TROPHIC TRANSFER AND BIOTRANSFORMATION OF POLYCHLORINATED BIPHENYLS IN ZEBRA MUSSEL, ROUND GOBY, AND SMALLMOUTH BASS IN LAKE ERIE, USA

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Abstract—Trophic transfer of polychlorinated biphenyl (PCB) congeners in zebra mussels (Dreissena polymorpha), round gobies (Neogobius melanostomus), and smallmouth bass (Micropterus dolomieu) were assessed in four sites along the south shore of the west and central basin of Lake Erie (all sites were in OH, USA). Total PCB levels in smallmouth bass (1.091–1.520 ng/g wet weight) and round gobies (118–256 ng/g wet weight) were similar among sites despite a west-to-east decrease in total PCB concentrations in zebra mussels (29–97 ng/g wet weight). At all sites, PCB body burden increased three- to fivefold at each successive trophic level, suggesting biomagnification in this nonnative food chain. Whereas fish species were dominated by the hexachlorine homologue, zebra mussels were dominated by penta- and hexachlorine homologues; the average degree of chlorination of PCBs was 56.1% for zebra mussels, 60.4% for round goby, and 59.9% for smallmouth bass bodies. Predictive structure-activity relationship based on chemical characteristics, such as the octanol-water partition coefficient (log Kow), had little predictive power on bioaccumulation and biotransformation of PCB congeners because of nonlinearity, threshold relationships, and species-specific differences. Calculated trophic transfer for the smallmouth bass-round goby linkage was higher than for the round goby–zebra mussel linkage. Only when PCB congeners were grouped by chemical structure first (vicinal [adjacent] H-atom position in the phenyl ring) were linear relationships achieved. It appeared that the chemical group to which each congener belonged influenced biotransformation more than species-specific (round gobies vs smallmouth bass) differences. Biotic changes at midtrophic levels, such as exotic species invasions, may have an increasingly important role in determining pollutant cycling and hence pollutant residues in top predators.

Keywords—Trophic transfer Exotic species Polychlorinated biphenyl Biomagnification Structure-activity relationship

INTRODUCTION

In aquatic ecosystems, strong evidence exists for trophic transfer of polychlorinated biphenyls (PCBs) [1,2]. Many factors influence the magnitude of trophic transfer of PCBs. These include PCB concentrations in water, sediment, and food [3]; feeding behavior and food composition of predators and prey [3,4]; food chain length [5]; species-specific, trophic-transfer efficiency [6,7]; uptake kinetics [8]; and metabolism of PCBs by organisms [9,10]. Often these food web properties have greater influence on PCB residues in upper trophic levels than source inputs into aquatic systems [5].

Invasions by exotic species can affect contaminant cycling in aquatic food webs if they can alter the flow of energy and material in food webs. As a result, contaminants that were previously sequestered in sediments could return to higher trophic levels, which include sport fish, fish-eating birds, and humans. Thus, if exotic species can link sediment sources to higher trophic levels, their introduction can affect cycling of PCBs. Many examples of organisms at midtrophic levels influencing PCB concentrations in higher trophic levels exist. These include opossum shrimp (Mysis relicta) and Diporeia spp. influencing PCB concentrations in salmonids [11]; alewife (Alosa pseudoharengus) causing fluctuations in herring gull (Larus argentatus) eggs [12]; and rainbow smelt (Osmerus mordax) increasing PCB concentrations in lake trout (Salvelinus namaycush) in lakes where they coexist [13]. If these exotic species that sequester contaminants are incorporated into predator diets, predators can be exposed and will assimilate their contaminant burdens. Thus, introductions of exotic species can change the ultimate fate of contaminants if they link benthic and pelagic food webs.

The recent invasion of zebra mussels (Dreissena polymorpha) could result in an efficient contaminant-transfer pathway from settling particulate matter to sport fishes. The recent invasion of these Ponto-Caspian organisms [14,15] has disrupted the native ecosystem, altering food web structure and competition among native species [16–18]. Zebra mussels are efficient filter feeders. The common sink for persistent contaminants in the aquatic environment is in sediments. However, because of the prodigious filtering ability of zebra mussels and their relatively high lipid content, contaminants sorbed to settling particulate matter, previously destined for sediments, can be redistributed into zebra mussels, increasing contaminant bioavailability and the likelihood of trophic transfer [10,19–22]. Of native fauna, diving ducks consume limited amounts of zebra mussels during migration [23], potentially transferring contaminants [24]; however, few native fish consume zebra mussels because of the unpalatable shell [25].

Since their first detection in Lake St. Clair (USA) in 1990,
round gobies (*Neogobius melanostomus*) have spread rapidly throughout the Great Lakes [17], potentially altering food web structure. Round gobies have coevolved to feed aggressively on zebra mussels [17,18]. Given that zebra mussels often occur in high densities (>5 × 10^3 individuals/m^2), zebra mussels can be an abundant food resource for round gobies. If round gobies are, in turn, preyed on by piscivorous fishes, including important commercial and recreational species such as walleye (*Stizostedion vitreum*), yellow perch (*Perca flavescens*), and smallmouth bass (*Micropterus dolomieu*), this completes an efficient pathway for trophic transfer of contaminants [26,27].

In fact, round gobies are becoming increasingly important in the diets of sport fish, especially smallmouth bass, where both smallmouth bass, where both field and experimental data suggest that round gobies are the preferred prey items (G. W. Kim, Ohio State University, Columbus, OH, USA). The purpose of this study was to quantify the influence of this exotic food chain on PCB cycling. First, zebra mussels were used as a sentinel species to assess the bioavailability of PCBs acquired from local PCB contamination. Second, biomagnification of PCBs in this exotic food chain (zebra mussel–round goby–smallmouth bass food chain) was quantified to assess risk to human consumers of sport fish. Because transfer and retention, in addition to biological activity, differ among PCB congeners, congener-specific analyses are needed to truly understand PCB cycling and risk from exposure. Third, using the distributions of PCB congeners in each species along with their chemical structures (vicinal [adjacent] H atoms in both the meta–para- and the ortho–meta-positions) and properties (octanol–water partition coefficient [log K_{ow}]), the ability of each organism to metabolize PCBs was inferred.

**MATERIALS AND METHODS**

**Materials**

Polychlorinated biphenyl standards, surrogate (PCB 14, 65, and 166) and internal standards (PCB 30, 204; >99% pure) were purchased from AccuStandard (New Haven, CT, USA). Each chromatographic peak was identified by the internal standard method. The calibration standard was donated, which is a mixture of Arochlor 1232, 1248, and 1262 as described by Mullin [28]. Chemstation software (Hewlett-Packard, Version B.00.00; Houston, TX, USA) was used for data collection and quantitation of chromatographic data.

**Sample collection**

During September 1996, organisms were collected from four nearshore sites in Lake Erie (all sites in OH, USA). These sites were Lakeside (Lakeside, OH, USA; 41°32’50”N, 82°44’53”W), Black River (Lorain, OH, USA; 41°28’30”N, 82°11’15”W), Grand River Harbor (Fairport, OH, USA; 41°45’53”N, 81°16’37”W), and Ashtabula River Harbor (Ashtabula, OH, USA; 41°55’0”N, 80°47’30”W).

At each site, zebra mussels were collected by diving in the littoral zone of each site, transported to the laboratory at The Ohio State University (Columbus, OH, USA), and stored at −70°C until extraction. Fish were caught by seines and angling. Fish were killed immediately on collection, then transported to the laboratory on ice. Thereafter, fish were stored at −70°C until extraction. Specific sizes were targeted from each site for analysis: 5 to 15 mm zebra mussels, 70 to 140 mm total length round gobies, and 200 to 400 mm total length smallmouth bass.

**Lipid analysis**

The method of van Handel [29] was used for lipid analysis with modifications. Whole bodies were homogenized, after which approximately 0.02 g of tissue were placed in a test tube containing 5 ml of chloroform/methanol (2:1 ratio). The tubes were sealed and refrigerated overnight. Then 0.5 ml of homogenate was analyzed for lipid content using the colorimetric method. These values were used to lipid normalize PCB concentrations using direct ratios.

**Sample preparation of organisms for gas chromatography**

Extraction and cleanup of samples were based on established methods [30–32], with slight modifications. Frozen samples were thawed at room temperature before analysis. Zebra mussels were shucked, and only the tissue was analyzed. Round gobies were analyzed as whole-body composites. To evaluate distribution of lipids and contaminants in top predators, smallmouth bass body and heads were analyzed separately because these may represent different destinations for PCBs, likely because of differences in lipid content. After homogenization in a blender, 10 g of homogenate from each species were ground with 40 g sodium sulfate (cleaned by baking at 400°C for 4 h; Sigma-Aldrich, St. Louis, MO, USA) that had been dried at 140°C overnight. The mixture was poured into a 2.5 × 60-cm glass column, plugged with glass wool, and filled with 70 ml of 1:1 dichloromethane/hexane. Samples were allowed to sit overnight (12 h), then eluted with 210 ml of 1:1 dichloromethane/hexane to complete the extraction. Following extraction, the sample was evaporated to (∼2 ml) by rotary evaporator (Yamato RE 200; San Francisco, CA, USA). The extract was further eluted through a Florisil column (6 g; 60–100 mesh; Sigma-Aldrich), activated, and cleaned by overnight drying at 130°C and running 15 ml hexane through the column (three times to yield 45 ml total). To minimize disturbance of the adsorbent and to remove moisture, a 3-cm layer of sodium sulfate was added to the top of the Florisil column. Target compounds (Table 1) were eluted with 50 ml hexane. The eluate was evaporated and then resuspended with 50 ml hexane on an activated silica-gel column (70–230 mesh) for a second cleanup step. The sample was eluted with 50 ml hexane and evaporated again. At the end of cleanup, samples were resuspended in 2 ml of isooctane for injection into gas chromatography–electron capture detector.

**Gas chromatography**

Both quantitative and qualitative analysis of PCB congeners (Table 1) were accomplished using a Hewlett-Packard 5890 Series II gas chromatograph (Houston, TX, USA) with an electron capture detector and a splitless injection port. The analytical column was a DB-5 (60 m × 0.25 mm, 0.25-μm film; J&W Scientific, Folsom, CA, USA). The injection port was set to 250°C, whereas the detector was set at 325°C. The oven was started at 100°C and increased (1°C/min) to 265°C, followed by 20°C/min to 300°C. Constant head pressure in the column was set at 65 psi. The carrier gas and makeup gas were hydrogen and nitrogen, respectively.

Quality assurance was achieved by sample replication and reagent blanks in each sample batch. Reagent blanks were prepared and analyzed identically to biotic samples. To evaluate the detection limit of the methods used for this study, method detection limits were calculated using a subset of congeners (PCBs 1, 6, 29, 49, 101, 141, 180, 194, and 206) repre-
representing specific homologue groups, replicated seven times in clean fish tissue. Values calculated by the method detection limit test were used to separate noise or background and response of the target compound. Method detection limits ranged from 0.02 to 2.05 ng/ml. A recovery test using a PCB mixture containing 99 congeners was performed. Recovery rate ranged from 75 to 120%.

Data handling

Transfer rates of PCBs were calculated for all site and species combinations. In order to assess trophic transfer of PCB congeners through the entire food chain and to determine whether hydrophobicity (measured as log $K_{ow}$) influences trophic transfer, lipid-normalized trophic transfer factors were calculated. Trophic transfer factors ($TTF_{lip}$), defined as the ratio of PCB concentration in the higher trophic level to the lower, were determined using means for each congener normalized by lipid content:

$$TTF_{lip} = \frac{C_p}{C_d}$$

In this equation, $C_p$ is the lipid-normalized concentration of congener $x$ (any given PCB congener) in the predator, and $C_d$ is the lipid-normalized concentration of congener $x$ in the prey.

A structure–activity-relationship model was used to estimate biotransformation potential on the basis of the position of vicinal H atoms in both the meta–para- and the ortho–meta-positions of the phenyl ring (Table 1). Each PCB congener was categorized into one of four groups on the basis of the criteria in Leonards et al. [33]. For each organism, all PCB congeners were converted from congener-specific concentration (wet-weight basis) into relative concentrations ($Cr$) by dividing that concentration by the concentration of reference PCB congener 180:

$$Cr = \frac{C_x}{C_{180}}$$

In this equation, $C_o$ is the concentration of congener $x$, and $C_{180}$ is the concentration of congener 180 in each sample. Congener 180 was used as a reference congener because it resists metabolism in most organisms [10].

These congener-specific patterns, normalized by PCB 180, were compared to those in higher trophic levels to evaluate the capacity of each species to biotransform PCBs:

$$Re = \frac{Cr}{Cr_{d}}$$

In this equation, $Re$ is the capacity of each species to biotransform PCBs, ($Cr$) is the $Cr$ value for the higher trophic level, and ($Cr_{d}$) is the $Cr$ values for the trophic level immediately below it for each congener. Therefore, low values (<1) reflect losses due to biotransformation and metabolism, high values (>1) reflect selective retention, and moderate values (=1) reflect persistent congeners [33,34].

Hypotheses and statistical analyses

Spatial differences in concentration of total lipids and PCBs. First, site differences in bioavailability of contaminants and risk to consumers of fish were tested. All organisms were assumed to feed and remain at each of the sites. This assumption was invoked for both sessile mussels and mobile fish. Round gobies are territorial within localized rocky habitats [35], and home ranges for field-tracked smallmouth bass are small, with fish moving only 1 to 3 km during multiple-season studies [36–38]. At each site, zebra mussels served as sentinel species of contaminant bioavailability from local suspended particulate material [19]. Because zebra mussels quickly accumulate and eliminate PCBs, they are immobile, and filter high volumes of water, they can reflect trends in local PCB contamination [22,39]. We assume that differences in PCB concentrations in smallmouth bass concentrations reflect risk to both human and avian consumers of these fish. Site and species differences were tested by first performing a two-way analysis of variance (ANOVA, unbalanced design; General Linear Model procedure, SAS version 8.02, 1999 release year; SAS Institute, Cary, NC, USA). If the interaction term was not significant, separate one-way ANOVAs (unbalanced design; General Linear Model procedure, SAS) were used to test main effects. For these analyses, PCB concentrations on a wet-weight basis were used.

Biomagnification of PCBs. Second, biomagnification was tested at each of our study sites, using a trophic-level model using lipid-normalized PCB concentration. To quantify biomagnification among our sites, analysis of covariance (General Linear Model procedure, SAS) was used to test for site differences in the relationship between total PCB concentrations and lipid levels, using trophic position as the covariate (zebra mussel = trophic level 2, round goby = trophic level 3, and smallmouth bass = trophic level 4). These categories correspond to the primary, secondary, and tertiary consumer trophic levels. Biomagnification was detected if PCB concentrations increased significantly with each trophic level, indicated by a positive slope [13]. Further, comparisons of slopes among sites allowed comparison of the relative degree of biomagnification at each site.

Structure–activity relationships and PCB trophic transfer. Third, structure–activity relationships were used to predict PCB congener distribution. The predictive power of hydrophobicity and chemical structure (vicinal H atom) were tested singularly and in concert. Hydrophobicity, measured as the log octanol–water partitioning coefficient ($K_{ow}$), was assessed.

Table 1. Four biotransformation groups of the analyzed polychlorinated biphenyls (PCBs) based of the presence or absence of vicinal H atoms. For these PCB congeners, the International Union of Pure and Applied Chemistry (IUPAC) classification scheme is used.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. ortho-C1</th>
<th>Meta–para</th>
<th>Ortho–meta</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 &lt; x &lt; 3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2 &lt; x &lt; 4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0 &lt; x &lt; 3</td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>1 &lt; x &lt; 4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

IUPAC numbers of PCB

| 1, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 16, 17, 18, 19, 22, 24, 25, 26, 27, 31, 33, 40, 41, 42, 43, 44, 45, 46, 49, 51, 56, 64, 70, 71, 76, 82, 83, 84, 87, 89, 91, 97, 110, 129, 131, 132, 134, 173 |
| 52, 53, 92, 95, 101, 135, 144, 149, 151, 176, 185, 199 |
| 15, 28, 37, 39, 47, 60, 63, 66, 74, 75, 81, 85, 99, 100, 105, 107, 114, 118, 119, 123, 128, 130, 137, 138, 156, 157, 158, 163, 170, 171, 177, 190, 195 |
| 146, 153, 167, 172, 175, 178, 180, 182, 183, 187, 189, 191, 193, 194, 196, 198, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209 |
as a predictor of $TTF_{lip}$ and $Re$ for smallmouth bass and round gobies. This relatively simple partitioning coefficient has yielded positive relationships between hydrophobicity and total PCB concentration in some organisms [4,33,40,41] however, other chemical properties also affect PCB uptake and biotransformation, resulting in different congeners patterns in predators and prey. Specifically, despite their high hydrophobicity, the uptake rate of larger molecules across biological membranes may be hindered (compared to smaller molecules), reducing the relative concentration of larger PCB congeners at higher trophic levels [33,39].

Elimination of PCBs is facilitated by biotransformation (metabolic processes) that results in less hydrophobic metabolites that can be more easily eliminated in urine and feces [21]. To assess metabolic potential, each PCB congener was assigned into one of four chemical groups on the basis of the position of vicinal hydrogen H-atom position (Table 1). Because eventual metabolism (and elimination) of PCB congeners begins with the insertion of an oxygen atom into the molecule by the cytochrome P450 system, differences in the position of vicinal H atoms (nonsubstituted C atoms) are hypothesized to drive biotransformation vulnerability for PCB congeners [10,34]. In general, PCB congeners with vicinal H atoms are most susceptible to metabolism [34]. Further, differences in the location of vicinal H atoms (vicinal H atoms in the ortho–meta-positions vs the meta–para-positions) can induce different isoenzyme pathways (CYP1A and CYP2B) in the organism, yielding dissimilar patterns among these PCB congener groups.

Further, the ability of each organism (smallmouth bass and round gobies) to metabolize PCBs was inferred from comparisons of metabolic slopes. Metabolic slopes were calculated by comparison of $Cr$ values between zebra mussels (x-axis) to round gobies or smallmouth bass (y-axis). This analysis assumes that zebra mussels are ideal reference organisms (they have low metabolism of PCBs), as are diatoms and a marine mussel, Crangon crangon [21]. A slope value of 1 suggests that round gobies or smallmouth bass have no biotransformation ability for PCBs. Values that approach zero indicate increasing biotransformation ability. Finally, structure–activity relationships were tested again between log $K_{ow}$ and $Re$ for both smallmouth bass and round gobies after first classifying congeners according to vicinal H atoms (Table 1). For these analyses, data from smallmouth bass heads were excluded because no significant difference was observed between $TTF_{lip}$ values in smallmouth bass heads and bodies.

Appropriate statistical comparisons were conducted. Total PCB concentrations were log$_{10}$ transformed to help normalize data and homogenize variance, and percentage data were arcsine transformed because variances are associated with mean values of proportional data [42]. Unless otherwise stated, all statistical analyses were conducted on lipid-normalized PCB concentrations (wt-weight basis). Because lipids have been found to correlate with fish size to varying degrees [6,43], using lipid-normalized PCB concentrations avoids this bias from organism size in addition to trophic position and lipid concentration. Bivariate correlations allowed determination of relationships between lipid and species, species and total PCBs, chlorination and total PCBs, and congener-specific concentration and total PCB data. Relationships between log $K_{ow}$ and both $Re$ and $TTF_{lip}$ were assessed by correlation analysis, except when a threshold relationship was apparent. In that case, a two-dimensional Kolmogorov–Smirnov test was used to detect thresholds at which variance changes [44]. By randomizing the original data 5,000 times, the randomly generated test statistic $D$ was compared to the $D$ value for the observed data pairs, allowing determination of exact $p$ values.

**RESULTS**

**Spatial differences in concentration of total lipids and PCBs**

Lipid levels in organisms varied with both species and collection site but not the interaction of the two variables (two-way ANOVA; site $df = 2, 52$, $p < 0.0001$; species $df = 3, 52$, $p < 0.0001$; site × species interaction $df = 6, 52$, $p = 0.2686$; Table 2). When organisms from each taxon were pooled across all sites, lipid levels increased with trophic level (smallmouth bass heads > smallmouth bass bodies > round gobies > zebra mussels; mean values were 7.4, 5.5, 2.9, and 1.5%, respectively; ANOVA, $df = 3, 87$; $p < 0.0001$). In general, fish collected from Lakeside had the lowest lipid levels, whereas fish collected from the Grand and Ashtabula sites had high lipid levels, especially for smallmouth bass (Table 2).

Although PCB concentrations showed a general decline in a west-to-east manner for zebra mussels and round gobies, the same was not evident for smallmouth bass. Zebra mussels, our surrogate measure of localized PCB contamination, had the highest lipid-normalized PCB concentration at Lakeside (Table 2). Lipid-normalized PCB levels in round gobies, our transfer link between benthic and pelagic food webs, showed a west-to-east decline (Table 2; Fig. 1); Lakeside levels were highest, Ashtabula levels were lowest, and the Black and Grand levels were intermediate. Despite these differences in PCB levels in lower trophic levels, smallmouth bass bodies and head (our top predator that reflects risk to fish-eating humans and birds) had similar total PCB concentrations among sites, except for Lakeside (Table 2; Fig. 1).

**Biomagnification of PCBs**

Total PCB concentration increased with increasing trophic level. When organisms of the same species were combined across all sites, the order of increasing PCB concentration was zebra mussels, round goby, smallmouth bass bodies, and smallmouth bass heads (82, 199, 929, and 1,209 ng/g wet weight, respectively; ANOVA; $df = 3, 87$; $p < 0.0001$). When organisms were compared within each site (Fig. 1), this stepwise increase was apparent, except for lack of differences between smallmouth bass bodies and heads. Although statistical comparisons among species could not be made for zebra mussels at the Ashtabula site (only one zebra mussel sample was analyzed at that site), the general trend was similar to the other sites.

When all organisms from different sites were combined, a high degree of individual variation in total PCB concentration could be explained by lipid concentration (Fig. 2). Data were combined to examine how lipids can predict PCB concentrations across species independent of site differences. In contrast, this predictive relationship was either nonexistent or weak (little explanatory ability) within each species. When all organisms were combined, regardless of species, total PCB concentration related positively with lipid content (Fig. 2). Marginal species-specific relationships between total PCBs and lipid content did not exist for round gobies ($n = 16$, $p = 0.29$) and smallmouth bass heads ($n = 37$, $p = 0.15$). However, marginal correlations were achieved between total wet-mass PCBs and lipids in zebra mussels ($n = 8$, $r = 0.391$, $p = ...
Table 2. Mean ± standard error values for organisms collected from four Lake Erie sites. The sites (listed from west to east) were Lakeside (Lakeside, OH, USA), Black River (Lorain, OH, USA), Grand River Harbor (Fairport, OH, USA), and Ashatabula River Harbor (Ashtabula, OH, USA). Letters that differ within a row denote mean values that differed among sites (analysis of variance; \( p \leq 0.05 \)). Parenthetical numbers indicate sample size.

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable</th>
<th>Lakeside</th>
<th>Black River</th>
<th>Grand River Harbor</th>
<th>Ashatabula River Harbor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zebra mussel</td>
<td>Total PCB/Lipid-normalized PCB</td>
<td>1.5A ± 0.1</td>
<td>1.6A ± 0.1</td>
<td>1.4A ± 0.1</td>
<td>1.1A (1)</td>
</tr>
<tr>
<td>Round goby</td>
<td>Total PCB/Lipid-normalized PCB</td>
<td>0.1 (3)</td>
<td>0.1 (3)</td>
<td>0.4 (5)</td>
<td>0.2 (5)</td>
</tr>
<tr>
<td>Smallmouth bass tissue</td>
<td>Total PCB/Lipid-normalized PCB</td>
<td>3.3A</td>
<td>3.3A</td>
<td>2.7AB</td>
<td>2.4B</td>
</tr>
<tr>
<td>Smallmouth bass head</td>
<td>Total PCB/Lipid-normalized PCB</td>
<td>1,520.3A</td>
<td>1,520.3A</td>
<td>1,091.8A</td>
<td>1,091.8A</td>
</tr>
</tbody>
</table>

Food chain biomagnification of total PCBs occurred at all four Lake Erie sites. Only at the Lakeside site was a difference in total PCBs between head and bodies of smallmouth bass apparent (Fig. 1). When all sites were averaged, total lipid-normalized PCB concentration increased with trophic level, indicating biomagnification (Fig. 3, regression equation). Patterns were more similar between the ranking of sites for round gobies and smallmouth bass (Lakeside was higher than Grand, Black, or Ashtabula) than for zebra mussels (Fig. 3).

Compared to the other species, congeners distribution for zebra mussels averaged across all sites accumulated lower-chlorinated homologues than other organisms (Fig. 4). Whereas fish species were dominated by the hexachlorine homologue, zebra mussels were dominated by penta- and hexachlorine homologues (Fig. 4). As a result, the average degree of chlorination of PCBs was 56.1% for zebra mussels, 60.4% for round goby, and 59.9% for smallmouth bass bodies. Congener distributions for PCBs among all species were dominated by a subset of congeners (28+31, 52, 47+48, 95, 84+92, 101, 99, 110, 123+149, 105+132+153, 138+163, 182+187, 180, 196+203).

Structure–activity relationships and PCB trophic transfer

In the zebra mussel–round goby linkage, thresholds appeared to exist above and below which simple linear patterns
PCB biomagnification in a Lake Erie exotic food chain

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Fig. 2. Relationship between total polychlorinated biphenyl (PCB) and lipid content (both on a wet-weight basis). The fitted line represents the correlation between % lipid and total PCB for all organisms pooled together from all sites. Within each taxon, this relationship was weak or not significant ($p > 0.05$).

Fig. 3. Biomagnification at four sites in Lake Erie. Biomagnification was defined as the relationship between trophic level and log-transformed lipid-normalized polychlorinated biphenyl (PCBlip) concentration. Trophic levels were assumed to be zebra mussels as primary consumers, round gobies as secondary consumers, and smallmouth bass as tertiary consumers. PCBlip concentration in smallmouth bass is the average of values for heads and bodies.

Fig. 4. Relative homologue proportions among species in Lake Erie (OH, USA), averaged across all four sites. Data for homologues are expressed as a proportion of the total, which was calculated by summing the individual congeners for each species. Error bars represent one standard error of the mean.

were absent. Thus, prediction of how hydrophobicity influences biotransformation and trophic transfer ability occurs in a nonlinear manner. Between round gobies and zebra mussels, $R_c$ value averaged 0.5, ranging from 0.1 (high biotransformation) to 1.1 (retention; Fig. 5A). Although a linear best-fit line could be forced through the data ($R_c = 0.253 \times [\log K_{ow}] - 1.14, r^2 = 0.61, p = 0.0001, n = 86$), a threshold was found at $\log K_{ow} = 6.3$ (vertical dashed line), above which variability increased (two-dimensional Kolmogorov–Smirnov test, $D = 0.19, p = 0.0002, n = 86$; Fig. 5A).

In a similar manner, variability in trophic transfer ($TTF_{lip}$) of PCB congeners increased above a threshold log $K_{ow}$ value (Fig. 5B). The average value for $TTF_{lip}$ our estimate of bioaccumulation, was 1.4, indicating high selective retention. Again, although a linear best-fit line could be forced through the data ($TTF_{lip} = 0.715 \times [\log K_{ow}] - 3.20, r^2 = 0.52, p = 0.0001, n = 84$), a threshold value of $\log K_{ow} = 6.3$ was detected, above which variability in $TTF_{lip}$ increased.

The pattern for biotransformation in the round goby–smallmouth bass linkage differed from the zebra mussel–round goby linkage. No overall correlation was found between log $K_{ow}$ and $R_c$ (Fig. 5A and C), and variability decreased at log $K_{ow}$ values greater than threshold values. Only smallmouth bass bodies are reported because no difference was found between heads and bodies ($p > 0.05$). For the round goby–smallmouth bass linkage, $R_c$ values were near or above 1 for a majority of the congeners (mean = 1.1; range = 0.3–1.8; Fig. 5C). This sug-
suggests lower biotransformation and retention of similar congener patterns between round gobies and smallmouth bass than between round goby and zebra mussels. Additionally, Rc was not linearly related to log $K_{ow}$, as was the case for the previous linkage. Instead, an opposite threshold was apparent. At values above log $K_{ow} = 6.8$ (Fig. 5C, vertical dashed line), variability decreased, and Rc values stabilized near 1.

Trophic transfer was variable until a threshold hydrophobicity value was reached, after which variability decreased (Fig. 5D). The $TTF_{lip}$ values averaged 2.8 (range: 0.5–4.6), suggesting higher bioaccumulation than the previous linkage. In a similar fashion to Rc for this linkage, $TTF_{lip}$ varied with log $K_{ow}$ in a nonlinear fashion. At values greater than a threshold value of log $K_{ow} = 6.8$, variation in $TTF_{lip}$ decreased.

Differences in the chlorine substitution in the ortho-, meta-, and para-positions were related to PCB biotransformation potential. The ability of each species to metabolically transform PCBs was related to compound structure and divided into four groups on the basis of the presence/absence and position of vicinal H atoms (Table 1). This analysis assumes that zebra mussels are ideal reference organisms (they had low metabolism of PCBs), as are diatoms and a marine mussel, C. cran- gon [21]. A slope value of 1 suggests that round gobies or smallmouth bass have no biotransformation ability for PCBs. Values that approach zero indicate increasing biotransformation ability. Differences in biotransformation appeared to be related to the structure of each PCB congener, suggesting different metabolic pathways.

Table 3. Relative metabolic slopes among zebra mussel–round goby (ZM-RG) and zebra mussel–smallmouth bass body (ZM-SBW) couples. Each polychlorinated biphenyl congener is assigned to these four chemical groups according to Table 1. In this table, ZM = zebra mussel, RG = round goby, and SBW = smallmouth bass body. Letters that differ within a row denote differences in metabolic ability ($p < 0.05$; analysis of variance) among the chemical groups.

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZM-RG</td>
<td>0.463A</td>
<td>0.352A</td>
<td>0.811B</td>
</tr>
<tr>
<td>ZM-SBW</td>
<td>0.526A</td>
<td>0.409A</td>
<td>0.831B</td>
</tr>
</tbody>
</table>

For round gobies and smallmouth bass, biotransformation depended on PCB congener composition. When congener concentrations of round gobies and smallmouth bass species were plotted against that of zebra mussels, no significant differences were observed among sites for the same species (ANOVA, $p > 0.05$; data not shown). When congeners were grouped (according to chemical structure; Table 1) and metabolic slopes measured, similar patterns were found between the round goby–zebra mussel and the smallmouth bass–round goby linkages. Metabolic slopes for groups 1 and 2 were lower (indicating high biotransformation) than for groups 3 and 4 (low biotransformation), indicating that chemical structure (vicinal H-atom position in the phenyl ring) can be an important determinant of biotransformation ability (Table 3).

When congeners were first grouped according to chemical structure (vicinal H), linear relationships between hydrophobicity and metabolic ability became evident. Rather than nonlinear relationships in the previous analysis (Fig. 5), positive, linear relationships between log $K_{ow}$ and Rc were found for nearly all comparisons (Fig. 6, rows A to C), indicating decreased biotransformation with increasing hydrophobicity. The exceptions to this generalization are in the smallmouth bass–round goby linkage (Fig. 6, row C); for this linkage, no relationship was found for congener in groups 1 and 4, and a negative relationship was found between log $K_{ow}$ and Rc for congeners in group 3. Also, group 4 congeners for the ZM-RG and ZM-SBW (rows A and B) had negative relationships between Rc and log $K_{ow}$. When zebra mussels were used as a reference organism (Fig. 6, rows A and B), metabolic slopes for smallmouth bass were nearly identical to round gobies, suggesting that the relative metabolic capacity of both fish are similar within each chemical group.

Finally, the last relationship (Fig. 6, row D) permits direct comparison of biotransformation between metabolic slopes for round gobies and smallmouth bass relative to concentrations in zebra mussels. In theory, if round gobies and smallmouth bass were to have identical biotransformation ability (relative to zebra mussels), the slope would equal 1 (the 1:1 line) and explain 100% of the variability in the relationship ($r^2 = 1$). If this slope is $<1$, this indicates that biotransformation ability is greater for smallmouth bass than for round gobies. For groups 1 and 4, slopes were roughly equal to 1 (Fig. 6, row D), indicating similar biotransformation between smallmouth bass and round gobies for these congeners; however, for congeners in group 2 and 3, some slight differences may exist. Round gobies may have slightly higher biotransformation ability for group 2 congeners, whereas smallmouth bass may have slightly better ability to biotransformation group 3 congeners (Fig. 6, row D).

Fig. 5. Relationships between log $K_{ow}$ and biotransformation capacity (Rc) and lipid-normalized trophic transfer factor ($TTF_{lip}$) for the zebra mussel–round goby (A, B) and round goby–smallmouth bass (C, D) linkage in Lake Erie (OH, USA). Although linear regressions could be forced through the data in panels A and B (see Results), violations of linearity prompted the use of two-dimensional Kolmogorov–Smirnov tests (shown as text in panels).
PCB biomagnification in a Lake Erie exotic food chain

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Fig. 6. Biotransformation relationship between trophic links and log $K_{ow}$ on the basis of the presence or absence of vicinal H atoms. Regression lines indicate significant relationships ($p < 0.05$). The values of $R_c$ are ng/g wet-weight polychlorinated biphenyl (PCB) congener values within each organism normalized to PCB 180 (a reference congener that resists metabolism in most organisms) to assess the capacity of each species to biotransform PCB. ZM = zebra mussels; RG = round goby; SBW = smallmouth bass body. The $x$- and $y$-axes in rows A to C differ from those in row D; within each of the two groupings, the same axes are used to allow direct comparison among graphs.

DISCUSSION

Our study answered our three general hypotheses. First, despite a general west-to-east decrease in bioavailability of contaminants (PCB concentrations in zebra mussels), PCB concentrations in smallmouth bass (risk to human and avian fish consumers) were similar among sites. Second, biomagnification occurred in our exotic zebra mussel-round goby-smallmouth bass food chain at all our sites. Third, structure-activity relationships based on chemical characteristics ($\log K_{ow}$) or chemical structure (vicinal H atom) alone had less predictive power than when both were considered the analysis.

Our study documented biomagnification in a new food chain in Lake Erie, which contains two exotic species at mid-trophic levels and a native terminal predator. In aquatic ecosystems, organisms can be exposed to hydrophobic contaminants through various routes; however, the most likely route for many organisms is through diet, resulting in the sequestration of hydrophobic contaminants in adipose tissue [3]. Similar trends were found for zebra mussels and round gobies in Michigan tributaries to the Great Lakes, where PCB concentrations were three to five times higher in round gobies than zebra mussels [45]. Unfortunately, in that study, skinless fillets of smallmouth bass were sampled, preventing direct comparison between our results and theirs. Whether on a wet-mass or lipid-normalized basis, PCB concentrations increased with trophic level in this Lake Erie food chain. If lipid-normalized PCB concentrations were similar among species, the major uptake pathway would be through the gills. However, lipid-normalized total PCB values differed, suggesting the relatively low importance of this gill uptake pathway. Our findings support general findings by Rasmussen et al. [1] where PCB body burden increased three- to fivefold per trophic level in the Lake Ontario food chain because of dietary uptake.

Spatial differences in PCB biomagnification

Zebra mussels appeared to be a good sentinel for local PCB bioavailability. Generally, PCB concentrations in zebra mussels decreased from west to east; however, the same longitudinal pattern was not evident for lipid content. This trend supports sediment PCB concentrations from the southern shore of Lake Erie in 1997–1998 [46]. Given that zebra mussel PCB concentrations matched the decreasing trend from west to east in sediment PCB levels [46] and did not simply correlate directly to lipid levels, zebra mussels appear to be a good sentinel species of PCB bioavailability, as was suggested by Cope et al. [19].

The PCB concentrations in our study zebra mussels (29.5–97.0 ng/g) were low relative to other Great Lakes samples. In
1994, total PCBs in zebra mussels collected at a reference site upstream of the impacted site in the Detroit River (Detroit, MI, USA) averaged 17.1 ng/g wet weight, whereas at the impacted site the zebra mussels averaged 301.2 ng/g wet weight [47]. In another tributary to Lake Erie (Raisin River, MI, USA), PCB concentrations in zebra mussels ranged from 31 to 2,920 ng/g wet mass [45]. Our values were intermediate between those river sites and open-water sites, likely reflecting proximity to point sources of PCBs. Total PCBs in zebra mussels in this study were higher than those from Middle Sister (Detroit, MI, USA), East Sister (Detroit, MI, USA), and Pelee Islands (Detroit, MI, USA; averaged 28.3, 23.8, 20.0 ng/g PCB wet weight, respectively). Although our values are higher than these open-water Lake Erie studies, a north-to-south increase in sediment PCB concentrations also exists [46], explaining some of the differences. Because these patterns in zebra mussel PCB concentration track local environments well, they again appear to be effective sentinels for local bioavailability of PCBs.

Our PCB residues in fish are low relative to fish in Michigan tributaries and high relative to fish found in Canadian waters of Lake Erie. Whereas total PCBs in our round gobies ranged from 118 to 256 ng/g wet weight, round gobies from Michigan tributaries, labeled as Areas of Concern by the International Joint Commission, ranged from 81 to 4,710 ng/g total PCBs [45]. Lipid-normalized total PCBs for our smallmouth bass bodies (14,560–23,346 ng/g, lipid corrected) were higher than smallmouth bass collected during 1991 from Middle Sister Island; those fish had muscle PCB concentrations of 12,268 µg/kg, lipid corrected [48].

Despite site differences in PCB concentrations in lower trophic levels, eventual body burden in top predators, smallmouth bass, did not differ among sites. Our study showed that lipid content is insufficient to explain the PCB congener accumulation patterns found among our food chains at different sites (Table 2). Although lipid levels were slightly higher in smallmouth bass from the eastern sites (Grand and Ashtabula), no such difference was found for PCB concentrations (on a wet-mass basis) at those sites, and no correlation was found between total lipid and PCB concentration in smallmouth bass. Although lipid concentration related positively to total PCB concentration when all taxa were combined (Fig. 2), this was not true within each taxon. Further, the sites with the highest lipid levels did not correspond to the highest total PCB concentration (Table 2). This assumption that PCBs correlate strongly with lipid content has been questioned [43]. In our study, the relationship between lipid content and total PCB concentration was either not statistically significant or yielded little explanatory power (<50% of variability of explained) within any given species.

When averaged across all sites (Fig. 4), our fish species exhibited similar, nearly identical PCB homologue distributions, whereas congener distributions in zebra mussel differed. Of the congeners studied, highly chlorinated congeners without vicinal H atoms and PCBs 153, 180, and 138±163 with vicinal H atoms in the ortho–meta-position were the dominant individual congeners in most species. In contrast, congeners from mono- to trichlorinated groups, excluding PCB 28±31, were found at significantly lower concentrations in all species.

Structure–activity relationships and PCB trophic transfer

Hydrophobicity (log Kow) alone did not yield simple, linear relationships to explain trophic transfer and biotransformation ability for the suite of PCB congeners in our study. This relatively simple partitioning coefficient has been used to predict total PCB fate [4,33,40,41]. Rather, trophic transfer and biotransformation ability appeared to depend on log Kow in nonlinear, threshold manners, which differed slightly between round gobies and smallmouth bass. Overall, the biotransformation ability of round gobies for PCBs was slightly higher (Rc values < 1 for most congeners) than smallmouth bass (Rc = 1 for most PCB congeners) for nearly the entire range of log Kow values.

For round gobies consuming zebra mussels, departures from trophic transfer/log Kow linearity reduced the explanatory ability of these structure–activity relationships. Rather than finding a strictly linear increase between log Kow and trophic transfer, linearity was interrupted at thresholds that correspond to pentachlorinated homologues, which average log Kow = 6.3 [49]. A trade-off between log Kow and molecule size [33] exists, which may, in part, explain the high variability in Rc and TTFtop for PCB congeners with log Kow values >6.3.

For smallmouth bass consuming round gobies, biotransformation ability for PCBs was variable at a higher log Kow value, which corresponds to hexachlorinated homologues, which average log Kow = 6.7 [49]. At log Kow values >6.3, roughly equivalent to homologues with five to six chlorines, biotransformation was negligible. This resulted in a stabilization of TTFtop at log Kow values >6.8; this value corresponds to homologues with five to eight chlorines [49]. These thresholds, above which trophic transfer stabilizes, support other studies. A similar threshold of trophic transfer occurs for PCBs in Lake Michigan coho salmon (Oncorhynchus kisutch) [2]. In that study, net trophic transfer efficiencies were high (43–56%) for PCB homologues with five to eight chlorines, compared to homologues with four chlorines (38%). Further, degree of chlorination and log Kow did not influence net trophic transfer efficiency for congeners with five to eight chlorines. Jackson et al. [11] found a similar relationship in Lake Michigan coho and chinook (Oncorhynchus tshawytscha) salmon, for which PCB congener concentration peaked at log Kow = 6.4. Maruya and Lee [9] have demonstrated a negative turnover of TTFtop with increasing log Kow in the trophic transfer of PCBs. Other factors, such as molecular weight, were suggested to be important for these higher-chlorinated congeners [33], reducing their presence and persistence in higher-trophic-level organisms.

Our metabolic slopes suggest that in addition to chemical properties (hydrophobicity), chemical structure (vicinal H atom) is important to understand biotransformation or trophic transfer of PCB congeners. When chemical structure was ignored, threshold relationships between hydrophobicity and biotransformation were nonlinear. When PCB congeners were first grouped according to vicinal H atoms, average biotransformation potential was similar between round gobies and smallmouth bass but related to hydrophobicity differently among structural groups. Comparison of calculated Rc values with log Kow shows that susceptibility to metabolism decreased with log Kow for groups 1 to 3. However, the trend was opposite for group 4, with an increase in biotransformation as hydrophobicity increased. Several investigators have reported that differences in metabolic slope among trophic links depended on susceptibility to metabolism [21,50] and that rate was constant within one group regardless of the chlorination [21]. Thus, metabolic potential may relate primarily to chemical structure, then secondarily to hydrophobicity.
CONCLUSION

Across all our nearshore sites in Lake Erie, PCB body burden increased with trophic level in this exotic food chain. Given the continued expansion by exotic species, the relative recalcitrance of sediment-sorbed PCBs (compared to PCBs in air, water, or biota), and importance of the sport fishery, this transfer pathway for PCBs will become increasingly important in the Great Lakes. The lack of continuing decreases in PCB residues in Great Lakes sport fishes may be a result of large pools of PCBs being recycled in the Great Lakes, resulting in stable levels in fishes, despite reductions in PCB inputs [5]. Another hypothesis is that PCBs are indeed declining in biota and sediments but at lower rates than fast-responding media such as water, sediment particles, or air [51]. Therefore, changes in biota from midtrophic levels may become more important in determining PCB residues in higher trophic levels (fish, fish-eating birds, or humans who consume fish) than source inputs of PCBs.

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