs, execute one injection into a GC with two different columns connected in series, or split the initial injection into two separate columns (Danielsson et al. 2005; Harju et al. 2003; Ruddy & Aldstadt 2007).

Popular detection methods for GCs are electron capture detection (ECD), mass spectrometry (MS), and tandem mass spectrometry (MS-MS).

Chemists conducting PCB analysis employ a variety of measures to ensure quality assurance (QA) and quality control (QC). Laboratories must demonstrate initial proficiency of their method (including PCB extraction, clean-up, and determination) through use of a standard reference material (SRM). Analysts typically acquire tissue from an agency such as the National Institute of Standards and Technology (NIST) containing certified concentrations of the analytes of interest. The SRM is then analyzed by their provisional method and results are compared to the known values in order to validate the method. Ideally, this process should be repeated whenever new technicians are trained or substantial changes in instrumentation occur. Once the method has been validated, samples can be analyzed with confidence. Each sample is typically spiked with a known concentration of a surrogate compound, thereby allowing the analyst to evaluate extraction efficiency for each particular sample. A chosen surrogate must mimic the chemical behavior of the analyte such that the surrogate's recovery percentage can be used to evaluate the extraction efficiency of that sample. Results for analytes of interest should be corrected based upon recovery percentage of the surrogate. Common surrogates for PCB analysis include tetrachlorometaxylene (TCMX), decachlorobiphenyl (PCB 209), or certain deuterated standards. Collectively, these QA/QC approaches validate the final PCB results for unknown samples.

1.6 Dreissenid Mussels: Engineering a New Ecosystem

Since consumption of aquatic fish is the primary mechanism of PCB exposure in humans, it is essential to view PCB dynamics within the context of the specific ecosystem under investigation. For Lake Michigan, ecological function has been fundamentally shaped by invasive species. Currently, 188 nonindigenous species inhabit the Laurentian Great Lakes (Sturtevant et al. 2019). With an invasion rate of about 1.3 – 1.8 new species per year, this freshwater system is one of the most heavily invaded in the world (GLRI Task Force 2010). In fact, many species most commonly recognized by the public and regarded as central to Great Lakes culture are non-natives, including chinook salmon, coho salmon, alewife, rainbow trout, and rainbow smelt. Mills et al. (1993) identified commercial shipping, deliberate release, accidental release, and migration up constructed canals as the primary mechanisms by which nonindigenous species have entered the Great Lakes.

When samples were collected in the early 2000s during the previous iteration of this study, most of the aforementioned non-native species had already been established. However, within the last two decades, a few new species have fundamentally altered ecological dynamics in Lake Michigan. No species has been more impactful than quagga mussels (*Dreissena rostriformis bugensis*). While zebra mussels (*Dreissena polymorpha*) had previously become established in shallow nearshore waters around the perimeter of Lake Michigan by 1993 (Nalepa et al. 1998), they have since been outcompeted and

nearly extirpated by their close phylogenetic relative, quagga mussels. During the first decade of the 21st century, quagga mussels' ability to colonize cold, deep water allowed them to proliferate in areas not suitable for zebra mussels (Bunnell et al. 2009). Coinciding with the transition between zebra mussel and quagga mussel dominance, Lake Michigan experienced a 17-fold increase in dreissenid biomass between 2003 and 2007 (Bunnell et. al 2009). By 2010, quagga mussels had blanketed nearly the entire lake bottom and reached densities as high as 10,000 per square meter (Nalepa et al. 2010; Figure 4).

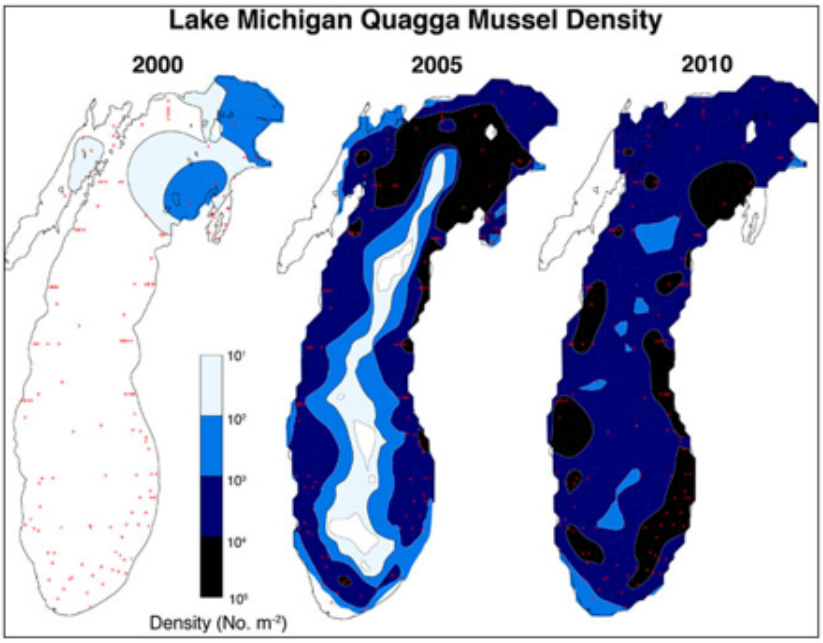


Figure 4. Depiction of the rapid spread of quagga mussels (Madenjian et al. 2015).

The establishment of an invasive species as prolific as quagga mussels necessarily causes dramatic ecological repercussions. In fact, their ability to craft a substantially different landscape has led to their labeling as "ecosystem engineers" (Hecky et al. 2004; Nogaro & Steinman 2014). Many of the mussels' ecological effects stem from their immense filtering capacity. A single mussel typically filters about 1 L of water per day, but that volume can approach as much as 7 L under ideal conditions and depending on the size of the individual (Gopalakrishnan and Kashian 2019). When filtering water,

dreissenid mussels intake phytoplankton, small zooplankton, and suspended particulates. They then egest feces and pseudofeces, thereby creating a nutrient-rich benthic zone (Vanderploeg et al. 2002). This redistribution of nutrients, in combination with deeper light penetration due to enhanced water clarity, creates ideal circumstances for nuisance *Cladophora* algae to grow in nearshore areas (Auer et al. 2010). In addition, mussel filtering and nutrient sequestration have substantially contributed to the loss of the spring phytoplankton bloom in Lake Michigan (Vanderploeg et al. 2010).

The abundance and diet of prey fish have also been dramatically affected by the cascade of effects set off by mussel activity. Turschak et al. (2014) documented a broad trend of many species relying more heavily on nearshore benthic energy sources, either directly through consumption of prey in this region or indirectly due to nearshore organic material being transported to offshore sediment. While alewife reproduction has remained stable (Tsehaye et al. 2014), their body condition has suffered, and their diet has somewhat shifted; alewife grazing on zooplankton has decreased by 37% due to competition with mussels (Pothoven & Madenjian 2008). To compensate, alewives now have a greater proportion of their diet devoted to *Mysis* than they did prior to the mussel invasion (Bunnell et al. 2015). *Mysis* have also become a more substantial component of the diets of deepwater and slimy sculpin (Mychek-Londer & Bunnell 2013). Increased predation on *Mysis* has, in turn, caused the abundance of this small crustacean to decline by 82% since 2002 (Pothoven & Vanderploeg 2017). The increasing scarcity of *Mysis* in combination with the extirpation of *Diporeia* has substantially contributed to decreased body condition for deepwater sculpin, who traditionally depend upon these species for

food (Pothoven et al. 2011). Competition for prey at the base of the food web has contributed to diet shifts and population declines for several fish species.

The changes at the base of the food web also have repercussions for fish of higher trophic status. Predation by salmonids historically has been, and continues to be, the main control on alewife abundance in Lake Michigan (Tsehaye et al. 2014). However, the reduced body condition of alewife has forced salmonids to consume a greater amount prey in order to obtain the same amount of energy as they did previously (Madenjian et al. 2006). A dramatic change in lake whitefish diet was the direct result of the mussel invasion. Lake whitefish used mussels to fill the gap in their diet left by the declines of *Mysis* and *Diporeia* (Pothoven & Madenjian 2008). In fact, dreissenids have been shown to make up nearly 40% of the lake whitefish diet (Madenjian et al. 2010a). This dietary shift is nontrivial; were it not for predation on mussels, dreissenids would otherwise be considered an energetic "dead-end" in the Lake Michigan food web (Madenjian et al. 2010a). The dietary plasticity of lake whitefish has made these predators a crucial energetic link in the altered Lake Michigan food web.

1.7 The Round Goby Invasion

Another species that has substantially affected bioenergetics in Lake Michigan over the last two decades is the round goby (*Neogobius melanostomus*). This species was unintentionally introduced into the Laurentian Great Lakes through contaminated ballast water of commercial shipping vessels (Brown & Stepien 2009). As early as 1997, round gobies had been reported in the southern basin of Lake Michigan (Clapp et al. 2001). By

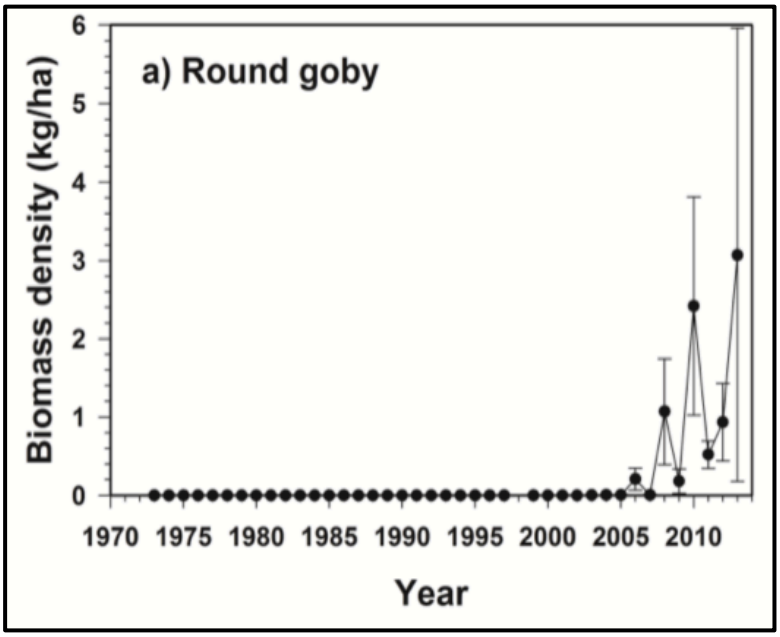


Figure 5. Graph depicting goby biomass estimated from Great Lakes Fisheries Commission annual bottom trawling surveys. The authors indicate that their sampling likely underestimates goby abundance because this species prefers areas shallower than their standardized sampling locations (Madenjian et al. 2014.).

the late 2000s, goby populations had increased dramatically (Madenjian et al. 2014; Figure 5). Since gobies prefer cobbled substrates for breeding, their spread has been primarily limited to the shallow, rocky zones along the perimeter of Lake Michigan (Young et al. 2010; Figure 6). However, there is evidence that gobies migrate to deeper waters during winter (Christoffersen et al. 2019). Competition with invasive gobies for these breeding sites has contributed to the near extirpation of Lake Michigan's native mottled sculpin population (Janssen & Jude 2001) and substantial declines in young-of-the-year yellow perch (Zuwerink et al. 2019).

The feeding ecology of round gobies has also substantially impacted other aquatic species. Gobies are known to prey upon the eggs and fry of many native fish including lake trout (Chotkowski & Marsden 1999) and lake sturgeon (Nichols et al. 2003). Perhaps

most interestingly, gobies often prey upon mussels (Ghedotti et al. 1995). In fact, predation by gobies has even been shown to significantly reduce mussel populations in eastern Lake Erie (Barton et al. 2005). Gobies prefer consuming mussels less than 11 mm (Andraso et al. 2011), and this differential predation has been shown to skew the size structure of nearshore mussel populations (Naddafi & Rudstam 2014). Lake Erie gobies are known to shift to consuming cladocerans and chironomids when appropriately sized mussels became scarce (Perello et al. 2015). Conversely, although gobies in Lake Huron's Saginaw Bay were found to regularly consume mussels, these dreissenids were rarely their preferred food source (Foley et al. 2017). Therefore, gobies' diets seem to shift somewhat based upon location; while the identity of prey items remains mostly the same, their dietary proportion is variable. Regardless, gobies' propensity to exploit mussels makes these invasive forage fish a vital energetic link between dreissenids and higher trophic fish.

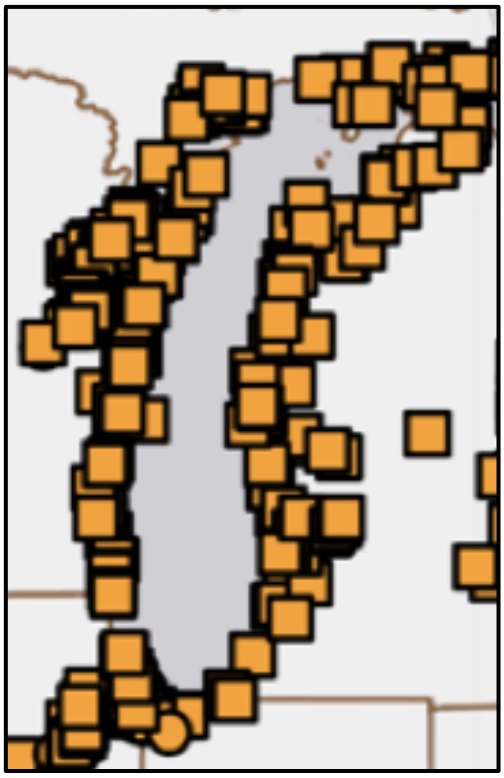


Figure 6. Locations of current round goby establishments as of August 2019 (NOAA 2019).

Many Great Lakes piscivores have adapted to consume the invasive round goby. For instance, as much as 15% of the Lake Erie walleye diet consists of gobies (Campbell et al. 2009). However, gobies were completely absent from the diet of chinook salmon according to gut content analysis of fish sampled in southern Lake Michigan (Savitz 2009).

During the winter and early spring, gobies generally occupy deeper waters, making them accessible to lake whitefish (Pothoven & Madenjian 2013). Of 83 lake whitefish collected during winter ice angling in Green Bay, 49% of their guts contained round gobies (Lehrer-Brey & Kornis 2014). Although not generally considered piscivores, lake whitefish therefore represent a notable energetic link due to wintertime predation. Even lake trout, a generally pelagic species, has been documented occasionally consuming gobies during gobies' winter migration to deeper waters (Jacobs et al. 2010). However, Johnson et al. (2005) indicated that gobies have a relatively low energy density in comparison to more traditional food sources such as alewife. Therefore, relying on gobies could contribute to the nutritional deprivation for some piscivores.

1.8 Stable Isotopes: A Valuable Ecological Tool

Quantifying the effects invasive species have had on the Lake Michigan food web can be achieved through analysis of stable isotopes in organisms. Preferential use of particular isotopes in biochemical reactions means that products typically have predictable, distinctive isotopic compositions. Isotopes of carbon and nitrogen can be used to characterize an organism's carbon source and trophic position, respectively. The ratio of ^{13}C:^{12}C relative to a carbon standard (hereafter $\delta^{13}C$) is typically greater in benthic and nearshore zones than it is in pelagic regions (Hecky & Hesslein 1995). ^{15}N:^{14}N (hereafter $\delta^{15}N$) increases in a stepwise fashion with increasing trophic level (Minagawa & Wada 1984). Both $\delta^{13}C$ and $\delta^{15}N$ are typically expressed on a per mil (‰) basis.

Disparities in $\delta^{13}C$ signatures between benthic and pelagic phytoplankton is primarily caused by primary producers discriminating against the heavier ^{13}C isotope during photosynthesis. This phenomenon occurs because ^{12}C is slightly more reactive than ^{13}C (Tykot 2004). The dissolved inorganic carbon (DIC) is isotopically distinct in nearshore and offshore water. Therefore, phytoplankton in these regions have unequal access to the kinetically favorable ^{12}C isotope, leading to different carbon isotope ratios in their respective sugars (France 1995). The organisms that feed upon phytoplankton generally retain the $\delta^{13}C$ signature of their food source with only a small increase of less than 1 ‰ (Vander Zanden & Rasmussen 1999). Consequently, carbon isotope ratios are a valuable metric to determine the main source (nearshore or offshore) of energy for an organism.

Differences in nitrogen isotopes between trophic levels is primarily attributed to preferential excretion of the lighter ^{14}N isotope during protein metabolism. This phenomenon biases the body tissue toward the heavier nitrogen isotope. A predator assimilates this prey tissue containing an elevated $\delta^{15}N$. Then, this predator itself preferentially excretes ^{14}N, further increasing the $\delta^{15}N$ of its own tissue. For each successive step up the food web, $\delta^{15}N$ is further enriched, with a typical increase of 3 – 5 ‰ between each trophic level (Peterson and Fry 1987). This predictable enrichment allows for $\delta^{15}N$ to be used as a continuous metric to measure the trophic position of organisms.

1.9 Objectives and Hypotheses

The invasions of dreissenid mussels and round gobies have certainly elicited dramatic changes in the abundance and diets of Lake Michigan fish. These shifts manifested in the form of substantial shifts in food web structure between 2002 and 2012 (Turschak & Bootsma 2015). Such trophic changes could have substantial ramifications for contaminants such as PCBs that are passed through feeding. The primary objective of this study is to investigate the extent to which changes in energy flows through the Lake Michigan food web have affected PCB trophic magnification dynamics.

Because the ecological effects of round gobies and quagga mussels on bioenergetics and trophic structure can be complex, nonintuitive, and location-specific, it is challenging to predict how these invasive species have altered PCB dynamics. Nevertheless, some particular traits may provide insight into potential PCB pathways in this altered Lake Michigan food web. For instance, mussels' filter feeding allows them to concentrate contaminants in their tissue (Lepak et al. 2015; Bai & Acharya 2019). Sediment has been shown to have over 100 times more PCBs than found in biota (Erickson 1997), and so mussels' filtering of sediment particles and general proximity to the lake bottom may make their PCB loads disproportionately high for their trophic level. By extending this logic to other benthivores, it is possible that lake whitefish and round gobies could also display elevated PCB concentrations. Fadaei et al. (2017) attributed a substantial portion of PCBs found in benthivorous fish to ingestion of contaminated sediment particles. Furthermore, since these gobies and whitefish species are known to

consume mussels, the PCBs concentrated in mussel tissue could conceivably pass directly into gobies and lake whitefish through predation.

PCB loads in apex species are likely to be directly related to the magnitude of the trophic link from mussels to lake whitefish and gobies. For instance, if predation causes substantial quantities of PCBs to be passed to gobies and whitefish, then they themselves could serve as a conduit of PCBs to apex predators. However, this linkage is ultimately dependent upon the fraction of a predator's diet originating from mussels, either through direct consumption or through multiple trophic linkages. In addition, invasive species may have altered food web length, causing species to increase or decrease in trophic level. If this is indeed the case, then PCBs (which magnify with each trophic level) would be expected to similarly have changed. Furthermore, changes in prey identity may affect PCB pathways. For instance, if a predator's diet has shifted to consist more of a fatty prey species, then the predator would be expected to contain elevated PCB concentrations. If lipid content of a species has fallen (perhaps due to food scarcity or malnourishment) since 2002-2003, then that species may be expected to itself carry fewer PCBs in its body tissue. Conducting the research described herein provides crucial insight into changes in Lake Michigan trophic structure and how those alterations affect PCB pathways.

Chapter 2: Methodology

2.1: Field Sampling

In accordance with federal regulations and university policy, approval must be obtained from the University of Wisconsin-Milwaukee Institutional Animal Care and Use Committee (UWM IACUC) prior to conducting research involving vertebrates. A protocol for humane collection and euthanasia of fish samples was defended before the IACUC on April 5, 2019 and officially approved on April 10, 2019 (Protocol #30 for the 2018-2019 academic year). An additional Animal Care Certification was obtained on July 8, 2019.

To construct a comprehensive picture of current trophic structure and PCB magnification in the Lake Michigan food web, it is necessary to sample a diversity of aquatic fauna. To do so, an effort was made to collect the same species as collected during a 2002-2003 sampling to allow for comparisons over time. With these objectives in mind, the following species were collected: lake trout (*Salvelinus namaycush*), lake whitefish (*Coregonus clupeaformis*), alewife (*Alosa pseudoharengus*), round goby (*Neogobius melanostomus*), chinook salmon (*Oncorhynchus tshawytscha*), slimy sculpin (*Cottus cognatus*), deepwater sculpin (*Myoxocephalus thompsonii*), opossum shrimp (*Mysis diluviana*), and quagga mussels (*Dreissena rostriformis bugensis*). Fish and mussel samples were collected between May and December 2019 from various sites in the southern basin of Lake Michigan (Table 3; Figure 7). Collection methods varied based on the target species. In total, 264 fish were collected in addition to several hundred mussels. Following collection, samples were frozen at -18°C until processing.

Table 3. Summary of 2019 field sampling.

Common Name	Genus and Species	Method of Collection	Location	Date
Quagga Mussel (nearshore)	*Dreissena bugensis*	Benthic Scrape	Atwater 10	6/18/19
Quagga Mussel (offshore)	*Dreissena bugensis*	Ponar grab	Atwater 55	7/1/19
Alewife	*Alosa pseudoharengus*	Dipnetting	Estabrook Falls	6/4/19
Round Goby	*Neogobius melanostomus*	Spear Fishing, Otter Trawling	Atwater 10	6/18/19, 7/25/19, 12/4/19
Lake Whitefish	*Coregonus clupeaformis*	Gill Netting	Green Can Reef	5/29/19
Lake Trout	*Salvelinus namaycush*	Gill Netting	Mid-Lake Reef	5/29/19
Chinook Salmon	*Oncorhynchus tshawytscha*	Angling	Varied	7/14/19 and 7/21/19
Slimy Sculpin	*Cottus cognatus*	Otter Trawling	Atwater 55, Atwater 75	11/4/19, 12/4/19
Deepwater Sculpin	*Myoxocephalus thompsonii*	Otter Trawling	Atwater 55, Atwater 75	11/4/19, 12/4/19
Opossum Shrimp	*Mysis diluviana*	Otter Trawling	Atwater 75	12/4/19

Figure 7. Map of approximate sampling locations.

2.2: Sample Preparation

PCB analysis is quite sensitive to contamination, especially by means of plastics. Therefore, contact with plastic materials following sample homogenization was limited as much as possible. Aluminum foil lined the inside of the caps of all vials to prevent plastic contamination. Furthermore, a rigorous glassware cleaning procedure was employed. All glassware and ceramics that came into contact with reagents or samples were soaked in a 5% HCl bath for at least 4 hours followed by a 5% HNO_3 bath overnight. Glassware was baked in a 250 °C oven for at least 15 minutes prior to use.

Differences in organism morphology necessitated processing samples in slightly different ways. Due to challenges with storage and homogenization, it was impractical to utilize the entirety of the larger fish species (lake trout, chinook salmon, and lake whitefish). Instead, for these samples a cross-sectional steak of about 3 cm thick was cut directly in front of the dorsal fin. Any organs present in this cross-section were retained, but partially digested chyme present in the stomach was removed. Individual Ziplock® bags held these cross-sections in a -18°C freezer until homogenization. Smaller fish (alewife, round goby, slimy sculpin, and deepwater sculpin) were frozen in their entirety in Ziplock® bags. Quagga mussel soft tissue was separated from the shells and placed in pre-cleaned glass petri dishes covered in foil.

"Homogenization groupings" were created based upon the length of each sample within a species. For instance, the three lake trout with the shortest body length were combined into the homogenization group LT1, while the next 3 shortest lake trout were grouped into LT2. This grouping method helps to reduce variability in data that may occur

if each sample were analyzed individually. Furthermore, this approach still allows for investigation of PCB content with respect to fish size.

Once samples were grouped on the basis of size, they were homogenized in an Oyster® kitchen blender. Scales and bones that were unable to blend were discarded. The wet homogenate was transferred from the blender to a pre-cleaned PYREX petri dish, spread to a uniform thickness on the petri dish, covered in aluminum foil, and placed in a -18°C freezer. Once the homogenate had frozen solid, it was placed in a freeze-dryer for 1-2 weeks or until completely dry. The lyophilized homogenate was removed from the freeze-dryer and further ground using a ceramic mortar and pestle. The homogenate was transferred from the mortar into a clean amber bottle, capped, and stored at -18°C until analysis for lipids, stable isotopes, and WHO PCB congeners.

2.3: Stable Isotope Analysis

Analysis of stable isotopes of nitrogen and carbon provide insight into the trophic position and energy source, respectively, of samples. About 1 mg of lyophilized tissue (weighted to the nearest thousandth mg) and a set of 5 acetanilide standards were packed into tin capsules. Analyses were conducted on an apparatus containing a gas chromatograph, an isotope ratio mass spectrometer (Delta V Plus, Thermo Fisher, Bremen), an elemental combustion system (ECS 4010, Costech Instruments, Valencia) and an autosampler. After every 12^{th} sample or standard, a blank tin capsule and an acetanilide bypass were run to verify instrument calibration. Results were expressed as per

mil (‰) differences between the isotope ratios of samples and those of a known reference material. High purity CO_2 and N_2 serve as respective the reference materials for C and N.

$$\delta^{15}N = \left(\frac{\left(\frac{^{15}N}{^{14}N}\right)_{sample}}{\left(\frac{^{15}N}{^{14}N}\right)_{reference\ material}} - 1 \right) * 1000\ ‰$$

$$\delta^{13}C = \left(\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{reference\ matieral}} - 1 \right) * 1000\ ‰$$

The ^{15}N:^{14}N ratio is utilized to determine the trophic position of organisms. To calculate trophic level, it is conventional to set an assumed primary consumer to a trophic level of 2 (Vander Zanden and Rasmussen 2001). Then, all other species are scaled accordingly based on a nitrogen enrichment factor, which is conventionally assumed to be 3.4 ‰ per trophic level (Post 2002). This approach accounts for temporal fluctuations in the $\delta^{15}N$ baseline at the bottom of the food web. Section 2.7 contains the specific equation used to determine trophic level.

Lipids have been shown to be depleted in ^{13}C relative to carbohydrates and proteins (Peterson and Fry 1987, Matthews and Mazumder 2005). Therefore, it is necessary to perform a correction to account for lipids, whose distinct isotopic signature can skew carbon isotope values. Table 4 lists the equations used to perform lipid corrections for carbon isotopes.

Table 4. List of species-specific lipid-correction equations. The C:N term signifies the mass ratio of carbon to nitrogen in tissue prior to lipid extraction. "Invertebrates" include dreissenid mussels, zooplankton, amphipods, and *Mysis*.

Species	Lipid-Correction Equation	Source
Alewife	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C + [(C:N - 3.1364)(1.2565) + 0.5828]$	Turschak et al. (2014)
Lake Trout	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C + [(C:N - 3.31)(0.65) + 0.6026]$	Turschak et al. (2014)
Lake Whitefish	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C - 3.32 + (0.99)(C:N)$	Post et al. (2007)
Chinook Salmon	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C - 3.32 + (0.99)(C:N)$	Post et al. (2007)
Round Goby	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C + [(C:N - 3.0324)(0.3992) + 0.3133]$	Turschak et al. (2014)
Slimy Sculpin	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C + [(C:N - 3.2412)(1.7659) + 0.2328$	Turschak et al. (2014)
Deepwater Sculpin	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C - 3.32 + (0.99)(C:N)$	Post et al. (2007)
Invertebrates	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C + [(C:N - 4.0755)(0.8842) + 0.2284]$	Turschak et al. (2014)
Yellow Perch	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C + [(C:N - 4.00)(0.8163) + 0.6623]$	Turschak et al. (2014)
Bloater Chub	$\delta^{13}C_{lipid\text{-}corrected} = \delta^{13}C - 3.32 + (0.99)(C:N)$	Post et al. (2007)

2.4: Lipid Extraction and Quantification

About 500 mg of lyophilized tissue (weighed to the nearest thousandth mg) was packed into 15 mL glass centrifuge tubes. 10 mL of a 2:1 chloroform:methanol extraction solution was added to each tube and shaken to suspend the tissue in solution. The tubes were periodically agitated to maximize solvent interaction with the tissue. After 30 minutes, the tubes were centrifuged at 4,000 rpm for 5 minutes. The supernatant was decanted into a pre-weighed aluminum weigh boat and the tissue pellet remained in the tube. An additional 10 mL of extraction solution was added, and the pellet was resuspended. After another 30 minutes of incubating, the tubes were again centrifuged,

and the solvent was decanted into the same respective weigh boats as previously used. This process was repeated yet another time for a total of three rounds. After the final round, the pellets were transferred to separate pre-weighed aluminum weigh boats. Tissue and lipid weigh boats were left in a fume hood overnight to dry, and they were weighed the following morning. The relative masses of lipid and lipid-extracted tissue were compared to determine the lipid percentage of the original sample.

2.5: PCB Extraction, Clean-Up, and Determination

PCBs were extracted from tissue samples using the microwave-assisted extraction (MAE) technique. MAE was conducted using a Mars 5 analytical microwave digestion oven (CEM Corporation, Matthews, NC, USA) and HP-500 Plus extraction vessels. Some samples underwent PCB extraction using a Mars 6 analytical microwave digestion oven (CEM) and EasyPrep Vessels. These instruments allow for superheating of solvents by exposing pressurized vessels to microwave radiation. The microwave digestion ovens were equipped with a carousel that could hold several vessels, allowing for simultaneous PCB extraction of multiple samples. In addition, a control vessel was placed on the carousel and fitted with an RTP-300 Plus temperature probe and ESP-1500 Plus pressure sensor. These devices collected continuous data, allowing the operator to monitor conditions inside the vessels throughout the extraction process. The control vessel contained all reagents without a tissue sample.

Previously, Beth Ruddy determined that the optimal water percentage for MAE was determined to be 15% (w/w) (Ruddy 2006). Therefore, lyophilized tissues needed to be

Figure 8. Photograph of Mars 5 analytical microwave digestion oven utilized for MAE.

supplemented with water to enhance extraction efficiency. Although the samples were previously lyophilized, there was latent water content present in the tissue due to absorption from the atmosphere during storage. To determine the latent water content of tissue prior to extraction, 3 separate subsamples of about 100 mg for each homogenization group were pre-weighted and then placed in a drying oven at 250 °C. After drying overnight, the samples were re-weighed, and the difference in mass corresponded with the sample's latent water content. A calculation was performed to determine what volume of water needed to be added to the sample prior to extraction to ensure a 15% (w/w) water content. Water added in this step was pre-washed with *n*-hexane to remove any residual organic compounds. To do this, *n*-hexane and DI water were added to a separatory funnel, and the funnel was inverted several times. Within a few minutes, the organic and aqueous layers separated, and the bottom aqueous layer contained purified DI water that could be utilized during MAE.

Extraction was carried out using a modified version of EPA Method 3546: Microwave Extraction. About 1000 mg of lyophilized tissue was weighed to the nearest thousandth mg and quantitatively transferred to vessels. 10 mL of acetone and 10 mL of *n*-

hexane were added to each vessel. These solvents were pipetted along the inside walls of the vessels to wash the tissue down to the bottom. The appropriate volume of *n*-hexane-washed DI water necessary to obtain 15% water (w/w) was added; this volume was typically about 150 µL. Vessels were then spiked with 10 µL of an acetone solution containing 0.2 mg/mL each of tetrachlorometaxylene (TCMX) and decachlorobiphenyl (PCB 209). These compounds function as surrogate standards used to determine extraction efficiency. Vessels were capped and placed in specialized frames. Screws on the top of the frames were hand-tightened and then further secured with a wrench to ensure that the vessels were completely sealed and therefore capable of being pressurized. Vessels were placed strategically on a carousel to ensure that it was balanced and microwave radiation would be distributed evenly to all vessels. Temperature and pressure sensors were affixed to the control vessel.

Once the vessels were in place, the MAE method was performed using a power of 1200 W (for the Mars 5) or 800 W (for the Mars 6). For 10 minutes, the microwave executed a gradual temperature ramp to 115°C. It then held this temperature for an additional 10 minutes followed by a cool-down period of about 45 minutes or until the vessels reached room temperature. Vessels were individually removed from the microwave and vented into a fume hood to safely release any built-up pressure.

Vessels were uncapped and the solvent from each vessel was decanted into a clean amber vial using a glass Pasteur pipette. The tissue on the bottom of each vessel was discarded. Some trace amounts of tissue were inadvertently transferred into the storage vial during decanting, but the subsequent clean-up stages removed any remaining tissue.

The sides of the vessels were rinsed 3 times with ~1 mL aliquots of *n*-hexane and this additional solvent was also added to the vial. The vials were capped and stored at -18°. Vessels were cleaned by rinsing 3 times with DI water and 3 times with acetone. They either air dried or were dried using a lint-free delicate task wipe. Once dry, the vessels were ready for the next round of MAE.

The crude extract underwent a series of clean-up techniques to remove interfering compounds that were extracted during MAE. The crude extract was poured into a round-bottom flask and the walls of the vessel were again rinsed with *n*-hexane to ensure quantitative transfer. A rotary evaporator (Heidolph Instruments, Schabach, Bavaria, Germany) operating at 100 rpm at 40°C for about 2 minutes concentrated the crude extract down to approximately 1 mL. In accordance with EPA Method 3630: Silica Gel Cleanup, a 6 mL LC-Si SPE cartridge containing 1 g of packing was conditioned using about 5 mL of *n*-hexane under gentle vacuum. The crude extract was added and flowed through the column at a rate of about 1 mL/minute under gravity flow. This slow flow rate was crucial to ensure that the PCB extract had ample opportunity to interact with the sorbent. The inside of the round bottom flask was rinsed three times with ~1 mL aliquots of *n*-hexane; these rinses were added to the column. The column was then eluted using 30 mL of *n*-hexane under gentle vacuum. Following elution, the cartridge was archived.

The contents of the vial were quantitatively transferred to another round bottom flask and evaporated down to about 500 µL. In accordance with EPA Method 3620: Florisil® Cleanup, a 6 mL ENVI Florisil SPE cartridge containing 1 g of packing was conditioned using *n*-hexane. The extract was transferred to the conditioned column, and

the flask was rinsed as previously described. The solution was eluted using 3 mL of *n*-hexane before being evaporated down to a final volume of slightly less than 1 mL. This purified extract was transferred to a small autosampler vial. The inside of the flask was rinsed with small amounts of *n*-hexane that were added to the vial until a final volume of 1.0 mL was achieved. Cleaned-up extracts were stored at 0°C.

Extracts were analyzed for WHO PCB congeners by the University of Wisconsin-Milwaukee's Shimadzu Laboratory for Advanced and Applied Analytical Chemistry. A GC-MS QP-2010 Ultra with an AOC-20i Auto Injector performed the determination. The mobile phase and make-up gas were helium and nitrogen, respectively. Both of these gases were ultrahigh purity and passed through gas line conditioners to remove any remaining impurities. The method detection limit (MDL) was 10 ppb. For the purposes of data analysis, samples below MDL were assigned a random value between 0 and 1/2*MDL for each congener, as done by Kidd et al. (2001).

PCB extraction and determination were validated by utilizing NIST SRM 1947: Lake Michigan Fish Tissue. This certified lake trout tissue was collected in October 1997 off the coast of Charlevoix, Michigan and analyzed for an expansive suit of contaminants. The tissue was initially stored at -80°C upon receipt but otherwise underwent lyophilization, extraction, and determination identical to that of all other samples.

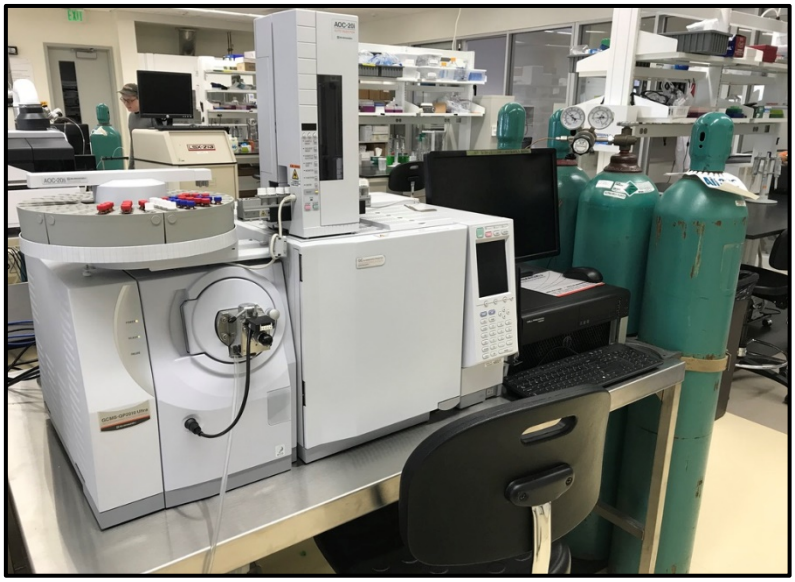


Figure 9. Photograph of the GC-MS system utilized for PCB determination.

2.6 Methodological Comparison between 2002-2003 and 2019 Studies

The specific method previously described was applied specifically in 2019, but it was developed to mirror that used in 2002-2003 (Ruddy 2006). Field sampling was generally conducted using similar techniques, but the species sampled were not completely identical due partially to changes in accessibility as a result of ecological shifts in Lake Michigan between the two sampling periods. For instance, round goby and chinook salmon were not sampled in 2002-2003, while yellow perch, bloater chub, and zooplankton were absent from the 2019 dataset. Stable isotope values from additional organisms sampled between 2010-2012 were included in data analysis to document shifts in trophic structure at the half-way point between the 2002-2003 and 2019 PCB surveys. While all fish sampling in 2002-2003 occurred in southwestern Lake Michigan, a

substantial portion of 2010-2012 sampling took place at Green Bay and in Sleeping Bear Dunes National Lakeshore. 2010-2012 fish were not analyzed for PCBs.

There were some slight extraction and analytical differences between the 2002-2003 and 2019 PCB surveys. The 2002-2003 method was validated using NIST SRM 1946: Lake Superior Fish Tissue, while NIST SRM 1947: Lake Michigan Fish Tissue was used in 2019. In addition, an 80:20 hexane:acetone extraction solution was utilized in 2002-2003, but a 50:50 ratio was used in 2019. This adjustment was made in 2019 due to recommendations from the analytical microwave manufacturer and in accordance to EPA Method 3546 (EPA 2007). During clean-up, the eluents in 2002-2003 and 2019 were *n*-pentane and *n*-hexane, respectively. Again, this modification was made to better align with EPA Method 3600 (EPA 1996). Cessation of production of the exact LC-Si cleanup cartridge (20 mL and 5 g of packing) used in 2002-2003 prohibited use of that product in 2019. Instead, a 6 mL LC-Si cartridge with 1 g of packing was utilized. Eluent volumes were scaled down proportionally. Final analysis in 2002-2003 was conducted using a GC tandem MS approach and a Varian Saturn 2000 system, while 2019 analysis occurred using a GC-MS technique and a Shimadzu QP-2010 Ultra system.

2.7: Data Analysis

Tests for statistical significance were conducted using JMP 14.1 statistical software. Data were initially visualized using a carbon and nitrogen isotope biplot to visualize shifts in trophic structure since 2002. One-way analyses of variance (ANOVAs) were run to compare 2019 stable isotope data with those of previous sampling efforts. Furthermore,

analyses of covariance (ANCOVAs) were used to detect changes in the fish length – stable isotope relationship over the sampling periods.

To determine the extent to which an organism relied upon pelagic carbon sources, the metric α was calculated using the formula

$$\alpha = \frac{(\delta 13\mathrm{C}_{sample} - \delta 13C_{amphipod})}{(\delta 13C_{profundal\ dreissenid} - \delta 13C_{amphipod})}$$

Similar to the approach used by Kidd et al. (2001). Comparisons between PCB trophic magnification in the offshore pelagic and nearshore benthic zones were made with organisms having an α value great than 0.7 or less than 0.3, respectively. These thresholds were defined by Kidd et al. (2001). All δ^{13}C values in this calculation had already been lipid-adjusted.

The following calculation for trophic level was slightly modified from that used by Post et al. (2000):

$$Trophic\ Level = \frac{(\delta 15N - [\delta 15N_{profundal\ dreissenid} * \alpha + \delta 15N_{amphipod} * (1 - \alpha)]}{3.4}$$

The denominator represents the assumed nitrogen enrichment factor of 3.4 ‰ (Vander Zanden & Rasmussen 2001). This calculation is sensitive to baseline changes in δ^{15}N for the energy sources upon which it relies. This formula or ones similar have been utilized in a number of other food web studies (Driscoll et al. 2015; Turschak & Bootsma 2015).

It is convention to adjust raw PCB concentrations by removing the effect of lipid. The method developed by Hebert and Keenleyside (1995) and applied to a number of contaminant studies (Kidd et al. 2001; Borga et al. 2004; Fliedner et al. 2018) was utilized. Residuals were calculated from the regression between % lipid and concentration of

PCBs. The grand mean of PCB concentration was added to these residuals to create the lipid-adjusted metric.

Multiple regressions analyses were conducted to determine the primary variables affecting PCB concentrations in Lake Michigan fauna. In addition, trophic magnification factors (TMFs) were calculated using the equation

$$TMF = \frac{[PCBs]_{TL}}{[PCBs]_{TL-1}}$$

where TL is any given trophic level and [PCB] is the predicted concentration of PCBs at that trophic level as determined by a regression between these two variables. TMFs exceeding 1 indicate that magnification has occurred. TMFs were also calculated for specific WHO congeners. There is a slight distinction between TMFs and BMFs (biomagnification factors); the former is calculated from a contaminant vs. trophic level regression line, while the latter is determined by dividing the mean contaminant load of a predator by that of an assumed prey species.

Chapter 3: Results

3.1: Shifting Energy Sources and Trophic Structure

Temporal comparisons of 2019 carbon and nitrogen stable isotope values with those of previous sampling efforts reveal substantial changes in Lake Michigan trophic structure. Between the 2002-2003 and 2010-2012 sampling periods, many of the species such as yellow perch, bloater chub, and round gobies shifted their energy reliance significantly towards nearshore benthic carbon sources (Turschak et al. 2014). However, the 2019 data appear to indicate a partial retreat toward the carbon values observed in 2002-2003 for a few species. For instance, after making a statistically significant shift toward nearshore benthic energy in the 2010-2012 sampling, round gobies have returned closer to $\delta^{13}C$ values observed in the early 2000s. A similar rebound was observed in *Mysis* when considering all three sampling periods (Figure 10).

Trophic values calculated from $\delta^{15}N$ indicate substantial shifts for many species. For instance, alewife, and slimy sculpin, and deepwater sculpin have all at least partially returned to 2002-2003 trophic levels after significant changes for the 2010-2012 sampling (Figures 11A, 11F, 11G). Meanwhile, lake trout have showed a statistically significant drop in trophic level for 2019 (Figure 11B). Lake whitefish significantly increased in mean trophic level between 2002-2003 and 2019, with data unavailable for the 2010-2012 sampling (Figure 11C).

Some species have shown minimal change in energy source over the last 2 decades. Chinook salmon and lake trout carbon sources stayed fairly constant between the sampling periods, with the latter's mean $\delta^{13}C$ fluctuating by less than 1 ‰ between

2002-2003 and 2019 (Figures 12B, 12D). Lake whitefish have also remained quite constant (Figure 12C). However, other bottom feeding fish have displayed shifts in energy sources; both slimy sculpin and deepwater sculpin have experienced a significant increase in $\delta^{13}C$ since the 2002-2003 sampling (Figures 12F, 12G).

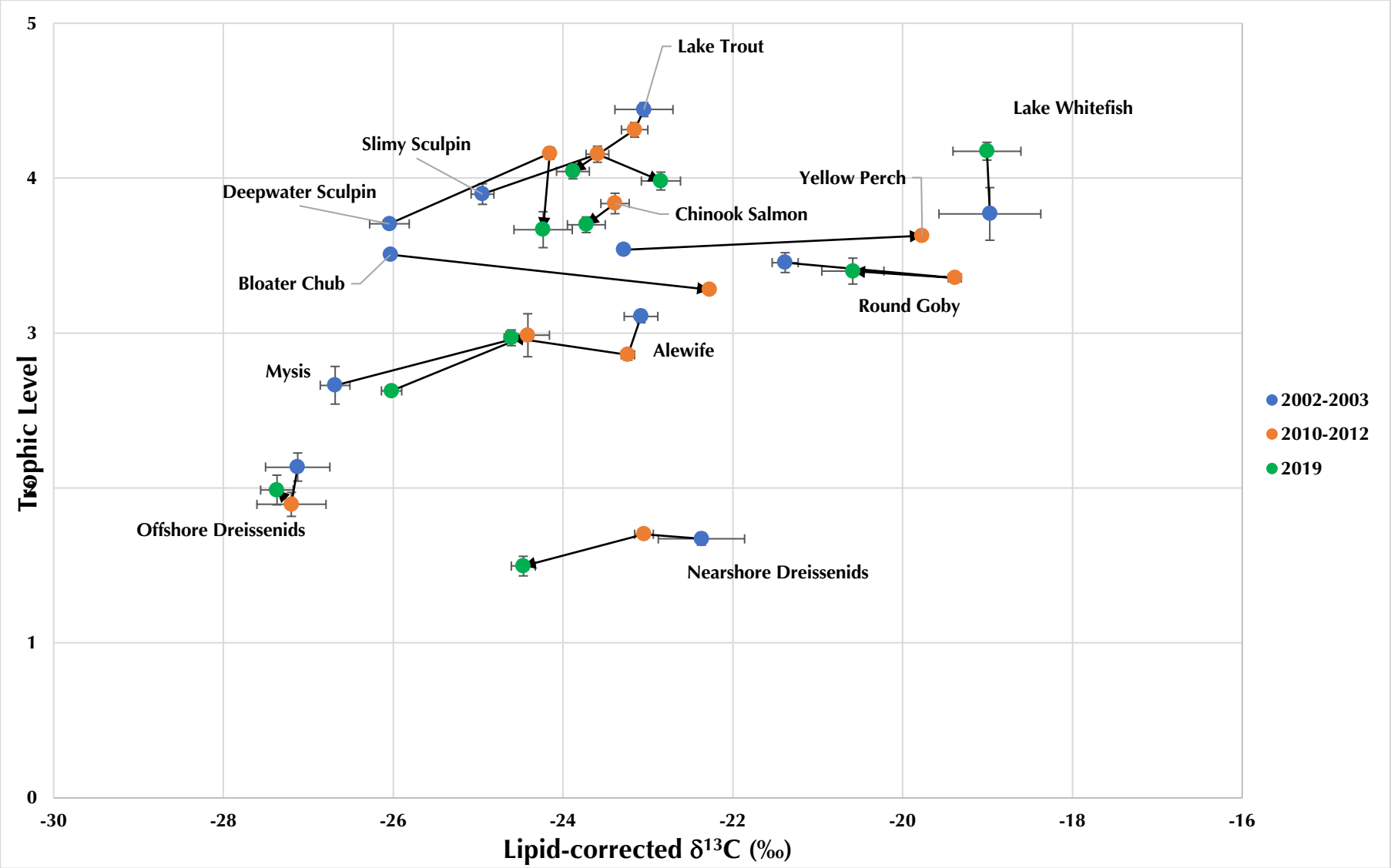
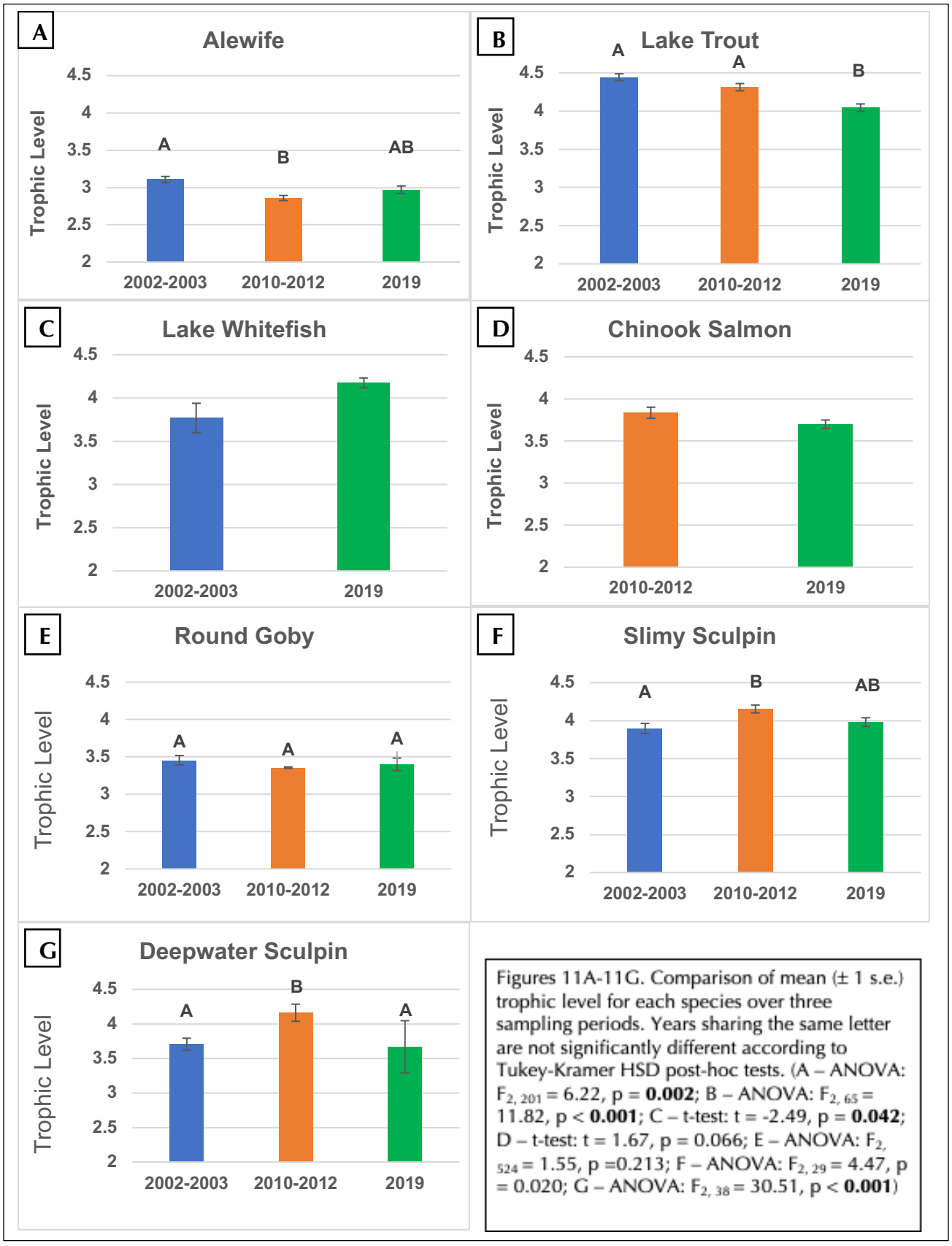
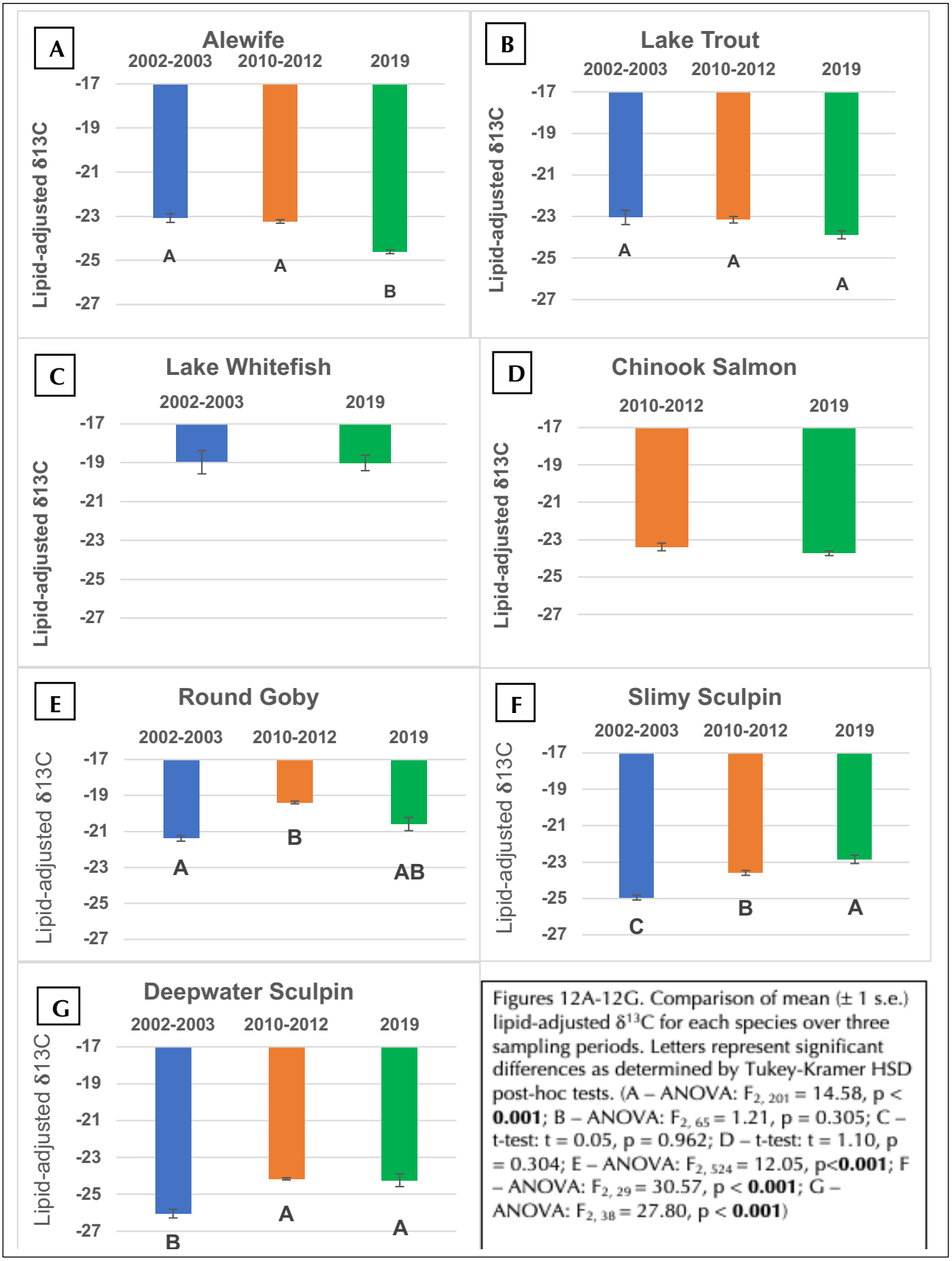


Figure 10. Stable isotope food web biplot depicting shifts in trophic structure among three time periods.



Figures 11A-11G. Comparison of mean (± 1 s.e.) trophic level for each species over three sampling periods. Years sharing the same letter are not significantly different according to Tukey-Kramer HSD post-hoc tests. (A – ANOVA: $F_{2, 201} = 6.22$, $p = \mathbf{0.002}$; B – ANOVA: $F_{2, 65} = 11.82$, $p < \mathbf{0.001}$; C – t-test: $t = -2.49$, $p = \mathbf{0.042}$; D – t-test: $t = 1.67$, $p = 0.066$; E – ANOVA: $F_{2, 524} = 1.55$, $p = 0.213$; F – ANOVA: $F_{2, 29} = 4.47$, $p = 0.020$; G – ANOVA: $F_{2, 38} = 30.51$, $p < \mathbf{0.001}$)

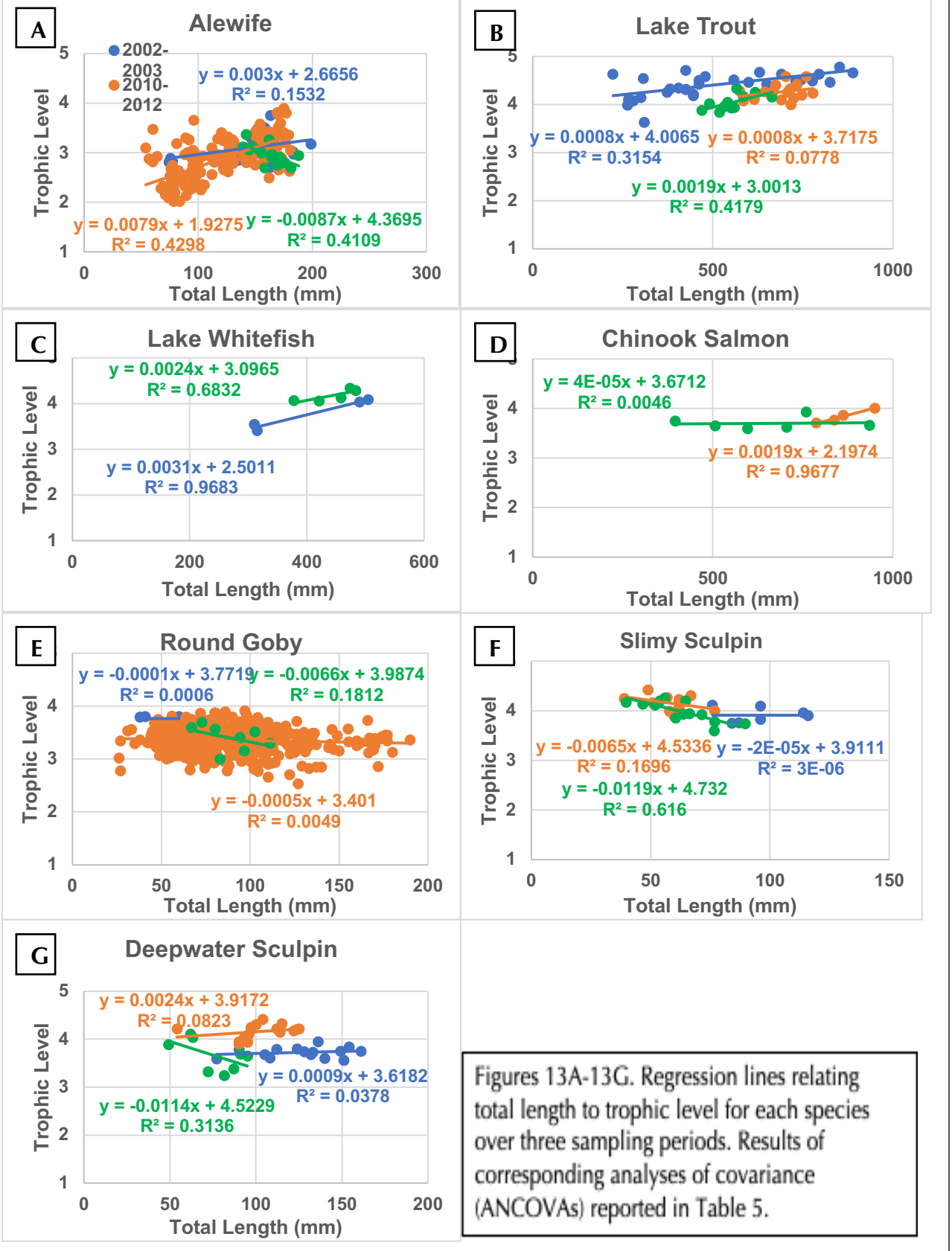


Figures 12A-12G. Comparison of mean (± 1 s.e.) lipid-adjusted $\delta^{13}C$ for each species over three sampling periods. Letters represent significant differences as determined by Tukey-Kramer HSD post-hoc tests. (A – ANOVA: $F_{2, 201} = 14.58$, $p <$ **0.001**; B – ANOVA: $F_{2, 65} = 1.21$, $p = 0.305$; C – t-test: $t = 0.05$, $p = 0.962$; D – t-test: $t = 1.10$, $p = 0.304$; E – ANOVA: $F_{2, 524} = 12.05$, $p <$ **0.001**; F – ANOVA: $F_{2, 29} = 30.57$, $p <$ **0.001**; G – ANOVA: $F_{2, 38} = 27.80$, $p <$ **0.001**)

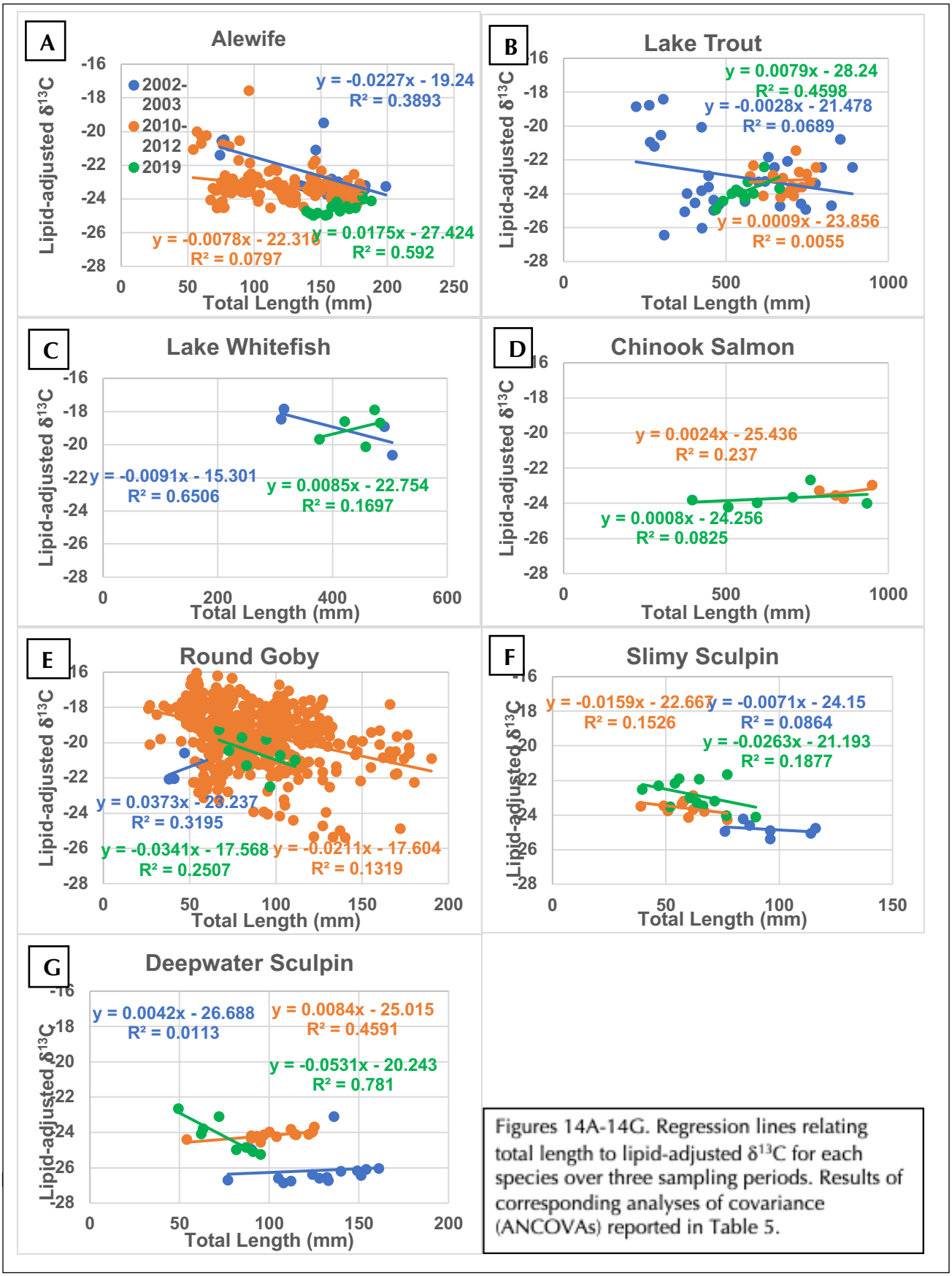
3.2 Intraspecific Factors Driving Stable Isotope Findings

A series of analyses of covariance was conducted for each species to discern any changes over time in the relationship between fish length and stable isotope composition. Fish total length was positively related to trophic level in lake whitefish (Figure 13B) and lake trout (Figure 13C). Slimy sculpin displayed a negative relationship between length and trophic level (Figure 13F). No species sampled showed a significant relationship between total length and lipid-adjusted $\delta^{13}C$. Round gobies were noticeably quite variable in $\delta^{13}C$ (Figure 14E).

The length – isotope relationships did not vary over time for most species. However, in alewife and deepwater sculpin the interaction term was significantly related to both trophic level and lipid-adjusted $\delta^{13}C$, meaning that the relationship between total length and these isotope signatures has changed over time. In 2002-2003 and 2010-2012, deepwater sculpin carbon source did not differ with age, but those sampled in 2019 became increasingly reliant on pelagic energy as their length increased. For all other species, this interaction showed no relationship with trophic level or lipid-adjusted $\delta^{13}C$ (Table 5).



Figures 13A-13G. Regression lines relating total length to trophic level for each species over three sampling periods. Results of corresponding analyses of covariance (ANCOVAs) reported in Table 5.



Figures 14A-14G. Regression lines relating total length to lipid-adjusted $\delta^{13}C$ for each species over three sampling periods. Results of corresponding analyses of covariance (ANCOVAs) reported in Table 5.

Table 5. Summary of ANCOVAs evaluating the effect of sampling year, fish total length, and the year*total length interaction on trophic level and lipid-adjusted $\delta^{13}C$. Statistically significant p-values are bolded.

Species	Dependent Variable	Degrees of Freedom			Year		Total Length		Year*Total Length	
		Year	**Total Length**	**Error**	**F**	**p**	**F**	**p**	**F**	**p**
Alewife	Trophic Level	2	1	178	2.419	0.092	0.131	0.718	6.468	**0.002**
	Lipid-Adjusted $\delta^{13}C$				15.277	**<0.001**	0.521	0.472	3.900	**0.022**
Lake Trout	Trophic Level	2	1	53	14.409	**<0.001**	5.602	**0.022**	0.428	0.654
	Lipid-Adjusted $\delta^{13}C$				0.542	0.585	0.258	0.961	0.765	0.471
Lake Whitefish	Trophic Level	1	1	5	27.910	**0.003**	30.217	**0.003**	0.475	0.521
	Lipid-Adjusted $\delta^{13}C$				0.000	0.984	0.003	0.961	2.289	0.191
Chinook Salmon	Trophic Level	1	1	6	0.548	0.487	3.799	0.099	3.467	0.112
	Lipid-Adjusted $\delta^{13}C$				0.002	0.965	0.491	0.510	0.118	0.743
Round Goby	Trophic Level	2	1	511	0.822	0.440	0.315	0.575	0.605	0.547
	Lipid-Adjusted $\delta^{13}C$				1.632	0.197	0.037	0.847	0.296	0.744
Slimy Sculpin	Trophic Level	2	1	24	2.265	0.126	6.876	**0.015**	2.690	0.088
	Lipid-Adjusted $\delta^{13}C$				6.223	**0.007**	2.990	0.097	0.430	0.656
Deepwater Sculpin	Trophic Level	2	1	32	29.958	**<0.001**	2.489	0.125	4.459	**0.020**
	Lipid-Adjusted $\delta^{13}C$				27.769	**<0.001**	4.081	0.052	6.157	**0.006**

3.3 PCB Results: Entire Food Web Analyses

The aforementioned evidence that trophic structure and energy pathways have continuously changed in Lake Michigan further underscores the importance of an updated assessment of PCB trophic magnification. Unfortunately, unforeseen circumstances have resulted in the 2019 PCB data being unavailable, prohibiting a temporal comparison. The analyses presented here will focus on the relationship between trophic structure and PCB load as revealed in the 2002-2003 dataset.

Recovery of PCB surrogates ranged from 71 – 82 % for 2002-2003 analyses. Congener 118 was the most frequently observed congener in this dataset; this congener accounted for 55% of the total concentration (ppb) of WHO congeners quantified (Figure 15). Congeners 77, 81, 126, 169 and 189 were typically below detection limit; congener 81 was never observed (Table 6). Since PCBs were anticipated to increase exponentially in the food web, PCB concentrations were log-transformed for the purposes of particular analyses such as some regressions.

When considered collectively, WHO congeners were shown to magnify in Lake Michigan fauna. A trophic magnification factor (TMF) of 3.1 was calculated when considering the raw ΣWHO congeners (Figure 16). Following lipid-correction, ΣWHO congeners displayed a TMF of 2.8 (Figure 17). TMFs were also evaluated on a congener-specific basis. However, only the three congeners that were present above detection limit in > 15% of samples were eligible for this calculation. Following lipid-correction congeners 105, 118, and 156 had TMFs of 3.5 (Figure 18), 3.3 (Figure 19), and 1.8 (Figure 20), respectively. ANCOVA revealed that the slopes of the lines used to calculate these

congener-specific TMFs differed significantly ($F_{5, 377} = 3.43$, $p = 0.033$). Samples were also designated as "nearshore benthic" or "offshore pelagic" based upon their $\delta^{13}C$ signatures. There was no evidence that lipid-corrected ΣWHO congeners magnified at different rates in nearshore and offshore regions of Lake Michigan (ANCOVA: $F_{3, 93} = 0.09$, $p = 0.760$, Figure 21).

A multivariate regression was performed to evaluate the effects of trophic level, lipid percentage, and lipid-corrected $\delta^{13}C$ on raw ΣWHO congener concentration. A significant regression equation was found ($F_{3, 129} = 30.82$, $p<0.001$). Trophic level and lipid content were significant predictors of concentration of ΣWHO congeners, but lipid-corrected $\delta^{13}C$ was not. Collectively, the model accounted 42% of the observed variability in ΣWHO congener concentration (Table 7).

In addition, multiple regression analyses evaluating the effects of this same suite of dependent variables on concentrations of specific congeners were performed. Only congeners 105, 118, and 156 were eligible for these congener-specific regressions, since all other who congeners were detected in < 15 % of samples analyzed. The regression model was statistically significant for all congeners eligible for this analysis (congener 105: $F_{3, 129} = 29.47$, $p<0.001$; congener 118: $F_{3, 129} = 33.57$, $p<0.001$; congener 156: $F_{3,129} = 3.02$, $p = 0.032$; Table 7). Trophic level and lipid content were significant predictors for concentrations of congeners 105 and 118. No individual parameters were significant for congener 156.

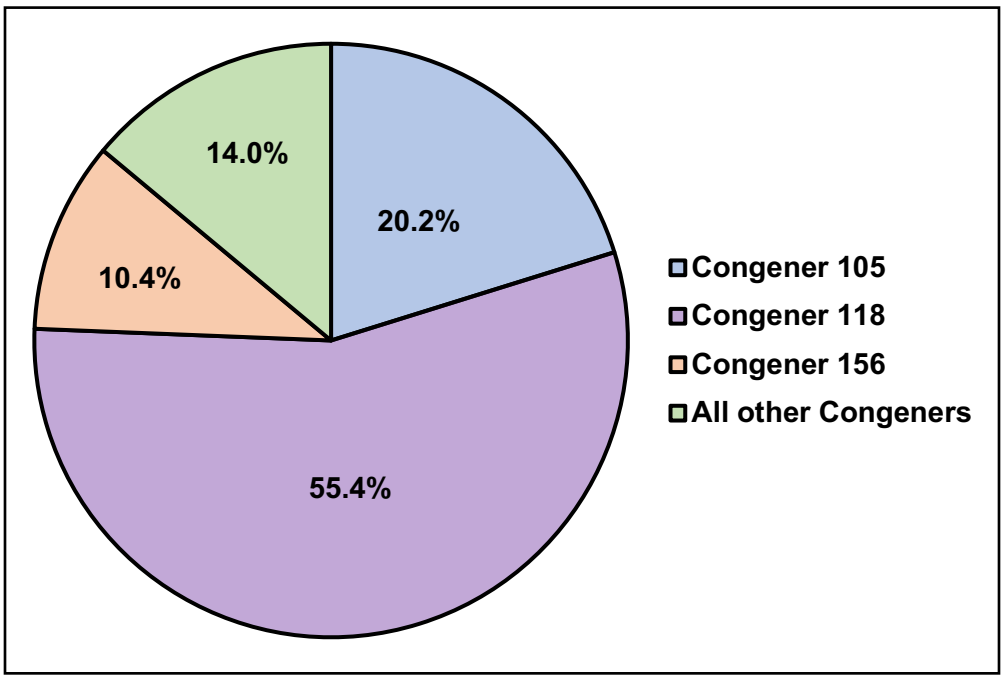


Figure 15. Of the total concentration of WHO PCBs quantified, the percent represented by each particular congener. Data includes randomized values assigned for samples < MDL.

Table 6. Summary of the percent of samples for which each congener was observed above the 10 ppb detection limit. Percentages rounded to nearest whole number. (n = 134)

Congener #	% of samples > MDL
77	1
81	0
105	78
114	11
118	93
123	8
126	1
156	48
157	11
167	10
169	6
189	2

77	1
81	0
105	78

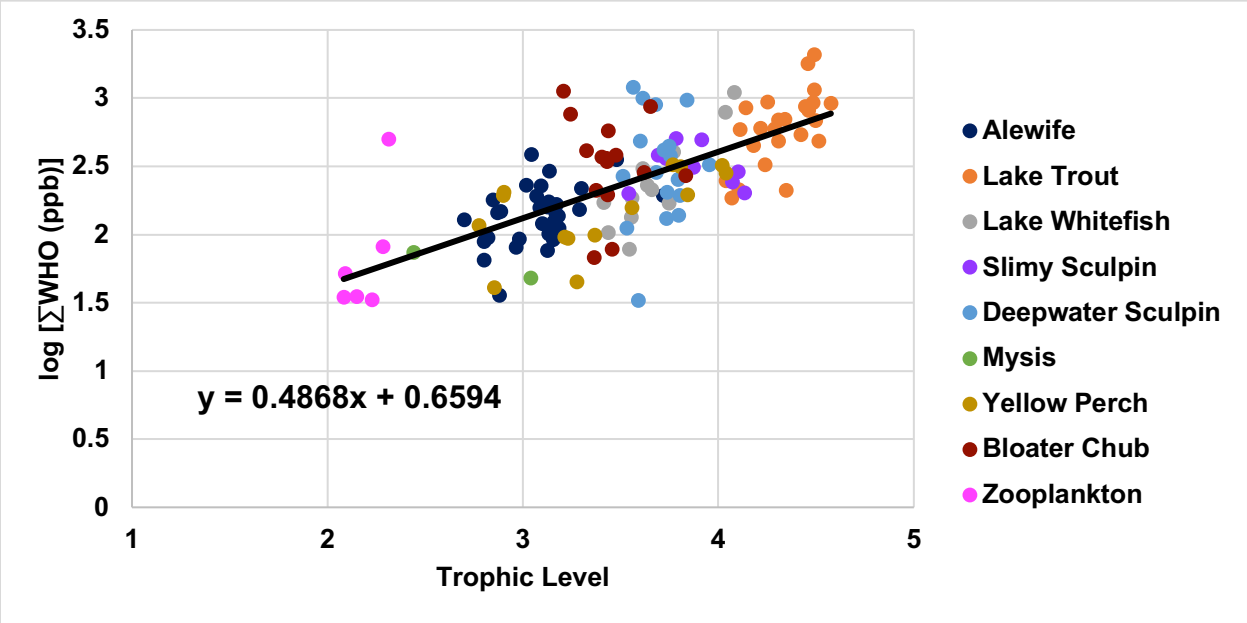


Figure 16. Relationship between trophic level and raw ΣWHO congeners (ppb) used to calculate a TMF of 3.1.

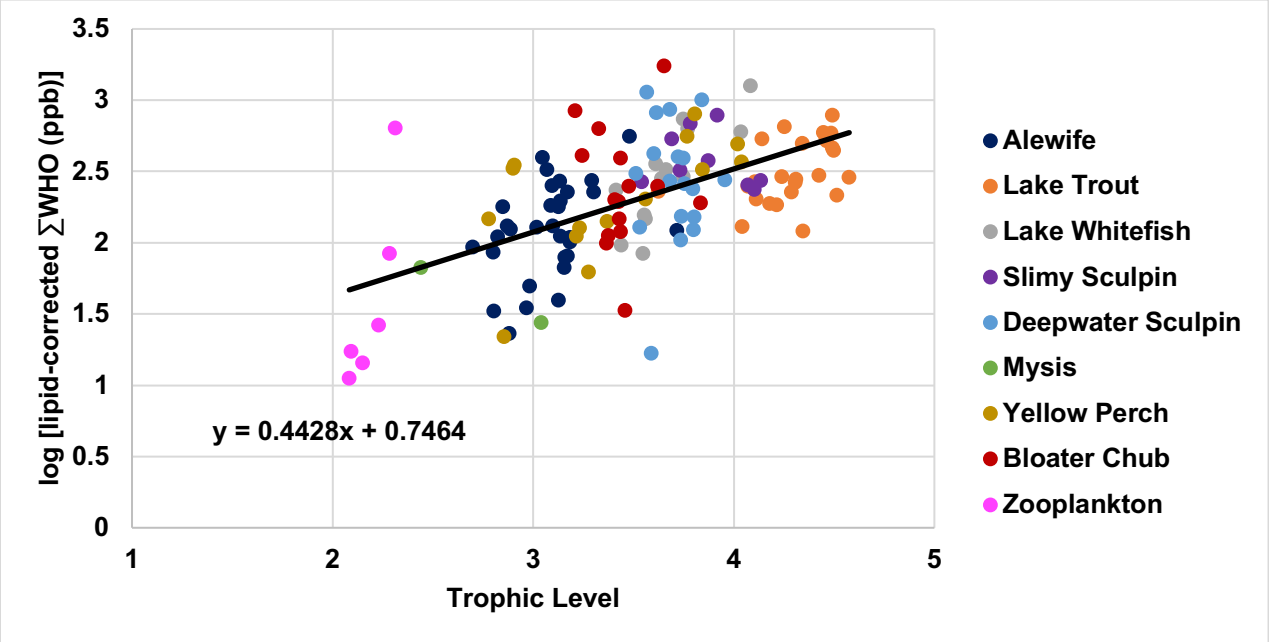


Figure 17. Relationship between trophic level and lipid-corrected Σ WHO congeners (ppb) used to calculate a TMF of 2.8.

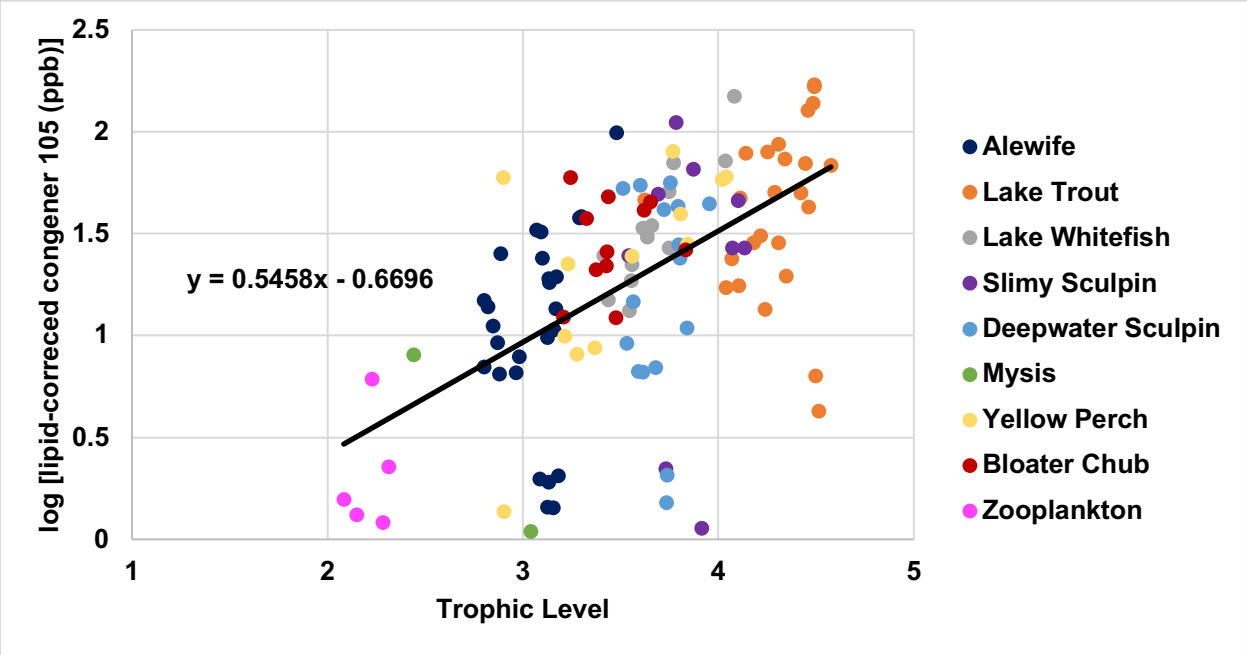


Figure 18. Relationship between trophic level and lipid-corrected congener 105 concentration (ppb) used to calculate a congener-specific TMF of 3.5.

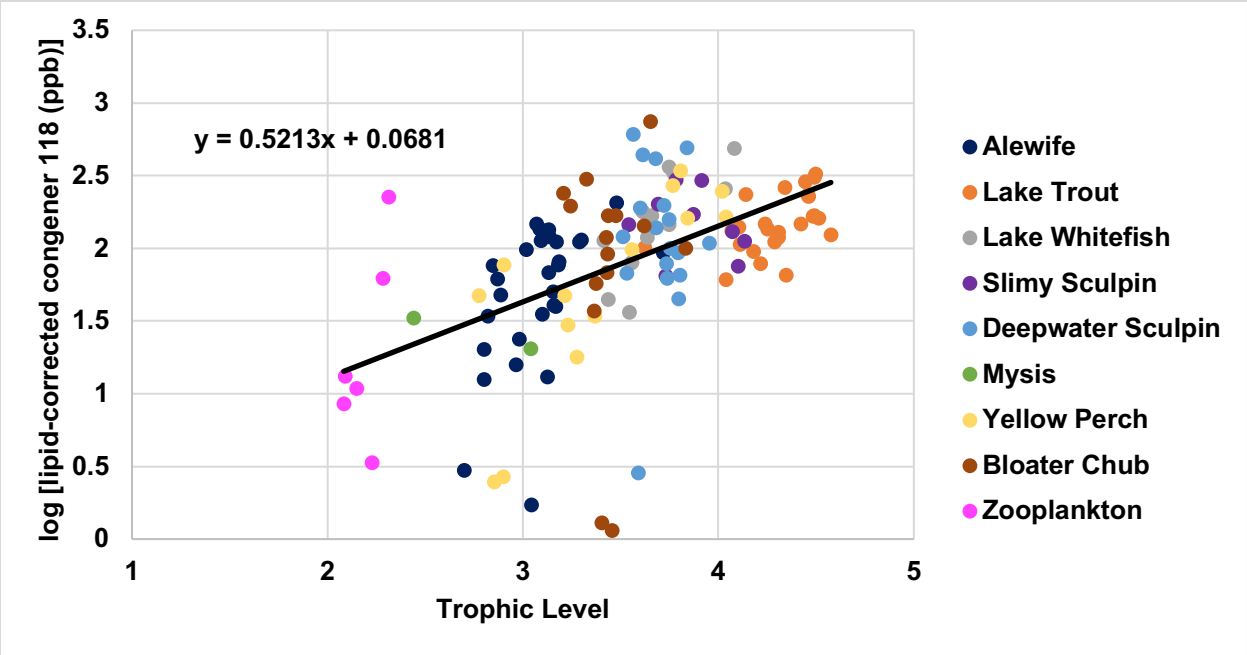


Figure 19. Relationship between trophic level and lipid-corrected congener 118 concentration (ppb) used to calculate a congener-specific TMF of 3.3.

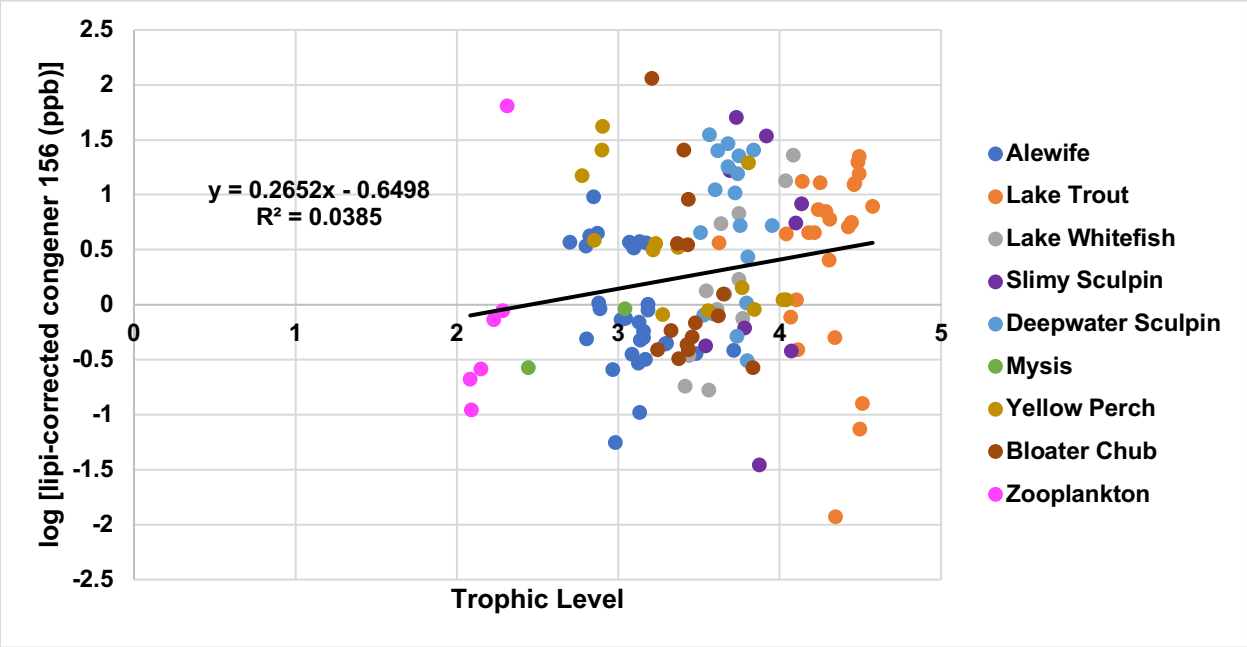


Figure 20. Relationship between trophic level and lipid-corrected congener 156 concentration (ppb) used to calculate a congener-specific TMF of 1.8.

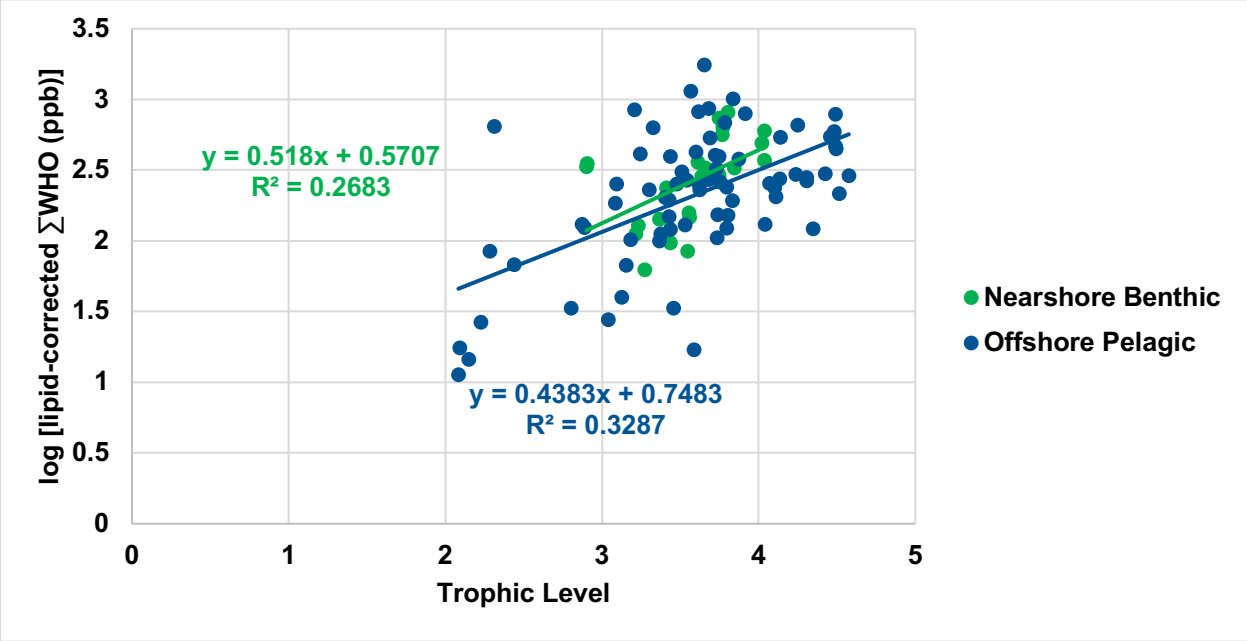


Figure 21. Comparison of magnification relationships between nearshore benthic and offshore pelagic food webs. There was no significant difference between slopes of these lines (ANCOVA: $F_{1, 71} = 0.09$, $p = 0.760$).

Table 7. Summary of the results of multiple regressions relating a suite of explanatory variables to the concentration of ΣWHO congeners ($F_{3, 129} = 30.82$, **p<0.001**), as well as the specific concentrations of congener 105 ($F_{3, 129} = 29.47$, **p<0.001**), congener 118 ($F_{3, 129} = 33.57$, **p<0.001**), and congener 156 ($F_{3,129} = 3.02$, **p = 0.032**). T-ratios represent the quotient of that parameter's regression coefficient divided by its standard error.

Response Variable	Explanatory Variable	t ratio	p value	R^2
[ΣWHO congeners] (ppb)	Trophic Level	6.22	**<0.001**	0.417
	% Lipid	2.99	**0.003**	
	Lipid-adjusted **δ**13C	-1.56	0.122	
	Intercept	-4.69	**<0.001**	
[Congener 105] (ppb)	Trophic Level	6.32	**0.001**	0.401
	% Lipid	3.18	**0.002**	
	Lipid-adjusted **δ**13C	0.80	0.426	
	Intercept	-3.28	**0.001**	
[Congener 118] (ppb)	Trophic Level	6.72	**<0.001**	0.438
	% Lipid	2.83	**0.005**	
	Lipid-adjusted **δ**13C	-1.75	0.082	
	Intercept	-5.15	**<0.001**	
[Congener 156] (ppb)	Trophic Level	0.92	0.360	0.066
	% Lipid	0.85	0.395	
	Lipid-adjusted **δ**13C	-1.81	0.072	
	Intercept	-1.77	0.080	

3.4 PCB Results by Species

Mean concentration of ΣWHO congeners differed among species, both before ($F_{8, 124} = 8.40$, $p<0.001$, Figure 22) and after lipid-correction ($F_{8, 124} = 2.77$, $p=0.008$, Figure 23). In both instances, *Mysis* displayed the lowest concentration of ΣWHO congeners. In general, lipid correction appeared to reduce some of the differences among species' raw PCB concentrations. For instance, the apparent difference between lake trout and other species is noticeably narrowed following lipid correction. However, it should be noted that both before and after lipid adjustment, lake trout PCB load was not significantly different from that of several other fish species.

Analyses were also run on a congener-specific basis. Only the 3 congeners that were observed above detection limit most frequently were eligible for congener-specific analysis. Species of high trophic level consistently showed elevated PCB concentrations, regardless of congener (Figures 24, 25). Relative concentrations among species were generally consistent across all congeners considered. Fish species did not differ in the concentration of congener 156 ($F_{8,124} = 1.38$, $p = 0.213$, Figure 26).

Relationships between total length and lipid-corrected Σ WHO congeners were investigated for each fish species sampled. In all instances, exponential curves fit this relationship better than linear regression did. Therefore, PCB data were log-transformed and regressed against total length. Lake trout and yellow perch displayed a statistically significant relationship between these two variables, with all other species failing to overcome the significance threshold (Figure 27A-G). Lake trout and yellow perch were

then chosen for a regression analysis relating total length to trophic level. These variables were significantly related in both species (Figure 28A-B).

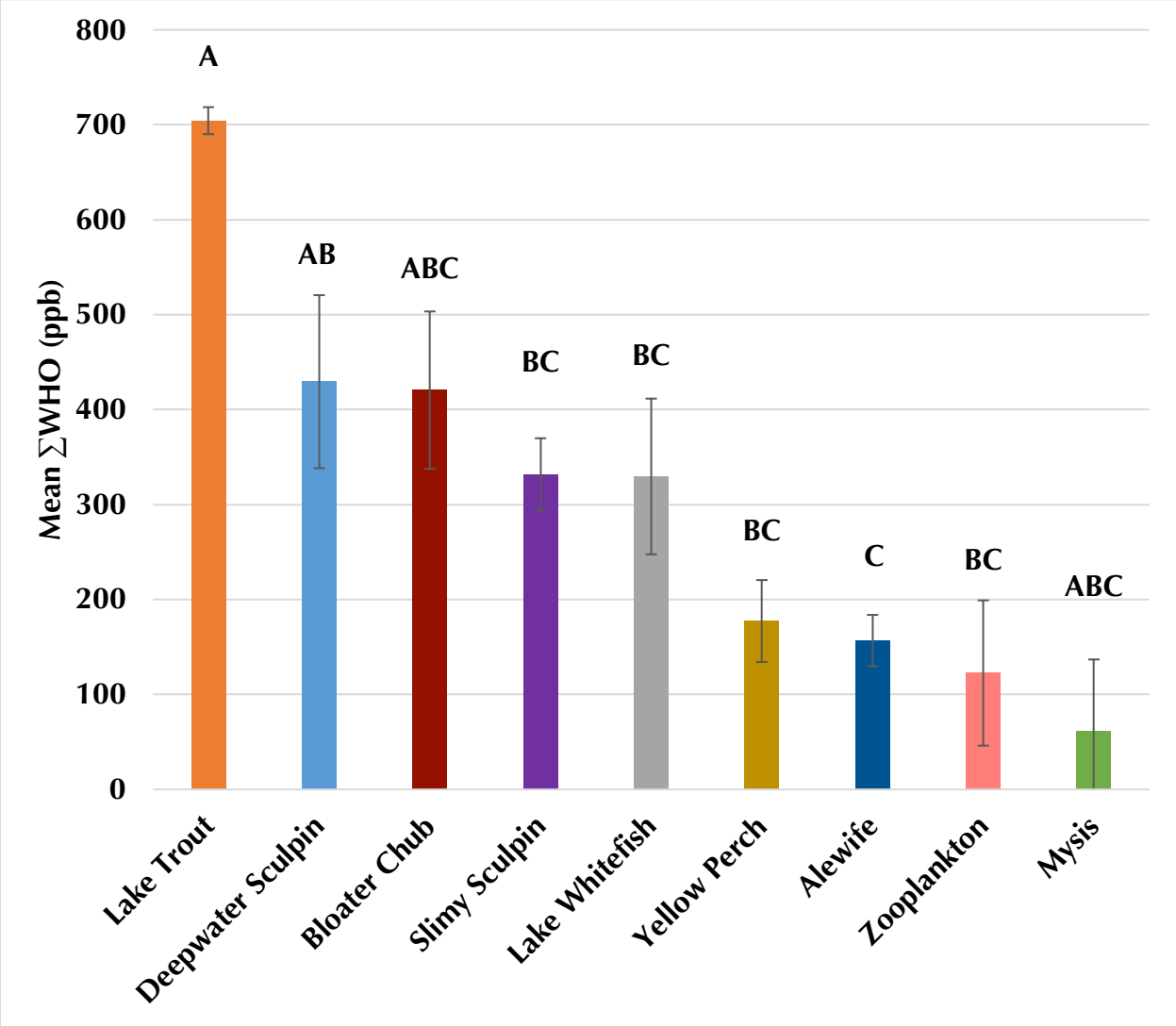


Figure 22. Comparison of mean concentration of raw ΣWHO congeners by species. Error bars represent ± 1 s.e. Species sharing a letter are not significantly different from each other as determined by a Tukey-Kramer HSD post-hoc test. One-way ANOVA: Table 9 = 8.40, p = **0.001**

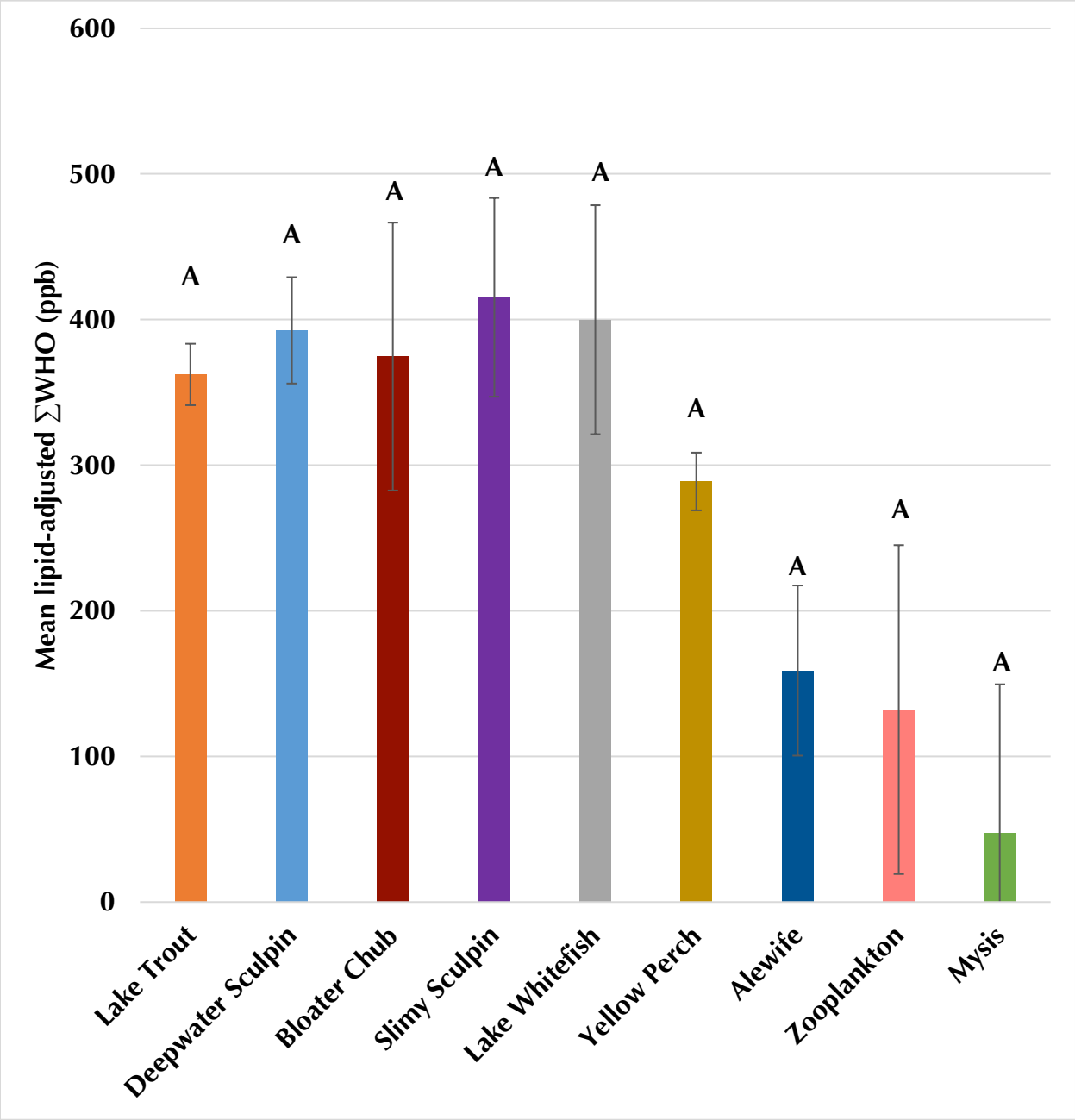


Figure 23. Comparison of mean concentration of lipid-adjusted ΣWHO congeners by species. Error bars represent ± 1 s.e. Species sharing a letter are not significantly different from each other as determined by a Tukey-Kramer HSD post-hoc test. No species' concentrations were significantly different from each other. One-way ANOVA: $F_{8, 124} = 2.770$, p = **0.008**

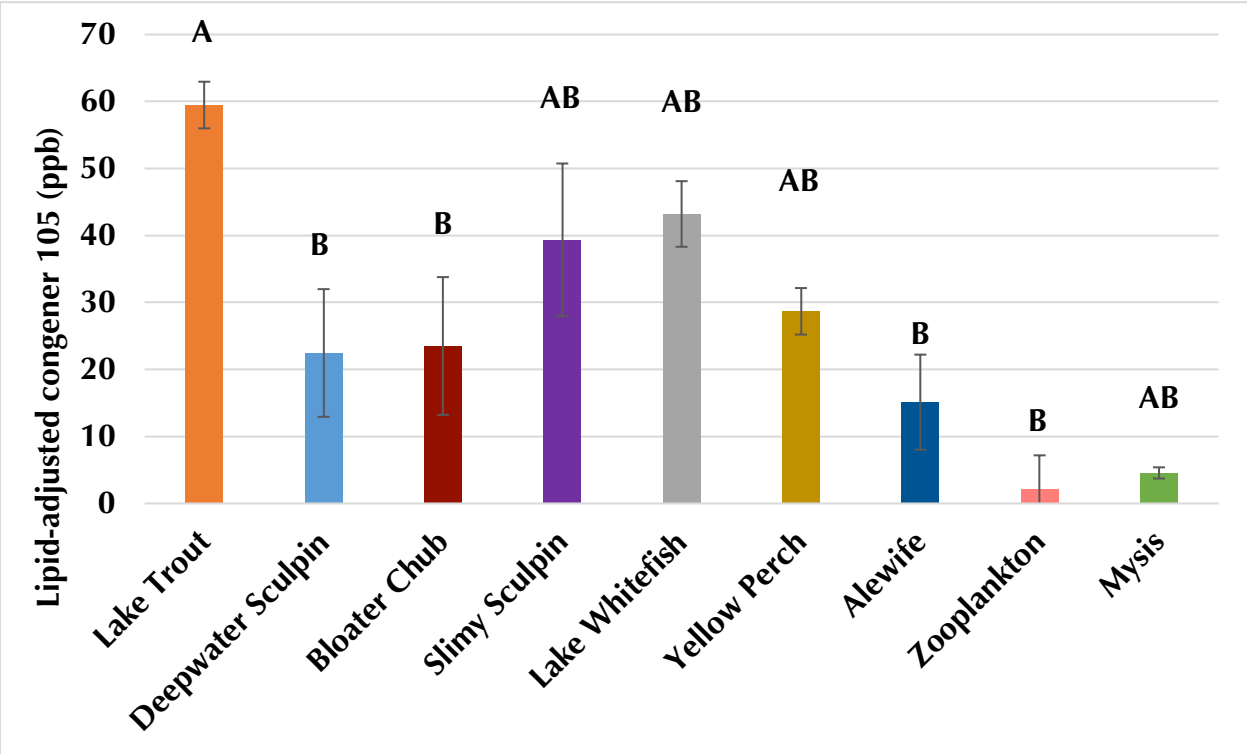


Figure 24. Comparison of mean concentration of lipid-adjusted congener 105 concentration by species. Error bars represent ± 1 s.e. Species sharing a letter are not significantly different from each other as determined by a Tukey-Kramer HSD post-hoc test. One-way ANOVA: $F_{8, 124} = 5.359$, p = **0.001**

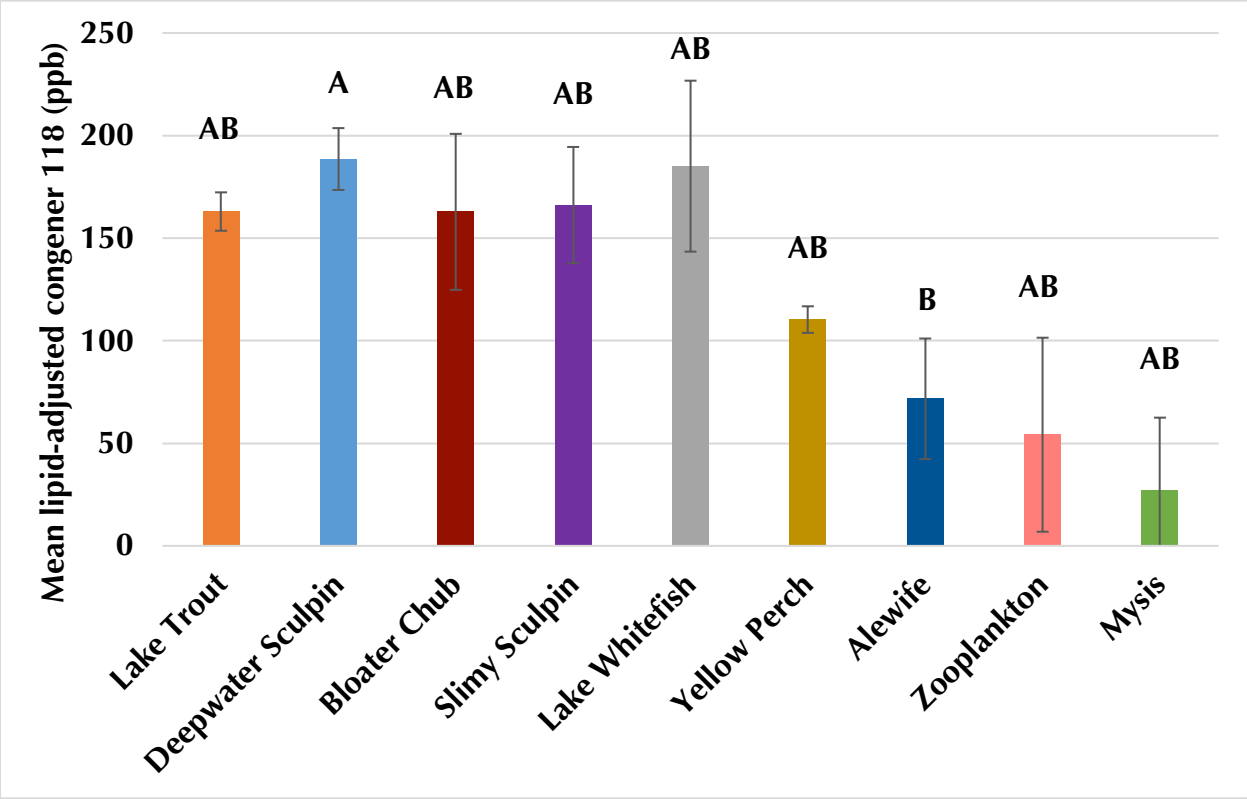


Figure 25. Comparison of mean concentration of lipid-adjusted congener 118 concentration by species. Species sharing a letter are not significantly different from each other as determined by a Tukey-Kramer HSD post-hoc test. One-way ANOVA: $F_{8, 124} = 2.918$, p = **0.005**

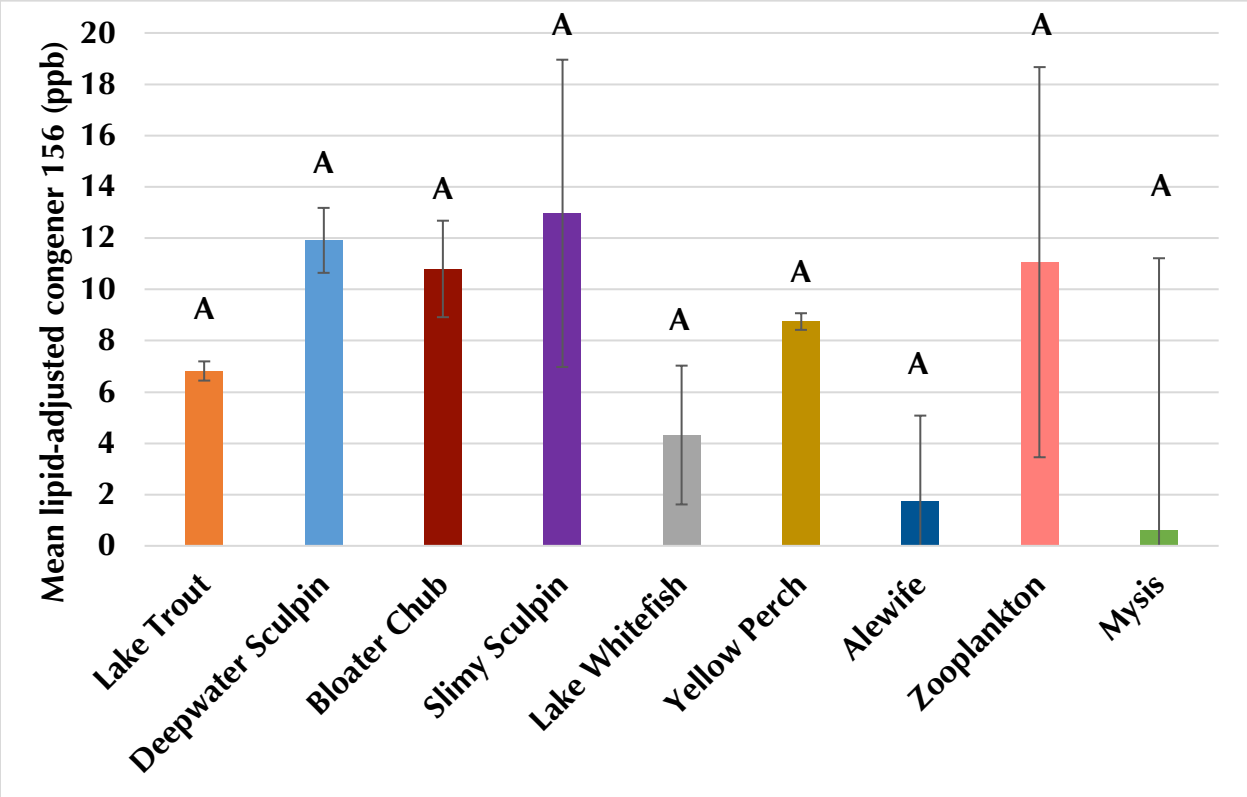
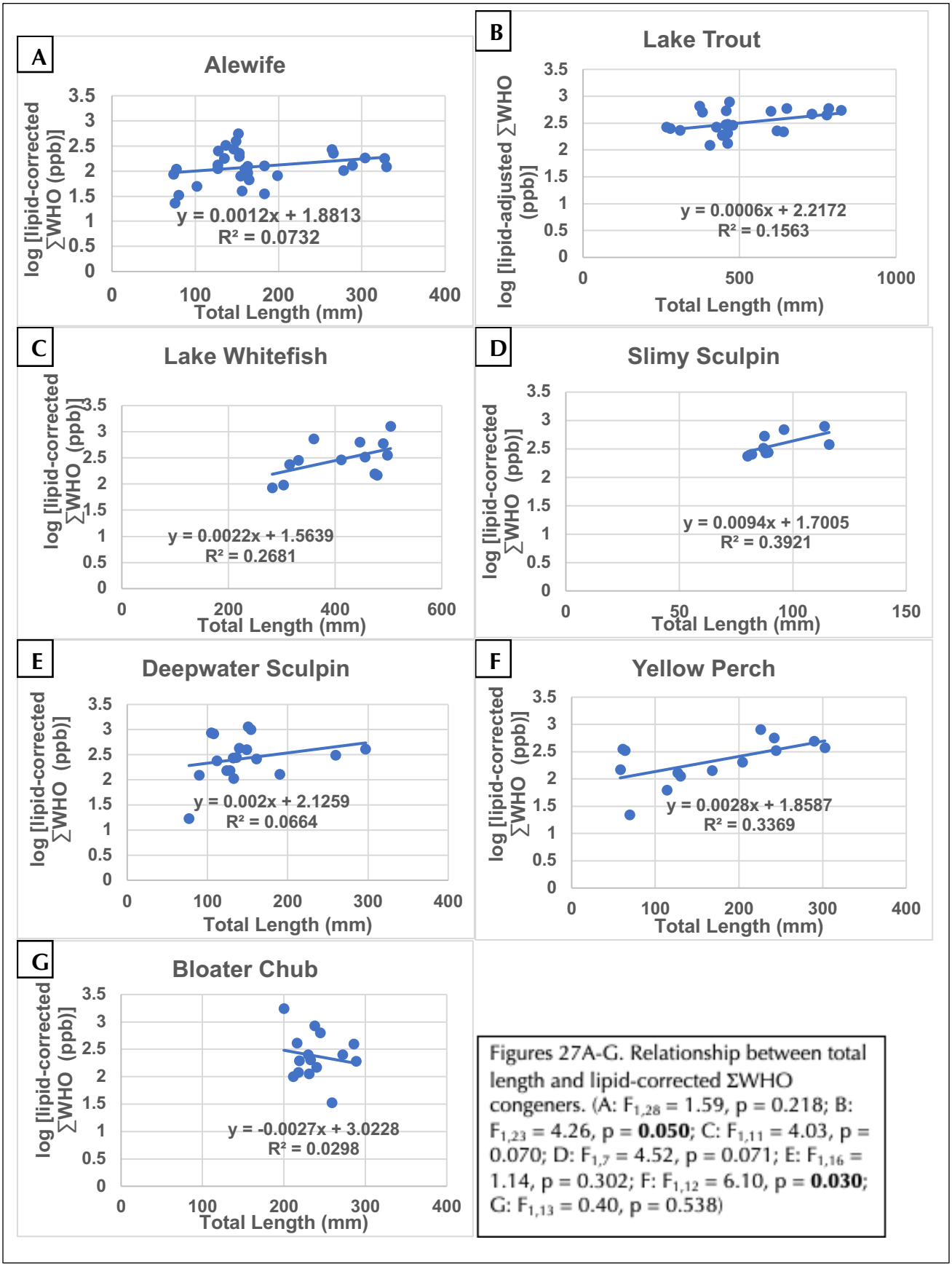
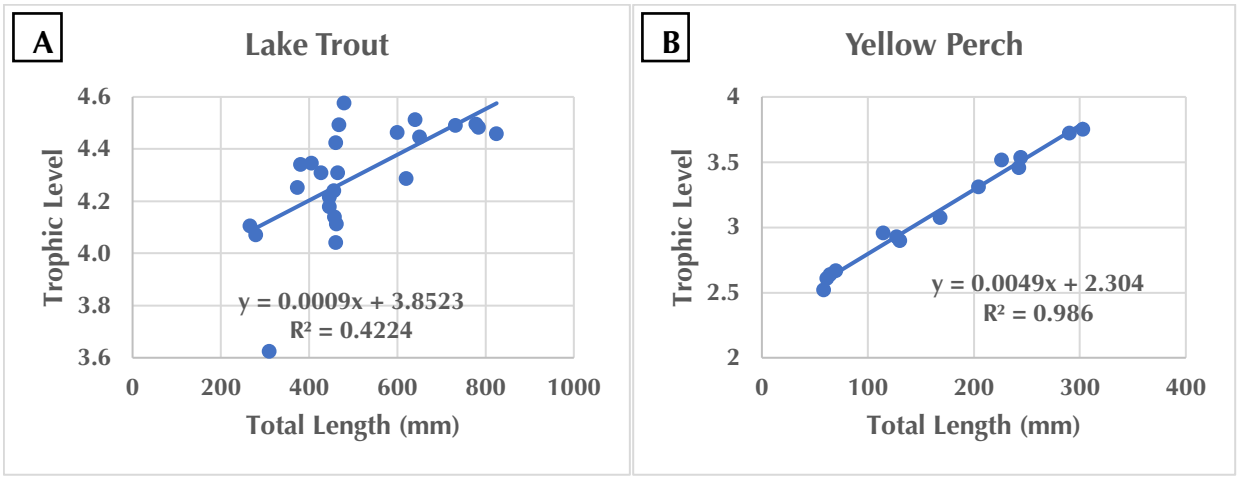


Figure 26. Comparison of mean concentration of lipid-adjusted congener 156 concentration by species. Species sharing a letter are not significantly different from each other as determined by a Tukey-Kramer HSD post-hoc test. One-way ANOVA: $F_{8, 124} = 1.38$, $p = 0.213$



Figures 27A-G. Relationship between total length and lipid-corrected ΣWHO congeners. (A: $F_{1,28} = 1.59$, $p = 0.218$; B: $F_{1,23} = 4.26$, $p = \mathbf{0.050}$; C: $F_{1,11} = 4.03$, $p = 0.070$; D: $F_{1,7} = 4.52$, $p = 0.071$; E: $F_{1,16} = 1.14$, $p = 0.302$; F: $F_{1,12} = 6.10$, $p = \mathbf{0.030}$; G: $F_{1,13} = 0.40$, $p = 0.538$)



Figures 28A-B. Regressions between total length and trophic level. (A: $F_{1,23} = 18.16$, $p <$ **0.001**; B: $F_{1,12} = 835.92$, $p <$ **0.001**)

Chapter 4: Discussion

4.1 Stable Isotopes Reveal Continued Shifts in Trophic Structure

Comparisons of 2019 stable isotope data with values from 2002-2003 and 2010-2012 underscore the dynamic nature of the Lake Michigan food web. Turschak et al. (2014) revealed that a number of fish species began relying more heavily on nearshore carbon sources between 2002 and 2012, coincident with the proliferation of quagga mussels. Since mussel grazing in the pelagic region had stripped the water column of nutrients and phytoplankton, some species were observed to depend instead on primary production from *Cladophora* and other nearshore energy.

According to the updated 2019 data, some species such as round goby and mysids show evidence of returning to 2002-2003 stable isotope values. Such changes may be attributed to seasonal sampling bias. Due to the logistical challenges of sampling a diverse assortment of species, 2019 samples were collected on an opportunistic basis and not necessarily at the same time of year as the previous sampling efforts. Driscoll et al. (2015) found evidence of seasonal shifts in trophic structure at the base of the Lake Michigan food web. If this seasonal variation is propagated farther up the food web, then it would be fair to also anticipate seasonal fluctuations in the stable isotope signatures of higher trophic fish. Seasonal fluctuations would likely have a larger effect for lower trophic fish, which generally have a higher rate of stable isotope turnover than that of apex predators (O'Reilly et al. 2002). Regardless, differences in sampling times during the 2002-2003, 2010-2012, and 2019 surveys could contribute to some perceived shifts in trophic structure among these time frames.

Comparisons of alewife total length and stable isotope ratios over time reveal some apparent changes for this species. In 2019, as alewives grew in length, they were shown to rely more on nearshore energy, the opposite relationship from that observed in 2002-2003 and 2010-2012. It is possible that such an energetic shift may be due to different alewife dietary preferences as they grew. Variation in the location of zooplankton prey might cause larger alewife to travel to nearshore areas inhabited by their preferred prey (Strus & Hurley 1992). However, the limited range of 2019 length data somewhat undermines this perceived relationship between alewife length and $\delta^{13}C$.

4.2 Interpretation of PCB Trophic Magnification Dynamics

The relative amounts of congeners observed in this study parallel the amounts initially manufactured by Monsanto. Congeners 77, 81, 114, 123, 126, 157, 167, 169, and 189 were quantified in fewer than 15 % of samples, and they were also found in quite low concentrations in the most commonly produced Aroclor mixtures (Table 8). Therefore, their absence in biota is likely due to the fact that these congeners simply have not been loaded into Lake Michigan in appreciable concentrations. Meanwhile, congener 118, which was the most commonly observed congener in our study, is also the WHO congener represented in Aroclor mixtures most abundantly. The distribution of congeners within the Aroclors can likely be explained by the manufacturing process; some positions on biphenyl are less thermodynamically or kinetically favorable to chlorination. Therefore, some particular congeners had a low probability of formation during manufacturing.

Table 8. Summary of the % by mass of each WHO congener in the most commonly produced Aroclors. Individual congener percentages greater than 1 are bolded. Aroclor 1248 and 1254 had slightly different "lot" formulas depending on the date and location of production. Source: United States Department of Health and Human Services (2000)

	Aroclor 1242	Aroclor 1248 "Lot A3.5"	Aroclor 1248 "Lot G3.5"	Aroclor 1254	Aroclor 1254 "Late"	Aroclor 1260
% Congener 77	0.31	0.41	0.52	0.03	0.2	0
% Congener 81	0.01	0.01	0.02	0	0	0
% Congener 105	0.47	1.6	1.45	**2.99**	**7.37**	0.22
% Congener 114	0.04	0.12	0.12	0.18	0.5	0
% Congener 118	0.66	**2.29**	**2.35**	**7.35**	**13.59**	0.48
% Congener 123	0.03	0.07	0.08	0.15	0.32	0
% Congener 126	0	0	0	0	0.02	0
% Congener 156	0.01	0.06	0.04	0.82	**1.13**	0.52
% Congener 157	0	0.01	0	0.19	0.3	0.02
% Congener 167	0	0.01	0.01	0.27	0.35	0.19
% Congener 169	0	0	0	0	0	0
% Congener 189	0	0	0	0.01	0.01	0.1
% WHO Congeners	1.53	4.58	4.59	11.99	23.79	1.53

As anticipated, the Lake Michigan food web displays a classic trophic magnification pattern, with PCB concentrations increasing exponentially with trophic level. It should be noted, however, that the magnification effect is slightly reduced when corrected for lipid content. This is due in part to the fact that, of the species sampled in this study, lake trout had the highest mean lipid content while also occupying the highest trophic level. Therefore, interpretation of lipid-adjusted values provides a clearer way to evaluate the impact of trophic level on PCB concentrations by reducing the influence of lipid content.

When considering different aquatic food webs, PCB TMFs appear to be somewhat variable. The calculated TMF of 2.8 in Lake Michigan is within the range of those calculated in other systems (Table 9). Perhaps the best comparison is with a study conducted in Lake Ontario by Helm et al. (2008), since this project also focused on the WHO congeners and the community composition of this lake is quite similar to that of Lake Michigan. They observed relative amounts of each congener very similar to those observed in this study, with congener 118 appearing most frequently and in the highest concentrations followed by congeners 105 and 156. Unfortunately, the lack of TMF calculations for these particular congeners prohibits a congener-specific TMF comparison with our findings.

Different levels of primary production in Lake Michigan and Lake Ontario could have contributed to different TMFs for these lakes. In systems where primary production is low, prey is typically scarce, leading to reduced body condition in some fish (Sommer et al. 1986). To offset the poor condition of their food source, a predator must eat more prey

to grow to a particular size. Eating more would expose the predator to more contaminants over the predator's lifespan. This turn-of-events may have been at place in Lake Michigan in the early 2000s, since whole-lake estimates of primary production were less than in Lake Ontario at that time (Carrick et al. 2001; Estepp & Reavie 2015). Alternatively, Borgmann & Whittle (1991) documented fluctuations in Lake Ontario populations of alewife, a key prey species, potentially contributing to TMF shifts. For these reasons, piscivorous fish may have had elevated PCB loads, contributing to a higher TMF in Lake Michigan than that observed in Lake Ontario by Helm et al. (2008).

Community composition and differences in metabolism could further explain some of the variability in TMFs across different ecological systems. As persistent organic pollutants (POPs), PCBs are generally considered resistant to environmental degradation. However, several members of the P450 superfamily of enzymes are known to detoxify PCBs (James 2001). For instance, deepwater sculpin have been shown to have substantial activity of the cytochrome P450-2B, which catalyzes the metabolism of particular congeners into methylsulfonyl-PCBs, which are readily excreted (Stapleton et al. 2001). These researchers estimated that up to 10% of the deepwater sculpin PCB burden is metabolized through this pathway. In the event that this enzyme is active in other species (perhaps close evolutionary relatives to deepwater sculpin), then it is possible that a substantial portion of the PCB load to a freshwater system could be metabolized. If ecological systems have different numbers of species utilizing this enzyme, then the trophic magnification rate could differ. It is reasonable to hypothesize that individual

congeners have slightly different affinities for the active site of p450 enzymes, further contributing to the observed differences in congener-specific TMFs.

Another reason for this aforementioned variability in TMFs across different ecological systems is that different studies investigate different collections of congeners. This is problematic since specific congeners have been shown to magnify at different rates, as was observed in this study. For instance, Zhou et al. (2019) observed TMFs ranging from 1.15 (congener 195) to 9.72 (congener 153). This example underscores that the overall TMF for Σ PCBs is highly dependent upon which congeners are considered. Zhou et al. (2019) also observed a weak positive link between log K_{ow} (octanal-water partition coefficient) and TMF. This relationship supports the intuitive hypothesis that the more lipophilic congeners will be less prone to excretion and therefore magnify at a higher rate. In addition, Walters et al. (2011) demonstrated that the strength of the relationship between K_{ow} and TMF varies by system, which is another possible explanation for the observed variability in TMFs in different studies. For this collection of reasons, comparisons of TMFs for ΣPCBs among different studies should generally be made with caution.

We observed no difference in trophic magnification of PCBs between Lake Michigan's benthic and pelagic food webs. This finding contrasts with that of Kidd et al. (2001), who observed such differences in Lake Malawi for DDT. The lack of agreement between these studies is perhaps explained by the disparate ecological characteristics of the two lakes. Lake Malawi, famous for its collection of colorful cichlids, has one of the greatest species richness measures in the world, due to the evolution of highly specialized

feeding niches. As a result, benthic and pelagic food webs are quite distinct. Relative to Lake Malawi, Lake Michigan has substantially lower richness, more overlap of niches, and greater seasonal changes in behavior. Collectively, these qualities cause trophic interaction between the benthic and pelagic regions. For instance, lake trout (a pelagic species by most definitions) frequently prey upon round gobies (a nearshore benthic species) during the latter's winter migration to offshore waters (Pothoven & Madenjian 2013). Interactions such as these indicate there may not be justification for segregation of Lake Michigan's fauna into two unique food webs.

Table 9. Comparison of lipid-adjusted TMFs calculated in various food webs around the world. *indicates the same collection of congeners was analyzed as in this study

Location	ΣPCB TMF	Reference
East China Sea	1.39	Zhou et al. (2019)
Lake Ontario*	1.44	Helm et al. (2008)
Omuta River (southern Japan)	1.52	Kobayashi et al. (2015)
Stream in South Carolina	1.6	Walters et al. (2007)
Lake Michigan	**2.8**	**This study**
Basin by e-recycling plant in Qinyuan, China	3.67	Wu et al. (2009)
Lake Baikal	3.8	Kucklick et al. (1996)
North Water Polynya	4.6	Fisk et al. (2001)

Table 9

4.3 Critical Factors Influencing PCB Concentration

As expected, trophic level was a significant predictor of ΣWHO congeners, as well as congeners 105 and 118. This relationship further underscores the trophic magnification effect which already been discussed at length. Total length was also significantly linked to ΣWHO congeners, congener 105, and congener 118. For a detailed analysis of species-specific trends relating total length to PCB concentrations, refer to section 4.4. The

multivariate regression model did a poor job of predicting concentration of congener 156. One likely explanation for this is that only 46% of the samples analyzed exceeded the method detection limit for this congener. Since these values that were below MDL were assigned a small random number (section 2.7), the quantified concentrations were essentially obscured by the randomness of values <MDL.

The multiple regression model (containing lipid content, trophic level, and lipid-adjusted $\delta^{13}C$ as explanatory variables) accounted for just under half of the overall variability in ΣWHO congeners, congener 105, and congener 118. The remaining variability could be explained by a number of ecological and chemical factors. For instance, the sex of the organisms could have an effect on their PCB concentrations. In a study investigating contaminant concentrations in Lake Michigan coho salmon, Madenjian et al. (2010b) found that male PCB concentrations were on average 20% greater than those of females, but the underlying reason behind this discrepancy was unknown. Larsson et al. (1993) showed that in female pike (*Esox lucius*), levels of PCBs peak just before their first reproductive cycle. The authors speculate spawning not only offloads eggs, but also a substantial amount of PCBs embedded in the fatty tissue of those eggs. No such effect was observed in males, whose germ tissue comprises a much smaller proportion of their body weight. If any species sampled experience a similar phenomenon as documented in female pike, then it is possible that time since spawning and sex of the organism could have explained some of the additional variability in PCB concentrations.

Another factor not accounted for in the multiple regression model is predator efficiency at assimilating PCBs from their prey. In a controlled feeding study, Liu et al.

(2010) found that the nutritional quality of prey was negatively associated with PCB assimilation in predator tissue. It is possible that this effect was observed because reduced nutritional quality in prey forces a predator to compensate by consuming more, thereby taking in more PCBs. In addition, the wide range of assimilation efficiencies for particular congeners (Nichols et al. 2001; Arnot & Gobas 2004) could further explain our observed differences in congener-specific TMFs. Assimilation of PCBs could also be affected by seasonal fluctuations of physical conditions in Lake Michigan. A decrease in water temperature reduces the fluidity of fatty acids, in turn reducing the functionality of P450 enzymes, which are typically found bound to phospholipid membranes (Das & Sligar 2009). Such scenarios hinder an organism's ability to biotransform PCBs and excrete their metabolites. Therefore, incorporation of collection date into the multiple regression models may have explained more of the variability observed in PCB concentrations

4.4 Interpretation of PCB Results by Species

As anticipated, species differed in both concentrations of ΣWHO congeners and individual congeners that were analyzed. Lake trout had the highest mean concentration of raw ΣWHO congeners. However, following lipid-correction lake whitefish, slimy sculpin, deepwater sculpin, and bloater chub all showed similar concentrations to lake trout. Lake trout's high fat content likely inflated its raw PCB concentrations, so once lipid had been accounted for, this species displayed PCB concentrations more typical for its trophic level. Sculpin had PCB concentrations about 9 times that of *Mysis*, closely

mirroring the biomagnification rate of 9.2 observed by Evans et al. (1991) for this trophic interaction.

One factor that could potentially complicate interpretation of PCB results by species is the composition of fatty acids. Little research has been done investigating preferential association of PCBs with particular types of fatty acids. While PCB data in this study were mathematically adjusted for lipid content of the sample, this correction was not sensitive to the particular types of fatty acids present in the samples. Given that the fatty acid profiles of Great Lakes fish are known to differ substantially by species (Dellinger et al. 2018), if PCBs preferentially associate with particular fatty acid subtypes, then this phenomenon would presumably affect the PCB burdens of certain species. Further research is necessary to elucidate any preference of PCBs for specific fatty acids.

Of the species sampled, deepwater sculpin showed one of the highest concentrations for congener 118 yet one of the lowest for congener 105. Interestingly, this trend was not observed for slimy sculpin, which inhabit slightly shallower waters but otherwise have a similar niche and comparable stable isotope signature. Since few researchers report congener-specific data, it is difficult to determine if others have observed a similar trend for deepwater sculpin. It is unlikely that deepwater sculpin simply encounter congener 118 more often than slimy sculpin do, as other studies have shown fairly uniform congener distributions in other lakes (Bentzen et al. 1999; Hurme & Puhakka 1999). One potential explanation is preferential metabolism of congener 105 relative to that of congener 118, particularly in light of the findings of Stapleton et al. (2010) that deepwater sculpin can metabolize a substantial portion of their PCB burden.

While these molecules are of the same homolog class, congener 105 has its chlorines unequally distributed to one side of the molecule. The empty space could allow for enhanced pi-pi interaction between this side of congener 105 and P450-2B's active site, which is rich in aromatic amino acid residues such as phenylalanine (Halpert 2011). The relatively uniform placement of chlorines around the phenyl rings in congener 118 may prohibit such pi-pi interactions for this isomer. While there appears to be no clear explanation for deepwater sculpin's relative concentrations of congeners 105 and 118, differential rates of metabolism could have contributed to this disparity.

Lake trout PCB concentrations documented in our study are somewhat lower than those observed in the mid 1990s in Lake Michigan (De Vault et al. 1996). After steady declines between 1974 and 1992, these decreases halted between 1993 and 1995. De Vault et al. (1996) speculate that a trophic level increase in lake trout counteracted the steady first-order decline in PCB concentrations observed over the previous two decades. Specifically, a strong alewife diet shift towards *Bythotrephes*, a predatory zooplankton, may have caused this prey fish to rise in trophic level. In turn, lake trout, whose diet consisted substantially of alewife, may have similarly rose in trophic level. This elevation of trophic level likely offset any anticipated drops in lake trout PCB concentrations due to burying in sediment, metabolism, or other factors that could remove these contaminants from the food web. The fact that our PCB concentrations in lake trout are slightly lower than those observed by De Vault et al. (1996) implies that any trophic level increase in lake trout in the mid-1990s was overcome by the first-order kinetics loss that had previously been reducing concentrations. Such a situation underscores the importance of

understanding the ecological context of PCB data, especially when conducting time-series comparisons.

Most species sampled in our study did not show a significant relationship between total length and PCB concentration. The exceptions were lake trout and yellow perch, which demonstrated significant exponential relationships. The observed increases in PCB load as these two species increase in length is likely linked to trophic level. Since total length and trophic level were significantly correlated in both species, then this apparent PCB-length link is simply a reflection of the effect of trophic level on PCB load. Older (and larger) perch and lake trout have different feeding behavior from the younger (and smaller) members of their species, such that the larger fish occupy a higher trophic level and therefore consume prey containing a higher PCB concertation.

Other researchers have also observed associations between total length and PCB concentrations for lake trout and yellow perch. Kidd et al. (1998) observed a similar trend when analyzing lake trout from a collection of subarctic lakes in Yukon territory. An exponential relationship is to be expected considering the growth rate of lake trout dramatically slows once individuals reach about 700 mm (Madenjian et al. 1998). After a lake trout hits this threshold, its PCB burden continues to rise through dietary sources, while its length remains relatively stagnant. As for perch, Olsson et al. (2000) found a similar link between total length and PCB concentration for moderate and large perch similar to the lengths represented in our sample. In accordance with our findings, a survey of several Ontario lakes found that lake whitefish rarely show a significant relationship between length and PCB burden (Gewurtz et al. 2011).

4.5: Key Points and Broader Implications

An intended emphasis of this study as it was initially designed was to investigate how recent shifts in trophic interactions and bioenergetics may have altered the PCB dynamics between the 2002-2003 and 2019 sampling efforts. For instance, the apparent drop in trophic level for species such as lake trout and slimy sculpin might be expected to coincide with a reduction in PCB concentrations for these species relative to the values reported in section 3.4. Alternatively, there may be some "lag time" before ecological shifts have the ability to manifest themselves in the form of altered PCB burdens, particularly for long-lived species. Once the final PCB analyses are complete for the 2019 sampling, proper comparisons can be made; any attempted comparisons in the meantime would be speculative.

Despite the current lack of PCB measurements for the 2019 sampling, the 2002-2003 dataset is valuable for determining the relationship between trophic structure and PCB loads. Few other PCB studies have been conducted on Lake Michigan with the same breadth of sampling as the research described herein. By sampling a rich diversity of Lake Michigan fauna, a wholistic view of PCB trophic magnification was developed. After accounting for the effect of lipids, PCBs were shown to magnify by a factor of 2.8 for each trophic level, a rate substantially higher than that observed in Lake Ontario (Helm et al. 2008). This disparity could potentially be attributed to the relatively lower primary productivity of Lake Michigan, forcing piscivores to consume more prey fish than they would in Lake Ontario. Alternatively, it could be explained by Lake Ontario fluctuations

in alewife, a crucial prey species. Considering that magnification factors are quite variable in different ecological systems, the specific TMF for Lake Michigan is valuable for limnologists and regional health officials alike. This trophic magnification factor can be used to approximate any organism's PCB burden if its general trophic position is known. In addition, this TMF may be valuable to researchers devising contaminant models in the Great Lakes. Meanwhile, this value can provide insight for public health officials formulating fish consumption advisories for the safety of the over 10 million residents of Lake Michigan's shores.

PCB findings for particular species also have broad implications. Understanding the typical PCB concentrations for certain species helps to determine the efficiency at which the species is assimilating these contaminants. Furthermore, these findings provide field context for researchers investigating molecular detoxification pathways in fish such as deepwater sculpin. In addition, species-specific PCB values for fish such as yellow perch, lake trout, and lake whitefish that are popular for human consumption help individuals adjust their diet to maintain their health. For instance, lake trout, an apex predator of the Lake Michigan food web, showed the highest PCB burden of all species sampled. The insight that lake trout PCB concentrations increase exponentially with length allows for a more nuanced view of estimating what the PCB load may be for a fish of this species.

While these findings are valuable, 2019 data will provide an updated understanding of PCB dynamics in the Lake Michigan food web. New calculations of trophic magnification factor and species-specific PCB concentrations will illuminate the effect dreissenid mussels and round gobies have had on this group of persistent organic

pollutants. This time-series comparison will be a unique opportunity to directly chronical the impact of ecological invasions on contaminant dynamics in large lake settings.

References

Andraso, G. M., Ganger, M. T. & Adamczyk, J. (2011). Size-selective predation by round gobies (Neogobius melanostomus) on dreissenid mussels in the field. Journal of Great Lakes Research 37, 298–304.

Arnot, J.A., Gobas, F.A.P.C. 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environmental Toxicology and Chemistry 23: 2343-2355.

Auer, M.T. Tomlinson, L.M., Higgins, S.N., Malkin, S.Y., Howell, E.T., Bootsma, H.A. 2010. Great Lakes *Cladophora* in the 21st century: same algae-different ecosystem. Journal of Great Lakes Research 26: 248-255.

Bai, X., & Acharya, K. 2019. Uptake of endocrine-disrupting chemicals by quagga mussels (*Dreissena bugensis*) in an urban-impacted aquatic ecosystem. Environmental Science and Pollution Research 26: 250-258.

Bahn A.K., Rosenwaike, I., Hermann, N., Grover, P., Stellman, J., & O'Leary, K. 1976. Letter: Melanoma after exposure to PCB's. New England Journal of Medicine 295:450.

Barton, D.R., Johnson, R.A., Campbell, L., Petruniak, J., & Patterson, M. 2005. Effects of round gobies (*Neogobius melanostomus*) on dreissenid mussels and other invertebrates in eastern Lake Erie, 2002-2004. Journal of Great Lakes Research 31: 252-261.

Bentzen, E., Mackay, D., Hickie, B.E., & Lean, D.R.S. 1999. Temporal trends of polychlorinated biphenyls (PCBs) in Lake Ontario fish and invertebrates. Environmental Reviews 7: 203-223.

Borga, K., Fisk, A.T., Hoekstra, P.F., & Muir, D.C.G. 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochloride contaminants in arctic marine food webs. Environmental Toxicology and Chemistry 23: 2367-2385.

Borgmann, U. & Whittle, D.M. 1991. Contaminant concentration trends in Lake Ontario lake trout (*Salvelinus namaycush*): 1977 to 1988. Journal of Great Lakes Research 17: 368-381.

Breivik, K., Sweetman, A., Pacyna, J.M., Jones, K.C. 2002. Towards a global historical emission inventory for selected PCB congeners — a mass balance approach. The Science of the Total Environment 290: 181-198.

Brown, J.E. & Stepien, C.A. 2009. Invasion genetics of the Eurasian round goby in North America: tracing sources and spread patters. Molecular Ecology 18: 64-79.

Bunnell, D.B. Madenjian, C.P., Holuszko, J.D., Adams, J.V., & French, J.R.P. 2009. Expansion of *Dreissena* into offshore waters of Lake Michigan and potential impacts on fish populations. Journal of Great Lakes Research 35: 74-80.

Bunnell, D.B., Davis, B.M., Chriscinske, M.A., Keeler, K.M., Mychek-Londer, J.G. 2015. Diet shifts by planktivorous and benthivorous fishes in northern Lake Michigan in response to ecosystem changes. Journal of Great Lakes Research 41: 161-171.

Cairns, T. and Siegmund, E.G. 1981. PCBs: Regulatory history and analytical problems. Analytical Chemistry 53: 1183A-1193A.

Calandra JC. 1975. Report to Monsanto Company: Two-year chronic oral toxicity study with Aroclor 1260 in albino rats. Industrial Bio-Test Laboratories.

Campbell, L.M., Thacker, R., Barton, D., Muir, D.C.G., Greenwood, D., & Hecky, R.E. 2009. Re-engineering the eastern Lake Erie littoral food web: the trophic function of non-indigenous Ponto-Caspian species. Journal of Great Lakes Research 35: 224-231.

Carpenter, D.O. 2006. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. Reviews on Environmental Health 21: 1-23.

Carrick, H., Barbiero, R.P., & Tuchman, M. 2001. Variation in Lake Michigan plankton: temporal, spatial, and historical trends. Journal of Great Lakes Research 27: 467-485.

Chotkowski, M.A. & Marsden, J.E. 1999. Round goby and mottled sculpin predation on lake trout eggs and fry: field predictions from laboratory experiments. Journal of Great Lakes Research 25:26–35.

Christoffersen, M., Svendsen, J.C., Behrens, J.W., Jepsen, N., & van Deurs, M. 2019. Using acoustic telemetry and snorkel surveys to study diel activity and seasonal migration of round goby (*Neogobius melanostomus*) in an estuary of the Western Baltic Sea. Fisheries Management and Ecology 26: 172-182.

Clapp, D.F., Schneeberger, P.J., Jude, D.J., Madison, G., & Pistis, C. 2001. Monitoring round goby (*Neogobius melanostomus*) population expansion in eastern and northern Lake Michigan. Journal of Great Lakes Research 27: 335-341.

Corsolini, S., Kannan, K., Imagawa, T., Focardi, S., & Giesy, J.P. Polychloronapthalenes and other dioxin-like compounds in Arctic and Antarctic marine food webs. 2002. Environmental Science & Technology 36: 3490-3496.

Corsolini, S., Covaci, A., Ademollo, N., Focardi, S., & Schepens, P. 2006. Occurence of organochloride pesticides (OCPs) and their enantiomeric signatures, and concentrations of polybrominated diphenyl ethers (PBDEs) in the Adelie penguin food web, Antartica. Environmental Pollution 140: 371-382.

Corsolini, S., Baroni, D., Martellini, T., Pala, N., & Cincinelli, A. 2019. PBDEs and PCBs in terrestrial ecosystems of the Victoria Land, Antarctica. Chemosphere 231: 233-239.

Danielsson, C., Wiberg, K., Korytar, P., Bergek, S., Brinkman, U.A.T., & Haglund, P. 2005. Trace analysis of polychlorinated dibenzo-*p*-dioxins, dibenzofurans and WHO polychlorinated biphenyls in food using comprehensive two-dimensional gas chromatography with electron-capture detector. Journal of Chromatography 1086: 61-70.

Das, A. & Sligar, S.G. 2009. Modulation of the cytochrome p450 reductase redox potential by the phospholipid bilayer. Biochemistry 48: 12104-12112.

Dasgupta, S., Peng, X., Chen, S., Li, J., Du, M., Zhou, Y.H., Zhong, G., Xu, H., Ta, K. 2018. Toxic anthropogenic pollutants reach the deepest ocean on Earth. Geochemical Perspectives Letters 7: 22-26.

De Vault, D.S., Rodgers, P.W., & Feist, T.J. 1996. Contaminant trends in lake trout and walleye from the Laurentian Great Lakes. Journal of Great Lakes Research 22: 884-895.

Dellinger, M.J., Olson, J., Holub, B., Ripley, M.P. 2018. Fatty acids in ten species of fish commonly consumed by the Anishinaabe of the Upper Great Lakes. Journal of Great Lakes Research 44: 521-526.

Diphenyl and Chlorinated Diphenyl Derivatives. 1935. Swann Chemical Company.

Drinker, C.K., Warren, M.F., & Bennett, G.A. 1937. The problem of possible systemic effects from certain chlorinated hydrocarbons. The Journal of Industrial Hygiene and Toxicology 19: 283-311.

Driscoll, Z.G., Bootsma, H.A., & Christiansen, E. 2015. Zooplankton trophic structure in Lake Michigan as revealed by stable carbon and nitrogen isotopes. Journal of Great Lakes Research 41: 104-114.

Engel L.S., Laden F., Andersen A., Strickland, P.T., Blair, A., Needham, L.L., Barr, D.B., Wolff, M.S., Helzlsouer, K., Hunter, D.J., Lan, Q., Cantor, K.P., Comstock, G.W., Brock, J.W., Bush, D., Hoover, R.N., & Rothman, N. 2007. Polychlorinated biphenyl levels in peripheral blood and non-Hodgkin's lymphoma: a report from three cohorts. Cancer Research 67:5545-52.

EPA. 1979. EPA bans PCB manufacture; phases out uses.

EPA. 1996A. Method 3540C: Soxhlet extraction.

EPA. 1996B. Method 3600C: cleanup.

EPA. 2007. Method 3546: microwave extraction.

Erickson, M.D. 1997. *Analytical Chemistry of PCBs, Second Edition*.

Eskilsson, C.S. and Bjorklund, E. 2000. Analytical-scale microwave-assisted extraction. Journal of Chromatography A 902: 227-250.

Estepp, L.R. & Reavie, E.D. 2015. The ecological history of Lake Ontario according to phytoplankton. Journal of Great Lakes Research 41: 669-687.

Evans, M.S., Noguchi, G.E., & Rice, C.P. 1991. The biomagnification of polychlorinated biphenyls, toxaphene, and DDR compounds in a Lake Michigan offshore food web. Archives of Environmental Contamination and Toxicology 20: 87-93.

Fadaei, H., Williams, E., Place, A.R., Connolly, J.P., & Ghosh, U. 2017. Assimilation efficiency of sediment-bound PCBs ingested by fish impacted by strong sorption. Environmental Toxicology and Chemistry 36: 3480-3488.

Fisk, A.T., Hobson, K.A., & Norstrom, R.J. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the North Water polynya marine food web. Environmental Science & Technology 35: 732-738.

Fliedner, A., Ruedel, H., Lohmann, N., Buchmeier, G., & Koschorreck, J. 2018. Biota monitoring under the Water Framework Directive: on tissue choice and fish species selection. Environmental Pollution 235: 129-140.

France, R.L. 1995. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series 124: 307-312.

Foley, C.J., Andree, S.R., Pothoven, S.A., Nalepa, T.F., & Hook, T.O. 2017. Quantifying the predatory effect of round goby on Saginaw Bay dreissenids. Journal of Great Lakes Research 43: 121-131.

Francis, E., 1998. Conspiracy of silence — how three corporate giants covered their toxic trail. Sierra.

Frederiksen, M., Meyer, H.W., Ebbehøj, N.E., & Gunnarsen, L. 2012. Polychlorinated biphenyls (PCBs) in indoor air originating from sealants in contaminated and

uncontaminated apartments within the same housing estate. Chemosphere 89: 473-479.

Gewurtz, S.B., Bhavsar, S.P., & Fletcher, R. 2011. Influence of fish size and sex on mercury/PCB concentration: importance for fish consumption advisories. Environmental International 37: 425-434.

Ghedotti, M.J., Smihula, J.C., &Smith, G.R. 1995. Zebra mussel predation by round gobies in the laboratory. Journal of Great Lakes Research 21: 665-669.

Gopalakrishnan, K.K. & Kashian, D.R. 2019. Identification of optimal calcium and temperature conditions for quagga mussel filtration rates as a predictor of invasion. Environmental Toxicology *in press*.

GLRI (Great Lakes Restoration Initiative) Task Force. 2010. GLRI Action Plan FY2010-FY2014.

Graham, R. 1970. Form letter to customers. Monsanto Chemical Company.

Gustafson, C. 1970. PCB's — prevalent and persistent. Environmental Science and Technology 4: 814-819.

Guzzella, L. Salerno, F., Freppaz, M., Roscioli, C., Pisanello, F., & Poma, G. 2016. POP and PAH contamination in the southern slopes of Mt. Everest (Himalaya, Nepal): long-range atmospheric transport, glacier shrinkage, or local impact of tourism? Science of the Total Environment 544: 382-390.

Halpert, J.R. 2011. Structure and function of cytochrome p450 2B: from mechanism-based inactivators to x-ray crystal structures and back. Drug Metabolism and Disposition 39: 1113-1121.

Harju, M., Danielsson, C., & Haglund, P. 2003. Comprehensive two-dimensional gas chromatography of the 209 polychlorinated biphenyls. Journal of Chromatography A 1019: 111-126.

Hawker, D.W. and Connell, D.W. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. Environmental Science & Technology 22: 382-387.

Hecky, R.E. & Hesslein, R.H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Bethological Society 14: 631-653.

Hecky, R.E. Smith, R.E.H., Barton, D.R., Guildford, S.J., Taylor, W.D., Charlton, M.N., Howell, T. 2004. The nearshore phosphorus shunt: a consequence of ecosystem engineering by dreissenids in the Laurentian Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 61: 1285-1293.

Helm, P.A., Gewurtz, S.B., Whittle, D.M., Marvin, C.H., Fisk, A.T., & Tomy, G.T. 2008. Occurrence and biomagnification of polychlorinated naphthalenes and non- and mono-*ortho* PCBs in Lake Ontario sediment and biota. Environmental Science & Technology 42: 1024-1031.

Hebert, C.R., & Keenleyside, K.A. 1995. To normalize or not to normalize? Fat is the question. Environmental Toxicology and Chemistry 14: 801-807.

Hurm, T.S. & Puhakka, J.A. 1999. Characterization and fate of polychlorinated biphenyl contaminants in Kernaalanjarvi sediments. Boreal Environment Research 4: 331-342.

IARC. 2013. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans. Volume 107: Polychlorinated biphenyls and polybrominated biphenyls Lyon: International Agency for Research on Cancer.

IRIS: Integrated Risk Information System. 2012. Polychlorinated Biphenyls (PCBs). U.S. Environmental Protection Agency.

Jacobs, G.R., Madenjian, C.P., Bunnell, D.B., & Holuszko, J.D. 2010. Diet of lake trout and burbot in northern Lake Michigan during spring: evidence of ecological interaction. Journal of Great Lakes Research 36: 312-317.

Jahns, L., Raatz, S.K., Johnson, L.K., Kranz, S., Silverstein, J.T., & Picklo, M.J. 2014. Intake of seafood in the US varies by age, income, and education level but not by race-ethnicity. Nutrients 6: 6060-6075.

James, M.O. 2001. PCB: metabolism and metabolites. In: Robertson, L.W., Hansen, L.G., ed. Recent advances in the Environmental Toxicology and Health Effects of PCB. Lexington, KY: University of Kentucky Press: 35-46.

Janssen, J. & Jude, D.J. 2001. Recruitment failure of mottled sculpin *Cottus bairdi* in Calumet Harbor, southern Lake Michigan. Journal of Great Lakes Research 27: 319-328.

Jensen, S. 1966. Report on a new chemical hazard. New Scientist 32: 612.

Johnson, T.B., Bunnell, D.B., & Knight, C.T. 2005. A potential new energy pathway in central Lake Erie: the round goby connection. Journal of Great Lakes Research 31: 238-251.

Kidd, K.A. Schindler, D.W., Hesslein, R.H., & Muir, D.C.G. 1998. Effects of trophic position and lipid on organochloride concentrations in fishes from subarctic lakes in Yukon Territory. Canadian Journal of Fisheries and Aquatic Sciences 55: 869-881.

Kidd, K.A., Bootsma, H.A., Hesslein, R.H., Muir, D.C.G., & Hecky, R.E. 2001. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. Environmental Science & Technology 35: 14-20.

Kobayashi, J., Imuta, Y., Komorita, T., Yamada, K., Ishibashi, H., Ishihara, F., Nakashima, N., Sakai, J., Arizono, K., & Koga, M. 2015. Trophic magnification of polychlorinated biphenyls and polybrominated diphenyl ethers in an estuarine food web of the Ariake Sea, Japan. Chemosphere 118: 201-206.

Kovacevic, R., Vojinovic-Miloradov, M., Teodorovic, I., & Andric, S. Effect of PCBs on androgen production by suspension of adult rat Leydig cells in vitro. 1995. Journal of Steroid Biochemistry & Molecular Biology 52: 595-597.

Kucklick, J.R., Harvey, H.R., Ostrom, P.H., Ostrom, N.E., & Baker, J.E. 1996. Organochloride dynamics in the pelagic food web of Lake Baikal. Environmental Toxicology and Chemistry 15: 1388-1400.

Kuratsune, M., Nakamura, Y., & Ikeda, M., 1987. Analysis of deaths seen among patients with Yusho – a preliminary report. Chemosphere 16: 2085-2088.

Larsson, P. Okla, L., Collvin, L. 1993. Reproductive status and lipid content as factors in PCB, DDT and HGH contamination of a population of pike (*Esox Lucius* L.). Environmental Toxicology and Chemistry 12: 855-861.

Lehrer-Brey, G. & Kornis, M.S. 2014. Winter distributional overlap facilitates lake whitefish (*Coregonus clupeaformis*) piscivory on invasive round gobies (*Neogobius melanostomus*) in Green Bay, Lake Michigan. Journal of Freshwater Ecology 29: 153-156.

Lepak, R.F., Krabbenhoft, D.P., Ogorek, J.M., Tate, M.T., Bootsma, H.A., & Hurley, J.P. 2015. Influence of *Cladophora*-quagga mussel assemblages on nearshore methylmercury production in Lake Michigan. Environmental Science & Technology 49: 7606-7613.

Liu, J., Haffner, G.D., & Drouillard, K.G. 2010. The influence of diet on the assimilation efficiency of 47 polychlorinated biphenyl congeners in Japanese koi (*Cyprinus carpio*). Environmental Toxicology and Chemistry 29: 401-409.

Lohmann, R., Breivik, K., Dachs, J., Muir, D. 2007. Global fate of POPs: current and future research directions. Environmental Pollution 150: 150-165.

Ma, R. and Sassoon, D.A. 2006. PCBs exert an estrogenic effect through repression of the *Wnt7a* signaling pathway in the female reproductive tract. Environmental Health Perspectives 114: 898-904.

Madenjian, C.P., DeSorcie, T.J., & Stedman, R.M. 1998. Ontogenic and spatial patterns in diet and growth of lake trout in Lake Michigan. Transactions of the American Fisheries Society 127: 236-252.

Madenjian, C.P., Pothoven, S.A., Dettmers, J.M., & Holuszko, J.D. 2006. Changes in seasonal energy dynamics of alewife (*Alosa pseudoharengus*) in Lake Michigan after invasion of dreissenid mussels. Canadian Journal of Fisheries and Aquatic Sciences 63: 891-902.

Madenjian, C.P., Pothoven, S.A., Schneeberger, P.J., Ebener, M.P., Mohr, L.C., Nalepa, T.F., & Bence, J.R. 2010a. Dreissenid mussels are not a "dead end" in Great Lakes food webs. Journal of Great Lakes Research 36: 73-77.

Madenjian, C.P., Schrank, C.S., Begnoche, L.J., Elliott, R.F., & Quintal, R.T. 2010b. Sexual difference in PCB concentrations of coho salmon (*Oncorhynchus kisutch*). Science of the Total Environment 408: 1719-1724.

Madenjian, C.P., Bunnell, D.B., Descorcie, T.J., Kostich, M.J., Armenio, P.M., & Adams, J.V. 2014. Status and trends of prey fish populations in Lake Michigan, 2013. Compiled reports to the Great Lakes Fisheries Commission of the annual bottom trawl and acoustics surveys, 2013. Great Lakes Fisheries Commission, Ann Arbor.

Madenjian, C.P., Bunnell, D.B., Warner, D.M., Pothoven, S.A., Fahnenstein, G.L., Nalepa, T.F., Vanderploeg, H.A., Tsehaye, I., Claramunt, R.M., & Clark, R.D. 2015. Changes in the Lake Michigan food web following dreissenid mussel invasions: a synthesis. Journal of Great Lakes Research 41: 217-231.

Matthews, B. & Mazumder, A. 2005. Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton $\delta^{13}C$. Freshwater Biology 40: 502-515.

McKinney, J.D. and Waller, C.L. 1994. Polychlorinated biphenyls as hormonally active structural analogues. Environmental Health Perspectives 102: 290-297.

Mead WB. EPA Vows to Curb Discharge of PCB. *Washington Post*. May 14, 1972, p.A-21

Mills, E.L., Leach, J.H., Carlton, J.T., & Secor, C.L. 1993. Exotic Species in the Great Lakes: a history of biotic crises and anthropogenic introductions. Journal of Great Lakes Research 19: 1-54.

Minagawa, M. & Wada, E. 1984. Stepwise enrichment of ^{15}N and animal age: further evidence and the relation between $\delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48: 1135-1140.

Monsanto Chemical Company. 1935. Letter to the Stockholders of Monsanto Chemical Company.

Monsanto Chemical Company. 1944. Salesmen's manual, Aroclor.

Monsanto Chemical Company 1956. Pydraul 150.

Monsanto Chemical Company. 1972. Contract with General Electric: Special undertaking by purchasers of polychlorinated biphenyls.

Monsanto Chemical Company. 1975. Report by PCB study group — confidential.

Monsanto Chemical Company. 1976. Open letter to consumers of polychlorinated biphenyls.

Muir, D.C.G., Wagemann, R., Hargrave, B.T., Thomas, D.J., Peakall, D.B., & Norstrom, R.J. 1992. Arctic marine ecosystem contamination. Science of the Total Environment 122: 75-134.

Mychek-Londer, J.G. & Bunnell, D.B. 2013. Gastric evacuation rate, index of fullness, and daily ration of Lake Michigan slimy (*cottus cognatus*) and deepwater sculpin (*Myoxocephalus thompsonii*). Journal of Great Lakes Research 39: 327-335.

Naddafi, R., & Rudstam, L.G. 2014. Does differential predation explain the replacement of zebra by quagga mussels? Freshwater Science 33: 895-903.

Nalepa, T.F., Hartson, D.J., Fanslow, D.L., Lang, G.A., & Lozano, S.J. 1998. Declines in macroinvertebrate populations in southern Lake Michigan, 1980-1993. 1998. Canadian Journal of Fisheries and Aquatic Sciences 55: 2402-2413.

Nalepa, T., Fanslow, D., Pothoven, S. 2010. Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan. Journal of Great Lakes Research 36: 5-19.

Negri, A., Burns, K., Boyle, S., Brinkman, D., Webster, N. 2006. Contamination in sediments, bivalves and sponges of McMurdo Sound, Antarctica. Environmental Pollution 143: 456-467.

Nichols, J.W. Fitzsimmons, P.N., Whiteman, F.W., Kuehl, D.W. Butterworth, B.C., & Jenson, C.T. 2001. Dietary uptake kinetics of 2,2',5,5'-tetrachlorobiphenyl in rainbow trout. Drug Metabolism & Disposition 29: 1013-1022.

Nichols, S.J., Kennedy, G., Crawford, E., Allen, J., French, J., Black, G., Blouin, M., Hickey, J., Chernyak, S., Haas, R., & Thomas, M. 2003. Assessment of lake sturgeon (*Acipenser fulvenscens*) spawning efforts in the lower St. Clair River, Michigan. Journal of Great Lakes Research 29: 383-391.

Nogaro, G. & Steinman, A.D. 2014. Influence of ecosystem engineers on ecosystem processes is mediated by lake sediment properties. Oikos 123: 500-512.

Pandini, A., Denison, M.S., Song, Y., Soshilov, A.A., & Bonati, L. 2007. Structural and functional characterization of the aryl hydrocarbon receptor ligand binding domain by homology modeling and mutational analysis. Biochemistry 46: 696-708.

Olsson, A., Valters, K., & Burreau, S. 2000. Concentrations of organochloride substances in relation to fish size and trophic position: a study on perch (*Perca fluviatilis* L.) Environmental Science & Technology 34: 4878-4886.

Papageorge, W.B. 1970. Letter to senior buyer of transformers. Monsanto Chemical Company.

Perello, M.M., Simon, T.P., Thompson, H.A., & Kane, D.D. 2015. Feeding ecology of the invasive round goby, *Neogobius melanostomus*, (Pallas, 1814), based on laboratory size preference and field diet in different habitats in the western basin of Lake Erie. Aquatic Invasions 10: 463-474.

Peterson, B.J. & Fry, B. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology, Evolution, and Systematics 18: 293-320.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703-718.

Pothoven, S.A. & Madenjian, C.P. 2008. Changes in consumption by alewives and lake whitefish after dreissenid mussel invasions in Lakes Michigan and Huron. North American Journal of Fisheries Management 28: 308-320.

Pothoven, S.A. & Madenjian, C.P. 2008. Changes in consumption by alewife and lake whitefish following dreissenid mussel invasions in Lake Michigan and Huron. North American Journal of Fisheries Management 28: 308-320.

Pothoven, S.A., Hondrop, D.W., & Nalepa, T.F. 2011. Declines in deepwater sculpin *Myoxocephalus thompsonii* energy density associated with the disappearance of *Diporeia* spp. in lakes Huron and Michigan. Ecology of Freshwater Fish 20: 14-22.

Pothoven, S.A. & Madenjian, C.P. 2013. Increased piscivory by lake whitefish in Lake Huron. North American Journal of Fisheries Management 33: 1194-1202.

Pothoven, S.A. & Vanderploeg, H.A. 2017. Changes in *Mysis diluviana* abundance and life history patterns following a shift toward oligotrophy in Lake Michigan. Fundamental and Applied Limnology 190: 199-212.

Portigal, C.L. Cowello, S.P., Feboruk, M.N., Butler, C.M., Rennie, P.S., & Nelson, C.C. 2002. Polychlorinated biphenyls interfere with androgen-induced transcriptional activation and hormone binding. Toxicological Applied Pharmacology 179: 185-194.

Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., & Montana, C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152: 179-189.

Puga, A., Barnes, S.J., Dalton, T.P., Chang, C.Y., Knudsen, E.S., & Maier, M.A. Aromatic hydrocarbon receptor interaction with the retinoblastoma protein potentiates repression of E2F-dependent transcription and cell cycle arrest. The Journal of Biological Chemistry 275: 2943–2950

Rahman, M.M., Abd El-Aty, A.M., Choi, J.H., Shin, H.C., Shin, S.C., & Shim, J.H. 2015. Basic overview on gas chromatography columns in *Analytical Separation Science*. Ed. Anderson, J.L. & Berthod, A. Wiley Publishers.

O'Reilly, C.M., Hecky, R.E., Cohen, A.S., & Plisnier, P.D. 2002. Interpreting stable isotopes in food webs: recognizing the role of time averaging at different trophic levels. Limnology and Oceanography 47: 306-309.

Risebrough, R.W., Rieche, P., Herman, S.G, Peakall, D.B., & Kirven, M.N. 1968. Polychlorinated biphenyls in the global ecosystem. Nature 220: 1098-1102.

Ruddy, B.A. 2006. Studies in gas chromatography-tandem mass spectrometry and absorbance spectroscopy for the determination of polychlorinated biphenyls in freshwater biota. University of Wisconsin-Milwaukee. Doctoral dissertation.

Ruddy, B.A. & Aldstadt, J.H. 2007. Challenges in the application of conventional PCB quantitation methods to studies of 'invasive' freshwater organisms. Journal of Environmental Monitoring 9: 260-265.

Ruddy, B.A., Qadah, D.T., Aldstadt, J.H., & Bootsma, H.A. 2008. Improving analytical confidence in the determination of PCBs in complex matrices by a sequential GC-MS/MS approach. International Journal of Environmental Analytical Chemistry 88: 337-351.

Ruder A.M., Hein M.J., Nilsen N., Waters, M.A., Laber, P., Davis-King, K., Prince, M.M., Whelan, E. 2006. Mortality among workers exposed to polychlorinated biphenyls (PCBs) in an electrical capacitor manufacturing plant in Indiana: an update. Environmental Health Perspectives 114:18-23

Savitz, J. 2009. Diets of Lake Michigan salmon and maximum size of alewife prey. Journal of Freshwater Ecology 24: 563-566.

Schulte, K.W., Green, E., Wilz, A., Platten, M., & Daumke, O. 2017. Structural basis for aryl hydrocarbon receptor-mediated gene activation. Structure 25: 1025-1033.

Sinks T., Steele G., Smith A.B., Watkins, K., & Shults, R.A 1992. Mortality among workers exposed to polychlorinated biphenyls. American Journal of Epidemiology 136:389-398.

Sommer, U., Glizicz, Z.M., Lampert, W., & Duncan, A. 1986. The PEG*-model of seasonal succession of planktonic events in fresh waters. Archive for Hydrobiologie 106: 433-471.

Stapleton, H.H., Letcher, R.J., & Baker, J.E. 2001. Metabolism of PCBs by the deepwater sculpin (*Myoxocephalus thompsoni*). Environmental Science & Technology 35: 4747-4752.

Stejskalova, L., Dvorak, Z., & Pavek, P. 2011. Endogenous and exogenous ligands of aryl hydrocarbon receptor: current state of art. Current Drug Metabolism 12: 198-212.

Stockholm Convention. 2016. Polychlorinated biphenyls (PCB) inventory guidance. PCB elimination network (PEN).

Strus, R.H. & Hurley, D.A. 1992. Interactions between alewife (*Alosa Pseudoharengus* [sic]), their food, and phytoplankton biomass in the Bay of Quinte, Lake Ontario. Journal of Great Lakes Research 18: 709-723.

Sturtevant, R.A., Mason, D.M., Rutherford, E.S., Elgin, A., Lower, E., & Martinez, F. 2019. Recent history of nonindigenous species in the Laurentian Great Lakes; an update to Mills et al. 1993 (25 years later). Journal of Great Lakes Research 45: 1011-1035.

Swann Use Codes. 1935. Aroclor use code.

Szostek, B., Tinklenberg, J.A., & Aldstadt, J.H. 1999. A simple method for the quantitative microextraction of polychlorinated biphenyls from soils and sediments. Chemosphere 38: 3131-3139.

Tharappel, J.C., Lee, E.Y., Robertson, L.W., Spear, B.T., & Glauert, H.P. 2012. Regulation of cell proliferation, apoptosis, and transcription factor activities during the promotion of liver carcinogenesis by polychlorinated biphenyls. Toxicology and Applied Pharmacology 179: 172-184.

Totland, C., Nerdal, W., Steinkopf, S. 2016. Effects and location of coplanar and noncoplanar PCB in a lipid bilayer: a solid-state NMR study. Environmental Science & Technology 50: 8290-8295.

Treon, J.F., Cleveland, F.F., Shaffer, F.E., Cappel, J., Beller, R.A., & Torbeck, J.P. 1955. The toxicity of the vapor of Aroclors 1242 and 1254. The Kettering Laboratory.

Tsehaye, I., Jones, M.L., Bence, J.R., Brenden, T.O., Madenjian, C.P., & Warner, D.M. 2014. A multispecies age-structured model to assess predator-prey balance: application to an intensively managed Lake Michigan pelagic fish community. Canadian Journal of Fisheries and Aquatic Sciences 71: 627-644.

Turschak, B.A., Bunnell, D., Czesny, S., Hook, T.O., Janssen, J., Warner, D., & Bootsma, H.A. 2014. Nearshore energy subsidies support Lake Michigan fishes and invertebrates following major changes in food web structure. Ecology 95: 1243-1252.

Turschak, B.A. and Bootsma, H.A. 2015. Lake Michigan trophic structure as revealed by stable C and N isotopes. Journal of Great Lakes Research 41: 185-196.

Tykot, R.H. 2004. Stable isotopes and diet: You are what you eat. Proceedings of the International School of Physics. IOS Press, Amsterdam 433-444.

US Department of Health and Human Services. 2000. Toxicological profile for polychlorinated biphenyls (PCBs).

Van den Berg, M. Birnbaum, L., Bosveld, A.T.C. Brunstrom, B. Cook, P. Feeley, M. Gisey, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T. 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environmental Health Perspectives 106: 775-792.

Vander Zanden, M.J. & Rasmussen, J.B. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. *Ecology* 80: 1395-1404.

Vander Zanden, J. & Rasmussen, J. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: implications for aquatic food web studies. American Society of Limnology and Oceanography 46: 2061-2066.

Vanderploeg, H.A., Nalepa, T.F., Jude, D.J., Mills, E.L., Holeck, K.T., Liebig, J.R., Grigorovich, I.A., & Ojaveer, H. 2002. Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences 59: 1209-1228.

Vanderploeg, H.A., Liebig, J.R., Nalepa, T.F., Fahnenstiel, G.L., & Pthoven, S.A. 2010. *Dreissena* and the disappearance of the spring phytoplankton bloom in Lake Michigan. Journal of Great Lakes Research 36: 50-59.

Vos, J.G. & Koeman, J.H. 1970. Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. Toxicology and Applied Pharmacology 17: 656-668.

Walters, D.M., Fritz, K.M., Johnson, B.R., Lazorchak, J.M., & McCormick, F.H. 2008. Influence of trophic position and spatial location on polychlorinated biphenyl (PCB) bioaccumulation in a stream food web. Environmental Science & Technology 42: 2316-2322.

Wu, J.P., Luo, X.J., Zhang, Y., Yu, M., Chen, S.J., Mai, B.X., Yang, Z.Y. 2009. Biomagnification of polychlorinated diphenyl ethers (PBDEs) and polychlorinated biphenyls in a highly contaminated freshwater food web from south China. Environmental Pollution 157: 904-909.

Yi, T., Wang, J., Zhu, K., Tang, Y., Huang, S., Shui, X., Ding, Y., Chen, C., & Lei, W. 2018. Aryl hydrocarbon receptor: a new player of pathogenesis and therapy in cardiovascular diseases. BioMed Research International 6058784: 1-11.

Young, J. A. M., Marentette, J. R., Gross, C., McDonald, J. I., Verma, A., Marsh-Rollo, S. E., Macdonald, P. D. M., Earn, D. J. D. & Balshine, S. (2010). Demography and substrate affinity of the round goby (Neogobius melanostomus) in Hamilton Harbour. Journal of Great Lakes Research 26: 115–122

Zhou, S., Zhu, H., Huange, S., Zhou, J., Zhang, S., & Wang, C. 2019. Biomagnification and risk assessment of polychlorinated biphenyls in food web components from Zhoushan fishing ground, China. Marine Pollution Bulletin 142: 613-619.

Zhu, C., Chen, Y.L., Wang, X.J., Hu, X.S., Yu, Z.B., & Han, S.P. ShRNA-mediated gene silencing of AHR promotes the differentiation of P19 mouse embryonic carcinoma cells into cardiomyocytes. Molecular Medicine Reports 6: 513–518.

Zuwerink, D.A. Jude, D.J., & Gannon, J.E. 2019. Behavioral interactions between the nonindigenous round goby and the young-of-the-year yellow perch. Biological Invasions 21: 3633-3639

APPENDIX A: Stable Isotope Means

Taxon	Year	n*	Mean $\delta^{15}N$	$\delta^{15}N$ s.e.	Mean Trophic Level	Trophic Level s.e.	Mean lipid-corrected $\delta^{13}C$	Lipid-corrected $\delta^{13}C$ s.e
Alewife	2002-2003	36	9.48	0.14	3.11	0.04	-23.08	0.20
	2010-2012	153	8.42	0.12	2.86	0.03	-23.24	0.08
	2019	15	9.13	0.18	2.97	0.05	-24.61	0.09
Lake Trout	2002-2003	36	14.01	0.15	4.44	0.04	-23.05	0.34
	2010-2012	21	13.33	0.16	4.31	0.05	-23.16	0.16
	2019	11	12.58	0.13	4.04	0.05	-23.88	0.19
Lake Whitefish	2002-2003	4	11.40	1.47	3.77	0.17	-18.97	0.60
	2010-2012							
	2019	5	11.71	0.15	4.17	0.06	-19.01	0.40
Chinook Salmon	2002-2003							
	2010-2012	4	11.79	0.20	3.84	0.07	-23.39	0.17
	2019	6	11.37	0.12	3.70	0.05	-23.73	0.22
Round Goby	2002-2003	15	10.52	0.22	3.45	0.06	-21.38	0.15
	2010-2012	504	8.75	0.03	3.36	0.01	-19.38	0.08
	2019	8	9.51	0.22	3.40	0.08	-20.59	0.37
Slimy Sculpin	2002-2003	9	12.31	0.23	3.90	0.07	-24.95	0.13
	2010-2012	10	12.94	0.16	4.15	0.05	-23.59	0.13
	2019	13	12.09	0.17	3.98	0.06	-22.85	0.23
Deepwater Sculpin	2002-2003	18	11.74	0.09	3.70	0.03	-26.04	0.23
	2010-2012	15	13.16	0.12	4.16	0.04	-24.16	0.06
	2019	8	11.40	0.38	3.67	0.12	-24.23	0.34

Yellow Perch	**2002-2003**	20	10.56	0.58	3.54	0.18	-23.28	0.52
	2010-2012	44	9.81	0.25	3.63	0.08	-19.77	0.19
	2019							
Bloater Chub	**2002-2003**	22	11.06	0.13	3.51	0.04	-26.03	0.17
	2010-2012	23	9.51	0.23	3.28	0.07	-22.28	0.35
	2019							
Mysis	**2002-2003**	5	8.25	0.42	2.66	0.12	-26.68	0.17
	2010-2012	6	9.26	0.44	2.99	0.14	-24.42	0.26
	2019	4	8.34	0.06	2.63	0.01	-26.02	0.12
Nearshore Dreissenids	**2002-2003**	10	4.10	0.12	1.67	0.04	-22.37	0.51
	2010-2012	219	4.41	0.05	1.70	0.02	-23.05	0.11
	2019	6	4.08	0.20	1.50	0.06	-24.47	0.14
Offshore Dreissenids	**2002-2003**	5	6.49	0.29	2.14	0.09	-27.13	0.38
	2010-2012	3	6.53	0.41	1.89	0.08	-27.20	0.41
	2019	4	6.53	0.36	1.99	0.10	-27.37	0.19

*Note: All 2019 samples were all homogenates. Thus, a single "sample" in 2019 was actually a pooled composite of at least 3 organisms. Some, but not all, of the 2002-2003 and 2010-2012 were similarly pooled.

APPENDIX B: 2002-2003 PCB Means

Taxon	n	Σ WHO congeners (ppb)		Σ Lipid-corrected WHO congeners (ppb)		Lipid-corrected congener 105 (ppb)		Lipid-corrected congener 118 (ppb)		Lipid-corrected congener 156 (ppb)	
		Mean	s.e	Mean	s.e.	Mean	s.e	Mean	s.e.	Mean	s.e.
Lake Trout	25	704.4	91.1	362.4	36.5	59.5	9.5	163.0	15.1	6.8	1.3
Deepwater Sculpin	18	429.5	82.0	392.6	78.6	22.5	4.9	188.6	41.7	11.9	2.7
Bloater Chub	15	420.5	76.5	374.6	112.9	23.5	5.0	162.8	47.3	10.8	7.6
Slimy Sculpin	9	331.7	38.2	415.3	68.3	39.4	11.4	166.1	28.3	13.0	6.0
Lake Whitefish	13	329.6	82.9	400.0	92.0	43.2	10.3	185.1	38.0	4.3	1.9
Yellow Perch	14	177.4	27.1	288.9	58.5	28.7	7.1	110.3	29.4	8.7	3.3
Alewife	31	156.7	14.2	159.0	21.1	15.1	3.5	71.7	9.4	1.8	0.4
Zooplankton	6	122.6	75.9	132.2	102.1	2.1	0.8	54.2	35.6	11.1	10.6
Mysis	2	61.0	43.2	47.4	19.8	4.6	3.5	26.9	6.5	0.6	0.3