CONTROLS OF CONTAMINANT BIOTRANSPORT BY PACIFIC SALMON TO
GREAT LAKES TRIBUTARIES

A Dissertation

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by

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My dissertation evaluated factors that influence the transfer and uptake of Pacific salmon (*Oncorhynchus* spp.) derived contaminants to stream-resident fish in tributaries of the upper Laurentian Great Lakes. This research has broad relevance to fisheries of the Great Lakes and beyond because introduced Pacific salmon are considered an important sport fish while the lakes are replete with contaminants, especially persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), and heavy metals such as mercury (Hg). My dissertation demonstrated that Pacific salmon transport contaminants to tributaries of the Great Lakes, which influence the contaminant burden of stream-resident fish. Using a combination of observational, experimental, and modeling
approaches, I identified the key factors related to how contaminants transported by salmon are transferred and accumulated by stream-resident fish. My research provides strong evidence for contaminant biotransport and transfer of POPs, but not Hg, by salmon. Therefore, not all contaminants are equivalent, and the trophic pathway to contamination is a primary driver of the impact of salmon-derived contaminants on stream-resident fish. First, I demonstrate how environmental context influences the transfer of PCBs biotransported by Pacific salmon. Specifically, the lake basin of origin for the salmon spawner, the flux of PCBs supplied by salmon, and the species identity of the resident fish strongly influenced the magnitude of contaminant biotransport and uptake. Differences among resident fishes are likely driven by differences in diet composition, consumption rate, and physiology. In contrast, the lack of a Hg effect suggests that salmon are not a significant source of Hg to resident fish. Moreover, because salmon eggs are enriched in POPs, but depleted in Hg, widespread consumption of eggs may be the primary pathway for uptake of salmon-derived contaminants by resident fish. Using experimental approaches, I then provide strong inference that egg consumption results in elevated POP concentrations, consumption of salmon tissue leads to increased Hg concentrations, and that consumption of salmon eggs but not salmon tissue enhances the growth of resident fish. The contrast in Hg response between my mesocosm (enhanced Hg) and salmon addition (suppressed Hg) experiments was likely driven by the forced consumption of salmon tissue in the mesocosms, suggesting that resident fish in streams receiving salmon runs rarely consume carcass material, especially if eggs are available. Finally, I integrate my results using a bioenergetics-bioaccumulation model that provides a mechanistic explanation of salmon-mediated
contaminant biotransport. The model that variation in the trophic pathway, individual diet and consumption rate, and location in which salmon accumulate their contaminant burden interact to regulate the transfer and uptake of salmon-derived contaminants by stream-resident fish. Overall, my dissertation demonstrates that the contaminant type, species identity, and trophic pathway to contamination determine the magnitude of salmon-mediated contaminant biotransport and uptake. Consequently, consideration of the recipient food web and route of exposure is critical to understanding the fate of biotransported contaminants in stream ecosystems.
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1.1 Background

To fully understand an event or phenomenon, an appreciation of context is required. In ecology, context dependency occurs when patterns or processes vary along biological, chemical, or physical gradients (Poff and Ward, 1990; Clements et al., 2012). Context dependency can obscure scientific inference due to high variation in community or ecosystem responses, which makes specific predictions challenging (Poff and Ward, 1990). Poor predicative ability precludes drawing generalizations about how ecosystems, communities, and populations function, which has consequences for both fundamental scientific understanding and applied natural resource management (Clements et al., 2012). Ecologists work in natural systems where idealized systems exhibiting low variation are seldom encountered. Rather, ecological research confronts broad gradients in physical (e.g., watershed characteristics, disturbance regime), chemical (e.g., pollution, physiochemical parameters), and biological (e.g., food web structure, ecosystem productivity) conditions. While this variation can be complex, it can serve as a powerful framework for identifying those controlling variables that determine ecological processes. Context dependency has been shown to strongly influence responses to disturbance (Menge and Sutherland, 1987), nutrient export (Mulholland et al., 2002), and
contaminant bioaccumulation (Borga et al., 2012; Clements et al., 2012). Context dependency also regulates the importance of resource subsidies in recipient ecosystems.

Resource subsidies have been shown to be a common and important ecological phenomenon. Resource subsidies occur when a donor ecosystem or organism moves nutrients or material across an ecosystem or habitat boundary, thereby influencing the function and structure of the recipient system (Polis et al., 1997; Polis et al., 2004). Pacific salmon (*Oncorhynchus* spp.) provide a compelling example of how migratory organisms create ecosystem linkages and act as resource subsidies. Pacific salmon return to natal freshwater streams to spawn and die (Schindler et al., 2003), depositing large quantities gametic tissue, carcass material, and excreted nutrients. This annual pulse of resources influences both ecosystem function and the productivity of resident organisms (Schindler et al., 2003; Wipfli et al., 2003). However, spawning salmon also act as ecosystem engineers capable of exerting a large disturbance effect on streams, thereby creating a tradeoff between enrichment and disturbance that is largely governed by environmental context (Janetski et al., 2009). Streams with low nutrient content and larger substrates have greater nutrient enrichment whereas streams with smaller substrates and higher background nutrients have higher levels of substrate disturbance during spawning. Moreover, the role of salmon in ecosystems can vary depending on both the community structure of the resident fish community (Scheuerell et al., 2007) and watershed land use (Tiegs et al., 2008). The resources transferred by salmon can also be replete with harmful anthropogenic toxicants, which have been dubbed the ‘dark side’ of ecosystem resource subsidies (Walters et al., 2008) because movement of contaminants can negatively impact ecosystem and human health (Blais et al., 2007).
Contaminant biotransport is a three-step process that includes the bioaccumulation of a contaminant by a migratory organism; the movement of the contaminant across an ecosystem boundary; and the release of the contaminant into the recipient habitat (Blais et al., 2007). Previous literature has demonstrated that the bioaccumulation of contaminants (Clements et al., 2012) and the ecological effects of salmon are influenced by the physical, chemical, and biological characteristics of the environment (Janetski et al., 2009). However, uncertainty exists as to whether contaminant biotransport by salmon is subject to context-dependent variation. For example, it is unclear whether contaminants delivered by salmon are taken up and incorporated by stream-resident fish and how this varies as a function of various biological, chemical, and physical factors.

The Laurentian Great Lakes are an appropriate setting to evaluate the role of context dependency on contaminant biotransport by Pacific salmon. Pacific salmon were introduced to the Great Lakes in the mid-1960s to exert biological control over invasive alewife (*Alosa psuedoharengus*; Dettmers et al., 2012). However, establishment of salmon coincided with peak concentrations of contaminants such as polychlorinated biphenyls (PCBs) and mercury (Hg), which bioaccumulate in aquatic food webs and have negative consequences for fish, wildlife, and human health (Stow et al., 1995; Murphy et al., 2013). The impact of Pacific salmon in the Great Lakes also extends to tributaries that are used during spawning by salmon that stray from the state hatcheries where they were reared. This phenomenon has resulted in many Great Lakes tributaries acquiring consistent annual runs of Pacific salmon. My dissertation will determine how context
dependency controls contaminant biotransport by Pacific salmon at multiple scales of inference.

1.2 Dissertation outline

For my dissertation, I adopted a multi-faceted approach to evaluate the impact of spawning salmon on resident fish within the Great Lakes. Specifically, I evaluated the dual role of Pacific salmon as a resource subsidy and contaminant biovector. My dissertation asked several questions that differ in their scale and rely on contrasting study designs (e.g., field surveys, mesocosm experiments, instream manipulations, and simulation models) and provide complementary inference to understand the role of salmon in Great Lakes tributaries. By combining these different approaches, I was able deconstruct the process of salmon contaminant biotransport to identify the driving factors controlling the uptake of contaminants by resident fish in Great Lakes tributaries.

In CHAPTER 2 (published as Gerig et al., 2016, in Environmental Science and Technology 50: 554-563), I used a hierarchical framework to determine whether introduced salmon were a source of POP contamination to stream-resident fish in Great Lakes tributaries. I first examined whether the PCB and PBDE congener pattern of Pacific salmon varied by Great Lakes basin, contaminant type, and tissue type. After documenting the pattern amongst salmon spawners, I determined whether the PCB and PBDE pattern of stream-resident fish differed between locations with and without salmon. I hypothesized that (1) salmon PCB and PBDE congener patterns would differ among basins, reflecting differing history, sources, and extent of pollution, and (2) that resident brook trout and mottled sculpin in stream reaches with salmon would have congener patterns more similar to salmon than to conspecifics in reaches lacking salmon.
Previous research has demonstrated that resident fish PCB concentrations (e.g., Merna, 1986; Janetski et al., 2012) are higher in locations with salmon spawners but the use of congener patterns to identify salmon as a source of contamination has not been conducted. Overall, this chapter presents a novel way to leverage existing POP data to determine the extent of ecosystem linkages created by spawning salmon between lake and tributary ecosystems.

In CHAPTER 3 (in review as Gerig et al. in the Journal of Applied Ecology), I evaluated whether environmental context mediates the transfer and uptake of salmon-derived contaminants to stream-resident fish across watersheds of the upper Laurentian Great Lakes. To meet this objective, I first quantified the PCB and Hg concentrations in tissue and gametes of Pacific salmon spawners, the putative source of contaminants. Second, I related stream-resident fish $\delta^{15}N$ to their PCB and Hg concentrations to compare rates of contaminant bioaccumulation between locations with and without salmon. Last, I used an information theoretic approach to assess the mediating influence of various components of the environmental context on the relationship between salmon contaminant flux and stream-resident fish contaminant concentrations. I hypothesized that (1) salmon would accumulate high concentrations of both PCBs and Hg; (2) stream-resident fish would exhibit a positive relationship between $\delta^{15}N$ and contaminant concentration reflecting salmon as a significant source of both PCB and Hg; and (3) attributes of environmental context, reflecting biological, chemical, and physical variables, would mediate the transfer of salmon-derived contaminants to stream-resident fish. Previous research has focused solely on assessing the direct effect of salmon on resident fish POP concentrations but has yet to consider how other biological, chemical,
and physical factors affect this transfer (e.g., Merna, 1986; Gregory-Eaves et al., 2007). My research provides insight into how the pathway to contamination, contaminant type, and diet quality interact to influence the magnitude of contaminant biotransport. Overall, this chapter provides a detailed view of the factors that drive and mediate the uptake of salmon-derived contaminants by resident fish.

In CHAPTER 4 (in press as Gerig et al., 2017, in the Canadian Journal of Fisheries and Aquatic Sciences), my overall objective was to evaluate the consequences of interactions between non-native and native fish species, in the context of a novel resource subsidy. For this study, I conducted a mesocosm experiment to determine the effects of a novel resource, salmon tissue, on native brook trout, and whether the provision of a salmon subsidy modulated interactions between brook and introduced brown trout. I hypothesized that brook trout with access to salmon material would exhibit (1) higher growth, due to consumption of high-quality salmon tissue; (2) altered isotopic ratios, reflecting incorporation of isotopically-enriched salmon-tissue; and (3) increased Hg concentrations, resulting from consumption of Hg-laden salmon tissue. In addition, I predicted that brook trout growth rates, isotopic ratios, and Hg concentrations would be lower in the presence of brown trout as a result of interspecific competition. To complement our experimental findings, I developed a coupled bioenergetics-bioaccumulation simulation model to determine how diet composition, energy density, and Hg content of diet items interacted to influence brook trout growth and mercury accumulation observed in our experiment. Previous salmon research has relied on mesocosm experiments (e.g., Chaloner et al., 2002; Wipfli et al., 2003) to understand growth and isotope responses of resident fish to salmon carcasses but has not been
extended to determine if salmon consumption mediates Hg accumulation or interactions between competing species. Overall, by coupling a controlled mesocosm experiment with a simulation model, I demonstrated that the influence of salmon resources on resident fish is dependent on the quantity and quality of food in the diet, contaminant partitioning in salmon tissue, and individual fish characteristics. Overall, my study highlights the complex nature of interactions between salmon and resident fish, which has important implications for fisheries management in the Great Lakes and elsewhere.

In CHAPTER 5 (in preparation as Gerig et al. for submission to Frontiers in Ecology and Evolution), my objective was to test the effect of a novel salmon carcass and egg addition on the organic contaminant and heavy metal burden of resident salmonids using a multi-year whole-stream experiment. I relied upon both gut content analysis and a suite of ecological tracers including stable isotopes of C and N, POPs including polychlorinated biphenyls (PCB), dichloro-diphenyl-dichloro-ethylene (DDE), polybrominated diphenyl ethers (PBDE), and mercury (Hg) to track the incorporation of salmon material into resident trout. We hypothesized that (1) resident trout diets would change, reflecting consumption of salmon material; (2) stable isotope ratios of resident trout would shift, indicating incorporation of salmon derived nutrients and energy; (3) contaminant levels of resident fish would increase following the addition of salmon material; and (4) isotopes, POPs, and Hg would provide complementary inference as ecological tracers of the influence of salmon on resident trout. This study is the first experimental evidence for the transfer of contaminants from salmon to stream-resident fish. Further, our experimental salmon addition provides insight into the timeline for
incorporation and elimination of salmon-derived contaminants from a resident fish population and the pathways of contaminant uptake by stream-resident fish.

In CHAPTER 6 (in preparation as Gerig et al. for submission to Science of the Total Environment) my broad objective was to understand the underlying mechanisms by which stream-resident salmonids acquire energy and accumulate contaminants transported by introduced Pacific salmon in the Laurentian Great Lakes. To meet my broader objective, I conducted a diet study to empirically measure the ration size and diet composition of resident salmonids in streams containing spawning salmon. I then used empirically collected data on the energy density and contaminant concentration of resident salmonid diet items to parameterize an individual-based bioenergetics-bioaccumulation model to simulate the influence of salmon contaminant biotransport on resident trout growth and bioaccumulation. Using this model, I established scenarios to predict how the trophic pathway to contamination, variation in salmon egg consumption, and environmental context influenced the uptake of salmon-derived resources and mediated contaminant biotransport to resident salmonids. This modeling framework provided a simple framework to identify the primary drivers of growth and contaminant bioaccumulation resulting from the uptake of salmon-derived energy and contaminants in resident salmonids in the Great Lakes, laying important groundwork for future studies.

In CHAPTER 7, I conclude my dissertation by providing a brief summary of my dissertation research, management implications, and future research directions.
1.3 Literature Cited


2.1 Abstract

In the Great Lakes, introduced Pacific salmon (*Oncorhynchus* spp.) can transport persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), to new environments during their spawning migrations. To explore the nature and extent of POP biotransport by salmon, we compared 58 PCB and 6 PBDE congeners found in spawning salmon directly to those in resident stream fish. We hypothesized that stream fish exposed to salmon spawners would have congener patterns similar to those of salmon, the presumed contaminant source. Using permutational multivariate analysis of variance (PERMANOVA) and non-metric multidimensional scaling (NMDS), we found that POP congener patterns of Pacific salmon varied among Great Lakes basin (i.e., lakes Huron, Michigan, or Superior), tissue type (whole fish or eggs), and contaminant type (PCB or PBDE). For

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1 This chapter was published in Environmental Science and Technology, volume 50, pages 554-563, with co-authors D. T. Chaloner, D. J. Janetski, R. R. Rediske, J. P. O'Keefe, A. H. Moerke, and G. A. Lamberti.
stream-resident fish, POP congener pattern was influenced by the presence of salmon, location (i.e. Great Lakes Basin), and species identity (i.e., brook trout \textit{Salvelinus fontinalis} or mottled sculpin \textit{Cottus bairdii}). Congener patterns of mottled sculpin sculpin either use salmon tissue to differing degrees, acquire POPs from different dietary sources, or bioaccumulate or metabolize POPs differently. Overall, our analyses identified the important role of salmon in contaminant biotransport but also demonstrated that the extent of salmon-mediated POP transfer and uptake in Great Lakes tributaries is location- and species-specific.

2.2 Introduction

Persistent organic pollutants (POPs) have been a major contaminant in aquatic ecosystems since the 1950s\textsuperscript{1} and include both legacy pollutants such as polychlorinated biphenyls (PCBs) and emerging pollutants such as polybrominated diphenyl ethers (PBDEs).\textsuperscript{2} Despite extensive regulation, POPs continue to be of concern due to their ability to bioaccumulate in food webs.\textsuperscript{3,4} Recently, attention has focused on understanding the role of organisms as biological vectors for contaminants to recipient ecosystems that do not have sources of these contaminants.\textsuperscript{5,6} Migratory organisms deliver resource subsidies and provide predictable and important pulses of nutrients and energy across ecosystem boundaries.\textsuperscript{7,8} However, migratory organisms, such as Pacific salmon \textit{(Oncorhynchus spp.)}, also transport contaminants.\textsuperscript{3,9} The contaminant burden and composition in an organism represents an integration of the specific suite of POPs to which they were exposed during their life cycle. Hence, the chemical ‘signature’ of an organism’s contaminant burden can be used to identify sources of contamination, track
the movement of material, and infer interactions between resident organisms and resource subsidies.\textsuperscript{10,11} Pacific salmon are ideal for studying contaminant biotransport and transfer. Salmon are anadromous and semelparous, grow to large sizes, occupy a high trophic position, and have a high lipid content.\textsuperscript{7,12} As a result, salmon can deposit contaminated tissue to tributaries and influence POP levels in the aquatic ecosystems where they spawn.\textsuperscript{9,10} For example, contaminated salmon material, especially eggs, can be directly ingested by fish and invertebrates\textsuperscript{13,14} or become incorporated and indirectly assimilated by primary producers and primary consumers through decomposition pathways.\textsuperscript{15,16}

The Laurentian Great Lakes provide an ideal setting to study contaminant biotransport by salmon. First, the Great Lakes have been extensively altered by introduced species and environmental contaminants that can magnify the role of salmon as a vector for pollutants.\textsuperscript{17,18} Second, the Great Lakes support large populations of naturally reproducing salmon that bioaccumulate POPs during their open-water phase and deposit body burdens to tributaries during their spawning run.\textsuperscript{18} Previous research has shown a positive relationship between POP burden and egg consumption in rainbow trout ($Oncorhynchus$ $mykiss$)\textsuperscript{13} and higher total POP concentrations in resident fish exposed to migratory fish in Great Lakes tributaries.\textsuperscript{19} Recently, Janetski et al.\textsuperscript{14} demonstrated a direct correlation between POP concentrations in stream-resident fish and the quantity of POPs delivered by salmon. Although associations between salmon and resident fish POPs have been documented in the Great Lakes, no study to date has directly linked the chemical composition of POPs in salmon to those in other organisms consuming salmon material.
The source of contaminants concentrated in biota can be identified using their specific chemical composition. PCBs and PBDEs consist of mixtures of congeners that differ in the position and number of chlorine or bromine additions, respectively, within their molecular structure. For instance, a total of 209 PCB congeners has been synthesized, thereby representing a diverse array of compounds in the environment. The variable structure of congeners, combined with their relative abundance, can be used as a fingerprint to identify sources of contamination and suggest the extent and pathway of ecosystem linkages. Such an approach has been used to identify sources of contaminants in tree swallows (Tachycineta bicolor), American dippers (Cinclus mexicanus), striped bass (Morone saxatilis), and sockeye salmon (Oncorhynchus nerka). To date, POPs have not been used similarly to trace the movement of material from migratory fish, including Pacific salmon (Oncorhynchus tshawytscha, O. kisutch) to stream-resident fish (brook trout [Salvelinus fontinalis], mottled sculpin [Cottus bairdii]) in the Great Lakes region.

We used a hierarchical framework to determine whether introduced salmon were a source of POP contamination to stream-resident fish in Great Lakes tributaries. First, we examined the PCB and PBDE congener patterns of salmon to determine if POP pattern varied by Great Lakes basin, contaminant type, and tissue type. Second, within each basin, we compared PCB and PBDE congener patterns of brook trout and mottled sculpin to determine if consumer species identity influenced POP congener pattern. Lastly, we determined whether resident fish POP pattern was directly related to salmon spawner biomass. We predicted that (1) salmon PCB and PBDE congener patterns would differ among basins, reflecting differing history, sources, and extent of pollution; (2)
within each lake basin, resident brook trout and mottled sculpin in stream reaches with salmon would have congener patterns more similar to salmon than conspecifics in reaches lacking salmon; and (3) as the amount of salmon spawner biomass increased, the congener patterns of stream-resident fish would become more similar to salmon.

2.3 Methods

2.3.1 Sampling protocol

We sampled Pacific salmon and resident fish during fall 2008 and fall 2009 from tributaries to Lake Michigan, Lake Huron, and Lake Superior (Figure 2.1). Each tributary received a fall spawning run of Chinook and/or coho salmon, and supported a resident fish community that included brook trout and mottled sculpin (Table 2.1). We selected brook trout and mottled sculpin because of differences in life history related to foraging mode and orientation, trophic position, and diet. In general, Lake Superior streams had small runs of coho salmon while Lake Michigan and Huron streams had small to large runs of Chinook salmon with occasional coho salmon present. We limited our study to streams with runs of semelparous Pacific salmon because salmon deposit all POPs accumulated in their bodies into streams as gametes and carcasses. Salmon were collected during early October and resident fish were collected about one month after the spawning run peaked. When salmon were collected, salmon spawner density was estimated by counting all live and dead salmon in the wetted stream channel over a 300-m reach. Stream-resident fish were captured using backpack electrofishing in stream reaches with salmon and in reaches upstream of a barrier to salmon (e.g., dam or waterfall). Persistent organic pollutant patterns in fish above barriers were assumed to
reflect an alternate source of contamination such as atmospheric deposition while those below the barrier reflected salmon and non-point contaminant sources.

Figure 2.1. Location of study streams within the upper Laurentian Great Lakes. Site characteristics are provided in Table 2.1.

2.3.2 Contaminant analyses

Procedures for tissue homogenization, extraction and clean-up, gas chromatography/negative chemical ionization mass spectrometry analysis, lipid determination, and quality control criteria used for contaminant analyses are described in
The relationship between total POP concentrations in salmon and resident fish was described in a previous publication.\textsuperscript{14} For salmon samples, we analyzed whole fish and eggs separately for PCB and PBDE congener pattern while for resident species, we only considered whole fish POP pattern. Mottled sculpin samples were composites of two or more individuals to obtain sufficient mass (>20 g) for POP analysis. Fifty-eight PCB congeners representing penta-deca PCB homolog groups and six PBDE congeners representing tetra-hexa PBDE homolog groups were quantified. The PCB and PBDE congeners assessed reflect congener mixtures present in the environment and commonly used in monitoring PCB and PBDE levels in the Great Lakes (Figure 2.2, Figure A.1).
TABLE 2.1.

SITE CHARACTERISTICS AND MEAN (STANDARD ERROR) AND SAMPLE SIZE (N) OF PERSISTENT ORGANIC CONTAMINANTS LEVELS FOR SALMON AND STREAM-RESIDENT FISH COLLECTED IN THIS STUDY.

<table>
<thead>
<tr>
<th>Lake Basin</th>
<th>Site Location</th>
<th>Stream Name</th>
<th>Species</th>
<th>Biomass (kg m$^{-2}$)</th>
<th>PCB</th>
<th>PBDE</th>
<th>N</th>
<th>Resident Fish</th>
<th>PCB</th>
<th>PBDE</th>
<th>N</th>
<th>Resident Fish</th>
<th>PCB</th>
<th>PBDE</th>
<th>N</th>
<th>Resident Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michigan</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Pine Creek, MI</td>
<td>CHS</td>
<td>0.305</td>
<td>390.97 (34.57)</td>
<td>34.38 (3.99)</td>
<td>8</td>
<td>BKBT</td>
<td>289.81 (39.23)</td>
<td>22.99 (3.32)</td>
<td>4</td>
<td>2.08 (0.34)</td>
<td>0.70 (0.20)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>Kids Creek, MI</td>
<td>CHS</td>
<td>0.190</td>
<td>456.39 (73.85)</td>
<td>56.35 (12.03)</td>
<td>2</td>
<td>BKBT</td>
<td>44.63 (15.17)</td>
<td>5.48 (1.39)</td>
<td>4</td>
<td>4.48 (0.70)</td>
<td>1.23 (0.29)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>Deer Creek, MI</td>
<td>CHS</td>
<td>0.160</td>
<td>197.63 (32.47)</td>
<td>21.59 (3.71)</td>
<td>4</td>
<td>BKBT</td>
<td>3.08 (1.34)</td>
<td>1.51 (1.1)</td>
<td>4</td>
<td>2.35 (0.34)</td>
<td>0.97 (0.16)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Boyne River, MI</td>
<td>CHS, COS</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>BKBT</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>2.40 (0.32)</td>
<td>1.37 (0.83)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td></td>
<td>Mosquito River, MI</td>
<td>COS</td>
<td>0.002</td>
<td>36.55 (13.83)</td>
<td>7.72 (2.40)</td>
<td>5</td>
<td>BKBT</td>
<td>74.00 (16.33)</td>
<td>5.04 (1.61)</td>
<td>5</td>
<td>6.15 (1.33)</td>
<td>1.13 (0.23)</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Pendills Creek, MI</td>
<td>COS, CHS</td>
<td>0.006</td>
<td>45.74 (12.13)</td>
<td>11.81 (2.57)</td>
<td>7</td>
<td>BKBT</td>
<td>2.53 (0.68)</td>
<td>0.56 (0.09)</td>
<td>3</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Huron</td>
<td></td>
<td>Garden River, ON</td>
<td>COS</td>
<td>0.042</td>
<td>495.67 (16.25)</td>
<td>50.18 (0.16)</td>
<td>2</td>
<td>BKBT</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>2.70 (0.48)</td>
<td>0.58 (0.16)</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>Crystal Creek, ON</td>
<td>COS</td>
<td>0.003</td>
<td>51.47 (2.55)</td>
<td>9.76 (0.04)</td>
<td>2</td>
<td>BKBT</td>
<td>18.81</td>
<td>5.41</td>
<td>1</td>
<td>1.91 (0.34)</td>
<td>0.79 (0.16)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>Elliot Creek, MI</td>
<td>CHS, COS</td>
<td>0.080</td>
<td>164.62 (75.65)</td>
<td>17.01 (6.31)</td>
<td>5</td>
<td>BKBT</td>
<td>55.19 (16.31)</td>
<td>5.63 (1.47)</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Live salmon were not collected from the Boyne River, but Chinook salmon carcasses provided evidence of a salmon run. CHS=Chinook salmon, COS=coho salmon, BKBT=brook trout; MTS=mottled sculpin. Order indicates which salmon species was dominant during the run. NA=not available due to absence of live salmon (e.g., Boyne River), lack of an upstream barrier (e.g., Elliot Creek), or species not captured at the site. Biomass estimate was based on a survey of 300 m of stream length. For more detailed information on salmon and resident fish contaminant burden see Janetski et al. 2012.
2.3.3 Statistical analyses

Prior to statistical analysis, congener concentration was converted to congener relative abundance because our primary interest was in differences in congener patterns rather than absolute concentrations. To address our first two predictions, we used permutational multivariate analysis of variance (PERMANOVA) and non-metric multidimensional scaling (NMDS) to compare (1) POP congener pattern of salmon among Great Lakes basins and tissue types and (2) POP congener pattern of brook trout and mottled sculpin between stream reaches within each individual lake basin. To address our third prediction of a biomass-congener relationship, we used a non-linear Michaelis–Menten model to explore if POP congener pattern was a function of salmon spawner biomass and a likelihood ratio test (LRT) to assess species-specific differences. We added one to each NMDS axis value as an offset prior to model fitting to ensure no data points were negative. Michaelis–Menten models are commonly used to assess asymptotic relationships, and have been applied widely in physiology (e.g., enzyme kinetics) and fisheries ecology (e.g., spawner-recruitment relationships).
Figure 2.2. PCB congener patterns (mean percentage ± standard error) of salmon eggs and whole fish taken from lakes Michigan (eggs N=16, tissue N=28), Huron (eggs N=4, tissue N=9), and Superior (eggs N=7, tissue N=12). PCB congeners quantified were: Penta-(CB82, CB83, CB85, CB87, CB97, CB99, CB105, CB107/123, CB110, CB118, CB124), Hexa-(CB130, CB132/153, CB134, CB137, CB138, CB141, CB144, CB146, CB149, CB151, CB156, CB157, CB158, CB163, CB164, CB167), Hepta-(CB170, CB171, CB172, CB174, CB175, CB176, CB177, CB178, CB179, CB180, CB183, CB185, CB187, CB189, CB190, CB191, CB193), Octa-(CB194, CB195, CB196, CB197, CB199, CB200, CB201, CB202, CB203, CB205), Nona-(CB206, CB207, CB208), and Deca-(CB209).
Permutational multivariate analysis of variance is a multivariate, non-parametric statistical method that partitions variance in a distance matrix by calculating a distance-based F-statistic.28 All PERMANOVA models were analyzed using a matrix of Bray-Curtis distances calculated from the congener pattern data. Similar to a Monte Carlo simulation, one thousand permutations of the original data were conducted to generate p-values to assess factor significance. Bray-Curtis distances are considered a robust metric for assessing dissimilarity in ecological data.29 When significant differences were identified for factors of interest, pairwise multiple PERMANOVA procedures were conducted to identify differences among factor levels.28 We controlled the procedure-wise error rate using a Bonferroni correction where our desired alpha level (0.05) was divided by the number of pairwise comparisons considered with a particular model.28 This resulted in an alpha level of 0.005 for salmon and 0.006 for stream-resident fish POP congener pairwise comparisons (non-significant indicated by NS in text). For the statistical analyses, non-detectable concentrations were treated as zero. Although the concentration of contaminants in non-detectable samples is seldom zero, this artifact did not influence our results because we used non-parametric, rank-based analyses.29

We used NMDS ordinations, in conjunction with pairwise PERMANOVA, to further explore how POP congener pattern differed among species, stream reaches, and lake basins. Non-metric multidimensional scaling was used to identify clusters among factors of interest by capturing variation in congener pattern along the two axes that explained the highest proportion of variance. All NMDS scores were calculated from a matrix of Bray-Curtis distances, and solutions were solved iteratively from 1,000 random starts to avoid local minima and ensure that a convergent solution was reached that
minimized model stress. Data points with close proximity in ordination space indicate similarity in congener patterns, whereas data points far away in ordination space indicate dissimilarity in congener pattern composition. The NMDS goodness-of-fit was assessed using the stress statistic where values from 0 to 10 indicate a good fit, 10 to 20 a moderate fit, and those over 20 a poor fit. All data were analyzed using R version 3.0.3; PERMANOVA and NMDS were calculated using the R package VEGAN.

2.4 Results

2.4.1 POP patterns of salmon

Salmon PCB patterns differed among lake basins (PERMANOVA, P=0.001) and between egg and whole-fish samples (PERMANOVA, P=0.001; Fig. 2.2). Specifically, salmon in Lake Superior were distinct from salmon in lakes Michigan and Huron (Pairwise PERMANOVA, P=0.001; Fig. A.1, Table A.1). Salmon PCB patterns were dominated by congeners CB132, CB138, and CB118 across all basins. Higher proportions of congeners CB85, CB87, CB97, CB99, CB110, CB118, CB146, and CB149 in lakes Michigan and Huron contributed to the observed dissimilarity in congener patterns from Lake Superior (Figure 2.2). In lakes Michigan and Huron, salmon eggs differed from homogenized whole-fish samples (Pairwise PERMANOVA, P=0.001; Fig. A.1, Table A.1). In general, egg samples exhibited higher proportions of CB110 and CB118, and lower proportions of CB180, CB187, and CB199 when compared with whole-fish samples (Figure 2.2). PBDE patterns in spawning salmon differed among lake basins (PERMANOVA, P=0.008) and between tissue types (PERMANOVA, P=0.003; Fig. A.1, Table A.1). However, the difference in salmon PBDE patterns was driven solely by egg samples from Lake Superior, which had a higher proportion of congener BDE47.
than Lake Michigan (pairwise PERMANOVA, $P=0.004$, Table A.1). Overall, PBDE patterns were similar between tissue types and among lake basins (Figure A.2).

2.4.2 Lake Michigan stream-resident fish POP pattern

PCB patterns in resident fish from Lake Michigan tributaries differed between stream reaches (PERMANOVA, $P=0.001$) and fish species (PERMANOVA, $P=0.001$). Brook trout and mottled sculpin in stream reaches with salmon spawners were distinct from conspecifics in streams lacking salmon (pairwise PERMANOVA, $P=0.001$; Fig. 2.3.A, Table A.2). Furthermore, brook trout PCB patterns in streams with salmon were similar to salmon spawners, and NMDS scores in ordination space overlapped considerably [pairwise PERMANOVA, $P=0.020$ (NS with Bonferroni correction); Fig. 2.3.A, Table A.2]. In brook trout, this pattern was driven by elevated proportions of PCB congeners CB82, CB83, CB87, CB97, CB99, CB110, CB118, and CB138 and lower proportions of congeners CB206, CB207, CB208, and CB209. In contrast, mottled sculpin in reaches with salmon spawners exhibited a pattern that was distinct from salmon or brook trout (pairwise PERMANOVA, $P=0.001$; Fig. 2.3.A, Table A.2). PBDE patterns in resident fish differed between reaches with and without salmon spawners (PERMANOVA, $P=0.001$) and between fish species (PERMANOVA, $P=0.001$). Brook trout exposed to salmon had a PBDE congener pattern similar to salmon [pairwise PERMANOVA, $P=0.170$ (NS with Bonferroni correction); Fig. 2.3.D, Table A.3] and differed from conspecifics in areas without salmon spawners, which had lower proportions of BDE99 (pairwise PERMANOVA, $P=0.001$; Fig. 2.3.D, Table A.3). As with PCBs, PBDEs in mottled sculpin in reaches with salmon spawners exhibited a pattern that was distinct from salmon or brook trout (pairwise PERMANOVA, $P=0.001$;
Fig. 2.3.D, Table A.3). Overall, PBDEs varied less among species and locations than did PCBs.

2.4.3 Lake Huron stream-resident fish POP pattern

Resident fish PCB patterns in Lake Huron tributaries differed between stream reaches (PERMANOVA, P=0.001) and between fish species (PERMANOVA, P=0.001). Brook trout in stream reaches with salmon present exhibited a congener pattern similar to salmon spawners [pairwise PERMANOVA, P=0.040 (NS with Bonferroni correction); Fig. 2.3.B, Table A.2] and differed from conspecifics in streams lacking salmon spawners (pairwise PERMANOVA, P=0.001; Fig. 2.3.B, Table A.2). Brook trout in reaches with spawning salmon had elevated proportions of PCB congeners CB82, CB83, CB87, CB97, CB99, CB118, and CB138 and reduced proportions of congeners CB206-209. Similar to Lake Michigan, mottled sculpin PCB patterns were distinct from brook trout and salmon (pairwise PERMANOVA, P=0.004; Fig. 2.3.B, Table A.2). Low sample sizes of mottled sculpin precluded detailed interpretation of their PCB patterns between reaches with and without salmon. PBDE patterns in stream-resident fish also varied between reaches with and without salmon spawners (PERMANOVA, P=0.002) and by fish species (PERMANOVA, P=0.004). Brook trout PBDE pattern in reaches with salmon spawners were similar to salmon [pairwise PERMANOVA, P=0.169 (NS with Bonferroni correction); Fig. 2.3.E, Table A.3] and different from conspecifics in reaches without salmon (pairwise PERMANOVA, P=0.003; Fig. 2.3.E, Table A.3). Low sample sizes of mottled sculpin precluded detailed interpretation of their PBDE patterns between reaches. Overall, PBDE patterns were less distinct compared with PCBs.
2.4.4 Lake Superior stream-resident fish POP pattern

Brook trout and mottled sculpin in Lake Superior tributaries exhibited species-specific PCB patterns (PERMANOVA, P=0.001) that did not vary between stream reaches (PERMANOVA, P=0.080). Brook trout and mottled sculpin in stream reaches with salmon were similar to conspecifics in streams lacking salmon (pairwise PERMANOVA, P>0.05; Fig. 2.3.C, Table A.2). Overall, brook trout and mottled sculpin PCB congener patterns were more variable than salmon (Fig. 2.3.C). PBDE patterns in stream fish did not differ between reaches (PERMANOVA, P=0.100) but did differ among fish species (PERMANOVA, P=0.001). Brook trout and mottled sculpin in stream reaches with salmon were similar to conspecifics in streams lacking salmon (pairwise PERMANOVA, P>0.05; Fig. 2.3.F, Table A.3). Overall, the patterns for PBDEs were less distinct among species and locations when compared with PCBs.
Figure 2.3. Non-metric multidimensional scaling (NMDS) plots of PCB (A-C) and PBDE (D-F) pattern for salmon spawners and resident fish in stream reaches with and without salmon from lakes Michigan (A, D), Huron (B, E), and Superior (C, F). Brook trout with salmon present are gold circles. Brook trout with salmon absent are red circles. Mottled sculpin with salmon present are blue squares. Mottled sculpin with salmon absent are green squares. Pacific salmon are pink triangles. Ellipses represent 95% confidence limits of the mean and ellipses are the same color as corresponding data points.
2.4.5 Relationship between salmon biomass and resident fish POP pattern

Brook trout and mottled sculpin PCB NMDS axis-1 scores were positively related to salmon biomass, suggesting that with increased salmon inputs, resident fish congener patterns became more similar to salmon (Fig. 2.4). The Michaelis-Menton model indicated that brook trout and mottled sculpin PCB patterns exhibited a positive relationship that saturated at high salmon spawner biomass. Including species (i.e., brook trout, mottled sculpin) in the PCB model improved model fit and estimated species-specific asymptotes (LRT, F=14.31, P=0.001). This result suggests that brook trout and mottled sculpin exhibit different NMDS scores but respond similarly to salmon inputs. Similar to PCBs, PBDE NMDS axis-1 scores were positively related to salmon biomass and saturated at high salmon biomass (Fig. 2.4). Unlike for PCBs, including species as a model variable did not improve model fit (LRT, F=1.46, P=0.24) suggesting that brook trout and mottled sculpin exhibit a similar range of PBDE scores and respond similarly to increased salmon inputs.
2.5 Discussion

2.5.1 Salmon contaminant biotransport to Great Lakes tributaries

By conducting a detailed analysis of POP congeners, we found direct evidence that Pacific salmon are a source of POPs to resident fish in Great Lakes tributaries. Our results are consistent with studies in the native range of salmon\textsuperscript{9,10} but represent the first study in the introduced range to demonstrate directly that congener patterns of stream-resident fish reflect the influx of salmon-derived contaminants. Our two focal resident
species of interest, brook trout and mottled sculpin, exhibited contrasting POP congener patterns between stream reaches with and without spawning salmon. However, these two species also differed from each other in POP congener pattern, suggesting that brook trout and mottled sculpin assimilate POPs differentially. Past studies have demonstrated that congener pattern can vary between species due to trophic position and individual physiology.\textsuperscript{31,32,33} In addition, our study clearly demonstrated that salmon in the Great Lakes have basin-specific PCB but not PBDE patterns. This result suggests that source and mode of transport as integrated by salmon differs between these two POP contaminant types. Overall, our data suggest that the specificity and diversity of POP congeners in the environment may make them a more sensitive tracer. Our research highlights the need to assess factors related to both location and species when assessing the extent of contaminant biotransport and transfer by Pacific salmon in the Laurentian Great Lakes.\textsuperscript{cf. 34}

2.5.2 Influence of environmental context on contaminant biotransport by Pacific salmon

Environmental context shapes the ecological role of salmon in stream ecosystems in their native\textsuperscript{34} and introduced\textsuperscript{35} ranges, and influences the pattern of contaminant dispersal in the Great Lakes. In particular, the muted response of stream-resident fish congener pattern to coho salmon inputs in Lake Superior tributaries reflects the interaction among lower historical inputs of pollutants,\textsuperscript{2,5} lower contaminant concentrations in salmon,\textsuperscript{14} and smaller salmon runs. In contrast, contaminant transport by Chinook salmon in Lake Michigan is magnified by their larger body size, higher contaminant burden, higher lipid content, and larger run sizes.\textsuperscript{14,36} In Lake Huron, we found evidence of contaminant transfer by salmon to stream fish, but large reductions in
salmon populations following an ecosystem-level change in food web structure appear to have reduced the risk of future contaminant biotransport by Pacific salmon. Thus, basin-specific differences among lakes Michigan, Huron, and Superior have the potential to influence the extent of contaminant biotransport to stream tributaries and transfer to stream-resident fish.

In the Lake Michigan basin, brook trout PCB and PBDE congener patterns closely reflected those of spawning salmon. Therefore, brook trout appear to be an important sentinel organism for assessing the extent of salmon-mediated contaminant transport. Brook trout are opportunistic stream predators that forage on aquatic and terrestrial insects, crustaceans, and fish. Similar to resident char (Salvelinus alpinus, S. malma) and grayling (Thymallus arcticus) in the native range of salmon, brook trout readily consume salmon eggs when they are available. This dietary plasticity can be directly linked to increased body POP concentrations via increased salmon egg consumption, trophic position, and salmon-mediated contaminant inputs. Within their native range, salmon spawner density and redd superimposition have been identified as key factors regulating the availability of resource subsidies (e.g., salmon eggs) to resident trout. In Lake Michigan tributaries, where stream sediments are relatively small and prone to disturbance, higher densities of spawning salmon increase egg availability for consumption by resident fish, and subsequent bioaccumulation of salmon-derived pollutants. This hypothesis of egg-mediated, pollutant transfer is supported by the increased similarity in brook trout congener patterns compared with salmon as salmon spawner biomass increases then saturates at higher spawner abundances. Similarly, resident fish stable isotope signatures reached an asymptote at high salmon spawner
densities in their native range. Taken together, these results indicate that an upper limit may exist which restricts the ability of stream-resident fish to bioaccumulate contaminants transported by salmon.

We found that mottled sculpin exhibited a PCB pattern that was distinct between stream reaches yet different from Pacific salmon or brook trout PCB patterns in the Lake Michigan basin. Mottled sculpin are small benthic fish with a relatively large gape that forage on salmon material, including eggs, when available. The dissimilarity in congener patterns between mottled sculpin and salmon coupled with differences in sculpin patterns between stream reaches with and without salmon spawners suggests that PCBs are expressed or accumulated differently in mottled sculpin when compared with brook trout. In addition, we found that mottled sculpin congener patterns exhibited less variation at high salmon spawner abundance, likely reflecting the incorporation of salmon into their diet. Previous research has shown that mottled sculpin have elevated POP concentrations in stream reaches receiving salmon runs, but a lower contaminant burden overall when compared to brook trout. These reach-specific differences suggest that salmon, in part, mediate this relationship. Many fish species assimilate POPs differently due to diet, trophic position, and individual physiology, which can lead to differential expression of POP patterns. For example, deepwater sculpin (Myxocephalus thompsonii) possess the unique ability to form methyl sulfone metabolites capable of reducing PCB body burdens by 10%.

2.5.3 Pacific salmon as integrators of basin-specific contamination

We found a strong contrast in PCB congener patterns of salmon between lakes Superior and Michigan. These differences reflect the extent of historical pollution,
differences in salmon species composition, and variation in salmon abundance among lake basins. Historically, Lake Michigan received 15 times the direct input of PCBs compared with Lake Superior, and continues to receive larger atmospheric inputs of POPs, especially at the southern terminus of Lake Michigan, along with higher rates of sediment re-suspension. Together, these factors contribute to greater availability of PCBs for uptake and bioaccumulation by Pacific salmon in Lake Michigan, resulting in the difference in PCB congener pattern among basins.

Similar to PCBs, PBDE concentrations are highest in Lake Michigan and lowest in Lake Superior. However, PBDE congener patterns were nearly identical among basins, suggesting that both basins are contaminated by atmospheric sources. Overall, our results are consistent with previous research that has identified BDE-47 as the primary PBDE congener associated with Pacific salmon from Lake Michigan. However, our samples had a much higher proportion of BDE-100 when compared with other published values. This difference may reflect changes in PBDE deposition patterns following legislative restrictions and bans that have reduced use. Overall, the congener pattern of salmon indicated that PCBs and PBDEs enter the environment by different mechanisms and from different sources that contribute to the contrasts observed among lake basins.

The similarity in PCB patterns of salmon in lakes Michigan and Huron suggests substantial inter-basin movement of fish during their open water life stage. Chinook salmon populations in Lake Huron collapsed in 2004 following a whole-ecosystem shift in food web structure due to changes in both bottom-up and top-down processes. This food web collapse led to the near extirpation of alewife (Alosa pseudoharengus) in Lake
Huron, the primary prey species for Chinook salmon in the upper Great Lakes.\textsuperscript{18} Such changes were important because levels of PCBs in upper trophic level fish are strongly affected by diet and the PCB congener pattern can be considered an integration of contamination from primary diet sources.\textsuperscript{4} Thus, the PCB congener patterns suggest that Lake Huron salmon move to Lake Michigan to forage where alewife are currently more abundant.\textsuperscript{18} Such behavior is consistent with large-scale mark-recapture studies that have found significant unidirectional movement of Chinook salmon from Lake Huron to Michigan in response to changing prey densities.\textsuperscript{18,48}

2.5.4 POPs as ecological tracers

Researchers increasingly are using chemical tracers, such as stable isotopes, to document flow of nutrients, energy, and contaminants through food webs.\textsuperscript{49} In ecology and ecotoxicology, different tracers can be useful in tracking the protein, lipid, and carbohydrate fractions in organisms to offer more complete profiles of consumer resource use.\textsuperscript{49} For instance, POPs generally track the lipid fraction of tissue,\textsuperscript{3} while heavy metals such as mercury are associated with the protein fraction of tissue.\textsuperscript{50} Non-traditional tracers, such as POPs, can offer additional insight when assessing ecosystem linkages and resource use when isotopic differentiation is not sufficient to identify prey items or when isotope data are not available. Pollutants have been used to document the export of POPs from streams via emergent insects,\textsuperscript{51} to assess the food web implications of heavy metal transport by colonial nesting birds,\textsuperscript{50} and to identify differences in foraging areas used by Atlantic salmon.\textsuperscript{32} Similarly, our data suggest that POP congener patterns can be used to assess interactions between migratory salmon and resident fish, infer basin-scale movement patterns, and reveal differential transport dynamics of PCBs and PBDEs.
However, much like stable isotopes, many factors influence pollutant patterns including habitat-specific foraging, location-specific contamination, individual physiology, and life history attributes (e.g., age, gender, body size) that can make the interpretation of tracer data challenging.\textsuperscript{49,52} While not perfect, POPs offer an additional tool for establishing pathways by which energy and contaminants move through and between ecosystems. In addition, POP data are routinely collected by state and federal agencies to inform consumption advisories across broad geographical areas and river networks. Large pollutant datasets, such as the US EPA Great Lakes Environmental Database (GLENDA, https://catalog.data.gov/dataset/great-lakes-environmental-database-glenda), could be leveraged in ecological and ecotoxicological studies to evaluate factors that influence contaminant concentration and pattern. When possible, future studies should incorporate both traditional isotope approaches and other non-traditional tracers such as POPs or heavy metals to help elucidate pathways of uptake and assimilation of resource subsidies.\textsuperscript{49,52}

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2.7 Literature Cited


3.1 Abstract

The extent to which environmental context mediates the uptake of biotransported contaminants by stream-resident organisms is not understood. For example, no clear understanding exists of the extent to which contaminant type, instream characteristics, or resident fish identity interact to influence the uptake of contaminants deposited by Pacific salmon (*Oncorhynchus* spp.) during their spawning runs. To address this uncertainty, we sampled four stream-resident fish species from 13 watersheds of the Laurentian Great Lakes in locations with and without salmon across a gradient of instream and watershed characteristics. We determined the polychlorinated biphenyl (PCB) and mercury (Hg) concentration along with the stable isotope ratio of carbon and nitrogen for each stream-resident fish. We found that stream-resident fish PCB concentrations were 24-fold higher in reaches with salmon and were positively related to δ<sup>15</sup>N. In contrast, stream-resident fish Hg concentrations were similar or lower in reaches with salmon and either exhibited

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a negative or no relationship with $\delta^{15}$N. Based upon AIC$_c$, stream-resident fish exhibited species-specific PCB concentrations that were positively related to salmon PCB flux. In addition, Hg burdens exhibited an interaction between fish length and salmon Hg flux; as salmon Hg inputs increased, Hg levels decreased with increasing resident fish length. We found no support for models that included the mediating influence of instream or watershed factors. Our results may be driven by the consumption of salmon eggs by stream-resident fish. Widespread egg consumption was observed for stream-resident fish; salmon eggs are enriched in PCBs but have very Hg concentrations. Our results highlight that contaminants bioaccumulate differently depending on contaminant type, species identity, and the trophic pathway of contamination. Consequently, consideration of the recipient food web and route of exposure is critical to understanding the fate of biotransported contaminants in ecosystems. Effective management of biotransported contaminants will require the establishment of threshold concentrations, delineated “hot-spots” of biotransport, and implementation of best management practices in watershed receiving contaminants from spawning salmon.

3.2 Introduction

Animal migrations are important ecological phenomena responsible for the transport of nutrients and energy across ecosystem boundaries (Bauer & Hoye, 2014). These predictable pulses of material strongly influence the structure and function of reciprocal habitats (Polis et al., 2004, Lamberti et al., 2010), mediating ecosystem stability and community biodiversity (Bauer & Hoye, 2014). In addition to these positive effects, migratory animals can also be a dispersal agent by which contaminants are relocated to new ecosystems (Blais et al., 2007). Pacific salmon (Oncorhynchus spp.)
provide a compelling example of animals controlling the flow of material to recipient ecosystems (Krummel et al., 2003, Schindler et al., 2003). Salmon are effective as both a resource subsidy and contaminant biovector because they exhibit a semelparous and anadromous life-history strategy, consequently, salmon deposit vast quantities of nutrient-rich but potentially contaminated carcass and gametic tissues in locations where they spawn and die (Gerig et al., 2016, Janetski et al., 2012). Salmon-derived material is then consumed by a variety of organisms, including stream-resident fish (Schindler et al., 2003, Janetski et al., 2009). However, as with all pulsed nutrient subsidies, the availability of salmon varies considerably across space and time due to environmental heterogeneity (Janetski et al., 2009).

Freshwater ecosystems exhibit extensive spatial variation in abiotic and biotic characteristics (Poff & Ward, 1990). This variability, or *environmental context*, can directly control community structure and ecosystem function (Poff & Ward, 1990). In streams receiving salmon spawners, this context can determine the broader influence of salmon (see meta-analysis by Janetski et al., 2009). For instance, background nutrient levels and stream sediment size determine the response of streams to nutrients and energy supplied by salmon (Janetski et al., 2014). Spawner biomass determines the amount of nutrients delivered and the disturbance imparted to the stream but background nutrient levels influence the magnitude of enrichment (Chaloner et al., 2004, 2007), while sediment size determines the susceptibility to disturbance (Moore et al., 2004, Tiegs et al., 2008). Environmental context may similarly influence the delivery and bioaccumulation of contaminants biotransported by Pacific salmon.
Environmental context in large part determines how contaminants bioaccumulate in freshwater food webs. Clements et al., (2012) presented a conceptual model, based upon the relationship between the nitrogen (N) stable isotope ratio and contaminant concentration of an organism, to understand food web bioaccumulation. This model reflects three potential mechanisms for how bioaccumulation varies among aquatic food webs. First, inputs of contaminants can differ among food webs as a result of landscape or instream factors, which influence contaminant bioavailability and bioaccumulation. Second, rates of food web bioaccumulation can differ as a function of variation in physicochemical variables, species-specific physiology, or species-specific behavior, which influences the extent and magnitude of bioaccumulation. Third, longer food chain length, reflecting differences in community structure, can increase bioaccumulation in food webs. Since contaminant concentrations are generally positively related to $\delta^{15}N$ (Cabana et al., 1994, Lavoie et al., 2013), drivers of bioaccumulation can be compared among food webs or among fish communities to elucidate factors influencing bioaccumulation. However, whether environmental context is a critical driver of salmon-mediated contaminant biotransport, in particular, is unknown.

The Laurentian Great Lakes serve as a unique setting to study the role of context on salmon-mediated contaminant biotransport. Pacific salmon were established in the Great Lakes in the 1960s to control invasive alewife populations (*Alosa pseudoharengus*) and rehabilitate predator populations decimated by invasive sea lamprey (*Petromyzon marinus*, Crawford 2001, Dettmers et al., 2012). Since then, salmon have become an important component of the multi-billion dollar Great Lakes recreational fishery (Dettmers et al., 2012). At present, salmon have established naturally reproducing
populations in many Upper Great Lakes tributaries (Kerns et al., 2016), while still being intensively managed by state resource agencies (Tsehaye et al., 2014). As a result, resource managers are concerned about factors that might compromise the sustainability and value of the fishery, such as the bioaccumulation of harmful contaminants (Stow et al., 1995, Murphy et al., 2012). Contaminants are of particular concern in the Great Lakes, which have an extensive legacy of industrial pollution, and include numerous Areas of Concern and EPA Superfund sites (Murphy et al., 2012). Hence, migratory species such as salmon that move between lake and stream environments represent a link for transferring contaminants between ecosystems. While transfer of salmon-derived persistent organic pollutants (POPs) to stream biota has been documented in the Great Lakes (e.g., Gerig et al., 2016, Janetski et al., 2012), the significance of salmon transfer of mercury (Hg), or how physicochemical and biological variables mediate the transfer and uptake of biotransported contaminants in general, is largely unknown.

Environmental context may play a key role in regulating the response of stream-resident fish communities to biotransported contaminants. Four processes may mediate interactions between environmental context and the uptake of salmon-derived contaminants by resident fish. First, watershed characteristics may modify background contaminant levels (Jardine et al., 2012, King et al., 2004), thereby minimizing or maximizing the accumulation of biotransported contaminants by stream-resident fish. Second, instream habitat (e.g. substrate type, large woody debris) may enhance the retention of salmon carcasses and eggs, thereby increasing the time that contaminated salmon material is available for direct consumption by stream-resident fish or indirect incorporation into the food web (Chaloner et al., 2002, Lisi et al., 2013). Third, species-
specific biological attributes of stream-resident fish may lead to differential contaminant uptake resulting from variation in diet, physiology, or trophic position (Clements et al., 2012). Last, salmon run characteristics may directly influence the quantity of salmon material available for direct uptake by resident fish (Gregory-Eaves et al., 2007, Janetski et al., 2012). Hence, interactions between salmon and environmental context may control the uptake of biotransported contaminants by resident fish, both where salmon are native and where they have been introduced, such as in the Laurentian Great Lakes.

In this study, we evaluated whether environmental context mediates the transfer and uptake of salmon-derived contaminants to stream-resident fish across watersheds of the upper Laurentian Great Lakes. We first quantified PCB and Hg concentrations in tissue and gametes of Pacific salmon spawners, the putative source of contaminants. Second, we assessed the uptake of salmon derived resources by stream-resident fish using stable isotope ratios of nitrogen ($\delta^{15}$N) and carbon ($\delta^{13}$C). Third, we related stream-resident fish $\delta^{15}$N to PCB and Hg concentrations to compare rates of contaminant bioaccumulation between locations with and without salmon. Last, we used an information theoretic approach to assess the mediating influence of various components of the environmental context on the relationship between salmon contaminant flux and stream-resident fish contaminant concentrations. We hypothesized, first, that salmon would accumulate high concentrations of both PCBs and Hg given contaminant levels in the Great Lakes. Second, we predicted that stream-resident fish in locations with salmon would exhibit isotopic enrichment reflecting the influence of salmon, given the documented response of fish to salmon runs elsewhere. Third, we hypothesized that stream-resident fish would exhibit a positive relationship between $\delta^{15}$N and contaminant
concentration reflecting salmon as a significant source of both PCB and Hg, given the known relationship between δ¹⁵N and contaminants. Last, we expected that attributes of environmental context, reflecting biological, chemical, and physical variables, will mediate the transfer of salmon-derived contaminants to stream-resident fish, given that environmental context has been shown to mediate the ecological influence of salmon on stream ecosystems.

3.3 Methods and materials

3.3.1 Study sites

We sampled Pacific salmon and stream-resident fish during fall of 2013 and 2014 from 13 watersheds in the Lake Michigan and Lake Huron drainage basins of the Upper Great Lakes (Fig. 3.1, Table 3.1). Within each watershed, we selected one 300-m stream reach accessible (salmon reach) and one 300-m reach inaccessible (reference reach) to Pacific salmon (cf. Chaloner et al., 2004, Janetski et al., 2012). Reference reaches were located in adjacent streams within the same watershed or upstream of dams within the same stream that served as salmon barriers. Reference sites were intended to control for non-salmon pollutant inputs. Sites represented a gradient of salmon spawner densities, stream characteristics, and landscape conditions representative of streams throughout the study area (Table B.1). Each tributary accessible to salmon received a fall spawning run of Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon. Chinook salmon were the predominant spawners in streams accessible to migratory fish. We sampled fish species representative of the cold-water fish community of upper Great Lakes streams, including brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*),
mottled sculpin (Cottus bairdii), and rainbow trout (Oncorhynchus mykiss), although they were not always present at each sampling location (Table 3.1). All fish were collected using standard fisheries techniques including backpack electrofishing (cf. Janetski et al., 2012, Pepino et al., 2012). Salmon were collected during the peak of the run in early-mid October and resident fish were collected 45-60 days after the spawning run peaked.

Figure 3.1. Locations of 13 watersheds sampled to evaluate the mediating role of environmental context on salmon contaminant biotransport. See Table 3.1 and Table B.1 for watershed covariates.
TABLE 3.1.

SITE CHARACTERISTICS OF WATERSHEDS SAMPLED TO EVALUATE THE EFFECT OF PACIFIC SALMON CONTAMINANT BIOTRANSPORT ON STREAM-RESIDENT FISH.

<table>
<thead>
<tr>
<th>Watershed</th>
<th>Basin</th>
<th>Watershed Area (km$^2$)</th>
<th>Salmon Biomass (kg/m$^2$)</th>
<th>PCB Flux (ng/m$^2$)</th>
<th>Hg Flux (ng/m$^2$)</th>
<th>Resident Species</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albany</td>
<td>Huron</td>
<td>103.26</td>
<td>0.15</td>
<td>5.74</td>
<td>8.09</td>
<td>BKT, MTS</td>
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<td>Betsie</td>
<td>Michigan</td>
<td>118.73</td>
<td>0.18</td>
<td>100.34</td>
<td>40.79</td>
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<td>Black</td>
<td>Michigan</td>
<td>86.64</td>
<td>0.02</td>
<td>7.63</td>
<td>5.81</td>
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</tr>
<tr>
<td>Boardman</td>
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<td>Boyne</td>
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<td>Carp</td>
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<td>0.01</td>
<td>2.06</td>
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<td>89.99</td>
<td>0.08</td>
<td>34.64</td>
<td>19.03</td>
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</tr>
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<td>Elliot</td>
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<td>Kalamazoo</td>
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<td>54.03</td>
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<td>14</td>
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<td>Manistee</td>
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<td>291.17</td>
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<td>14</td>
</tr>
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</table>

Note: Sample size refers to the total number of stream-resident fish sampled. BKT=brook trout, BNT= brown trout, RBT=rainbow trout, MTS=mottled sculpin. The order of resident species present reflects abundance in each watershed.
3.3.2 Environmental site characterization

A suite of biological, chemical, and physical variables was measured during stream habitat surveys conducted in September 2014 (Table B.1). To determine watershed characteristics, a Geographic Information System (GIS) was used to measure watershed land cover (% Forested, % Wetland, % Developed) and watershed area (km², Table S1) from the National Land Cover Database 2011 using the HUC-12 watershed delineation (Homer et al., 2015). Additional watershed attributes were extracted from state geodatabases (e.g., Michigan Geographic Data Library, www.mcgi.state.mi.us/mgdl/). Instream habitat was assessed using both instream and GIS-based metrics (Table B.1). For instream measurements, we established 30 lateral transects within each 300-m sampling reach. At each transect, we randomly measured five substrate particles for size and assessed the total volume of large wood (m³/m²) that intersected the lateral transect (Lamberti & Gregory, 2006). In addition, physicochemical variables were measured at three locations within each reach using a multi-parameter sonde (YSI, Yellow Springs, OH). At one location per reach, we collected filtered water samples (n=3) to determine dissolved inorganic nutrient concentrations. Stream gradient (m/km) was derived from a digital elevation model related to stream flow path in GIS. Water temperature was estimated from a regional groundwater model for mean July stream temperatures in Michigan (Wang et al., 2011). To assess biological context, we measured attributes related to both the salmon run and individual stream-resident fish. For salmon, we estimated salmon spawner density by counting all live and dead salmon in the wetted stream channel within each 300-m sampling reach accessible to salmon (cf. Chaloner et al., 2004, 2007). We multiplied counts of live and dead salmon by the mean
salmon weight and mean salmon contaminant load in a given stream to estimate the total flux of contaminants delivered by salmon to the stream (cf. Janetski et al., 2012). For stream-resident fish, biometric data were measured, including species identity, length, and weight. Fish condition was determined from length and weight measurements (Bervoets & Blust, 2003).

3.3.3 Analytical chemistry

To determine stream nutrient status, water samples collected from each stream reach, filtered through a Whatman GF/F (0.7 μm) into polyethylene bottles, and stored frozen at -20 °C for later laboratory analysis of soluble reactive phosphorus (SRP), nitrate (NO$_3^-$-N), and ammonium (NH$_4^+$-N) (cf. Tiegs et al., 2008). A Lachat QC8500 Flow Injection Autoanalyzer (Lachat Instruments, Loveland, CO) was used to determine SRP, NO$_3^-$-N, and NH$_4^+$-N concentrations using standard methods. DOC samples were filtered through a Whatman GF/F (0.7 μm) into amber glass bottles, immediately acid stabilized with hydrochloric acid, and later measured on a Shimadzu TOC-5000 (Shimadzu Corp., Tokyo, Japan) at the Center for Environmental Science and Technology (CEST) of the University of Notre Dame.

Polychlorinated biphenyl (PCB) concentrations of homogenized whole fish samples were determined for Pacific salmon and stream-resident fish using EPA method 1668 (USEPA Method 1668). Individual PCB congeners were quantified using an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a micro–electron capture detector. Instrumental conditions were previously described (Stapanian et al., 2013). This method determined a total of 89 PCB congeners. Total PCB concentration was calculated by summing the concentration of all 89 congeners and
reported as ng/g wet weight. The instrument was calibrated with individual congener standards at 5 concentration levels (beginning at 0.10 ng/g) from AccuStandard (New Haven, CT). The West Coast Fish Studies standard (AccuStandard) was analyzed for calibration verification. Method blanks were run at a frequency of 1 per 20 samples, and the mean concentration (± SE) was 0.41 (± 0.08 ng/g) ng/g. Matrix spikes and matrix-spiked duplicates were also performed at a 5% frequency, with mean recovery and mean relative percentage difference equal to 81% (± 5%) and 11% (± 3%), respectively. Surrogate recoveries averaged 86 ± 9%. Detection limits for the individual congeners were set at 3 times the baseline noise (=0.01 ng/g). Detection limits were verified by analyzing a low-level standard at 0.03 ng/g, which yielded a signal to noise ratio between 12 and 15. See Table B.2 for a list of PCB congeners measured.

Mercury concentrations of homogenized whole fish samples for salmon and stream-resident fish were determined using a Direct Mercury Analyzer 80 (DMA-80, Milestone S.r.l., Sorisole, Italy), located at CEST. Prior to analysis, all samples were freeze-dried, homogenized into a fine powder, and stored at -20°C (cf. Abma et al., 2015). For analysis, 0.02 g of homogenized sample was weighed into ashed nickel boats, placed into the DMA-80, and analyzed via fixed wavelength atomic absorption spectrophotometry (cf. Abma et al., 2015). The DMA-80 was calibrated using standard reference materials (National Research Council of Canada, DORM-4, Ottawa, ON). Dry weight Hg concentration was converted to wet weight concentrations using the percent water content of each homogenized sample and expressed as ng/g wet weight. Quality control measures, including blanks, matrix spikes, matrix spiked duplicates, and
standards were analyzed to ensure precision and accuracy of analyses. Percent recovery from DORM-4 standard was 100.6 ± 6.8% and the detection limit was 0.2 ng/g.

Carbon and Nitrogen stable isotope and C:N ratios were measured for homogenized whole body samples of salmon and stream-resident fish using an Elemental Analyzer (Costech, Valencia, CA) coupled to a Delta Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Waltham, MA) located at CEST. All samples were prepared for isotope analysis in the same manner as for Hg analyses. Isotope ratios were corrected within each individual run using a 3-point standard curve developed from known isotope standards (EA Consumables, Pennsauken, NJ). Standards used to develop the standard curve included wheat flour, sorghum, and protein. Stable isotope ratios ($\delta^{15}N$, $\delta^{13}C$) were expressed as:

$$\delta^{15}N\ or\ \delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 1000$$

(Equation 3.1)

where $R$ is the ratio of $^{15}N$ to $^{14}N$ or $^{13}C$ to $^{12}C$. Tissue C:N data was determined using an acetanilide standard (cf. Chaloner et al., 2002, Reisinger et al., 2013). Data were deemed acceptable if the standard deviation of acetanilide standards during the run was <0.2 per mil ($\%o$). The standard deviations for acetanilide standards were 0.14 and 0.09$\%o$ for N and C, respectively. All $\delta^{13}C$ values were lipid-corrected using individual C:N ratios (cf. Post et al., 2007).

3.3.4 Statistical analyses

Two-way analysis of variance (ANOVA, $\alpha = 0.05$, Zar, 2010) was used to analyze salmon contaminant concentrations. The main factors were (1) contaminant type (PCB or Hg), and (2) tissue type (whole fish or eggs). We interpreted a significant interaction as evidence that contaminant accumulation was dependent on the contaminant and tissue
type combination under consideration. Two-way ANOVA was also used to determine whether stream-resident fish δ\textsuperscript{15}N and δ\textsuperscript{13}C differed between the factors of location (salmon present or absent) and stream-resident species identity (brook trout, brown trout, mottled sculpin, rainbow trout). Analysis of covariance (ANCOVA) was used to assess whether the relationship between stream-resident fish concentrations and δ\textsuperscript{15}N ratio was influenced by the factors of location (salmon present or absent), species identity (brook trout, brown trout, mottled sculpin, or rainbow trout), or contaminant type (PCB or Hg) (cf. Clements et al., 2012). An interaction between salmon presence, species identity, and contaminant type was used as the justification to conduct individual ANCOVAs for each species-contaminant combination. Assumptions of ANOVA and ANCOVA were assessed visually using plots of residuals (Zar, 2010), and all data was log-transformed prior to statistical analysis due to heteroscedasticity. All statistical analyses were performed using the R software platform (https://cran.r-project.org/).

To assess whether uptake of salmon-derived contaminants by stream-resident fish was influenced by environmental context, we used a generalized linear mixed modeling (GLMM) approach coupled with Akaike Information Criterion with correction for small sample sizes (AIC\textsubscript{c}, cf. Johnson & Olmland, 2004, Bolker et al., 2009). For each model, stream-resident fish contaminant concentration was considered the response variable and predictor variables included instream variables (substrate size, coarse wood debris volume, stream gradient, mean water temperature, DOC concentration, nutrient concentrations [NO\textsubscript{3}−-N, NH\textsubscript{4}+ -N, SRP], pH), watershed characteristics (percent forested, percent wetland, percent urban, watershed area), and biological variables (length, condition, δ\textsuperscript{15}N, δ\textsuperscript{13}C). In all models, watershed was treated as a random effect to account
for non-independence of fish sampled within the same watershed (cf. Swain et al., 2014). Stream-resident fish contaminant concentrations were log-transformed prior to analysis (cf. Bolker et al., 2009). We evaluated the relative support for GLMMs using an automated model selection procedure that solved all permutations of a global model (model fit with all predictor variables, Barton, 2012). To avoid overparameterization, we restricted our analysis by including a maximum of 4 predictor variables per model and only tested the interaction between salmon contaminant flux and individual variables related to instream, watershed, and biological characteristics (cf. Swain et al., 2013). Model performance and uncertainty were assessed $\text{AIC}_c$, which ranks models based upon the principle of parsimony (Burnham & Anderson, 2002). The lower the $\text{AIC}_c$ score for a given model, the better the trade-off in complexity and more optimal the fit between the model and data. Distinguishing between high-ranking models based on $\text{AIC}_c$ values alone can be difficult, so we calculated model-averaged parameter estimates and relative variable importance (sum of $\text{AIC}_c$ weights from all models containing a variable of interest) for fixed effects from models with a $\Delta \text{AIC}_c$ of less than 10 (Bolker et al., 2009). Last, we calculated a pseudo-$R^2$ for all models with $\Delta \text{AIC}_c$ less than 10; this metric represents the variance explained by fixed and random effects, and provides complimentary inference to the AIC analysis (Barton, 2012). Model selection was performed using the MuMIn package in the R software platform (Barton, 2012; https://cran.r-project.org/).
3.4 Results

3.4.1 Contaminant source: salmon isotope and contaminant patterns

Pacific salmon eggs and tissue had elevated $\delta^{15}$N and $\delta^{13}$C relative to stream-resident fish, irrespective of location (ANOVA, $\delta^{15}$N $p<0.001$, $\delta^{13}$C $p<0.001$, Table 3.2). Contaminant accumulation in Pacific salmon exhibited a strong interaction between contaminant and tissue type (ANOVA, $p<0.001$, Fig. 3.2). Salmon eggs had 1.2-fold higher PCB concentrations relative to whole body concentrations. In contrast, Hg concentrations in salmon eggs were 15-fold lower than were whole body Hg concentrations. Overall, our results suggest that contaminant accumulation in salmon is dependent on the contaminant and tissue type considered.

Figure 3.2. PCB and total Hg concentrations (ng/g wet weight) in whole body and egg samples of spawning Chinook and Coho salmon collected from tributaries of the Upper Great Lakes.
3.4.2 Contaminant recipient: stream-resident fish isotope and contaminant patterns

The isotopic composition of stream-resident fish varied among species and between locations with and without salmon. Stream-resident fish in locations accessible to salmon exhibited higher isotope values than conspecifics in locations without salmon (ANOVA, δ\(^{15}\)N p<0.001, δ\(^{13}\)C p<0.001, Table 3.2). On average, brook trout, rainbow trout, and brown trout exhibited isotopic enrichment of 0.7‰, 1.8‰, and 1.8‰, respectively, for δ\(^{15}\)N, and 1.5‰, 1.8‰, and 1.8‰, respectively, for δ\(^{13}\)C, in locations with salmon compared to those without salmon. In contrast, mottled sculpin exhibited smaller increases of 0.2‰ for δ\(^{15}\)N and 0.5‰ for δ\(^{13}\)C in locations having salmon spawners.

### TABLE 3.2.

STREAM-RESIDENT FISH ISOTOPIC COMPOSITION IN LOCATIONS WITH AND WITHOUT PACIFIC SALMON.

<table>
<thead>
<tr>
<th>Species</th>
<th>Salmon Presence</th>
<th>δ(^{15})N</th>
<th>δ(^{13})C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brook trout</td>
<td>Salmon Absent</td>
<td>8.0 (0.26)</td>
<td>-27.8 (0.45)</td>
</tr>
<tr>
<td></td>
<td>Salmon Present</td>
<td>8.7 (0.22)</td>
<td>-26.3 (0.50)</td>
</tr>
<tr>
<td>Brown trout</td>
<td>Salmon Absent</td>
<td>8.5 (0.22)</td>
<td>-27.3 (0.24)</td>
</tr>
<tr>
<td></td>
<td>Salmon Present</td>
<td>10.3 (0.18)</td>
<td>-25.5 (0.33)</td>
</tr>
<tr>
<td>Mottled sculpin</td>
<td>Salmon Absent</td>
<td>8.4 (0.22)</td>
<td>-28.2 (0.53)</td>
</tr>
<tr>
<td></td>
<td>Salmon Present</td>
<td>8.6 (0.26)</td>
<td>-28.8 (0.63)</td>
</tr>
<tr>
<td>Rainbow trout</td>
<td>Salmon Absent</td>
<td>7.3 (0.23)</td>
<td>-27.6 (0.15)</td>
</tr>
<tr>
<td></td>
<td>Salmon Present</td>
<td>9.0 (0.39)</td>
<td>-25.9 (0.39)</td>
</tr>
<tr>
<td>Pacific salmon whole body</td>
<td>Salmon Absent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Salmon Present</td>
<td>11.1 (0.07)</td>
<td>-23.1 (0.05)</td>
</tr>
<tr>
<td>Pacific salmon eggs</td>
<td>Salmon Absent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Salmon Present</td>
<td>11.7 (0.13)</td>
<td>-24.5 (0.06)</td>
</tr>
</tbody>
</table>

Note: All values represented as mean (standard error).

The relationship between stream-resident fish contaminant concentration and δ\(^{15}\)N ratio was dependent upon salmon presence, species identity, and contaminant type (ANCOVA, p<0.001, Fig. 3.3). As predicted, stream-resident fish PCB concentration was
positively related to $\delta^{15}$N (Fig. 3.3, Table 3.2). However, only brown trout exhibited a
significant positive interaction between $\delta^{15}$N and salmon presence (Fig. 3.3, Table 3.2).
Mean stream-resident fish PCB concentrations in locations with salmon were $205.4 \pm 
23.4$ ng/g (mean $\pm$ SE) compared to $7.4 \pm 0.5$ ng/g in locations without salmon. The
magnitude of this effect differed strongly among species (Table 3.2). Brown trout were
57-fold, rainbow trout were 29-fold, brook trout were 18-fold, and mottled sculpin were
8-fold more contaminated with PCBs in locations with salmon runs (Fig. 3.3).

Contrary to PCBs, we found no consistent relationship between Hg concentration
and $\delta^{15}$N, either among locations or among stream-resident fish species (Fig. 3.3, Table
3.2). Brown trout again exhibited a significant interaction between $\delta^{15}$N and salmon
presence. In locations with salmon, Hg concentrations in brown trout decreased as $\delta^{15}$N
increased, in locations without salmon, brown trout Hg concentrations increased with
increasing $\delta^{15}$N (Fig. 3.3, Table 3.2). Brook trout Hg concentrations exhibited a similar,
albeit non-significant, pattern to brown trout (Fig. 3.3). Furthermore, we found no
evidence that salmon spawners increased stream-resident fish Hg concentrations. All
stream-resident fish exhibited similar or lower Hg concentrations in locations with
salmon ($70.8 \pm 3.54$ ng/g, mean $\pm$ SE) compared to locations without salmon ($83.1 \pm 4.1$
ng/g, Fig. 3.3).
TABLE 3.3.

RESULTS OF ANCOVA ASSESSING IF SALMON PRESENCE INFLUENCES THE RELATIONSHIP BETWEEN STREAM-RESIDENT FISH CONTAMINANT CONCENTRATION AND $\delta^{15}$N ($\alpha=0.05$).

<table>
<thead>
<tr>
<th>Effect</th>
<th>PCB</th>
<th></th>
<th>Hg</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>P</td>
<td>df</td>
</tr>
<tr>
<td>Brook Trout</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>1, 48</td>
<td>4.3</td>
<td><strong>0.040</strong></td>
<td>1, 48</td>
</tr>
<tr>
<td>Salmon Presence</td>
<td>1, 48</td>
<td>51.0</td>
<td><strong>0.001</strong></td>
<td>1, 48</td>
</tr>
<tr>
<td>$\delta^{15}$N *Salmon Presence</td>
<td>1, 48</td>
<td>0.1</td>
<td>0.810</td>
<td>1, 48</td>
</tr>
<tr>
<td>Brown Trout</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>1, 35</td>
<td>562.2</td>
<td><strong>0.001</strong></td>
<td>1, 35</td>
</tr>
<tr>
<td>Salmon Presence</td>
<td>1, 35</td>
<td>405.0</td>
<td><strong>0.001</strong></td>
<td>1, 35</td>
</tr>
<tr>
<td>$\delta^{15}$N *Salmon Presence</td>
<td>1, 35</td>
<td>5.7</td>
<td><strong>0.022</strong></td>
<td>1, 35</td>
</tr>
<tr>
<td>Mottled Sculpin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>1, 37</td>
<td>9.2</td>
<td><strong>0.004</strong></td>
<td>1, 37</td>
</tr>
<tr>
<td>Salmon Presence</td>
<td>1, 37</td>
<td>48.0</td>
<td><strong>0.001</strong></td>
<td>1, 37</td>
</tr>
<tr>
<td>$\delta^{15}$N *Salmon Presence</td>
<td>1, 37</td>
<td>0.3</td>
<td>0.587</td>
<td>1, 37</td>
</tr>
<tr>
<td>Rainbow Trout</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>1, 14</td>
<td>43.0</td>
<td><strong>0.001</strong></td>
<td>1, 14</td>
</tr>
<tr>
<td>Salmon Presence</td>
<td>1, 14</td>
<td>6.8</td>
<td><strong>0.020</strong></td>
<td>1, 14</td>
</tr>
<tr>
<td>$\delta^{15}$N *Salmon Presence</td>
<td>1, 14</td>
<td>0.3</td>
<td>0.890</td>
<td>1, 14</td>
</tr>
</tbody>
</table>

Note: Significant relationships shown in bold.
Figure 3.3. Relationship between contaminant concentration (PCB or Hg, ng/g wet weight) and $\delta^{15}$N in stream-resident fish from reaches with and without salmon. Color denotes salmon presence (orange) or absence (green). Lines are least-squares regressions.
3.4.3 Contaminant biotransport in relation to environmental context

Specific biological characteristics appeared to modulate the uptake and incorporation of salmon-derived contaminants by stream-resident fish. Stream-resident PCB concentrations were best explained by the interaction between species identity and salmon-derived PCB flux, and $\delta^{13}C$ (Table 3.4, Table B.3). The top model with only PCB flux and $\delta^{13}C$ received a high AIC$_c$ $w_i$ and explained a large proportion of variation in stream-resident fish PCB concentrations (Table 3.4, pseudo $R^2 = 0.85$). Furthermore, PCB concentrations of all stream-resident fish species exhibited a positive, saturating relationship with salmon PCB flux into the system (Fig. 3.4). Stream-resident fish PCB concentrations were also positively related to $\delta^{13}C$, which better explained patterns than $\delta^{15}N$ (Table 3.4). The second ranked model also received a high AIC$_c$ $w_i$, and was similar to the top model except that the instream volume of large wood was included as a covariate rather than $\delta^{13}C$ (Table 3.4). Overall, every model with an AIC$_c$ less than 10 included the species identity-salmon PCB flux interaction. Physical or chemical variables including watershed area, stream temperature, % forested land cover, and stream substrate size, were selected in 4 of 6 models with an AIC$_c < 10$ (Table 3.4). However, each of these models had low AIC$_c$ $w_i$, low variable importance (0.02-0.04), and did not improve the proportion of variance explained by the model (Table B.3), suggesting that their inclusion in the model did not substantially increase the model’s explanatory power.

Factors that influenced Hg concentrations were distinct from those that affected PCB concentrations in stream-resident fish. The Hg concentration of stream-resident fish was best explained by the interaction between fish length and salmon-derived Hg flux, species identity, and the random effect of location (Table B.3). The top Hg model
received a high AIC\textsubscript{c}, but explained a much smaller proportion of variance when compared to the top PCB model (Table 3.4, Table B.3; pseudo $R^2 = 0.32$). In contrast to the PCB model, salmon-mediated Hg flux did not directly influence stream-resident fish Hg concentrations (Table 3.4, Fig 3.4). The interaction between stream-resident fish length and salmon-derived Hg flux indicated that at low Hg fluxes, stream-resident fish Hg concentrations increased with increasing fish length (Fig. 3.5). However, as the flux of Hg supplied by salmon increased, Hg concentration in stream-resident fish decreased with increasing fish length. Other models that included biological, chemical, or physical covariates received low AIC\textsubscript{c} and were not considered for additional inference (Table 3.4).
TABLE 3.4.

AIC<sub>C</sub> MODEL SELECTION TABLE FOR GENERALIZED LINEAR MIXED MODELS USED TO EXPLAIN STREAM-RESIDENT FISH PCB AND Hg CONTAMINANT CONCENTRATIONS.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Model</th>
<th>K</th>
<th>Δ AIC</th>
<th>AIC w_i</th>
<th>pseudo-R&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB</td>
<td>Species x PCB flux, δ&lt;sup&gt;13&lt;/sup&gt;C</td>
<td>11</td>
<td>0.00</td>
<td>0.578</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, Large wood volume</td>
<td>11</td>
<td>1.27</td>
<td>0.307</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, Watershed area</td>
<td>11</td>
<td>5.53</td>
<td>0.036</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, Temperature</td>
<td>11</td>
<td>5.61</td>
<td>0.035</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, % Forested land cover</td>
<td>11</td>
<td>6.74</td>
<td>0.020</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, Substrate size</td>
<td>11</td>
<td>7.23</td>
<td>0.016</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>11</td>
<td>10.08</td>
<td>0.004</td>
<td>0.83</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, SRP (ug/L)</td>
<td>11</td>
<td>11.47</td>
<td>0.002</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux</td>
<td>10</td>
<td>11.47</td>
<td>0.002</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Species x PCB flux, Length</td>
<td>11</td>
<td>12.13</td>
<td>0.001</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>Length x Hg flux, Species</td>
<td>9</td>
<td>0.00</td>
<td>0.977</td>
<td>0.32</td>
</tr>
<tr>
<td>Hg</td>
<td>Species, Length, Hg flux</td>
<td>8</td>
<td>11.04</td>
<td>0.004</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Species, Length, Hg flux, % Forested land cover</td>
<td>9</td>
<td>11.08</td>
<td>0.004</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Species, Length, Hg flux, Watershed area</td>
<td>9</td>
<td>11.60</td>
<td>0.003</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Species, Length</td>
<td>7</td>
<td>11.96</td>
<td>0.002</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Species, Length, Hg flux, δ&lt;sup&gt;13&lt;/sup&gt;C</td>
<td>9</td>
<td>12.20</td>
<td>0.002</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Species, Length, Watershed area</td>
<td>8</td>
<td>12.32</td>
<td>0.002</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>Species, Length, Hg flux, % Wetland land cover</td>
<td>9</td>
<td>12.42</td>
<td>0.002</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Species, Length, δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>8</td>
<td>12.49</td>
<td>0.002</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>Species, Length, Hg flux, δ&lt;sup&gt;15&lt;/sup&gt;N</td>
<td>9</td>
<td>12.54</td>
<td>0.002</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Note: K=number of parameters in model. pseudo-R<sup>2</sup> represents the proportion of variance explained by fixed effects in the GLMM model.
Figure 3.4. Relationship between log PCB concentrations (ng/g) in stream-resident fish and salmon-mediated PCB flux (ng/m²) supplied by spawning salmon. PCB flux calculated from visual abundance estimates, biometric data, and mean PCB concentration of salmon from a given watershed.
In contrast to the PCB model, salmon-mediated Hg flux did not directly influence stream-resident fish Hg concentrations (Fig 3.4, Table 3.4). The interaction between stream-resident fish length and salmon-derived Hg flux indicated that at low Hg fluxes, stream-resident fish Hg concentrations increased with increasing fish length (Fig. 3.5). However, as the flux of Hg supplied by salmon increased, Hg concentration in stream-resident fish decreased with increasing fish length. Other models that included biological, chemical, or physical covariates received low AICc $w_i$ and were not considered for additional inference (Table 3.4).

Figure 3.5. Interaction plot between the salmon-mediated Hg flux (ng/m²) and stream-resident fish total length. Log of stream-resident fish Hg concentration (ng/g) is represented within the heat map. Note that stream-resident fish mercury concentrations increase with increasing fish length at low salmon-mediated Hg fluxes. At high salmon-mediated Hg fluxes, stream-resident mercury concentrations decrease with increasing fish length.
3.5 Discussion

Our study is the first to simultaneously use both a persistent organic pollutant and a heavy metal to understand the influence of migratory fish mediated contaminant biotransport on the bioaccumulation of stream-resident fish. Consistent with results from both the native and non-native range of salmon, stream-resident fish from locations receiving salmon runs had higher PCB concentrations (Gregory-Eaves et al., 2007, Janetski et al., 2012) and exhibited isotopic enrichment for both C and N (Schludt & Hershey, 1995, Chaloner et al., 2002). Moreover, PCB levels of stream-resident fish were strongly linked to the PCB flux delivered by migrating salmon, although this relationship was variable among species and individuals (SD=224, range=10-880 ng/g PCB). PCB concentrations in our study were 30-fold higher than results from the native range (cf. Gregory-Eaves et al., 2007, this study) but similar to other reports from the Great Lakes (Janetski et al., 2012). In stark contrast, salmon-mediated transport of Hg was associated with decreases in Hg concentrations of stream-resident fish. Our study represents the first assessment of Hg transport by salmon in the Great Lakes but results correspond to past studies from the native range where resident fish have lower Hg concentrations in locations with salmon relative to control sites (Baker et al., 2009, Cyr et al., 2016). Overall, our findings suggest that different classes of contaminants behave differently in recipient ecosystems, and that complex responses by resident organisms should be expected among contaminants.
3.5.1 Contaminant partitioning and diet influence bioaccumulation in stream-resident fish

Environmental tracers, including POPs, trace metals, and stable isotopes, can provide a powerful tool to understand the movement of nutrients and energy, including resources and contaminants delivered by migratory fishes (Kennedy et al., 2004, Ramos & González-Solis, 2012). Partitioning of contaminants between different tissue types may help explain the patterns of PCB and Hg concentrations in stream-resident fish. Our data indicate that salmon eggs have high concentrations of PCBs but low concentrations of Hg. PCBs are highly lipophilic and accumulate readily into lipid-rich tissues, such as fish eggs (Blais et al., 2007, Walters et al., 2016). In contrast, Hg accumulates by forming a strong bond with cysteine in muscle tissue (Kuwabara et al., 2007), which is largely absent from eggs. Hence, Hg tends to be low in salmon eggs (Zhang et al., 2001). As such, variation in egg consumption may drive a tradeoff in the bioaccumulation of PCBs and Hg in stream-resident fish. This hypothesis is particularly striking when considering brown trout from this study, where δ¹⁵N and PCBs were positively related but δ¹⁵N and Hg were negatively related in locations with salmon spawners. Taken together, our findings suggest that differential partitioning of contaminants between salmon tissue types coupled with preferential consumption of salmon eggs over tissue may determine the magnitude of bioaccumulation of biotransported contaminants by stream-resident fish.

An indirect trophic pathway (i.e., via the food web) appears to play a minor role in the bioaccumulation of biotransported contaminants in tributaries we studied. In Lake Ontario tributaries, PCB concentrations in water increased by 50% during the salmon run but this event was short-lived and small relative to concentrations observed in salmon
tissue or eggs (O’Toole et al., 2006). Similarly, PCBs deposited via carcass
decomposition do not significantly increase sediment PCB levels in upper Great Lake
tributaries (Janetski et al., 2012). Dynamics of Hg display contrasting patterns. In a Lake
Ontario tributary, water column methyl-mercury (MeHg) increased, and aquatic
invertebrates had 25-fold higher Hg concentrations in locations with decomposing
salmon compared to reference sites (Sarica et al., 2004). However, if salmon-derived
contaminants were strongly following an indirect pathway, then a concomitant increase in
both PCB and Hg concentrations would be predicted for stream-resident fish (Christensen
et al., 2005, Gerig et al., 2017). Instead, we found that as salmon contaminant flux
increased, Hg in stream-resident fish decreased with increasing fish length while PCB
concentrations increased, irrespective of fish length. These results suggest that a shift to
consumption of salmon eggs by resident fish, which are high in PCBs but low in Hg, may
have a disproportionate impact on bioaccumulation, thereby controlling PCB
accumulation and indirectly mediating reductions Hg concentration. This finding has
significant implications for the broader, conceptual understanding of how contaminants
bioaccumulate in food webs.

3.5.2 Conceptual model of contaminant bioaccumulation

The conceptual model proposed by Clements et al. (2012) provides a useful
construct to consider how bioaccumulation of biotransported contaminants differs from
the traditional model of food web bioaccumulation. Our PCB results conform to the
Clements et al. (2012) model, with PCB concentrations being positively related to $\delta^{15}$N
and the magnitude of this effect being mediated by the flux of PCBs supplied by salmon.
Counter to the model, the relationship between $\delta^{15}$N and Hg was inconsistent between
species and locations. In the presence of salmon, Hg concentrations either decreased with increasing $\delta^{15}$N (e.g., brown trout, rainbow trout), or exhibited no relationship (e.g. brook trout, mottled sculpin), while stream fish from locations without salmon exhibited a positive relationship between $\delta^{15}\text{N}$ and Hg. These findings in salmon streams differ from the Clements et al., (2012) model and a global meta-analysis (Lavoie et al., 2013), which found a ubiquitous positive relationship between $\delta^{15}\text{N}$ and Hg in aquatic systems, across both biomes (e.g., arctic, temperate, tropical) and ecosystems (e.g., streams, lakes, estuaries). We posit that widespread consumption of salmon eggs restructures how contaminants are bioaccumulated, creating a trade-off between PCB and Hg bioaccumulation. As a result, the influence of watershed or instream characteristics, which typically mediate the bioavailability, and accumulation of contaminants are overshadowed by the salmon effect (King et al., 2004, Jardine et al., 2012).

3.5.3 Biological context overshadows physical or chemical variables

Biological variables appear to overwhelm the influence of instream or watershed characteristics on stream-resident fish bioaccumulation, likely due to the predominance of direct pathways of contaminant uptake and incorporation from salmon (cf. Scheuerell et al., 2007, Moore et al., 2008). In particular, we found that salmon-mediated PCB flux and stream-resident species identity to be the most important variables explaining PCB accumulation in resident fish. Variation in the relationship between PCB concentration and salmon PCB flux is likely a function of the degree of egg consumption, physiology, and habitat use (McGill et al., 2017).

Contaminant bioaccumulation in fish is strongly controlled by diet (Gerig et al., 2017, Madenjian et al., 2016). Stream-dwelling salmonids exhibit dietary plasticity and
adaptive ration size that allows them to exploit rare, but large resource pulses, such as salmon eggs (Armstrong et al., 2013, Jaecks et al., 2014) thereby increasing their PCB exposure (Janetski et al., 2011, Merna, 1986). Differences among resident salmonids in our study may therefore reflect different consumption rates of salmon eggs. Merna (1986) found a significant relationship was found between PCB concentrations and number of salmon eggs consumed by brown and rainbow trout. Salmon eggs also have a high-energy density and, when available, are preferentially consumed over other items by stream-resident fish (B. Gerig, personal observation). As a result, egg consumption likely promotes somatic growth dilution (Ward et al., 2010), whereby consumption of salmon eggs leads to increased growth, higher PCB accumulation, but reduced Hg bioaccumulation in stream-resident fish (Gerig et al., 2017). In the future, this phenomenon should be modeled using individual-based bioaccumulation models to understand the interacting mechanisms of diet, metabolism and species-identity (cf. McGill et al., 2017).

Mottled sculpin contaminant burdens exhibited the weakest relationship with salmon PCB flux. Freshwater sculpin are small benthic fish with a relatively large gape that forage on salmon material, including eggs, when available (Swain et al., 2014). However, sculpin may lack the dietary plasticity or gut capacity of stream salmonids, which limits their ability to gorge on salmon eggs (Armstrong & Schindler, 2013). In addition, as a result of physiological differences, sculpin may have higher elimination rates of PCBs than salmonids (Stapleton et al., 2001). A related freshwater species, deepwater sculpin (*Myoxocephalus thompsonii*) can metabolize PCBs, thereby reducing their contaminant burden (Stapleton et al., 2001). Last, the benthic orientation of sculpin
may make them susceptible to displacement during redd construction when salmon
spawn, reducing access to salmon material, especially eggs (Moore et al., 2004).

Differences in spatial distributions among species may also mediate the uptake of
salmon-derived contaminants. For instance, we observed that PCB contamination was
higher in brown trout (387.5 ± 196.4 ng/g PCB, mean ± SD) than in brook trout (173.4 ±
176.4 ng/g PCB). Our sampling revealed that brown trout were spatially segregated from
brook trout, generally occupying habitats lower in watersheds with larger salmon runs
(mean salmon biomass in reaches occupied by only brown trout=0.22 kg/m², mean
salmon biomass in reaches occupied by only brook trout=0.05 kg/m²). Spatial segregation
between brook and brown trout populations has been shown previously, with brown trout
being implicated in the decline of brook trout through direct predation and competition
(Fausch & White, 1981, Waters 1999). Consequently, brown trout and spawning salmon
may have a higher probability of interacting, presumably enabling brown trout to
consume more salmon eggs than brook trout and influencing contaminant uptake.

3.5.4 Contaminant biotransport models revised

The process of contaminant biotransport is defined by several steps including: (1)
the contaminant is bioaccumulated by a migratory organism; (2) the contaminant is
transported across an ecosystem boundary; and (3) the contaminant is deposited into the
recipient ecosystem (Blais et al., 2007, Kallenborn & Blais, 2015). We propose that this
model should include an additional step focused on the mechanisms by which
biotransported contaminants are taken up by resident organisms in the recipient
ecosystem. Specifically, many factors determine the effect that migratory animals will
have on contaminant bioaccumulation by resident organisms in the recipient ecosystem,
either magnifying or modulating those effects. First, the background environment in which the migratory animal matures determines the contaminant load transported across an ecosystem boundary and the severity of the potential impact of that contaminant (Gregory-Eaves et al., 2007, Janetski et al., 2012). Second, the abundance and contaminant load of the migratory animal determines the flux of pollutants available for uptake by resident organisms (Janetski et al., 2012, this study). Third, the trophic pathway by which biotransported contaminants are taken up within the recipient ecosystem will influence the overall magnitude of contaminant bioaccumulation by resident organisms (Cyr et al., 2016, this study). Fourth, the contaminant legacy of the recipient ecosystem; ecosystems that are highly contaminated may be less susceptible to biotransport. Last, individual traits (e.g., species identity, diet, physiology, depuration, spatial distribution) that vary among resident organisms will interact to determine the magnitude of bioaccumulation of biotransported contaminants (Clements et al., 2012, Michelutti et al., 2010). Refinement of the individual factors that contribute to this broader conceptual model provides insight into the consequences of the bulk movement of material and associated contaminants carried by migratory organisms.

The migratory organism transporting the contaminants also impacts the magnitude of contaminant biotransport (Michelutti et al., 2010). Blais et al., (2007) suggest that iteroparous fish, such as steelhead, or Atlantic salmon (Salmo salar), represent a diminished risk for contaminant biotransport because they only deliver gametic tissues along with excretory products to recipient ecosystems as compared with semelparous species, such as Pacific salmon, that also deliver carcasses. Arguably, because the flux of material is relatively small with iteroparous species, the
corresponding impact on resident fish contaminant burdens is diminished compared to semelparous species. However, our results suggest that egg consumption may disproportionately drive PCB accumulation by stream resident-fish, and therefore migratory species that survive reproduction may also pose a risk to resident organisms. For example, over 50 fish species exhibit a migratory life history and use tributaries for spawning in the Great Lakes (Lane et al., 1996). These species differ markedly in traits related to fecundity, spawning mode, run timing, energy density, and mobility, which may all interact to influence the flux of material to recipient ecosystems (Childress et al., 2015, Janetski et al., 2012). Knowing the risk imparted by different migratory fish runs is important to managing inputs of biotransported contaminants.

Management of biotransported contaminants will likely require different mitigation strategies than those already used to manage environmental contamination (cf. Qi et al., 2014). At present, contaminant biotransport by salmon is not monitored by state or federal management agencies, in part because biotransported contaminants often defy the conventional paradigm of pollution flowing from upstream to downstream. However, literature on managing non-point sources of nutrients may be relevant to reducing contaminant biotransport by migratory fish. For instance, non-point sources can be effectively managed when threshold levels are defined, “hot-spots” are identified, and best management practices are implemented (Carpenter et al., 1998). This approach translates well to the challenges of contaminant biotransport, where managers could (1) set threshold contaminant concentrations for streams receiving migratory fish; (2) identify locations with large salmon runs and high resident fish contaminant burdens for potential advisories; (3) use adaptive stocking strategies to minimize contaminant transfer.
to sites of conservation concern; and (4) implement seasonal removable barriers that limit the upstream flux of contaminants by spawning salmon. Our research therefore facilitates a greater awareness of the need for management of contaminant biotransport by state and federal regulatory agencies.

The delivery of resource subsidies has been widely recognized as critical to ecosystem structure and function (Bauer & Hoye, 2014, Polis et al., 2004). Our study similarly demonstrates that ecosystem linkages created by spawning salmon have significant consequences for resident fishes in tributaries. Previous work on migrations has emphasized the translocation of nutrients and energy (e.g., Schindler et al., 2003, Janetski et al., 2009). By contrast, the movement of contaminants across ecosystem boundaries, called the ‘dark side of ecosystem resource subsidies’ (after Walters et al. 2008, Kraus et al. 2016), has been understudied relative to subsidy effects (Blais et al., 2007, Walters et al., 2008). Overall, our results highlight that contaminant transport by a migratory organism can have large consequences for recipient ecosystems with the effects being mediated by the contaminant being transported, the stream-resident fish being considered, and the pathway of trophic transfer.

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3.7 Literature Cited


United States Environmental Protection Agency Method 1668, Revision A: Chlorinated Biphenyl Congeners in Water, Soil, Sediment, and Tissue by HRGC/HRMS (EPA 821-R-00-002)


4.1 Abstract

Pacific salmon and brown trout are introduced species stocked in the Laurentian Great Lakes. In their native range, salmon deliver material that enhances growth, alters isotopic ratios, and increases contaminant burdens of resident fish. However, whether salmon subsidies mediate interactions between competing species is unknown. Here, we employed a mesocosm experiment and a simulation model to determine if salmon tissue consumption influences brook trout growth, isotopic ratios, and mercury concentrations, and whether these were modified by brown trout. Our results indicate that brook trout growth did not increase with provision of salmon tissue and was not reduced by brown trout. However, brook trout exhibited isotopic enrichment and increased Hg concentrations suggesting dietary intake of salmon tissue. Because salmon eggs have a higher energy density and lower mercury concentration compared to salmon tissue, our simulation model suggests that consumption of salmon eggs rather than tissue can

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increase growth while reducing mercury accumulation. Overall, our results suggest that the role of introduced Pacific salmon is dependent on both food quantity and quality along with diet contaminant concentrations.

4.2 Introduction

The introduction of species outside their native range is an ecological problem of global significance (Gozlan et al. 2010). While most focus is on harmful invasions, intentional species introductions can bring economic benefits while also resulting in ecological impacts that are complex and difficult to predict (Horan and Lupi 2010). Salmonid fishes have been widely introduced because of their economic and recreational value (Korsu et al. 2009), but their impacts on native fish have been significant (Baxter et al. 2004; Yard et al. 2011). Moreover, anthropogenic factors, such as pollution, can interact with non-native species introductions to influence populations of native species (Kolar and Lodge 2002; Gozlan et al. 2010). The Laurentian Great Lakes are a nexus for interactions between native species and environmental change, having an extensive legacy of industrial pollution along with decades of species introductions (Allan et al. 2013).

Numerous fish species have been intentionally introduced to the Great Lakes. Non-native Pacific salmon (*Oncorynchus* spp.) have been stocked for over five decades while European brown trout (*Salmo trutta*) have been established in tributaries for over a century (Crawford 2001). In the Great Lakes, salmon are potadromous and semelparous, accumulating nutrients and contaminants in the lakes, and then transferring resource subsidies, in the form of excretory products, carcasses, and eggs, to tributaries during spawning migrations and subsequent death in tributaries (Cederholm et al. 1999;
Pacific salmon were initially introduced to control invasive alewife and quickly became an economically valuable component of lake and tributary-based recreational fisheries (Dettmers et al. 2012). Several studies have assessed the effects of non-native salmon in the Great Lakes (Crawford 2001; Tsehaye et al. 2014), but comparatively little is known about their effects in tributaries (but see Ivan et al. 2011; Janetski et al. 2011, 2014). In addition, previous studies have documented the decline of native brook trout (*Salvelinus fontinalis*) as a result of competitive and predatory interactions with introduced brown trout (Fausch and White 1981; Waters 1999) but it is unclear how salmon spawning may alter this relationship.

The ecological influence of spawning salmon on stream ecosystems may reflect the environmental context. The importance of salmon resource subsidies (cf. Polis et al. 2004) can reflect spawner biomass, background nutrient levels, and stream sediment size (Janetski et al. 2009, 2014). Spawner biomass determines the amount of nutrients delivered and the disturbance imparted to the stream by redd construction; in turn, background nutrients regulate the enrichment response, and sediment size determines susceptibility to disturbance (Janetski et al. 2009). Nutrients supplied by salmon can increase stream productivity (e.g., Wipfli et al. 2003) while disturbance can increase or decrease the availability of invertebrates for resident fish consumption (e.g., Scheuerell et al. 2007). In addition, stream-resident fish readily consume salmon tissue and eggs (Moore et al. 2008; Scheuerell et al. 2007), increasing their growth rates (Bilby et al. 1998; Wipfli et al. 2003) and altering their isotopic composition (Chaloner et al. 2002; Reisinger et al. 2013). Consequently, different mechanisms may explain the resident fish response to spawning salmon across a gradient of biological, physical, and chemical
characteristics (Janetski et al. 2009) that vary between native and introduced ranges (Janetski et al. 2011, 2014).

In tributaries of the Great Lakes, our knowledge of the influence of spawning salmon on stream-resident fish is limited. Previous research has shown that stream-resident fish in Great Lakes tributaries readily consume salmon eggs (Ivan et al. 2011; Johnson et al. 2016), but no experimental evidence exists to establish if consumption of salmon material confers a growth benefit. Similarly, stable isotopes have been used to track movement of salmon-derived material in the native range of salmon (cf. Bilby et al. 1998; Chaloner et al. 2002), but seldom in the Great Lakes (but see Schuldt and Hershey 1995).

Previous research has also shown that the body burden of persistent organic pollutants (POPs) in stream-resident fish is determined by the contaminant flux supplied by spawning salmon (Janetski et al. 2012; Gerig et al. 2016). However, whether salmon spawners biotransport heavy metals, such as mercury, is uncertain. Despite these uncertainties, recent studies suggest that common environmental contaminants, such as mercury, could be used as an ecological tracer to establish pathways by which salmon-derived resources are incorporated into stream food webs and how species compete for and utilize those resources (Ramos and Solis 2012; Gerig et al. 2016).

Interactions between introduced and native fish species are complex (Korsu et al. 2009) and can be modulated by resource subsidies (Baxter et al. 2007). In their native range, spawning salmon alter diets of co-occurring rainbow trout (Oncorhynchus mykiss) and arctic grayling (Thymallus arcticus), positively influencing the growth of both species (Scheuerell et al. 2007). Rainbow trout growth increased from direct consumption
of salmon carcasses and eggs, while grayling growth increased from consumption of invertebrates dislodged by spawning salmon (Scheuerell et al. 2007). In contrast, introduced rainbow trout negatively impacted the growth of native Dolly Varden \textit{(Salvelinus malma)} through competitive interactions by disrupting their access to terrestrial invertebrate prey (Baxter et al. 2007). In Great Lakes tributaries, the presence of spawning salmon could either confer bioenergetic benefits for both brook and brown trout, or result in increased competition for resources (cf. Fausch and White 1986; Ivan et al. 2011).

Our objective was to evaluate the consequences of interactions between non-native and native fish species, in the context of a novel resource subsidy. For this study, we first conducted a mesocosm experiment to determine the effects of a new resource, salmon tissue, on native brook trout, and whether those effects are modulated by the presence of introduced brown trout. We hypothesized that brook trout with access to salmon material would exhibit (1) higher growth, due to consumption of high-quality salmon tissue; (2) altered isotopic ratios, reflecting incorporation of isotopically-enriched salmon-tissue; and (3) increased mercury concentrations, resulting from consumption of mercury-laden salmon tissue. We further expected that brook trout growth rates, isotopic ratios, and mercury concentrations would be lower in the presence of brown trout because inter-specific competition would reduce the consumption of salmon tissue. We then developed a coupled bioenergetics-bioaccumulation model to determine specifically how diet composition, energy density, and mercury content of diet items could interact to influence brook trout growth and mercury accumulation observed in our experiment.
4.3 Methods

4.3.1 Mesocosm experimental setup

We conducted a mesocosm experiment from 11 June to 26 July, 2014, at the Hunt Creek Fisheries Research Station in Lewiston, Michigan (see Grossman et al. 2012 for detailed site information on Hunt Creek). Experimental mesocosms consisted of 16 flow-through polyurethane tanks (1.0 m diameter, 0.5 m height). Tanks were supplied with gravity-fed water from an artesian well, which was then split from a main water supply pipe to four secondary spouts. Each secondary spout supplied water to four tanks. At each secondary spout, an inline filter removed flocculent iron. Each mesocosm was aerated continuously with air stones to ensure an adequate supply of oxygen. During the experiment, water temperature (10.9 ± 1.8°C; mean ± SD) and oxygen (9.8 ± 0.7 mg/L; mean ± SD) was measured twice daily.

Young-of-the-year (age-0) brook trout and brown trout were obtained from the Marquette and Oden State Fish Hatcheries, Michigan Department of Natural Resources, one week prior to the start of the experiment. Hatchery fish were fed a maintenance ration of bloodworms prior to the start of the experiment, and 5 brook trout and 5 brown trout were sacrificed to determine initial stable isotope values and mercury concentrations. At the start of the experiment, fish were divided into three size classes (small: 50-59 mm; medium: 60-69 mm; and large: ≥70 mm), and initial individual length (mm) and mass (g) measured. Each fish received a unique fin clip in order to assess individual growth over the experiment. During the experiment, fish length and mass were measured weekly while mesocosm tanks were checked daily for mortalities. The mortality rate during the experiment was low (<15%) and similar to other published studies (cf. Wipfli et al.)
2003). If a mortality occurred, it was replaced with a fish of the same species of similar length and mass to maintain experimental conditions. Prior to analysis, we censored our data to only include fish that had been in the experiment for more than 35 days to ensure that replacement fish did not bias our inference. At the end of the experiment, all experimental fish were euthanized and then stored frozen at -20°C for later stable isotope and mercury analyses.

Brook trout were subjected to four treatments (Fig. 4.1): (1) salmon tissue absent and brown trout absent; (2) salmon tissue present and brown trout absent; (3) salmon tissue absent and brown trout present; and (4) salmon tissue present and brown trout present. The experiment was fully crossed, with four replicates (i.e., tanks) of each treatment. For treatments without brown trout, two brook trout from each size class were placed into tanks. For treatments with brown trout, one brook trout and one brown trout from each size class were placed into tanks. Regardless of treatment, each tank held 6 fish to maintain equal densities and total biomass. For treatments without salmon tissue, tanks received 5.0 g of chironomid midge larvae twice daily. For treatments with salmon tissue, tanks received 2.5 g of chironomids and 2.5 g of salmon tissue twice daily. Thus, each tank received the same wet mass of food, and any uneaten food was removed daily. Salmon tissue used in the experiment was fall run Chinook salmon (O. tshawytscha) collected from the Little Manistee River (MI) weir by the Michigan Department of
Figure 4.1. Mesocosm experimental design, which consisted of 16 flow-through tanks, with 4 randomized treatments per block (1-4). Brook trout were present in all tanks.

Natural Resources during fall 2013. Prior to the experiment, salmon were homogenized whole, excluding head and gametes, and stored frozen. Salmon tissue was used as a food source to simulate carcass tissue that is putatively available for consumption during natural salmon runs (cf. Cederholm et al. 1999; Wipfli et al. 2003). Note that salmon eggs were not provided to salmon treatments due to availability, but were included as a diet item in subsequent model simulations due to previous studies demonstrating resident fish consumption of salmon eggs (Ivan et al. 2011). Data for salmon eggs used in the model were obtained from freshly spawned Chinook salmon from the Little Manistee River. Chironomid midge larvae (*Chironomus spp.*) were obtained from JEHM Co. (jehmco.com). Chironomid larvae were chosen as an alternative food item because they are often found in the diet of trout in tributaries of the Great Lakes (Wills et al. 2006).
4.3.2 Stable isotope analyses

Stable carbon (δ\textsuperscript{13}C) and nitrogen (δ\textsuperscript{15}N) isotope ratios were measured for whole fish homogenized tissue and diet sources (chironomids, provisioned salmon tissue, non-provisioned salmon eggs) using an Elemental Analyzer (Costech, Valencia, USA) coupled to a Delta Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Waltham, USA) located in the Center for Environmental Science and Technology (CEST) at the University of Notre Dame. Prior to analysis, all samples were oven dried at 60°C, homogenized into a fine powder, and stored at -20°C. Data were included in subsequent analyses if the standard deviation of the acetanilide standard was <0.2‰ (cf. Chaloner et al. 2002). The standard deviations for acetanilide standards were 0.08‰ and 0.06‰ for N and C, respectively. Stable isotope ratios of N (δ\textsuperscript{15}N) and C (δ\textsuperscript{13}C) were expressed as:

\[
\delta^{15}N \text{ or } \delta^{13}C = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000 \quad \text{(Equation 4.1)}
\]

where \( R \) is the ratio of \(^{15}\text{N} / ^{14}\text{N} \) or \(^{13}\text{C} / ^{12}\text{C} \). All δ\textsuperscript{13}C values were lipid-corrected using individual C:N ratios (cf. Post et al. 2007).

4.3.3 Mercury analyses

Total mercury concentrations of brook and brown trout and diet sources (chironomids, provisioned salmon tissue, non-provisioned salmon eggs) were determined using a Direct Mercury Analyzer 80 (DMA-80, Milestone S.r.l., Sorisole, Italy), also located at CEST. All samples were prepared for Hg analysis in the same manner as for stable isotope analyses. Prior to analysis, 0.02 g of homogenized sample was weighed into ashed nickel boats, placed into the DMA-80, and analyzed via fixed wavelength atomic absorption spectrophotometry (cf. Abma et al. 2014). The DMA-80 was calibrated using standard reference materials (National Research Council of Canada, DORM-4, 410
± 55 Hg ng/g) and all results were expressed in parts per billion (ng/g) wet weight.

Standard reference materials, instrument and method blanks, duplicates, and matrix spikes were incorporated into each run to ensure data quality. Percent recovery from DORM-4 standard was 99.2 ± 2.2% (n = 11) and the detection limit was 0.141 ng/g.

4.3.4 Statistical analyses

We used a randomized block analysis of variance with a split plot design (rb ANOVA, α = 0.05; Zar 2010) to analyze the experimental results. Our main treatment factors were (1) salmon tissue (presence or absence) and (2) brown trout (presence or absence). Size class (small, medium, large) was considered a sub-treatment across blocks (cf. Wipfli et al. 2003). We interpreted a significant interaction between salmon tissue and brown trout treatments as evidence that the presence of brown trout mediated the response of brook trout to salmon tissue. All treatments were randomly assigned within each block, each of which was fed by a different output spout from the same water source. Response variables were growth rate (change in length or mass over the experimental period [mm/day or g/day]), stable isotope ratio (δ¹⁵N and δ¹³C [%o]), and total mercury concentration (Hg [ng/g]). We also used an analysis of covariance (ANCOVA, α = 0.05; Zar 2010) to assess if variation in growth rate with respect to mass was related to variation in isotope ratios and total mercury among treatments. Assumptions of ANOVA and ANCOVA were assessed visually using QQ and plots of residuals. All statistical analyses were performed using the R software platform (https://cran.r-project.org/).

We used two different yet complementary approaches to link the consumption of salmon tissue to variation in brook trout Hg concentrations. First, we used ANCOVA to
establish if $\delta^{13}\text{C}$ was related to mercury concentration ($\text{Hg [ng/g]}$) among treatments. Second, for fish from salmon treatments we estimated the extent of salmon consumption using a Bayesian stable isotope-mixing model (MixSiar in R V3.0.2; Stock and Semmens 2015). This model estimated how variation in the dietary contribution of salmon mediated Hg accumulation among individual brook trout. This model directly accounts for uncertainty in diet isotope ratios and trophic discrimination factors (e.g., standard deviation). Tissue discrimination factors used were 3.4 (SD = 1.0) for $\delta^{15}\text{N}$ and 1.0 (SD = 0.5) for $\delta^{13}\text{C}$ (cf. Reisinger et al. 2013). The model was fit using an iterative Markov Chain Monte Carlo fitting routine. Chain length was set to 100,000 with a burn in of 50,000 and residual-only error structure (cf. Stock and Semmens 2015).

4.3.5 Bioenergetics model

To better understand the coupling of fish growth and contaminant burden, we used a simulation model to assess how energy density and mercury concentration of diet items could explain brook trout growth and mercury accumulation. To do so, we modified a time dynamic bioenergetics model (cf. Hanson et al. 1997; Rashleigh and Grossman 2005), and parameterized it using species-specific physiological parameters (Hartman and Cox 2008) for brook trout. Through this energetics-based approach, consumed energy is first allocated to catabolic processes and then to waste losses; remaining energy is allocated to growth (Hanson et al. 1997; Rashleigh and Grossman 2005). This individual-based bioenergetics model was defined as follows:

$$\frac{dM}{dt} = (C – Eg – Ex) * EDp – (ACT*R + SDA) * JO2 / EDbkt$$

(Equation 4.2)

where $dM/dt$ is the organism’s change in mass over time; $C$ is consumption; $Eg$ is egestion; $Ex$ is excretion; $EDp$ is the energy density of the prey; $ACT$ is the activity rate
multiplier; $R$ is respiration; $SDA$ is specific dynamic action; $JO2$ is the oxycalorific coefficient; and $EDbkt$ is the energy density of the brook trout (cf. Hanson et al. 1997). Model inputs include empirically derived daily mean water temperatures, diet proportions, diet energy density, and brook trout energy density. Water temperature values reflected those obtained from the mesocosm study. Energy density ($J/g$ wet mass) for salmon tissue, chironomids, salmon eggs, and brook trout were measured empirically using a bomb calorimeter (cf. Glover et al. 2010; Parr Instrument Co. Moline, IL, USA; Table 4.1, Table 4.2). Following convention, the model was fit to observed weekly growth data for brook trout using a maximum likelihood approach to determine what proportion of maximum consumption realized ($P$) best fit the observed data. The parameter $P$ was determined from brook trout growth data that were pooled across treatments. The value of $P$ was determined to be 0.53, and was used for all model scenarios (see below for scenario description, Fig. C.1).

4.3.6 Bioaccumulation model

Brook trout growth predictions were coupled to a dynamic bioaccumulation model based on the model of Arnot and Gobas (2004). The model was defined as follows:

$$\frac{dM}{dt} = [Mbkt \ast (kD \Sigma wiCD,i)] - (ke) \ast MHg$$  \hspace{1cm} (Equation 4.3)

where $dM/dt$ is the change in the mass of the contaminant in the brook trout over time; $Mbkt$ is the mass of the brook trout obtained from the bioenergetics model; $kD$ is the uptake efficiency of the contaminant; $\Sigma wiCD,i$ is the product of diet proportion and contaminant concentration of a given diet item; $ke$ is the elimination rate; and $MHg$ is the mass of the contaminant in the brook trout (cf. Arnot and Gobas 2004). Diet mercury
concentrations used in the model were measured empirically (Table 4.2). Our bioaccumulation model differs from the work of Arnot and Gobas (2004) in that the model components dealing with contaminant uptake via the gills and metabolic transformations were removed (Trudel and Rasmussen 2006). In addition, we used a fixed rate of mercury loss based upon previous research (Trudel and Rasmussen 1997, Madenjian et al. 2012). Given that greater than 99% of contaminant uptake in fish comes from diet, and the duration of our simulation was only 50 days, these simplifying assumptions were deemed reasonable (Trudel and Rasmussen 2006). Brook trout growth and mercury accumulation was modeled across five scenarios related to variability in diet: (1) 100% chironomids; (2) 50:50 chironomids:salmon tissue; (3) 100% salmon tissue; (4) 50:50 chironomids:salmon eggs; and (5) 100% salmon eggs. For each scenario, the simulation lasted 50 days to mimic the duration of the mesocosm experiment. Starting mass of brook trout for the simulation was 2.5 g, which approximated the median mass of individuals at the beginning of the experiment. All modeling was conducted using the deSolve package in R (https://cran.r-project.org/web/packages/deSolve/index.html).

4.4 Results

4.4.1 Mesocosm experiment

During our 7-week experiment, brook trout exhibited positive growth rates, increasing in length and mass irrespective of treatment. Overall, brook trout grew at an average rate of 0.4 mm (SD=0.1, range=-0.1-0.6 mm) and 0.07 g (SD=0.03, range=0.01-0.16 g) per day. Contrary to our hypothesis, brook trout growth with respect to length or mass was not influenced by the provision of salmon tissue (Length ANOVA, F\textsubscript{1,68}=0.35, p=0.55; Weight ANOVA, F\textsubscript{1,68}=0.15, p=0.69; Fig. 4.2.A) or the presence of brown trout.
(Length ANOVA, $F_{1,68}=0.001$, $p=0.98$; Weight ANOVA, $F_{1,68}=0.06$, $p=0.79$, Fig. 4.2.B). However, large fish were found to grow at higher rates with respect to mass but not length (Length ANOVA, $F_{2,68}=2.9$, $p=0.06$; Weight ANOVA, $F_{2,68}=6.72$, $p=0.002$; Fig. C.2.A). Consistent with our hypothesis, brook trout isotopic ratios differed in the presence of salmon tissue ($\delta^{15}N$ ANOVA, $F_{1,68}=81.2$, $p<0.001$; $\delta^{13}C$ ANOVA, $F_{1,68}=15.1$, $p<0.001$) but this result was not affected by the presence of brown trout ($\delta^{15}N$ ANOVA, $F_{1,68}=1.01$, $p=0.31$; $\delta^{13}C$ ANOVA, $F_{1,68}=0.21$, $p=0.64$). In treatments with salmon tissue, brook trout were significantly enriched in $\delta^{15}N$, which was 20% higher relative to non-salmon treatments (Fig. 4.2.C; Table 4.1) and significantly depleted in $\delta^{13}C$, which was 3% lower relative to non-salmon treatments (Fig. 4.2.D; Table 4.1). Large fish were found to have higher $\delta^{15}N$ but lower $\delta^{13}C$ relative to fish from medium and small size classes ($\delta^{15}N$ ANOVA, $F_{2,68}=4.5$, $p=0.010$, Fig. C.2.B; $\delta^{13}C$ ANOVA, $F_{2,68}=5.1$, $p=0.008$, Fig. C.3.C). Consistent with our hypothesis, brook trout mercury concentrations were higher in salmon treatments (Hg ANOVA, $F_{1,68}=164.75$, $p<0.001$) but once again this result was not affected by brown trout (Hg ANOVA, $F_{1,68}=1.2$, $p=0.28$). We observed no size response in mercury concentration (Hg ANOVA, $F_{2,68}=2.4$, $p=0.09$). Overall, brook trout mercury concentrations exhibited a 9-fold increase in salmon relative to non-salmon treatments (Fig. 4.2.E).
TABLE 4.1.

STABLE ISOTOPE RATIO, MERCURY CONCENTRATION, AND SAMPLE SIZE OF BROOK AND BROWN TROUT AT THE END OF THE MESOCOSM EXPERIMENT.

<table>
<thead>
<tr>
<th>Species</th>
<th>Treatment</th>
<th>δ¹⁵N</th>
<th>δ¹³C</th>
<th>Mercury (ng/g)</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brook Trout</td>
<td>Initial Pre-Experiment</td>
<td>11.3±0.1</td>
<td>-19.3±0.2</td>
<td>24.3±1.7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>No Salmon, BNT absent</td>
<td>9.1±1.1</td>
<td>-20.1±0.3</td>
<td>18.1±3.0</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Salmon, BNT absent</td>
<td>10.9±0.6</td>
<td>-20.8±0.5</td>
<td>159.1±57.2</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>No Salmon, BNT present</td>
<td>9.2±1.0</td>
<td>-20.1±0.3</td>
<td>17.8±5.4</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Salmon, BNT present</td>
<td>10.5±0.4</td>
<td>-20.9±0.3</td>
<td>162.5±39.2</td>
<td>12</td>
</tr>
<tr>
<td>Brown Trout</td>
<td>Initial Pre-Experiment</td>
<td>11.6±0.2</td>
<td>-19.5±0.1</td>
<td>31.8±3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>No Salmon, BNT present</td>
<td>9.43±1.0</td>
<td>-19.8±0.1</td>
<td>24.1±2.8</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Salmon, BNT present</td>
<td>10.3±1.0</td>
<td>-20.2±0.3</td>
<td>87.6±34.2</td>
<td>13</td>
</tr>
</tbody>
</table>

Note: Initial stable isotope ratio and mercury concentration for fish used in the experiment are also reported. Mercury concentration reported as ng/g wet weight. Values reported as mean (standard deviation).
Figure 4.2. Response of brook trout to provision of salmon tissue and presence of brown trout (BNT). (A.) length growth rate (mm/day), (B.) mass growth rate (g/day), (C.) tissue $\delta^{15}$N (‰), (D.) tissue $\delta^{13}$C (‰), and (E.) total mercury concentration (ng/g). Values reported are medians with upper and lower quartiles to illustrate variability. Beige boxplots are brook trout and navy boxplots are brown trout.
Given the variability in growth among treatments for brook trout, we explored how growth rate with respect to mass was related to isotopic composition and total mercury concentration. We found a significant relationship between brook trout growth rate and δ\textsuperscript{15}N (ANCOVA, $F_{1,64}$=7.8, $p<0.001$), with treatments provisioned with salmon tissue having higher δ\textsuperscript{15}N relative to non-salmon treatments (ANCOVA, $F_{1,64}$=72.0, $p<0.001$). However, this relationship was not influenced by brown trout (ANCOVA, $F_{1,64}$=1.2, $p=0.27$; Fig. 4.3.A). Similarly, brook trout growth rate was related to δ\textsuperscript{13}C (ANCOVA, $F_{1,64}$=9.3, $p=0.003$; Fig. 4.3.B), with treatments provisioned with salmon having lower δ\textsuperscript{13}C relative to non-salmon treatments (ANCOVA, $F_{1,64}$=80.7, $p<0.001$). This relationship was not influenced by brown trout (ANCOVA, $F_{1,64}$=0.42, $p=0.52$; Fig. 4.3.B). No interactions were observed between growth rate and salmon indicating that provision of salmon was not driving growth rates (δ\textsuperscript{15}N ANCOVA, $F_{1,64}$=0.16, $p=0.69$; δ\textsuperscript{13}C ANCOVA, $F_{1,64}$=0.62, $p=0.43$).
TABLE 4.2.

STABLE ISOTOPE RATIO, MERCURY CONCENTRATION, AND ENERGY DENSITY OF DIET ITEMS FED TO BROOK AND BROWN TROUT IN THE MESOCOSM EXPERIMENT AND USED TO PARAMETERIZE THE BIOENERGETICS-BIOACCUMULATION MODEL.

<table>
<thead>
<tr>
<th>Diet Item</th>
<th>$\delta^{15}$N</th>
<th>$\delta^{13}$C</th>
<th>Mercury (ng/g)</th>
<th>Energy Density (Joules/g)</th>
<th>Sample Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chironomids</td>
<td>4.6±3.1</td>
<td>-20.7±3.8</td>
<td>17.1±25.3</td>
<td>4265.7±515.2</td>
<td>5</td>
</tr>
<tr>
<td>Salmon Tissue</td>
<td>11.8±0.3</td>
<td>-23.2±0.6</td>
<td>193.7±25.9</td>
<td>4806.4±457.0</td>
<td>5</td>
</tr>
<tr>
<td>Salmon Eggs</td>
<td>-</td>
<td>-</td>
<td>14.6±5.8</td>
<td>6548.6±119.8</td>
<td>5</td>
</tr>
<tr>
<td>Hatchery Feed</td>
<td>7.5±0.2</td>
<td>-20.2±0.1</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
</tbody>
</table>

Note: Isotope values of hatchery feed is also included for reference. Energy density (Joules/g wet weight) was determined by bomb calorimetry and mercury concentration (ng/g wet weight) was determined using atomic absorption spectrophotometry. Values reported as mean (standard deviation).
Figure 4.3. Relationship between brook trout (A.) mass growth rate (g/day) and tissue δ¹⁵N (‰), (B.) mass growth rate (g/day) and tissue δ¹³C (‰), (C.) mass growth rate (g/day) and total mercury concentration, and (D.) tissue δ¹³C (‰) and total mercury concentration among treatments. Regression line represents line of best fit for salmon and non-salmon treatments. BNT=Brown Trout. P-value and r² statistic are from overall ANCOVA model.
In contrast to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, the relationship between brook trout growth rate and total mercury concentration displayed a significant interaction between treatments provisioned with salmon (ANCOVA, $F_{1,64}=4.9$, $p=0.03$; Fig. 4.3.C). We observed a strong positive relationship between total mercury and growth rate in salmon treatments; faster growing fish increased their mercury concentrations more quickly than slower growing fish. In contrast, in treatments without salmon tissue, mercury accumulation decreased with increasing growth rates; slower growing fish had higher mercury concentrations than faster growing fish. Moreover, brook trout mercury levels exhibited a significant interaction with $\delta^{13}\text{C}$ ratio between salmon treatments (ANCOVA, $F_{1,44}=50.4$, $p<0.001$; Fig. 4.3.D), suggesting that brook trout mercury content increased as they became depleted in $\delta^{13}\text{C}$. Results from the Bayesian stable isotope model also suggested that brook trout Hg concentration increased as salmon became more prevalent in their diet (Fig. C.3).

Contrary to our original hypothesis, the presence of brown trout did not affect brook trout growth, isotope ratios, or mercury concentration. Brook trout growth rates were higher than co-occurring brown trout, with respect to both length (ANOVA, $F_{1,44}=36.9$, $p<0.001$; Fig. 4.2.A) and mass (ANOVA, $F_{1,44}=45.5$, $p<0.001$; Fig. 4.2.B). Brown trout responded similarly to brook trout in the presence of salmon tissue (ANOVA, $F_{1,44}=0.06$, $p=0.80$), and were enriched in $\delta^{15}\text{N}$ (ANOVA, $F_{1,44}=18.4$, $p<0.001$; Fig. 4.2.C). Overall, brook and brown trout from salmon treatments were more depleted in $\delta^{13}\text{C}$ compared to non-salmon treatments (ANOVA, $F_{1,44}=25.9$, $p<0.001$), but brown trout were more enriched in $\delta^{13}\text{C}$ relative to brook trout (ANOVA, $F_{1,44}=39.7$, $p<0.001$; Fig. 4.2.D). Similar to brook trout, brown trout mercury concentrations increased in
salmon treatments (ANOVA, $F_{1,44}=219.4$, $p<0.001$; Fig. 4.2.E). However, an interaction was observed between the provision of salmon tissue and species identity (ANOVA, $F_{1,44}=33.8$, $p<0.001$), suggesting that brown trout were less contaminated with mercury than brook trout in the presence of salmon, but more contaminated with mercury in the absence of salmon (Fig. 4.2.E).

4.4.2 Bioenergetics-bioaccumulation modeling

In our bioenergetics-bioaccumulation model, diet items consumed by brook trout differed in their energy content (ANOVA, $F_{2,12}=43.6$, $p<0.001$; Table 4.1) and mercury concentration (ANOVA, $F_{2,12}=246.2$, $p<0.001$; Table 4.2). We used the empirical estimates of energy density and mercury concentration of diet items to parameterize our model. Salmon tissue has a similar energy density to chironomids (Tukey HSD, $p=0.12$) whereas salmon eggs have a higher energy density than either salmon tissue or chironomids (Tukey HSD, $p<0.001$). For mercury, salmon tissue was significantly higher compared to salmon eggs or invertebrates (Tukey HSD, $p<0.001$), whereas no difference was observed between salmon eggs and invertebrates (Tukey HSD, $p=0.84$).

Variation in prey energy densities had a moderate effect on brook trout growth, whereas mercury accumulation was strongly influenced by mercury concentration in food. Our model predicted that consumption of salmon tissue would result in a modest 3% increase in growth for the 50:50 chironomids:salmon tissue scenario and a 7% increase in growth for the 100% salmon tissue scenario, relative to the 100% chironomids scenario (Fig. 4.4.A). By contrast, consumption of salmon eggs, which have a higher energy density than salmon tissue, resulted in a 14% increase in growth for the 50:50 chironomids:salmon egg scenario and a 26% increase in growth for the 100% salmon egg scenario.
scenario, relative to the chironomids only scenario (Fig. 4.4.A). Overall, consumption of 100% salmon eggs resulted in a 19% increase in growth relative to the 100% salmon tissue scenario. Brook trout mercury accumulation also differed considerably depending upon the diet consumed. Our model predicted that consumption of salmon tissue would result in a 4.6-fold increase in mercury concentration for the 50:50 chironomids:salmon scenario and a 8-fold increase in mercury concentrations for the 100% salmon tissue, relative to the chironomids-only scenario (Fig. 4.4.B). In contrast, consumption of salmon eggs, which have lower mercury concentration than salmon tissue, resulted in a 3.7% decrease in mercury concentration for the 50:50 chironomids:salmon egg scenario and a 7% decrease in mercury concentration for the 100% salmon egg scenario, relative to the chironomids-only scenario (Fig. 4.4.B). Overall, consumption of 100% salmon tissue resulted in a 8.5-fold higher mercury concentration relative to the 100% salmon egg scenario.
Figure 4.4. Modeled change in brook trout (A.) mass and (B.) total mercury concentrations over a 50-day simulation under five scenarios reflecting different diet sources and proportions.

4.5 Discussion

Our mesocosm experiment suggested that brook trout growth and mercury accumulation were much more influenced by provision of salmon material than by the presence of brown trout. Moreover, our model demonstrated that diet can mediate both growth and mercury bioaccumulation in brook trout. Consistent with our hypothesis, we showed that consumption of salmon tissue strongly increased mercury levels in brook trout. However, contrary to our hypothesis, provision of salmon tissue did not increase growth despite assimilation as indicated by isotopic ratios. Further, brook trout exhibited higher growth rates than co-occurring brown trout, an unexpected result based on past studies (e.g., Dewald and Wilzbach 1992; Waters 1999). Our simulation model complemented our mesocosm experiment by elucidating the role of diet in regulating growth and mercury accumulation. Specifically, our model suggests that consumption of
salmon eggs rather than salmon tissue moderately increases growth while reducing mercury accumulation in brook trout. Taken together, our experiment and model highlight that the bioenergetic influence of introduced salmon on brook trout is dependent on the type and amount of salmon tissue consumed.

4.5.1 Role of introduced salmon as a resource subsidy

Salmon material has been shown to stimulate productivity, including the growth of resident fish. For example, a meta-analysis found that the presence of salmon spawners increased resident fish growth, and this relationship was strongly driven by spawner biomass (Janetski et al. 2009). The positive response of resident fish has been attributed to direct ingestion of salmon material (Moore et al. 2008), increased invertebrate production (Chaloner and Wipfli 2002), or increased invertebrate drift (Scheuerell et al. 2007). However, the primacy of these trophic pathways remains uncertain (Janetski et al. 2009).

In our mesocosm experiment, we showed that brook trout exhibited equivalent growth rates among treatments regardless of whether they were or were not provisioned with salmon tissue. Thus, consumption of salmon material did not elicit strong subsidy effects (cf. Harvey and Wilzbach 2010). However, in salmon treatments, analysis of covariance revealed that brook trout growth rate was positively correlated with mercury concentration and that $\delta^{13}C$ was negatively correlated to Hg concentrations while salmon dietary proportion was positively related to Hg levels. These findings indicate that brook trout with increased reliance on salmon tissue had concomitant increases in both growth rate and mercury concentrations. These observations suggest that the response of brook trout to a novel resource subsidy is dependent on their ability to increase their rate of
consumption and energy intake when resource availability is high (Moore et al. 2008).

Thus, the response of resident fish to salmon subsidies may be most pronounced when
background resource availability is low relative to the flux of material delivered (Flecker
et al. 2010; Marcarelli et al. 2011). Therefore, locations with low in situ food availability
may experience the largest benefits from salmon resources by alleviating nutritional
limitation (Wipfli and Baxter 2010).

Diet quality may also mediate the growth response of organisms provisioned with
a resource subsidy. The putative subsidy effect is strongest when resource quality differs
among diet items and high quality diet items are selected for in greater proportion than
their availability (Marcarelli et al. 2011; Polis et al. 2004). Empirically, we demonstrated
that salmon eggs are more energetically dense than salmon tissue or aquatic invertebrates,
which resulted in enhanced growth in our model. This finding is similar to previous
empirical studies, where resident fish growth increased as a result of consumption of
salmon eggs (Moore et al. 2008; Scheuerell et al. 2007). In contrast, we did not observe
growth differences in salmon treatments within our mesocosm experiment. Similarly, the
addition of salmon carcasses without eggs did not increase growth of resident fish in a
carcass addition experiment (Harvey and Wilzbach 2010). This suggests that resource
quantity may interact with resource quality to magnify the effects of salmon subsidies on
resident fish. Future research should validate our experimental results in natural streams
receiving salmon spawners throughout the introduced range of salmon in the Great
Lakes.
4.5.2 Insights from experiment-model integration

Bioenergetics modeling can provide a mechanistic understanding of how fish growth impacts population dynamics, ecosystem function, and contaminant accumulation within aquatic food webs (Madenjian et al. 2000). Integrating experiments with bioenergetic models can allow for greater inference by exploring potential causal mechanisms driving observed patterns. For example, Madenjian et al. (1994) used a bioenergetics-bioaccumulation model to explain variation in the PCB concentrations of Lake Michigan salmonines as a function of diet, consumption rate, and growth efficiency. Chemical tracers are particularly effective in fish for understanding energy flow and food web dynamics because more than 99% of their contaminant burden is obtained from dietary sources (Trudel and Rasmussen 2006).

Our study has important implications for the role of salmon tissue and egg consumption in brook trout growth and mercury accumulation. First, our study demonstrated that when dietary resources have different mercury concentrations and are consumed in different proportions, mercury is an effective tracer for incorporation of salmon-derived material. Previously, mercury has been shown to elucidate trophic level, food sources, and inter-ecosystem habitat use (Ramos and Solis. 2012), while in the Great Lakes, mercury was an effective tracer of salmon consumption in aquatic invertebrates (Sarica et al. 2004). Second, our model demonstrated that diet composition can mediate both growth and mercury bioaccumulation. In particular, modeled consumption of salmon eggs resulted in increased growth (cf. Scheuerell et al. 2007) and reduced mercury accumulation (cf. Cyr et al. 2016). Third, certain individual brook trout in the mesocosm experiment grew to sizes larger than predicted under any of our modeled
scenarios. One explanation for this finding is that brook trout with high growth rates had larger than average consumption rates irrespective of treatment (e.g., salmon or no salmon). Fourth, we found that across treatments, fish from the large size class exhibited higher growth rates and $\delta^{15}N$ ratios than fish from small or medium size classes. Dominance hierarchies have been observed within stream salmonid populations where larger fish increase in mass more rapidly than smaller fish (Wipfli et al. 2003). Thus, large fish are able to exert competitive dominance and thereby maximize foraging efficiency (Ahrens et al. 2012). When taken together, our results suggest that fish growth in response to salmon is controlled by the interactions between resource quantity and quality, along with individual factors including fish size and behavior that regulate foraging opportunities and energy acquisition.

4.5.3 Implications for contaminant accumulation and biotransport

Variation in the diet and energy intake of individual fish may also have implications for contaminant bioaccumulation and biotransport (Gerig et al. 2016). Our study showed that mercury burden, while primarily controlled by diet, can be influenced by growth. Somatic growth dilution occurs when organisms dilute a contaminant into a larger body mass; the growth dilution hypothesis holds that the faster an individual grows, the more the contaminant burden is diluted by increasing body mass (Trudel and Rasmussen 2006). In our mesocosm, we found that faster growing brook trout in non-salmon treatments exhibited lower mercury concentrations, conforming to the growth dilution hypothesis. In contrast, we observed that faster growing brook trout provisioned with salmon had higher mercury concentrations than slower growing individuals, opposite to the predictions of the growth dilution hypothesis (Trudel and Rasmussen...
This suggests that consumption of a highly contaminated food source can override the influence of growth efficiency and dilution (Madenjian et al. 1994, Trudel and Rasmussen 2006). Additionally, we found with our model that brook trout mercury accumulation was slightly lower in fish that consumed salmon eggs compared to chironomids, despite these diet items having similar mercury content. This suggests that consumption of energy dense salmon eggs resulted in increased growth thereby diluting the Hg burden in brook trout. However, consumption of eggs may lead to potential tradeoffs in bioaccumulation with other pollutants, such as PCBs, which accumulate in lipid-rich tissues.

Pacific salmon deliver contaminants to ecosystems that often lack direct point sources of pollution (Blais et al. 2007). Previous research, in both the native (Gregory-Eaves et al. 2007) and non-native (Janetski et al. 2012, Gerig et al. 2016) range of salmon, has shown that organisms that reside where salmon spawn exhibit higher body burdens of POPs. Moreover, the magnitude of uptake is linked to the flux of pollutants supplied by salmon (Gregory-Eaves et al. 2007, Janetski et al. 2012). Our study demonstrates that consumption of salmon tissue increases the mercury load in brook and brown trout. At present, no study has assessed salmon-mediated mercury transport to resident fish in the Great Lakes. However, a previous study found that spawning salmon increased mercury concentrations in aquatic invertebrates by more than 10 times as a result of carcass consumption (Sarica et al. 2004). We expect that resident fish will be similarly impacted if they consume significant quantities of salmon tissue. However, whether spawning salmon provide enough mercury to induce behavioral or physiological effects will depend upon the flux of mercury supplied by salmon, and the degree to which
resident fish consume salmon tissue over food from other sources (Krauss et al. 2014, Scheuhammer et al. 2007). Dietary exposure to mercury can result in behavioral changes in fish when tissue concentrations exceed 200 ppb (Beckvar et al. 2005), which is within the upper range of tissue concentrations observed in this study. Further research is needed to quantify the impact of salmon-mediated mercury transport in natural streams, especially on the stream-resident fish community.

4.5.4 Interactions between brook and brown trout

Contrary to our expectation, we found no evidence that brown trout adversely affected brook trout growth or altered their use of introduced salmon tissue. In fact, brook trout grew faster than brown trout when held together. These results are surprising given that introduced brown trout have been implicated in the decline of native brook trout in North America (Fausch and White 1981, Waters 1999). Several potential explanations exist. First, the mesocosms may have been saturated with food, thereby reducing interspecific competition for resources (Korsu et al. 2009). Second, competition between brook and brown trout varies with size and age; therefore in our mesocosm juvenile brook trout may outcompete brown trout due to aggressive foraging behavior (Fausch and White 1986). Third, the negative influence of brown trout on brook trout may be most apparent in natural systems where multiple age-classes of trout co-occur; brown trout grow larger and live longer than brook trout, and thus older brown trout may detrimentally impact smaller brook trout through direct predation and competition for optimal foraging locations (Waters 1999). These interactions may explain the observed decline in brook trout populations in portions of their native range (Hudy et al. 2008). Last, mesocosm water temperatures of ~10 °C may have favored growth of brook trout,
which prefer cooler water temperatures than brown trout, although water temperatures in the mesocosms were intentionally within the suitable feeding range for both species (Dewald and Wilzbach 1992). In total, the unexpected response of brook trout that we observed highlights the need for evaluation of competitive mechanisms across varying conditions, using both controlled laboratory and natural experiments (Pine et al. 2009).

Our mesocosm study demonstrated that provision of salmon resources did not modulate interactions between brook and brown trout, but that consumption of salmon tissue by both species increased mercury accumulation. Our simulation model suggested that consumption of salmon eggs increases growth and limits mercury accumulation, but tradeoffs in bioaccumulation of other pollutants may exist. Therefore, by coupling a controlled mesocosm experiment with a simulation model, we were able to demonstrate that the influence of salmon resources on resident fish is dependent on the quantity and quality of food in the diet, contaminant allocation to salmon tissues, and individual fish characteristics. Overall, this study highlights the complex nature of interactions between salmon and resident fish, which have important implications for fisheries management in the Great Lakes and elsewhere.
4.6 Acknowledgments

We thank James Aho and Dan Sampson, hatchery managers of the Michigan DNR Marquette State Fish Hatchery and Oden State Fish Hatchery, respectively, for providing the brook trout and brown trout used in this study. We also thank personnel from the Michigan Department of Natural Resources Alpena Fisheries Research Station including James Johnson, Dave Fielder, and Bill Wellenkamp for access to Hunt Creek Fisheries Research Station. Michael Dodrill of the USGS Grand Canyon Monitoring and Research Center assisted with the maximum likelihood estimator within the bioenergetics model. Doug Miller of UND assisted with the bomb calorimetry analysis. Funding was provided by the Great Lakes Fishery Trust (grant #2012.1244), UND College of Science Summer Undergraduate Research Fellowship program, UND Environmental Change Initiative, and the UND Center for Undergraduate Scholarly Engagement. In addition, BSG was supported by a STAR Fellowship (#F13F11071) from the United States Environmental Protection Agency. This research was conducted under the animal handling protocols of UND IACUC (#14-05-1773). Authors Gerig and Weber equally contributed to the paper.
4.7 Literature Cited


Hanson, P.C., T.B. Johnson, D.E. Schindler, and J.F. Kitchell. 1997. Fish Bioenergetics 3.0 for Windows. University of Wisconsin-Madison Center for Limnology and University of Wisconsin, Sea Grant Institute, Madison, WI.


5.1 Abstract

Pacific salmon transfer large quantities of nutrients and energy and sometimes contaminants to tributaries during their spawning migrations. Such ecosystem linkages can increase the contaminant burden of resident fish but the mechanisms causing this change are unclear. We conducted a before-after-control-intervention salmon addition experiment in a stream that had never received spawning salmon. Specifically, we added salmon carcasses and eggs in two consecutive years to Hunt Creek, a second-order tributary to Lake Huron, and then assessed the change in resident trout diet, isotopic composition, and contaminant burden. Resident trout diets shifted considerably after salmon tissue was added, with isotopically-enriched eggs representing 60% of their diet. However, this dietary shift did not change the $^{15}$N or $^{13}$C isotopic composition of resident trout, likely due to the short-term availability of salmon eggs and tissue turnover rate. In contrast, the introduction of salmon tissue increased the persistent organic pollutant (POP) burden of resident fish, with PCBs exhibiting a 21-fold increase. Mercury (Hg)
concentrations did not increase with the introduction of salmon tissue and were lower in the treatment reach for the entirety of the experiment. Our study suggests that the consumption of salmon eggs drives the increase in POP burden of resident trout but that Hg uptake was likely controlled by watershed sources. Moreover, differences in physicochemical properties of the contaminants and rates of tissue turnover influence the value of different ecological tracers. Overall, our study provides new understanding of processes that can induce variation in contaminant biotransport and uptake.

5.2 Introduction

Migratory organisms, such as Pacific salmon (*Oncorhynchus* spp.), create ecosystem linkages facilitated by their directional migration, which can have consequences for organisms and ecosystems (Schindler et al. 2003, Bauer and Hoye 2014). As adults, Pacific salmon occupy a high trophic position, accumulating not only energy but also contaminants such as persistent organic pollutants (POPs) and heavy metals, from lake or ocean environments as they mature. Pacific salmon are anadromous and semelparous, which facilitates a large transfer of energetically dense, but sometimes contaminated carcass and gametic tissue to streams where they spawn (Krummel et al. 2003, Schindler et al. 2003). While the ecosystem resource subsidies that salmon provide to freshwater habitats can have beneficial effects (e.g., Schindler et al. 2003, Lamberti et al. 2010), the transfer of contaminants by salmon represents a potential impact to both ecosystem (Blais et al. 2007) and human health (Hites et al. 2004).

Pacific salmon have been widely introduced outside of their native range (Crawford 2001). In particular, salmon have been established in the Laurentian Great Lakes since the 1960s with consequences for both the lakes and their tributaries
The watersheds of the Great Lakes contain a vast network of tributaries that provide essential contributions of water, nutrients, and organic matter to the lakes. However, these tributaries also act as bidirectional conduits for both biological (e.g., non-native species) and chemical (e.g., PCBs, mercury) pollution (Murphy et al. 2012). Pacific salmon represent a unique pollution source to Great Lakes tributaries due to their ability to transfer contaminants accumulated from the lake to streams during their spawning migration (Janetski et al. 2012, Gerig et al. 2016). This upstream movement of contaminants defies the usual pattern of downstream transport of pollution. The role that migratory species, especially salmon, play as a vectors for contaminant transfer has received recent attention (Bauer and Hoye 2014, Kallenborn and Blais 2015, Gerig et al. 2016) but many questions remain about the underlying mechanisms by which contaminant biotransport impacts ecosystems.

Contaminant biotransport consists of multiple linked components. Fundamentally, contaminants are bioaccumulated by a migratory organism, transferred across an ecosystem boundary via migration, and then deposited in a recipient ecosystem via excretion, egestion, spawning, or death (Blais et al. 2007, Kallenborn and Blais 2015). Previous research has focused on the process of bioaccumulation and bulk movement of contaminants by migratory organisms (Krummel et al. 2003, Baker et al. 2009) without consideration of the consequences of contaminant transfer for organisms within the recipient ecosystem (but see Morrissey et al. 2011, Janetski et al. 2012). In addition, studies of contaminant biotransport have relied primarily on survey-based approaches (reviewed in Kallenborn and Blais 2015) rather than direct experimental studies that are common in the salmon-resource subsidy literature (e.g., Wipfli 1999, Chaloner et al.
2002, Claeson et al. 2006). Furthermore, ecosystem manipulation has been rare in the contaminant literature (but see Sarica et al. 2005) but has the potential to inform the effect of contaminants on an ecosystem and community processes (Carpenter et al. 1995). Consequently, experiments may elucidate the circumstances influencing the mode and magnitude of contaminant biotransport, how biotransport and uptake varies among different contaminant types, and the dynamics of uptake and elimination of biotransported contaminants by resident organisms.

Using a two-year whole-stream experiment, we evaluated the effects of a novel salmon carcass and egg addition on the diet composition, stable isotope signature, and contaminant burden of resident trout in a Michigan stream. We used a suite of ecological tracers including stable isotopes of carbon (C) and nitrogen (N), POPs including polychlorinated biphenyls (PCB), dichloro-diphenyl-dichloro-ethylene (DDE,), polybrominated diphenyl ethers (PBDE), and mercury (Hg) to track the incorporation of salmon material into resident trout, coupled with gut content analysis. We hypothesized that (1) resident trout diets would change reflecting consumption of salmon material; (2) stable isotope ratios of C and N resident trout would exhibit enrichment reflecting incorporation of salmon-derived nutrients and energy; (3) contaminant levels of resident fish would increase following the addition of salmon material; and (4) isotopes, POPs, and Hg would provide complementary inference as ecological tracers of the influence of salmon on resident trout.
5.3 Methods

5.3.1 Site characteristics

We conducted a multi-year salmon carcass addition at the Hunt Creek Fishery Research Station (HCFRS) in Michigan, USA. Hunt Creek is a groundwater-fed second-order stream in the Thunder Bay River watershed located in the northeastern portion of the lower peninsula of Michigan. Hunt Creek is located above a series of mainstem dams on Thunder Bay River, and has never received Great Lakes salmon runs. Common resident fish species in Hunt Creek include native brook trout (*Salvelinus fontinalis*), introduced brown trout (*Salmo trutta*), and mottled sculpin (*Cottus bairdi*). The resident trout population has been continuously monitored at HCFRS since 1939, and the station has been the location of several seminal inland fisheries studies, including fish population responses to fisheries exploitation, flow alteration, and introduced species (Willis et al. 2006, Grossman et al. 2012).

5.3.2 Experimental design

We used a Before-After-Control-Intervention (BACI) study design (after Stewart-Oaten et al. 1986) to evaluate the contaminant responses of resident trout to an experimental carcass and egg addition. In Hunt Creek, we established 90-m treatment and 90-m control reaches (Fig 5.1). The control reach was located 600 m upstream of the treatment zone, separated by two impassible weirs that prevented upstream fish movement between reaches. Salmon carcasses and eggs were added to Hunt Creek in October of 2014 and 2015. Loading rates of salmon carcasses and eggs approximated that of a typical salmon run in a Lake Michigan tributary (Janetski et al. 2012) along with associated contaminant concentrations and flux (Table 5.1). Introduced carcasses were
staked using rebar in locations where they are commonly retained, including pools, undercut banks, and debris jams (Tiegs et al. 2011). To simulate spawning, salmon were introduced at the upstream extent of the treatment area and allowed to passively drift downstream and settle. Eggs were retained in substrate interstices and depositional areas of the treatment zone for ~10 days following the addition (B. Gerig, pers. obs.). The source of Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) carcasses used in the experiment were state fish hatcheries operated by the Michigan Department of Natural Resources. Prior to being introduced to Hunt Creek, carcasses were tested for bacterial and viral pathogens to prevent inadvertent disease introduction.

![Diagram showing experimental layout of control and treatment reaches in Hunt Creek.](image)

Fig. 5.1 Experimental layout of control and treatment reaches in Hunt Creek.
Resident trout were sampled one day prior to the addition of salmon carcasses in October 2014 and sampled again 49 days after the salmon material addition commenced. The salmon addition was replicated in 2015, albeit at a somewhat lower loading rate (Table 5.1). In 2015, resident trout were sampled one day prior to the addition of salmon and resampled 44 days after the second salmon addition commenced. All fish collected during the salmon addition experiment were sampled using backpack electrofishing. Upon collection, individual fish were identified to species and measured for length and weight. Resident trout diets were collected from a subsample of fish in 2015 using gastric lavage (Ivan et al. 2011). Diet material were preserved in ethanol and stored in a freezer until samples could be processed in the laboratory. Each component of the diet was classified into 6 categories: aquatic invertebrates, terrestrial invertebrates, non-insect invertebrates, fish, amorphous detritus, and salmon eggs. For each diet, frequency of occurrence of each prey item was determined.

### TABLE 5.1.

CHARACTERISTICS OF SALMON EXPERIMENTALLY ADDED TO HUNT CREEK.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Tissue Type (ng/g)</th>
<th>Contaminant flux (ng/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carcass</td>
<td>Eggs</td>
</tr>
<tr>
<td>PCB</td>
<td>394</td>
<td>547</td>
</tr>
<tr>
<td>DDE</td>
<td>31</td>
<td>41</td>
</tr>
<tr>
<td>PBDE</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Hg</td>
<td>233</td>
<td>15</td>
</tr>
</tbody>
</table>
5.3.3 Analytical chemistry

Carbon and nitrogen stable isotope ratios were determined for homogenized whole-body samples of resident trout, salmon tissue, and eggs using an Elemental Analyzer (Costech, Valencia, CA) coupled to a Delta Plus Isotope Ratio Mass Spectrometer (Thermo Scientific, Waltham, MA) located in the Center for Environmental Science and Technology (CEST) at UND. Prior to analysis, all samples were freeze-dried, homogenized into a fine powder, and stored at -20°C (cf. Gerig et al. 2017). Isotope ratios were corrected within each individual run using a 3-point calibration curve developed from known isotope standards (EA Consumables, Pennsauken, NJ). Wheat flour, sorghum, and protein standards were used to build the calibration curve. Stable isotope ratios of N ($\delta^{15}$N) and C ($\delta^{13}$C) were expressed as:

$$\delta^{15}N \text{ or } \delta^{13}C = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000 \quad \text{(Equation 5.1)}$$

where $R$ is the ratio of $^{15}$N to $^{14}$N or $^{13}$C to $^{12}$C. Tissue C:N was determined using an acetanilide standard (cf. Chaloner et al. 2002, Reisinger et al. 2013). Data were deemed acceptable if the standard deviation of acetanilide standards during the run was <0.2 per mil ($\‰$). The standard deviations for acetanilide standards were 0.12 and 0.08$‰$ for N and C, respectively. All $\delta^{13}$C values were lipid-corrected using individual C:N ratios (cf. Post et al. 2007).

Concentrations of the POPs polychlorinated biphenyl (PCB), dichloro-diphenyl-dichloroethylene (DDE), and polybrominated diphenyl ether (PDBE) in homogenized whole-fish samples were determined for Pacific salmon and resident trout using EPA methods 1668, 608, and 1614, respectively. All POPs were quantified using an Agilent 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a
micro–electron capture detector. Instrumental conditions were similar to Stapanian et al. (2013). Our analytical method assessed a total of 89 PCB congeners and 6 PBDE congeners. DDE was the only dichloro-diphenyl-trichloro-ethane (DDT) metabolite quantitatively assessed. Total PCB concentration for each sample was calculated by summing the concentrations of all 89 congeners. Total PBDE concentration was calculated by summing the concentration of all 6 congeners. All POPs are reported as ng/g wet weight. The instrument was calibrated with individual congener standards at five concentration levels (beginning with 0.10 ng/g) obtained from AccuStandard (New Haven, CT). The West Coast Fish Studies standard (AccuStandard) was analyzed for calibration verification. Method blanks were run at a frequency of 1 per 20 samples, with a mean concentration (± SE) of 0.41 (± 0.08) ng/g. Matrix spikes and matrix-spiked duplicates were also performed at a 5% frequency, with mean recovery and mean relative percentage difference equal to 81 (± 5)% and 11 (± 3)%, respectively. Surrogate recoveries averaged 86 (± 9)%. Detection limits for the individual congeners were set at 3 times the baseline noise (≈0.01 ng/g). Detection limits were verified by analyzing a low-level standard at 0.025 ng/g, which yielded a signal to noise ratio of between 12 and 15.

Mercury concentrations of homogenized whole fish samples for salmon and stream-resident fish were determined using a Direct Mercury Analyzer 80 (DMA-80, Milestone S.r.l., Sorisole, Italy) located at CEST. All samples were prepared for Hg analysis in the same manner as for isotope analyses. For analysis, 0.02 g of homogenized sample was weighed into ashed nickel boats, placed into the DMA-80, and analyzed via fixed wavelength atomic absorption spectrophotometry (cf. Gerig et al 2017). The DMA-80 was calibrated using standard reference materials (National Research Council of
Canada, DORM-4, Ottawa, ON). Dry weight Hg concentration was converted to wet weight concentrations using the percent water content of each homogenized sample and expressed as ng/g wet weight. Quality control measures, including blanks, matrix spikes, matrix spiked duplicates, and standards were analyzed to ensure precision and accuracy of analyses. Percent recovery from a DORM-4 standard was 99.2 ± 7.1% and the detection limit was 0.2 ng/g.

5.3.4 Statistical analyses

The response of resident trout isotope and contaminant concentrations to the salmon addition was assessed using a three-way analysis of variance (ANOVA, $\alpha=0.05$, Zar 2010). In this analysis, we evaluated the main effects of stream reach (Control or Treatment), time period (Before or After salmon addition), and Year (2014 or 2015). This analysis was conducted for each isotope ($\delta^{15}$N, $\delta^{13}$C) and contaminant (PCB, DDE, PBDE, Hg). We interpreted a significant Reach x Time interaction as evidence for the salmon addition influencing resident trout isotopic signatures or contaminant concentrations. A Reach x Time x Year interaction was interpreted as evidence that response of resident trout differed between the 2014 and 2015 experiments. To facilitate interpretation of the outcome of the experiment, we conducted post-hoc Tukey’s multiple comparison tests ($\alpha=0.05$, Zar 2010) to assess differences between treatment combinations. We did not evaluate species-specific differences in this analysis to maximize our statistical power for evaluating treatment effects. Prior to statistical analysis, all contaminant data were log-transformed to meet the assumption of normality and equal variance among residuals (Zar 2010), while isotope data met the assumption of normality and equal variance and were not transformed. For the gut content analysis, we
used a permutational analysis of variance (PERMANOVA) to assess whether diet composition changed significantly between reach (Control, Treatment) and time periods (Before, During, After) as a result of the salmon addition (cf. Gerig et al. 2016).

5.4 Results

5.4.1 Diet response to salmon addition

Resident trout diet composition exhibited a large shift that coincided with the introduction of salmon material to Hunt Creek (PERMANOVA, Reach x Time Interaction, P<0.001). Prior to the salmon addition, resident trout diets were similar between the treatment and control reaches and consisted primarily of aquatic and terrestrial insects and non-insect invertebrates (Fig 5.4). One week after the introduction of salmon eggs and carcasses, resident trout diets in the treatment reach were composed primarily of salmon eggs, which represented more than 60% of their diet, whereas trout diets in the control reach were largely unchanged. One week after the introduction of salmon, all resident trout sampled in the treatment reach had eggs in their diet but the frequency of occurrence varied widely (mean=16.1 eggs/diet, SD=16.7 eggs/gut, range: 2-56 eggs/gut). Forty-four days after introduction, salmon eggs represented only 4% of the diet, and aquatic invertebrates returned to being the predominant diet item in both the control and treatment reaches. Only two resident trout after salmon addition were observed to have small quantities of carcass tissue in their diet.
5.4.2 Stable isotope response to salmon addition

Resident trout isotope ratios did not shift following the salmon addition (Fig 5.3, Table 5.2). In 2014, an effect of salmon addition was not observed in stable isotope ratios between time periods or reaches, and was consistently a trophic level lower than the introduced salmon (Table 5.2). We did find evidence of isotopic enrichment in $\delta^{15}N$ after the salmon addition in 2015. However, this result should be interpreted with caution as the $\delta^{15}N$ ratio after the 2015 salmon addition did not differ from the control or treatment $\delta^{15}N$ from the 2014 before time period (Fig 5.3). We found no evidence of a salmon effect in $\delta^{13}C$ in either 2014 and 2015 (Fig 5.3, Table 5.2). Therefore, our salmon
addition was unable to significantly shift stable isotope ratios for this resident trout population.

Figure 5.3. Response of resident trout stable isotope ratios to an experimental salmon addition to Hunt Creek.
TABLE 5.2.
RESULTS OF ANOVAS ASSESSING WHETHER EXPERIMENTAL SALMON ADDITIONS INFLUENCED THE STABLE ISOTOPE RATIOS OF RESIDENT TROUT.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>F-stat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{15}$N</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>11.1</td>
<td>0.002</td>
</tr>
<tr>
<td>Time</td>
<td>9.1</td>
<td>0.004</td>
</tr>
<tr>
<td>Year</td>
<td>0.004</td>
<td>0.95</td>
</tr>
<tr>
<td>Reach $\times$ Time</td>
<td>3.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Reach $\times$ Year</td>
<td>3.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Time $\times$ Year</td>
<td>15.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Reach $\times$ Time $\times$ Year</td>
<td>0.89</td>
<td>0.35</td>
</tr>
<tr>
<td>$\delta^{13}$C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>0.4</td>
<td>0.51</td>
</tr>
<tr>
<td>Time</td>
<td>2.1</td>
<td>0.16</td>
</tr>
<tr>
<td>Year</td>
<td>0.04</td>
<td>0.84</td>
</tr>
<tr>
<td>Reach $\times$ Time</td>
<td>0.12</td>
<td>0.73</td>
</tr>
<tr>
<td>Reach $\times$ Year</td>
<td>16.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Time $\times$ Year</td>
<td>0.07</td>
<td>0.78</td>
</tr>
<tr>
<td>Reach $\times$ Time $\times$ Year</td>
<td>1.73</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Note: Factors include reach (treatment, control), time (before or after salmon addition), and Year (2014 or 2015). Significant Tukey relationships shown in bold ($\alpha=0.05$).

5.4.3 Contaminant response to salmon addition

The addition of salmon carcasses and eggs led to a rapid and large increase in PCB, DDE, and PBDE concentrations in resident trout as evidenced by the significant Reach $\times$ Time $\times$ Year interaction for each POP (Fig. 5.4, Table 5.3). For all POPs, a clear treatment effect was observed when comparing the before and after time period between the control and treatment reach in 2014 (Fig. 5.4), However, the magnitude of this increase varied among contaminants, with PCB, DDE, and PBDE exhibiting a 21-fold, 9-fold, and 8-fold increase respectively. POPs accumulated during the first year of the
salmon addition were retained between the after 2014 and before 2015 time period (Fig 5.4). The response of resident trout to the 2015 salmon addition differed depending on the POP considered (Fig 5.4, Table 5.3). PCB concentrations in resident trout did not increase in response to the second salmon addition but remained similar to the after 2014 and before 2015 PCB concentrations (Fig. 5.4). In contrast, DDE and PBDE concentrations increased by 1.9-fold and 1.2-fold respectively, from the after 2014 to the after 2015 time period. However, the magnitude of the 2015 increase was much smaller when compared to the large increase observed after the 2014 addition (Fig. 5.4). Overall, PCBs increased from 7.1 to 148 ng/g, DDE from 2.2 to 20.9 ng/g, and PBDE from 2.3 to 19.6 ng/g between control to treatment reaches across time periods.

We observed no effect of salmon addition on resident trout Hg concentrations (Fig. 5.4, Table 5.2). Overall, resident trout located in the upstream control reach had 32% higher Hg concentrations, with a mean concentration of 109.1 ng/g in the control and 83.8 ng/g in the treatment reach respectively. This result was not driven by fish size, which was not statistically different among both control and treatment reaches during each sampling event (length ANOVA, p=0.83; weight ANOVA, p=0.91).
Figure 5.4. Response of resident trout contaminant concentrations to an experimental salmon addition. All concentrations are in ng/g wet weight and reported on a log scale. Letters represent significant differences between reaches/time points based upon Tukey multiple comparison tests.
TABLE 5.3.
RESULTS OF ANOVAS ASSESSING WHETHER EXPERIMENTAL SALMON ADDITIONS INFLUENCED THE CONTAMINANT CONCENTRATIONS OF RESIDENT TROUT.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>F-stat</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>193.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Time</td>
<td>9.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Year</td>
<td>19.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Reach × Time</td>
<td>2.5</td>
<td>0.04</td>
</tr>
<tr>
<td>Reach × Year</td>
<td>5.9</td>
<td>0.02</td>
</tr>
<tr>
<td>Time × Year</td>
<td>21.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Reach × Time × Year</td>
<td>21.6</td>
<td>0.001</td>
</tr>
<tr>
<td>DDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>137.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Time</td>
<td>8.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Year</td>
<td>42.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Reach × Time</td>
<td>9.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Reach × Year</td>
<td>15.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Time × Year</td>
<td>5.8</td>
<td>0.02</td>
</tr>
<tr>
<td>Reach × Time × Year</td>
<td>9.4</td>
<td>0.001</td>
</tr>
<tr>
<td>PBDE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>142.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Time</td>
<td>1.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Year</td>
<td>54.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Reach × Time</td>
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<td>0.004</td>
</tr>
<tr>
<td>Reach × Year</td>
<td>15.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Time × Year</td>
<td>13.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Reach × Time × Year</td>
<td>6.5</td>
<td>0.013</td>
</tr>
<tr>
<td>Hg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reach</td>
<td>15.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Time</td>
<td>0.1</td>
<td>0.72</td>
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<tr>
<td>Year</td>
<td>10.7</td>
<td>0.001</td>
</tr>
<tr>
<td>Reach × Time</td>
<td>2.1</td>
<td>0.15</td>
</tr>
<tr>
<td>Reach × Year</td>
<td>2.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Time × Year</td>
<td>0.2</td>
<td>0.65</td>
</tr>
<tr>
<td>Reach × Time × Year</td>
<td>0.1</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Note: Factors include reach (treatment, control), time (before or after salmon addition), and Year (2014, 2015). Significant Tukey relationships shown in bold (α=0.05).
5.5 Discussion

We conducted the first experimental manipulation quantifying the transfer and uptake of salmon-derived contaminants to stream-resident trout. Our study provides experimental confirmation of previous observations that stream-resident fish exposed to salmon have higher POP burdens (Janetski et al. 2012, Gerig et al. in review, Chapter 3). Stream-resident trout exposed to salmon additions increased their POP body burden but the magnitude of the response was dependent on the specific contaminant, with PCBs exhibiting the largest increase. Moreover, our results show that the transfer of salmon-derived pollutants to stream-resident fish occurred rapidly (<40 days) and then persisted over the two years of the experiment. We posit that the swift uptake of salmon-derived contaminants is likely driven by consumption of salmon eggs. In contrast, the addition of salmon material did not increase Hg concentrations of resident trout, suggesting that salmon are not a significant source of Hg to resident trout. Similar to Hg, the stable isotope ratios of C and N in resident trout did not change, suggesting that resident fish incorporate salmon nitrogen or carbon at a different temporal scale than POPs. Our findings suggest that POPs may be a more sensitive ecological tracer than stable isotopes when seeking to understand the flow of salmon-derived material to stream-resident fish. Overall, our experimental manipulation suggests that the contaminant considered and trophic pathway to contamination are the primary drivers of contaminant biotransport by salmon and subsequent uptake.

Salmon egg consumption by resident trout appeared to drive the increase in resident trout POP concentrations. Past studies have found that the resident fish increase their ration size and consumption rate when salmon eggs are available, thereby increasing
their energy intake by greater than 10-fold (Ivan et al. 2011, Jaecks et al. 2014).

Moreover, previous studies have found that salmon eggs have higher POP concentrations when compared to whole fish samples (Gerig et al. in review, Ch. 3). This finding is attributable to the high lipid content of salmon eggs compared to whole fish tissue (Janetski et al. 2012), which as a function of toxicokinetics leads to higher POP concentrations (Niimi and Oliver 1983, Arnot and Gobas 2004). To illustrate the influence of salmon egg consumption on resident trout PCB concentrations; consider this back of the envelope calculation (cf. Harte 1988). If a 40 g resident trout with a PCB concentration of 8 ng/g prior to the salmon run consumes 50 salmon eggs with an average weight of 0.25 g (Kerns et al. 2016) and a PCB concentration of 550 ng/g (Gerig et al. in review, this study), the PCB concentration of the resident trout would increase by 21-fold. We observed a similar increase in resident trout PCB concentrations in our experiment. While this scenario is a simplification of the process of contaminant uptake and incorporation, and does not account for the physiology of growth or contaminant bioaccumulation (Niimi and Oliver 1983, Arnot and Gobas 2004), it illustrates how changes in diet can greatly increase the overall PCB burden of resident trout when the diet item consumed is highly contaminated. Thus, the high POP concentrations of salmon eggs along with their preferential consumption interact to amplify the capacity of salmon resources to increase resident trout contaminant concentrations.

The POP burden accumulated by resident trout in 2014 was retained over the following year, suggesting that, resident trout do not substantially reduce their POP burden via metabolism or growth (Arnot and Gobas 2004, Paterson et al. 2007). Paterson et al. (2007) demonstrated through bioenergetics modeling that PCB elimination by
yellow perch (*Perca flavescens*) varied seasonally as a function of temperature but was markedly reduced for highly lipophilic and more chlorinated congeners (log $K_{ow}$ >6.5). Furthermore, in a laboratory-based experiment with rainbow trout (*Oncorhynchus mykiss*), the half-life for elimination of highly chlorinated PCB congeners exceeded 1000 days (Fisk et al. 1998). Andersson et al. (2001) found that Arctic char (*Salvelinus malma*), a species closely related to brook trout, lacked the ability to substantially metabolize or eliminate PCBs. In a previous study, Gerig et al. (2016) showed that salmon transfer a specific mixture of PCB congeners to resident trout that consists primarily of the highly lipophilic and bioaccumulative penta, hexa, and hepta congener homolog groups. Taken together, resident trout appear to have little ability to eliminate PCBs accumulated from salmon. High variability in resident fish POP concentrations (Gerig et al. in review, Chapter 3), particularly at low salmon run sizes, may therefore be related to both variation in egg consumption and among-year variation in salmon run size.

We saw no additive effect between the first and second year of our experiment that resulted in higher POP concentrations in resident trout after the second salmon addition. In a modeling study, McGill et al. (2017) predicted that PCB concentrations in brook and brown trout would increase over the course of multiple salmon runs to equilibrium concentrations, as fish get larger and older. The difference between studies may reflect the size or age class of fish sampled in our experiment. In our study, we only sampled fish that were between 140-180 mm (i.e., 1+ fish) to minimize any variation associated with fish size (Schneider 2001). The lack of a stepwise increase in POP concentrations may reflect this focus on the same age class of fish through time. If larger
and older fish larger had been sampled, we may have seen a stepwise increase in the 
second year of salmon addition. This finding suggests that the age structure of fish 
populations is an important consideration in contaminant studies (McGill et al. 2017).

We found no evidence that salmon are a significant source of Hg to resident trout. 
In fact, trout in the upstream control reach had higher Hg body burdens. Moreover, the 
lack of an increase in Hg following the salmon addition coupled with our diet analysis 
suggests that consumption of carcass tissue, the presumed pathway for salmon-derived 
Hg, is uncommon. This finding confirms field studies from both the introduced (Gerig et 
al. in review, Ch. 3) and native range of salmon (Baker et al. 2009, Cyr et al. 2016) where 
resident fish in locations with salmon had lower Hg concentrations compared to upstream 
locations. These findings may suggest that other covariates such as water temperature, 
pH, and dissolved organic carbon more strongly control rates of Hg bioaccumulation in 
fish (Ward et al. 2010, Driscoll et al. 2013).

Previous studies have demonstrated that Hg concentrations decreased with 
consumption of salmon eggs (Cyr et al. 2016) and increased salmon spawning biomass 
(Gerig et al. in review). The discrepancy between our salmon addition and these studies 
may result from differences between natural and simulated salmon runs (Janetski et al. 
2009). Specifically, our experiment relied on a single pulse of salmon eggs to Hunt Creek 
executed in both 2014 and 2015, whereas field-based surveys integrate egg consumption 
over the entirety of the salmon spawning run across multiple years. As a result, the period 
that salmon eggs were available for consumption by resident trout was much shorter in 
our experiment when compared to observational surveys. In addition, in streams 
receiving natural salmon runs, competition for spawning habitat can be high which
results in redd superimposition (Moore et al. 2008). As a result, the availability of eggs for consumption increases as spawning habitat becomes limiting. The effect of redd superimposition is likely magnified in the Great Lakes, where salmon have a large disturbance effect on the streambed as a result of small, glacially derived sediments (Janetski et al. 2014). Overall, the lack of a Hg effect is consistent with the lack of isotopic enrichment observed in this study as compared to the substantial enrichment seen with natural salmon runs (Chaloner et al. 2002, Cyr et al. 2016, Gerig et al. in review). In contrast, a mesocosm study showed that brook trout provisioned with salmon tissue but not eggs exhibited both isotopic enrichment and large increases in Hg concentration (Gerig et al. 2017). This difference highlights that resident salmonids in streams receiving natural salmon runs do not consume large quantities of decomposing carcass material.

Resident trout in our upstream control reach had significantly more Hg despite being separated from the treatment reach by only 600 m. This finding may be related to a large beaver dam located 100 m upstream from our control reach. Beavers modify stream ecosystems by creating impoundments that alter stream flow and capture large quantities of organic matter (Naiman et al. 1988, Jones et al. 1994). These impoundments also serve as ideal bioreactors for sulfate-reducing bacteria, which increase the bioavailability of Hg for food web bioaccumulation (Painter et al. 2015). Prior research has shown that Hg can be higher in basal resources and aquatic invertebrates in stream locations downstream of beaver dams when compared to upstream reference sites (Painter et al. 2015). Our findings suggest that this effect may also extend to upper trophic level organisms such as resident trout. Why this upstream Hg effect dissipates over a short distance between the control and treatment reach is unknown. Potential mechanisms to explain this pattern
may include that Hg derived from the beaver impoundment is demethylated and lost to the atmosphere or accumulated into biota over a relatively short stream reach, and thus there is limited spatial extent of those effects (Driscoll et al. 2013). We posit that resident trout in the control reach consume a large proportion of secondary production derived from the control reach, including from areas where mercury levels are higher (cf. Huryn 1996). The higher Hg concentration in trout from the control reach may then reflect dietary reliance on reach-scale, instream invertebrate production. Overall, this unexpected finding suggests that contaminant bioaccumulation can differ across small spatial scales. This finding reinforces the importance of robust sampling designs such as BACI to account for potential confounding variables.

Ecological tracers, including stable isotopes and anthropogenic contaminants, are increasingly used to understand food web linkages and trace the movement of energy in ecosystems (Ramos and González Solís 2012, McLeod et al. 2015). Traditionally, stable isotopes have been used trace the nutrient and energy contributions of salmon to ecosystems (e.g., Bilby et al. 1996, Chaloner et al. 2002). In this study, we used three different tracers to document the flow of salmon material and its contaminants to resident trout, each with contrasting conclusions. Stable isotopes, the standard currency for tracking salmon nutrients in food webs, were ineffective tracers in our system. The efficacy of isotopes in salmon studies is dependent on the isotopic enrichment of both C to N when comparing salmon to the stream ecosystems they subsidize. In contrast, PCBs were highly effective tracers of the uptake of salmon-derived resources, likely because of the high PCB concentration of salmon eggs relative to other dietary sources (McGill et al. 2017) and their high assimilation efficiency of lipids that bind PCBs (Niimi and Oliver
The efficacy of PCBs as a salmon tracer is enhanced by their persistence, stability, and slow elimination rate (Paterson et al. 2007, McLeod et al. 2015). In contrast, bioaccumulative Hg was a poor tracer of the uptake of salmon-derived resources, likely due to other sources of Hg.

These unexpected results demonstrate the importance of understanding the biochemistry and spatiotemporal scale for tracer uptake into the organism. For example, isotopic enrichment of resident trout following a natural or experimental salmon addition is dependent upon both a dietary shift to isotopically-enriched salmon material and the tissue turnover rate within the resident trout (Jardine et al. 2006). Isotopic change in whole fish is dependent on the metabolic turnover of both protein and lipid, and is a relatively slow (i.e., months) process compared to other tissue types such as liver or plasma (i.e., days). Similarly, Hg accumulates by complexing with cysteine in the muscle matrix and is more closely linked with protein turnover. In contrast, POPs are absorbed directly from lipid-rich diet items and partition rapidly into tissue during digestion (Arnot and Gobas 2004). Thus, the variation among the isotope, POP, and Hg results suggests that the switch to salmon eggs by resident trout was reflective of a short-term change in diet that led to a strong POP signal without a corresponding shift in Hg concentration or isotopic composition. Understanding the complex routing of tracers into particular tissue types a priori will increase the likelihood that the tracer will provide meaningful information regarding the ecological history and past diet of the organism.
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5.7 Literature Cited


6.1 Abstract

Polychlorinated biphenyls (PCBs) and mercury (Hg) are contaminants of concern for ecosystems and ultimately for human health. Meanwhile, resource subsidies derived from donor ecosystems, in which those contaminants may accumulate, can have significant influences on recipient ecosystems. Pacific salmon (*Oncorhynchus* spp.) introduced into the Laurentian Great Lakes can accumulate large contaminant burdens that they disperse to streams during spawning in the form of valuable resources, such as tissue and eggs, with uncertain consequences. We used a combination of empirical and literature data to parameterize a coupled bioenergetics-bioaccumulation model to understand the bioaccumulation of salmon-mediated contaminants and how this is balanced by positive resource subsidy effects. More specifically, we assessed how the trophic pathways of contamination, level of salmon egg consumption, and environmental context affected growth and contaminant accumulation in resident trout. Using our model, we demonstrated that salmon egg consumption strongly controls the growth and

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PCB bioaccumulation of resident trout, and indirectly mediates a reduction in Hg through somatic growth dilution. Other trophic pathways, including carcass consumption and the indirect food web pathways did not significantly increase growth or contaminant bioaccumulation. In addition, we demonstrated that species identity and the environment where salmon accumulate their contaminant burden strongly influences the uptake of salmon-derived contaminants by resident trout. Overall, our model highlighted that migratory fish transfer both energy and contaminants but this balance is dictated by the food web pathway and attributes of the resident trout’s diet including energy density, ration size, and contaminant concentration.

6.2 Introduction

Contamination of aquatic ecosystems with persistent organic pollutants (POPs) has important consequences for ecological and human health. Contaminants of particular concern include polychlorinated biphenyls (PCBs), along with heavy metals, such as mercury (Hg) (Blais et al., 2007; Murphy et al., 2012). PCBs historically were used in a number of industrial applications but were banned in the late 1970s as part of the Stockholm Convention (Blais, 2005). Polychlorinated biphenyls remain a concern due to their persistence and resistance to degradation in the environment (Blais, 2005). Unlike PCBs, Hg is naturally occurring but concentrations in the environment are increasing due to fossil fuel combustion and food web transfers (Johnson et al., 2015; Blukacz-Richards et al., 2017). The bulk of previous research on POPs has focused on understanding the chemical and physical processes responsible for their cycling in the environment, or their bioaccumulation in food webs (Blais, 2005; Clements et al., 2012). However, a more recent focus of research has been on the ability of migrating organisms to transport...
Contaminant biotransport is a complex, multi-stage process. The four-step process involves: first, bioaccumulation of a contaminant in the body of a migratory organism; second, movement of the contaminated organism across an ecosystem boundary; third, deposition of the contaminant into a recipient ecosystem; and fourth, the uptake of the biotransported contaminant by a different, resident organism (Blais et al., 2007; Clements et al., 2012; Gerig et al., in review). The magnitude of contaminant biotransport is dependent upon the environmental context (Clements et al., 2012). This context may include traits (e.g., run size, life-history, contaminant burden) of the migratory organism, the contaminant being transported, and the characteristics of the resident community in which the contaminants are deposited (Gerig et al., in review). The biotransport of contaminants has been dubbed the ‘dark side of ecosystem resource subsidies’ because the normal controls and resistance to bioaccumulation can be circumvented through direct consumption of contaminated tissue (Walters et al., 2008; Gerig et al., in review).

Pacific salmon (Oncorhynchus spp.) provide an ideal model to study the process of contaminant biotransport. Pacific salmon accumulate large contaminant burdens, and then migrate and die after a single spawning event (Blais et al., 2007). Through spawning and senescence, salmon also deposit large quantities of energetically dense (Gende et al., 2002; Schindler et al., 2003), but potentially contaminated (Sarica et al., 2004; Janetski et al., 2012) carcass and egg material that is readily utilized by resident organisms including fish (Chaloner et al., 2002; Scheuerell et al., 2007). Thus, salmon can act as both a resource...
subsidy and a contaminant biovector that can influence overall ecosystem productivity and health (Krummel et al., 2003). Considerable uncertainty exists about the synergistic and antagonistic interactions between the effects of material delivered as a resource that is also contaminated (Gerig et al., 2017). This research considering the influence of introduced Pacific salmon on Great Lakes tributaries addresses those uncertainties.

The Great Lakes and their tributaries provide a unique context in which to study contaminant biotransport by Pacific salmon. Salmon were introduced during the mid-1960s to control invasive alewife and rehabilitate predator populations. Since being introduced, salmon have become an integral component of the Great Lakes recreational fishery (Dettmers et al., 2012). At present, salmon populations are maintained through natural reproduction and hatchery supplementation (Dettmers et al., 2012; Kerns et al., 2015). In addition, the Great Lakes have suffered from a legacy of industrial pollution that has elicited contaminant bioaccumulation and widespread fish consumption advisories based upon high concentrations of PCBs and Hg (Stow et al., 1995; Murphy et al., 2012). As such, Great Lakes salmon represent a vector by which lake-derived contaminants are transferred and deposited in tributary streams. While transfer of salmon-derived POPs to stream biota has been documented in the Great Lakes (e.g., Gerig et al., 2016; Janetski et al., 2012), little is known about the mechanisms regulating the biological uptake of salmon-derived contaminants (Gerig et al. 2017; Gerig et al., in review). Such insights could also improve our understanding of the influence of resource subsidies on recipient ecosystems by providing insight into dominant trophic pathways which salmon derived energy is incorporated into resident fish. Given these uncertainties and knowledge gaps, we reasoned that an individual-based modeling approach may be a
useful and informative way to evaluate and predict the influence of spawning salmon on resident fish.

Individual-based models are increasingly used to understand ecological phenomenon. This, in part, is due to their ability to model complex system dynamics and multiple state variables. As such, individual-based models can be used to predict factors influencing growth and contaminant accumulation patterns in fish (Hanson et al., 1997; Arnot and Gobas, 2004). For example, bioenergetic and bioaccumulation models can be linked to a dynamic model of how changes in diet composition, feeding rate, and contaminant concentration influence both the short-term and lifetime rates of growth and bioaccumulation (Johnson et al., 2015). As diet is the primary pathway of contaminant uptake by fish (Trudel and Rasmussen, 2006), as such, modeling approaches may reveal the underlying mechanisms by which salmon, influence the uptake and accumulation of contaminants by resident fish in recipient ecosystems (McGill et al. 2017, Gerig et al., in review).

The main objective of this study was to use a modeling framework to better understand and integrate the underlying mechanisms by which stream-resident salmonids acquire energy and accumulate contaminants transported by introduced Pacific salmon in the Laurentian Great Lakes. To meet this objective, we first conducted a diet study to empirically measure the ration size and diet composition of resident salmonids in streams where spawning salmon were present. Second, we used empirical information on the energy density and contaminant concentration of resident salmonid diet items to parameterize an individual-based, bioenergetics-bioaccumulation model. Third, we used the model to assess the influence of salmon contaminant biotransport on resident trout
growth and bioaccumulation. More specifically, we established scenarios to predict how the trophic pathway to contamination, variation in salmon egg consumption, and environmental context influenced the uptake of salmon-derived resources and mediated contaminant biotransport to resident salmonids. This modeling framework allowed us to identify the primary drivers of contaminant bioaccumulation of salmon-derived contaminants in resident fish in the Great Lakes, laying important groundwork for future studies and ecosystem management.

6.3 Methods

6.3.1 Diet Analysis

We sampled the diet of stream-resident salmonids during the fall of 2016 from six streams in the Lake Michigan basin. Three streams were accessible to spawning salmon while three streams were upstream of impassible barriers that made them inaccessible to salmon migration. We sampled resident salmonid populations prior to the arrival of salmon in mid-September and during the peak of the salmon run in mid-October. Each tributary accessible to salmon received a fall spawning run of Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon. Chinook salmon were the predominant spawner in streams accessible to migratory fish. We sampled resident salmonids that are common components of the cold-water fish community of Great Lakes tributaries, including brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*), although not all species were present at each location (Gerig et al., in review). All fish were collected using standard fisheries techniques, including backpack electrofishing (Gerig et al., in review). Upon collection,
individual fish were identified to species, measured for length and weight, and had their stomach contents purged via gastric lavage (cf. Ivan et al., 2011). Each diet sample was preserved in ethanol and stored in a freezer until samples could be processed in the laboratory. We classified resident salmonid diets into six categories: aquatic invertebrates, terrestrial invertebrates, non-insect invertebrates, fish, amorphous detritus, and salmon eggs. For each diet, the ration size (wet mass in g) and frequency of occurrence was determined. To assess the role of Pacific salmon on resident salmonid diets we used a two-way analysis of variance (Zar 2010, $\alpha=0.05$) to assess how the factors of sampling location (salmon spawners present or absent) and time (before or during salmon run) influenced ration size.

6.3.2 Bioenergetics model

We used a coupled bioenergetics-bioaccumulation model to predict the response of resident salmonid growth and contaminant burden to energy and contaminants transported by spawning salmon over the lifespan of a resident salmonid (cf. Johnson et al., 2015). Our model was based upon a time-dynamic bioenergetics model (cf. Hanson et al., 1997; Rashleigh and Grossman, 2005), and parameterized using species-specific physiological parameters (Hartman and Cox, 2008) for brook trout. We selected brook trout as our focal species for modeling because they are common throughout the introduced range of Pacific salmon and readily consume salmon material when available (Janetski et al., 2012; Gerig et al., 2016). In the model, energy consumed through diet is allocated to catabolic processes (e.g., respiration, specific dynamic action) and then to waste production (e.g., egestion, excretion); any remaining energy is allocated to growth
(Hanson et al., 1997; Rashleigh and Grossman, 2005). The bioenergetics portion of the model was defined as:

\[
d\frac{M}{dt} = (C - Eg - Ex) \times ED_p - (ACT \times R + SDA) \times J_{O2} / ED_{bkt} \tag{Equation 6.1}
\]

where \(dM/dt\) is the organism’s change in mass over time; \(C\) is consumption; \(Eg\) is egestion; \(Ex\) is excretion; \(ED_p\) is the energy density of the prey; \(ACT\) is the activity rate multiplier; \(R\) is respiration; \(SDA\) is specific dynamic action; \(J_{O2}\) is the oxycalorific coefficient; and \(ED_{bkt}\) is the energy density of the brook trout (cf. Hanson et al., 1997, Fig. 6.1). The model was integrated on a day time-step for 1460 days; this time period approximates the lifespan of a resident brook trout. Model inputs included empirically derived mean daily water temperatures, diet proportions, diet energy density, and brook trout energy density. Daily water temperature was summarized from a USGS gauge located on the Upper Manistee River (USGS Gauge 04124000, Sherman, Michigan, USA) and averaged across a 10-year time series from 2005-2015 on a daily time step from January 1 to December 31 (Fig. 6.2A). The temperature time series was repeated in the model for each successive year of the simulation. We assumed that the modeled resident salmonid only ate salmon material or aquatic invertebrates, and used our empirical estimates of diet proportion to inform our model. We simplified our assumption about resident trout diets because we were only interested in the influence of salmon material; the similarity in energy density among non-salmon prey types; and uncertainty in seasonal diet composition (cf. Moore et al., 2008). Energy density (J/g wet mass) for salmon tissue, salmon eggs, aquatic invertebrates, and brook trout were measured empirically using a bomb calorimeter (cf. Gerig et al., 2017; Parr Instrument Co. Moline, IL, USA).
Figure 6.1. Conceptual model of growth and bioaccumulation based upon the bioenergetics model for a resident salmonid. Diet items are consumed and then allocated to metabolism or lost via excreted or egested waste. Leftover energy is allocated to growth. Contaminant uptake is a function of both the contaminant burden of the diet and the assimilation efficiently of contaminant from the diet. A small constant portion of the diet is lost via egestion. Two bioaccumulation models were simultaneously fit for change in PCB and Hg concentrations as a function of salmon spawning. Image used with permission from Joe Tomelleri®.

To ensure that our bioenergetics model was able to reasonably approximate brook trout growth, we fit the bioenergetics model to observed weight-at-age data for brook trout in Michigan streams not accessible to salmon. To obtain weight-at-age data, we first assembled a dataset of brook trout length-at-age data from an online database (Michigan Department of Natural Resources, https://www.mcgi.state.mi.us/fishpop/). We then used coefficients from brook trout length-weight relationships in Michigan tributaries to convert length to weight (Schneider, 2000). Next, a maximum likelihood approach was used to establish what value for parameter P (i.e., proportion of maximum consumption
realized) minimized the variance between model fit and observed data for each year of
growth (Gerig et al., 2017).

6.3.3 Bioaccumulation model

Brook trout growth predictions were coupled to a dynamic bioaccumulation
model based on the model of Arnot and Gobas (2004) to predict PCB and Hg
concentrations in a resident salmonid. The model was defined as follows:

$$\frac{dM}{dt} = [M_{bkt} \cdot (k_D \cdot \Sigma w_i C_{D,i})] - (k_e) \cdot M_{\text{Contaminant}}$$  \hspace{1cm} (Equation 6.2)

where $dM/dt$ is the change in the mass of the contaminant in the brook trout over time;
$M_{bkt}$ is the mass of the brook trout obtained from the bioenergetics model; $k_D$ is the
uptake efficiency of the contaminant; $\Sigma w_i C_{D,i}$ is the product of diet proportion and
contaminant concentration of a given diet item; $k_e$ is the elimination rate; and $M_{\text{Contaminant}}$
is the mass of the contaminant in the brook trout (cf. Arnot and Gobas, 2004, Fig. 6.1).

An individual differential equation was parameterized for both PCB and Hg to predict
change over time. For both PCBs and Hg, change in concentration was calculated by
dividing the observed contaminant mass (ng PCB or Hg) in the bioaccumulation model
by the observed growth (g of biomass) from the bioenergetics model on a daily time step.
Similar to the bioenergetics model, the bioaccumulation model was run for 1460 days to
approximate the typical lifespan of a brook trout in the Upper Great Lakes.

Our bioaccumulation model differs from that of Arnot and Gobas (2004) in
several ways. First, contaminant uptake and loss via the gills and metabolic
transformations were eliminated from the model (Trudel and Rasmussen, 2006;
Madenjian et al., 2012). Second, we used a fixed rate of PCB and Hg loss based upon
previous model parameterization (Gobas, 1993; Madenjian et al., 2012). Third, we
assumed that a constant proportion of PCBs were assimilated from the diet rather than using a more complex model parameterization based upon the physicochemical properties of the contaminant. We empirically measured the PCB and Hg concentration of resident salmonids and their diet items to parameterize our simulation model. Details regarding analytical methods, instrumental conditions, and quality control measures are reported elsewhere (Gerig et al., 2017). Results for PCB and Hg are reported as ng/g wet weight.

6.3.4 Model scenarios

We quantified three scenarios reflecting how the trophic pathway to contamination, level of salmon egg consumption, and the origin and species-identity of the salmon spawner influence the uptake of salmon-derived contaminants by stream-salmonids to understand the underlying mechanisms of contaminant biotransport. To assess the trophic pathway to contamination we assumed three likely pathways by which resident salmonids can take up salmon-derived contaminants, given prior studies in the non-native and native range of salmon (cf. Chaloner et al., 2002). These pathways included: (1) direct consumption of salmon eggs (cf. Janetski et al., 2012; Gerig et al., in review); (2) direct consumption of salmon carcass tissue (Gerig et al., 2017); and (3) consumption of invertebrates that are contaminated by salmon, termed an “indirect” pathway here (Sarica et al., 2004). In this scenario, we assumed that salmon eggs were available for two weeks during spawning while salmon carcass material was available for one month based upon observed salmon runs in the Great Lakes (B. Gerig, personal observation). For time periods in which salmon were present in the simulation, we used our empirical estimates of mean ration size and dietary proportion of salmon eggs rather
than the predicted model-based consumption rate. We used data from a previous diet study to inform the dietary proportion and ration size of salmon carcasses by resident salmonids (Johnson et al., 2016). To assess the indirect pathway, we assumed that invertebrates increase their PCB and Hg burden two-fold to reflect contamination via salmon (cf. Sarica et al., 2004 for Hg; Janetski et al., unpublished data, for PCBs). In addition, we used our empirical estimate of invertebrate ration size for the two weeks when salmon were actively spawning. Each trophic pathway was modeled individually to assess the magnitude of the salmon effect.

In our second scenario, we evaluated how variation in salmon egg consumption mediated growth and bioaccumulation of salmon-derived contaminants. First, we determined the empirical percentiles of egg consumption by resident salmonids from our diet study. We then substituted the ration size from each percentile of egg consumption into our model during the two-week time period when salmon eggs were available for consumption by resident trout. We simulated variation in egg consumption for the 10th, 25th, 50th, 75th, and 97.5th percentile of empirically observed egg consumption. In this scenario, we assumed that salmon eggs were the only source of salmon-derived contaminants.

In our third scenario, we modeled how environmental context as a function of spawner identity and origin influenced the bioaccumulation of salmon-derived contaminants by resident salmonids. In this scenario, we considered how Chinook salmon from Lake Michigan, and coho salmon from Lakes Michigan and Superior, influenced the growth and bioaccumulation of salmon-derived contaminants based on a stream-resident brook trout. For this simulation, we used location and species-specific PCB and
Hg concentrations. We assumed that resident salmonids consumed the average amount of salmon eggs for Lake Michigan Chinook based upon our diet study. We did not have egg consumption data for Lake Michigan or Lake Superior coho. However, Janetski et al., (2012) found that coho had 10-fold lower spawning populations in Lake Michigan than Chinook and that spawning salmon populations in Lake Superior were 100-fold lower than in Lake Michigan. Thus, we assumed that resident salmonids consumed 10-fold fewer coho eggs in Lake Michigan and 100-fold fewer coho eggs in Lake Superior. Similar to the egg consumption scenario, we assume that salmon eggs were the only source of salmon-derived contaminants. For all scenarios, we assumed that the influence of salmon followed a uniform distribution such that forcing functions simulated the uptake of salmon material as a constant pulse (Fig. 6.2B-D).
6.3.5 Sensitivity analysis

All parameters in Table 6.3 were varied individually by ±10% to evaluate the sensitivity of model outcomes to variation in initial parameter values following Hamby (1994). In addition, we varied the consumption of salmon eggs by the standard deviation of egg consumption to account for the wide range of variation observed in this parameter in our diet study. The sensitivity of the model to parameter variation was assessed for
both PCB and Hg concentration and evaluated based upon the average level of egg consumption for Lake Michigan. Model sensitivity was calculated as:

$$SI = \left[ D_{\text{max}} - \left(\frac{D_{\text{min}}}{D_{\text{max}}}\right) \right] \times 100 \quad \text{(Equation 6.3)}$$

where $D_{\text{max}}$ and $D_{\text{min}}$ represent the maximum and minimum values of PCB and Hg concentration at the end of the simulation as a result of parameter variation (Hamby, 1994; Wallace and Blersh, 2015).

6.4 Results

6.4.1 Diet composition

Overall, we observed a large shift in the diet of resident salmonids that coincided with the spawning of Pacific salmon in tributaries of Lake Michigan (Fig. 6.3). Before the salmon run, resident salmonid ration size was small (<0.25 g) and comprised primarily of aquatic (34% by mass) and terrestrial invertebrates (33% by mass). During the salmon run, a 14-fold increase in ration size was observed (Two-way ANOVA, $F_{1,101}=14.29$, $P<0.001$), driven primarily by the consumption of salmon eggs, which became the dominant diet item (68% by mass, Fig. 5.4). However, resident salmonids exhibited considerable variation in their degree of egg consumption (mean=2.73 g eggs/fish, SD=3.14), suggesting individual responses to the pulse of resources supplied by salmon. Interestingly, when we evaluated how ration size changed by removing the influence of egg consumption, we found that in locations with salmon, ration size increased 2-fold from pre-run masses (Two-way ANOVA, $F_{1,101}=6.6$, $P=0.011$), driven by increased consumption of aquatic invertebrates. Small proportions of amorphous detritus, non-insect invertebrates, and fish were consumed during both sampling periods.
Figure 6.3. Mean ration size of resident salmonids in Great Lakes tributaries before and during the Pacific salmon run.

Figure 6.4. Mean diet proportions of resident salmonids in Great Lakes tributaries before and during the Pacific salmon run.
6.4.2 Influence of trophic pathway to contamination

The growth and contaminant response of brook trout to the provision of salmon resources depended on the trophic pathway. We observed no difference in growth or PCB accumulation with the no-salmon, indirect pathway, or direct carcass consumption scenarios over the duration of the simulation (Table 6.1). We observed small differences in Hg accumulation among scenarios with the indirect pathway, resulting in a modest 3.3 to 6.6% increase when compared to the carcass consumption scenario across age-classes. The direct egg consumption scenario resulted in large increases in both growth and PCB accumulation, and indirectly mediated reductions in resident salmonid Hg concentrations. Brook trout exhibited a 34- to 57-fold increase in growth when compared to the no-salmon scenario across age-classes (Table 6.1). This sharp increase in growth reflects both the increased energy density of the salmon eggs and increased ration size resulting from egg consumption. Similarly, lifetime PCB concentrations of the resident salmonid increased by more than 90-fold when compared to the other two trophic pathways, reflecting both the high PCB content of eggs and the increased ration size observed in our field study. In contrast, consumption of salmon eggs led to a 25- to 50-fold reduction in Hg concentrations across year classes when compared to the other three trophic pathways (Table 6.1), which was mediated by the increased consumption of salmon eggs that were low in Hg while having high energy density.
TABLE 6.1.

INFLUENCE OF TROPHIC PATHWAY ON THE GROWTH (g), PCB BIOACCUMULATION (ng/g), AND TOTAL MERCURY BIOACCUMULATION (ng/g) OF A BROOK TROUT OVER A 4-YEAR SIMULATION.

<table>
<thead>
<tr>
<th>Time</th>
<th>Scenario</th>
<th>Mass (g)</th>
<th>PCB (ng/g)</th>
<th>Hg (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 365</td>
<td>No Salmon</td>
<td>68.4</td>
<td>7.1</td>
<td>83.1</td>
</tr>
<tr>
<td></td>
<td>Carcass only</td>
<td>68.4</td>
<td>7.5</td>
<td>83.2</td>
</tr>
<tr>
<td></td>
<td>Indirect Pathway</td>
<td>68.4</td>
<td>7.9</td>
<td>86.0</td>
</tr>
<tr>
<td></td>
<td>Egg Consumption</td>
<td>104.4</td>
<td>146.4</td>
<td>65.9</td>
</tr>
<tr>
<td>Day 730</td>
<td>No Salmon</td>
<td>82.2</td>
<td>6.6</td>
<td>87.3</td>
</tr>
<tr>
<td></td>
<td>Carcass only</td>
<td>82.2</td>
<td>7.2</td>
<td>87.4</td>
</tr>
<tr>
<td></td>
<td>Indirect Pathway</td>
<td>82.2</td>
<td>8.0</td>
<td>91.9</td>
</tr>
<tr>
<td></td>
<td>Egg Consumption</td>
<td>153.9</td>
<td>196.0</td>
<td>62.2</td>
</tr>
<tr>
<td>Day 1035</td>
<td>No Salmon</td>
<td>94.4</td>
<td>6.4</td>
<td>90.0</td>
</tr>
<tr>
<td></td>
<td>Carcass only</td>
<td>94.4</td>
<td>7.1</td>
<td>90.2</td>
</tr>
<tr>
<td></td>
<td>Indirect Pathway</td>
<td>94.4</td>
<td>8.1</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>Egg Consumption</td>
<td>201.4</td>
<td>223.6</td>
<td>60.1</td>
</tr>
<tr>
<td>Day 1460</td>
<td>No Salmon</td>
<td>105.6</td>
<td>6.2</td>
<td>92.0</td>
</tr>
<tr>
<td></td>
<td>Carcass only</td>
<td>105.6</td>
<td>7.0</td>
<td>92.2</td>
</tr>
<tr>
<td></td>
<td>Indirect Pathway</td>
<td>105.6</td>
<td>8.2</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>Egg Consumption</td>
<td>247.6</td>
<td>241.8</td>
<td>58.7</td>
</tr>
</tbody>
</table>

6.4.3 Variation in the effects of egg consumption

Over the duration of the model, the resident salmonid exhibited consistent increases in growth and PCB concentrations in proportion to the amount of eggs consumed. Moreover, the resident salmonid exhibited increased or reduced Hg concentrations as the degree of egg consumption varied in the simulation. Resident salmonid body mass exhibited a 5%, 94% and 176% increase reflecting the 25\textsuperscript{th}, 50\textsuperscript{th}, and 75\textsuperscript{th} percentile of observed egg consumption when compared to the no-salmon egg consumption simulation (Figure 6.5A). Similarly, resident salmonid PCB concentrations exhibited an 8-, 44-, and 56-fold increase reflecting the 25\textsuperscript{th}, 50\textsuperscript{th}, and 75\textsuperscript{th} percentile of
observed egg consumption (Figure 6.5B). The large increase in PCB concentrations, even at low consumption rates, reflects the high PCB content of salmon eggs relative to other diet items. Additionally, resident salmonid exhibited an 9%, 45% and 57% decline in Hg concentrations reflecting the 25th, 50th, and 75th percentile of observed egg consumption (Figure 6.5C). The variation in our PCB and Hg responses to salmon egg consumption is similar to the variation observed in our basin-wide survey (Figure 6.5D).

6.4.4 Influence of spawner identity and lake basin

Variation in environmental context, as a function of salmon spawner identity and where salmon accumulate their contaminant burden, has consequences for the magnitude of contaminant biotransport to resident salmonids. When evaluating the species-specific impact of different salmon spawners on resident salmonids, we found that Chinook salmon from Lake Michigan had a much larger impact on the PCB burden of resident salmonids compared to coho spawners from either Lake Michigan or Lake Superior (Table 6.2). Over the duration of the simulation, resident salmonid growth was 2-fold lower for fish that consumed eggs deposited by Lake Superior or Lake Michigan coho compared to Lake Michigan Chinook. In addition, resident salmonids receiving contaminants biotransported by Lake Superior coho had 31-fold lower PCB concentrations while consumption of eggs supplied by Lake Michigan coho resulted in an 8-fold lower PCB burden in resident salmonids compared to fish that consumed Lake Michigan Chinook eggs (Table 6.2). As observed previously, Hg accumulation declined with increased levels of salmon egg consumption.
Figure 6.5. Influence of variation in salmon egg consumption on lifetime growth (A), PCB concentration (ng/g; B.), and total mercury bioaccumulation (ng/g; C.) in a brook trout over a 4-year simulation. Panel D. reflects empirical variation in resident salmonid PCB and Hg concentrations in locations with (orange box) and without (green box) salmon.
TABLE 6.2.

INFLUENCE OF SPAWNER IDENTITY AND LOCATION ON GROWTH (g), PCB BIOACCUMULATION (ng/g), AND TOTAL MERCURY BIOACCUMULATION (ng/g) OF A BROOK TROUT OVER A 4-YEAR SIMULATION.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Mass (g)</th>
<th>PCB (ng/g)</th>
<th>Hg (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Salmon</td>
<td>105.6</td>
<td>6.2</td>
<td>92.0</td>
</tr>
<tr>
<td>Lake Michigan Chinook</td>
<td>243.8</td>
<td>319.0</td>
<td>44.6</td>
</tr>
<tr>
<td>Lake Michigan coho</td>
<td>118.3</td>
<td>40.7</td>
<td>79.1</td>
</tr>
<tr>
<td>Lake Superior coho</td>
<td>105.8</td>
<td>10.2</td>
<td>87.0</td>
</tr>
</tbody>
</table>

6.4.5 Sensitivity analysis

Most parameters in the bioenergetics model had little impact on the final PCB and Hg concentration of the modeled brook trout (Table 6.3). For example, changes to parameters related to respiration were invariant to the predicted PCB or Hg concentration. Similarly, parameters related to specific dynamic action, egestion, and excretion only had a small effect (-2% to +9%) on modeled bioaccumulation. In contrast, parameters related to the assimilation efficiency of PCBs and Hg and the degree of salmon egg consumption led to large changes in modeled contaminant accumulation (Table 6.4). For example, variation in salmon egg consumption resulted in a 92% increase and a 51% reduction in PCB and Hg accumulation, respectively. Similarly, changes in the assimilation efficiency resulted in an 18% and 16% change in PCB and Hg accumulation.
# TABLE 6.3.

SENSITIVITY ANALYSIS FOR SELECTED PARAMETERS IN THE MODEL.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCB</th>
<th>Hg</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSal</td>
<td>92.87</td>
<td>-51.9</td>
<td>Consumption of salmon eggs</td>
</tr>
<tr>
<td>CnonSal</td>
<td>-4.23</td>
<td>9.26</td>
<td>Consumption of invertebrates</td>
</tr>
<tr>
<td>RA</td>
<td>0.00</td>
<td>0.00</td>
<td>Intercept of mass dependent function for respiration</td>
</tr>
<tr>
<td>RB</td>
<td>0.00</td>
<td>0.00</td>
<td>Exponent of the mass dependence function for respiration</td>
</tr>
<tr>
<td>RQ</td>
<td>0.00</td>
<td>0.00</td>
<td>Slope of the respiration function at low water temperature</td>
</tr>
<tr>
<td>RTO</td>
<td>0.00</td>
<td>0.00</td>
<td>Optimal temperature for respiration</td>
</tr>
<tr>
<td>RTM</td>
<td>0.00</td>
<td>0.00</td>
<td>Lethal temperature for respiration</td>
</tr>
<tr>
<td>P</td>
<td>5.13</td>
<td>8.39</td>
<td>Proportion of consumption realized</td>
</tr>
<tr>
<td>SDA</td>
<td>0.30</td>
<td>0.02</td>
<td>Specific dynamic action</td>
</tr>
<tr>
<td>ACT</td>
<td>0.00</td>
<td>0.00</td>
<td>Activity rate multiplier</td>
</tr>
<tr>
<td>FA</td>
<td>5.19</td>
<td>2.42</td>
<td>Intercept of the temperature/ration dependence for egestion</td>
</tr>
<tr>
<td>FB</td>
<td>-2.69</td>
<td>-1.14</td>
<td>Exponent of the temperature dependence function for egestion</td>
</tr>
<tr>
<td>FG</td>
<td>3.31</td>
<td>1.53</td>
<td>Coefficient for the feeding level dependence of egestion</td>
</tr>
<tr>
<td>UA</td>
<td>1.86</td>
<td>0.91</td>
<td>Intercept of the temperature/ration dependence for excretion</td>
</tr>
<tr>
<td>UB</td>
<td>2.68</td>
<td>1.44</td>
<td>Exponent of the temperature dependence function for excretion</td>
</tr>
<tr>
<td>UG</td>
<td>-0.56</td>
<td>-0.27</td>
<td>Coefficient for the feeding level dependence of excretion</td>
</tr>
<tr>
<td>EdPCB</td>
<td>18.17</td>
<td>0.00</td>
<td>Assimilation efficiency of PCB from diet</td>
</tr>
<tr>
<td>keCPCB</td>
<td>-0.01</td>
<td>0.00</td>
<td>Elimination rate of PCB</td>
</tr>
<tr>
<td>EdHg</td>
<td>0.00</td>
<td>16.04</td>
<td>Assimilation efficiency of Hg from diet</td>
</tr>
<tr>
<td>keCHg</td>
<td>0.00</td>
<td>-0.01</td>
<td>Elimination rate of Hg</td>
</tr>
</tbody>
</table>

Note: We based our model on the average level of salmon egg consumption for the sensitivity analysis. Parameters were varied by ±10% with the exception of consumption of salmon eggs (CSal), which was varied by one standard deviation to reflect the observed variation in egg consumption.
6.5. Discussion

In this study, we developed a coupled bioenergetic-bioaccumulation model to predict the influence of salmon-derived resource subsidies and contaminants on resident trout in Great Lakes tributary streams. Insights provided by our model parallel inference from our watershed survey and experimental approaches detailed in Chapters 1-4 of this dissertation. Our individual-based model builds upon a previous ecosystem modeling effort by McGill et al., (2017) in which stream-resident fish PCB bioaccumulation was controlled by salmon inputs but subject to species-specific variation. Through simulation modeling in this study, we found that egg consumption was the strongest determinant of PCB accumulation and the degree of egg consumption (which are low in Hg) indirectly diluted the mass of Hg in brook trout. Moreover, salmon eggs were an important resource that increased the growth in brook trout. Our results are consistent with previous research conducted in the Great Lakes basin (Merna 1986; Janetski et al., 2012) and in the native range of Pacific salmon (Krummel et al., 2003; Gregory-Eaves et al., 2007). This research demonstrated that spawning salmon increase the growth and PCB loads but reduce the Hg burdens of resident fish (Cyr et al., 2016). Overall, our modeling results provide insight into resource subsidies and contaminant biotransport by evaluating how trophic pathway, diet variation, and environmental context interact to influence the lifetime growth trajectory and magnitude of contaminant bioaccumulation in resident salmonids.
6.5.1 Trophic pathway to contamination

In our model, trophic pathway was a primary determinant of resident trout contaminant levels. Consumption of eggs resulted in a much larger increase in PCB concentration compared to the carcass or food web pathways. In addition, Hg concentrations decreased with egg consumption but exhibited small increases in the indirect and carcass consumption scenarios. Previous research has documented both dietary shifts and increases in ration size when salmon eggs were available in Great Lakes tributaries (Merna, 1986; Ivan et al., 2011). Furthermore, Merna (1986) showed that PCB and DDT concentrations in brown trout were directly correlated to the number of salmon eggs consumed. Similarly, Cyr et al., (2016) showed that egg consumption was inversely related to tissue Hg concentrations in Dolly Varden char in SE Alaska, a closely related species to brook trout. This suggests that egg consumption mediates a trade-off between the bioaccumulation of PCBs and Hg dilution. Based upon our study, we conclude that salmon eggs are the predominant pathway by which resident salmonids are influenced by spawning salmon.

We observed that resident salmonids increased their consumption of non-salmon diet items when salmon were spawning. In both the native and non-native range, salmon can have a significant disturbance effect on stream substrates through redd construction (Moore et al., 2004; Janetski et al., 2014), but the consequences are very different between those two locations. The disturbance effect is especially magnified in the Great Lakes tributaries with smaller substrates that are more prone to disturbance (Collins et al., 2011; Janetski et al., 2014). Consequently, invertebrate abundance declines as a function of redd digging (Janetski et al., 2014). Our findings suggest that salmon-spawner
mediated-disturbance may indirectly increase the ration size of resident trout through higher invertebrate drift. Similarly, Arctic grayling (*Thymallus arcticus*) increase their consumption of aquatic insects because of higher invertebrate drift following salmon disturbance, resulting in increased growth (Scheuerell et al., 2007). However, in our model, increased consumption of invertebrates during spawning only resulted in a small increase in growth. We hypothesize that the small growth response in our model results from the shorter temporal duration of salmon spawning in the Great Lakes (e.g. days) compared to salmon runs in the native range, which can persist for months (Scheuerell et al., 2007; Janetski et al., 2014).

Direct consumption of salmon carcass material is hypothesized to control the growth of stream-resident salmonids (Gende et al., 2002; Wipfli et al., 2003). However, consumption of salmon tissue had a negligible role on the growth and contaminant accumulation of brook trout in our model. In our diet study, we failed to identify salmon carcass tissue in any resident salmonid diets, despite abundant decomposing salmon in the study streams (cf. Janetski et al., 2012; Gerig et al., in review). In our model, we found that carcass consumption did not confer a growth benefit or strongly increase contaminant concentrations in brook trout because of the low occurrence of salmon tissue in trout diets. Previous experimental studies display conflicting results related to the effect of salmon carcasses on stream-resident fish growth and contaminant accumulation. Our model mirrors experiments where resident fish growth did not increase in the presence of salmon carcasses (Harvey and Wilzbach; 2010, Gerig et al., 2017) but contrast with other studies that found increased growth rates when salmon material in present in gut contents (e.g., Collins et al., 2016), or salmon-mediated stable isotope
enrichment (e.g., Chaloner et al., 2002). With respect to contaminants, Sarica et al., (2004) concluded that salmon carcasses are a significant source of Hg in a Lake Ontario tributary based upon the large increase in invertebrate Hg concentrations following salmon spawning. In contrast, we have found that resident trout exhibit large increases in PCBs and modest declines in Hg following salmon spawning in Great Lakes tributaries (Gerig et al., in review). If salmon tissue were consumed in large quantities, we would expect to see increases in both brook trout PCB and Hg concentrations. Taken together, these results suggest that in Great Lakes streams, salmon carcass material is consumed infrequently by fish, and is not a dominant pathway for energy acquisition and contaminant uptake for resident salmonids.

6.5.2 Implications of variation in egg consumption

Variation in the consumption of salmon eggs controlled the growth and contaminant bioaccumulation of resident salmonids in our model. At low levels of egg consumption, our model resulted in small increases in trout growth. However, when egg consumption increased, reflecting the 50\textsuperscript{th}, 75\textsuperscript{th}, and 97.5\textsuperscript{th} percentile of observed egg consumption, 2- to 5-fold increases in growth were found. This result is similar to studies from the native range of Pacific Salmon where consumption of salmon eggs is strongly linked to increases in the growth of resident fish (Moore et al., 2008; Baldock et al., 2016; Jaecks et al., 2014). This growth response may be linked to diet plasticity in stream salmonids when resource pulses are supplied by salmon (Armstrong and Schindler, 2013). For instance, piscine predators have physiological adaptations to effectively double or triple gut capacity to take advantage of spatially and temporally heterogeneous prey resources (Armstrong and Schindler, 2013). Thus, resident salmonids may have
adaptive capacity to cope with a feast or famine of temporally variable resource subsidies, such as those associated with salmon runs that are limited to 1-2 months each year. As such, resident salmonids exposed to salmon runs likely exhibit individual foraging strategies that enable them to maximize energy intake. This variability is evident across both the native and introduced range of salmon where stream-resident fish can exhibit a large increase in energy intake from eating salmon eggs (Ivan et al., 2011; Jaecks et al., 2014; Moore et al., 2008; this study).

In our model, consumption of salmon eggs by stream-resident fish resulted in large increases in PCBs but somatic growth dilution of Hg, which occurs when positive growth dilutes the existing mass of a contaminant in the body of a fish, thereby reducing their contaminant burden (Trudel et al., 2006; Ward et al., 2010). The observed trade-off in PCB and Hg accumulation is governed by the interaction among the high PCB concentration but low Hg concentration in salmon eggs, the high-energy density of salmon eggs, and the ration size of the resident salmonid. In our model, fish with the highest level of egg consumption exhibited the greatest growth dilution. Gerig et al. (in review) noted a similar response in a field study, where brown trout PCB and Hg concentrations in Dolly Varden were inversely related. In the native range, Hg concentrations declined with increased δ\(^{15}\)N, where δ\(^{15}\)N enrichment was used as an index of salmon egg consumption (Cyr et al., 2016). This contrast in PCB and Hg loads likely reflects the differential partitioning of PCBs and Hg between salmon eggs and carcass tissue. Mercury accumulates in the muscle matrix of fish by forming strong bonds with cysteine (Kuwabara et al., 2007). In contrast, PCBs are lipophilic and accumulate in tissues with high lipid content, such as salmon eggs (Harmelin-Vivien et al., 2012). Thus,
consumption of high quality diet items can facilitate both increases and decreases in specific contaminants as a function of dietary contaminant concentrations, diet quality, partitioning between tissue types, and consumption rates. Moreover, the PCB burden of a resident trout may be the integration of multiple salmon runs.

Our model also indicated that a significant proportion of the PCB burden accumulated from salmon egg consumption was retained over the course of a year and carried over between spawning runs. This result suggests that the PCB burden of a fish can integrate contaminants delivered by multiple salmon runs. As such, inter-annual variation in salmon run sizes and egg consumption rates can contribute to the high variation observed in the relationship between stream-resident fish PCB concentration and salmon PCB flux (Gerig et al., in review). Our model of life-time contaminant accumulation also highlights that growth dilution is unable to significantly reduce the PCB burden accumulated as a result of salmon egg consumption over the course of a year. Further, variation in the degree of egg consumption, coupled with the contaminant burden of the salmon spawner, may drive variation in resident fish PCB concentrations across the introduced range of salmon in the Great Lakes.

6.5.3 Influence of basin-specific factors on contaminant biotransport

In our model, the consumption of Chinook salmon eggs from Lake Michigan resulted in a larger increase in PCBs than consumption of Lake Superior coho eggs. This increase mirrors a basin-wide comparison where resident fish from Lake Michigan streams had 31-fold higher PCB concentrations when compared to Lake Superior tributaries (Janetski et al., 2012). These differences reflect the interaction between historic pollution, salmon species identity, and salmon run sizes. Historically, Lake
Michigan received higher inputs of persistent PCBs than Lake Superior (Golden et al., 1993), and continues to have higher PCB concentrations resulting from internal PCB cycling (Hornbuckle et al., 2006). In addition, Chinook salmon have larger population sizes, occupy higher trophic positions, and grow to larger size (Madenjian et al., 2004), which lead to higher PCB loads than for coho. Together, our findings highlight that spawner species identity, the environment in which the contaminant bioaccumulates, and spawner density are all important factors that determine the impact of salmon-mediated contaminant biotransport.

Anadromous life history strategies have been documented in greater than 110 fish species from 18 families (McDowall, 1988). In the Great Lakes, several common fish species exhibit migrations including steelhead (*Oncorhynchus mykiss*), suckers (*Catastomous* spp.), and walleye (*Sander vitreus*). Moreover, migratory fish assemblage in the Great Lakes varies considerably based upon life history (semelparous, iteroparous), origin (native, introduced), spawning mode (broadcast, redd building), fecundity (gonadosomatic index), phenology (spring, summer, fall), abundance (run size), and biochemical makeup (lipid content, contaminant burden). These characteristics likely interact with spatial variation in legacy contamination and tributary accessibility to influence whether migratory fish are a significant energy and contaminant source to tributaries. As such understanding the broader temporal and spatial characteristics of migratory fish runs will help determine the significance of migratory fish runs to the production of resident fish populations. We posit that salmon, as part of the broader portfolio of migratory fish runs in the Great Lakes, may represent a large and novel source of both energy and contaminants for resident fish populations (cf. Schindler et al.,
The importance of other migratory fish subsidies to resident fish production in the Great Lakes should be evaluated in the future.

6.5.4 Model limitations and future directions

In this study, we demonstrated the value of a modeling approach to understanding the potential impact of energy and contaminants transferred from salmon to resident trout. Our work also revealed some limitations of our model and future work needed to refine this approach to understanding contaminant biotransport. Future research should focus on better understanding how salmon run dynamics interact with resident fish diet to control the uptake of salmon-derived energy and contaminants. Information on how resident fish diets change over the course of a salmon run is currently limited and variation in this parameter caused large variation in model outcomes based upon our sensitivity analysis. Given the lack of information on the temporal duration of salmon runs and availability of salmon eggs, we assumed that eggs were consumed at a constant ration over a two-week period in the model. While the quantiles of salmon egg consumption were derived from empirical field estimates, we were unable to assess whether this assumption was correct over the duration of the salmon run. We suspect that this approximation does not adequately capture the heterogeneity in run timing or the response of resident fish to this dynamic resource pulse (ct. Moore et al., 2008). Understanding how egg availability and egg consumption vary across space, time, and spawner gradients would inform the duration of time and in what quantity salmon eggs are consumed, providing a more detailed view of the consequences of salmon-mediated contaminant biotransport (cf. Clements et al., 2012).
In our model, we made necessary simplifications regarding the process of metabolism and contaminant bioaccumulation that may have consequences for PCB and Hg bioaccumulation. Specifically, we assumed a constant rate of PCB and Hg assimilation from the diet, a constant rate of PCB and Hg elimination, and we borrowed all parameters from previous studies to parameterize the bioenergetics portion of the model. While these parameters did not appear to strongly influence the outcome of our simulation based upon sensitivity analysis, future laboratory experiments would yield insights into how physiology influences the uptake of biotransported contaminants. Specifically, laboratory-based estimates of assimilation efficiencies, metabolism, and elimination rates may markedly improve the performance and realism of this type of individual-based model (Wallace and Blersh, 2015).

6.5.5 Conclusions

Our bioenergetic-bioaccumulation model allowed us to partition the uptake and incorporation of contaminants and resources supplied by salmon to evaluate the role of trophic pathway, variation in egg consumption, spawner identity, and spawner origin in the bioaccumulation of PCBs and Hg in resident salmonids. Based on our model, we demonstrated that salmon egg consumption strongly controls the growth and PCB bioaccumulation rate of a resident salmonid, and indirectly mediates reductions in Hg through somatic growth dilution. Other trophic pathways, including carcass consumption and food web transfers, did not appear to facilitate increased PCB contamination in resident salmonids. In addition, we demonstrated that species identity and location strongly influence the uptake of salmon-derived contaminants, suggesting that regional differences in the legacy of contamination and abundance of biovectors can strongly
influence the magnitude of contaminant biotransport and uptake. Our results provide a
detailed understanding of the process of contaminant biotransport by migratory fish that
can be incorporated into future management activities aimed at reducing the transfer of
contaminants between ecosystems.

6.6 Acknowledgments

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States Environmental Protection Agency.

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CHAPTER 7:
SYNTHESIS

7.1 Overview of findings

My dissertation has clarified the processes by which migratory fish, such as Pacific salmon, can transport contaminants to tributaries of the Great Lakes and influence the contaminant burden of stream-resident fish. By using a combination of survey, experimental, and modeling approaches, I was able to identify the key factors involved in the transfer of salmon contaminants to stream-resident fish (Fig 7.1). Across chapters of this dissertation, I provide strong evidence for salmon-mediated contaminant transfer of POPs, but not Hg, suggesting that not all contaminants are equal and that the trophic pathway followed by contaminants is a primary driver by which salmon influence the contaminant burden of stream-resident fish.

In CHAPTERS 2 and 3, I found that environmental context influenced the transfer of PCBs transferred from Pacific salmon to resident fish. Specifically, the lake basin of origin from which the salmon spawner originated, the flux of PCBs supplied by salmon, and the species identity of the resident fish all strongly influenced the magnitude of contaminant transfer with resident trout being much more contaminated than mottled sculpin. Differences among resident species for PCBs are likely driven by differences in diet composition, consumption rate, and physiology. The strong evidence of POP biotransfer between salmon and resident fish reflects the large POP load that salmon supply to streams relative to much smaller atmospheric and watershed sources.
The lack of a mercury effect associated with salmon suggests they are not a large source of Hg to resident fish. Moreover, because salmon eggs are enriched in POPs but depleted in Hg suggests that widespread egg consumption could lead to increased POPs but decreased Hg concentrations in resident fish. In contrast, background sources of mercury appear to be a more important determinant of stream-resident fish mercury concentrations than do salmon (Baker et al. 2009).

The experimental approaches utilized in CHAPTERS 4 and 5 largely agree with inferences drawn from my survey-based studies, which suggest that salmon egg consumption is a primary driver of salmon-mediated contaminant biotransfer. The contrast in Hg response between our mesocosm (CHAPTER 4) and in situ salmon-material addition experiment (CHAPTER 5) is likely driven by which salmon materials were consumed in each experiment (i.e., only salmon tissue in the mesocosm experiment versus both salmon tissue and eggs for the in situ experiment). Furthermore, such a contrast suggests that resident fish in streams receiving salmon runs do not always consume carcass material, especially if eggs are abundant (cf. Wipfli et al. 2003; Collins et al. 2016).

I integrated inferences from CHAPTERS 2-5 in CHAPTER 6 to parameterize a bioenergetics-bioaccumulation model that provides a mechanistic explanation of salmon-mediated contaminant biotransport. This effort demonstrated that variation in trophic pathway, salmon egg consumption, and location at which salmon accumulate their contaminant burden interact to regulate the transfer and uptake of salmon-derived contaminants by stream-resident fish. Through my dissertation research, I was able to enhance the current model of contaminant biotransport developed by Blais et al. (2007).
Previously, contaminant biotransport has been described as a three-step process that includes the bioaccumulation of a contaminant by a migratory organism, the movement of the contaminant across an ecosystem boundary, and the release of the contaminant into the recipient habitat (Blais et al. 2007). My revised model includes a fourth step focused on factors influencing the uptake of the biotransported contaminant (Fig. 7.1). This revised model highlights that the extent of contaminant biotransport is not only dependent on the contaminant burden and abundance of the biovector but also attributes of fish community and contaminant considered.

Figure 7.1 Conceptual model of the influence of context on contaminant biotransport by Pacific salmon. The process of contaminant biotransport is described by Blais et al. (2007) as a three-step process whereby a contaminant is bioaccumulated, moved, and deposited into a recipient ecosystem by a migratory organism. Here we revise this model and add a fourth step focused on the processes that facilitate contaminant uptake by resident organisms. Uptake can be modified by attributes of the salmon, run dynamics, type of tissue deposited, and characteristics of the resident fish community.
7.2 Dissertation in context

In CHAPTER 2, I showed that POP congener patterns can provide many insights into the relationship between the contaminant burden of salmon and that of stream-resident fish (see Gerig et al. 2016). This study demonstrated that salmon spawners have a distinct congener profile that they transfer to stream-resident fish. This research also showed that the congener pattern of salmon differed between Great Lake basins, suggesting regional differences in the extent and magnitude of contaminants in the environment. Similarly, stream-resident fish exhibited basin-specific patterns with fish from Lake Michigan streams displaying clear evidence of biotransport by salmon whereas such a pattern was not evident in tributaries of Lake Superior. The strong contrast in congener patterns between Lake Michigan and Lake Superior streams reflects contrasts in historical pollution, salmon abundance, and species composition between locations. For instance, Lake Michigan received 15 times more PCBs when compared to Lake Superior (Golden et al. 1993), and continues to receive larger atmospheric inputs of POPs (Li et al. 2009). In addition, Lake Michigan has a medium to large population of highly contaminated Chinook whereas Lake Superior has smaller populations of less contaminated coho (Dettmers et al. 2012, Janetski et al. 2012). Overall, this research highlights the utility of using non-traditional ecological tracers (e.g., PCBs) to infer ecological linkages related to the transfer of resources between ecosystems (Ramos and González Solís 2012).

In CHAPTER 3, my basin-wide survey generated insights into how environmental context potentially influences the transfer and uptake of salmon-derived contaminants to stream-resident fish in the upper Great Lakes. This research
demonstrated that the magnitude of contaminant biotransport by salmon to stream-
resident fish differed among contaminants. I demonstrated that resident fish in reaches
with salmon had 24-fold higher PCB concentrations, compared to non-salmon reaches,
but exhibited either a reduction or no response in Hg concentrations. Strong salmon egg
consumption may indirectly mediate a reduction in Hg because eggs are enriched in
PCBs but depleted in Hg (Cyr et al. 2016, Ch. 3). The variation in the PCB concentration
of resident fish exposed to salmon runs suggests that variation in run size and egg
consumption may control this pattern. Contrasts between contaminants appear to reflect
differences in the influence of environmental context. In general, biological factors, such
as species identity, salmon contaminant flux, and resident fish size were more important
for explaining variation in the contaminant burden of resident fish than were physical and
chemical factors, such as landscape features or instream characteristics. Previous studies
have found PCB and Hg concentrations in fish to vary with physical and chemical factors
such as water chemistry and land cover (King et al. 2004, Driscoll et al. 2013), but
salmon deliver such a large pollutant flux that other factors are overshadowed. The
stronger influence of biological over physico-chemical factors parallels recent research
that considered how salmon spawners influence the stable isotope composition of sculpin
(Swain et al. 2013) and juvenile coho salmon (Reisinger et al. 2013), in which the most
important factor was salmon run size. The response of in stream-resident fish to salmon
PCB flux exhibited a logarithmic relationship between salmon biomass and stream-
resident fish contaminant burden, suggesting limits on uptake and incorporation of
salmon-derived contaminants by resident fish; similar saturation patterns have been
observed previously with salmon-derived nutrients (e.g., Chaloner et al. 2002, Wipfli et
al. 2003). The high level of variation around the logarithmic relationship, particularly for brook trout, suggests individual variation in exposure to contaminants, likely as a function of variation in egg consumption and carry-over of contaminants accumulated from past salmon runs.

In CHAPTER 4, I combined a mesocosm experiment with a coupled bioenergetics-bioaccumulation model to explore the influence of salmon on brook trout growth and mercury accumulation, especially the role of the type of salmon material. My results demonstrated that consumption of salmon tissue did not enhance growth, but shifted stable isotope ratios and led to large increases in total mercury concentrations. Given this counterintuitive growth response, I used the bioenergetics-bioaccumulation model parameterized with empirically measured energy density and contaminant concentration of diet items to show the consequences of salmon egg versus tissue consumption, with the former resulting in both improved growth and lower total mercury concentrations in brook trout. The opposite patterns for brook trout total mercury concentrations in our large-scale survey and whole-stream experiment reaches suggests that brook trout do not consume large quantities of contaminated tissue, but rather focus on egg consumption in the field. My results differ from the literature, especially in the native range of salmon (e.g., Bilby et al. 1998, Wipfli et al. 2003), which often implies that the major source of nutrition from salmon is from carcass tissue. This interpretation has led to the addition of carcasses where salmon runs have been extirpated or are much lower than they were historically (Cederholm et al. 1999). In fact, provision of eggs may be equally or more important. Overall, my results highlight that growth and contaminant
bioaccumulation are influenced by both resource quantity and quality (cf. Johnson et al. 2015).

In CHAPTER 5, I show that the results from a whole-stream experimental salmon addition were largely consistent with those of the field survey. Results were also consistent with a strong influence of salmon contaminants on stream-resident fish but not on stable isotopes of those fish, as has been shown within and outside the native range of Pacific salmon (Gregory-Eaves et al. 2007, Janetski et al. 2012, Merna 1986). Several key insights can be drawn from this experiment. For instance, the POP contaminant burden in resident fish increased rapidly after the salmon material addition, but dynamics differed between years and between POPs and mercury, as well as among types of POPs. The continued increase in DDE and PBDE, but not PCB concentrations, in the second year of my salmon addition may result from resident fish not yet exhibiting equilibrium concentrations or related to variation in DDE and PBDE concentrations in salmon eggs. The much smaller magnitude of increase in year two of the salmon addition may also reflect the nature of our sampling. We intentionally sampled the same size-age class of fish, which means that in both years the resident fish sampled were likely only exposed to a single year of salmon additions. The lack of correspondence between POPs and stable isotopes suggests that the timing for incorporation of POPs and isotopes into the tissue of the stream-resident fish differs between tracers. For instance, POPs partition rapidly into fat during digestion (Arnot and Gobas, 2004) whereas change in isotopic composition is governed by growth and turnover of both protein and lipid fractions of the body (Jardine et al., 2006). Overall, this research highlights the need to understand the dynamics of
tissue turnover and routing when using ecological tracers to infer ecological linkages between ecosystems (Ramos and González Solís, 2012).

In CHAPTER 6, I used a bioenergetics-bioaccumulation model to understand the mechanisms driving contaminant biotransport and uptake. The range of modeled PCB concentrations corresponded to that of the empirical survey, suggesting that individual variation in both individual ration size and level of egg consumption are important determinants of the extent to which fish incorporate certain salmon-derived contaminants. The model also demonstrates that fish are unable to reduce their PCB burden via metabolism or growth over time. This insight may explain the large amount of variation we have observed, and suggests that interactions between growth rate, consumption rate, and contaminant concentrations in the diet can strongly influence the bioaccumulation rate. Moreover, these complexities may obscure the relationship between salmon contaminant flux and stream-resident fish contaminant burden, given that fish are likely integrating PCBs from multiple salmon runs. In addition, our model clearly demonstrated that the contaminant burden of the spawner strongly influences the magnitude of bio-uptake of salmon-derived contaminants. Overall, the model provides a simple tool that can be used by managers to determine the likely consequence of salmon spawning on resident fish contaminant burdens.

7.3 Management implications of dissertation findings

Introduced Pacific salmon populations are intensely managed in the Great Lakes as an economically important open-water and riverine recreational fishery. Pacific salmon were introduced in the mid-1960s as a biocontrol agent for invasive alewife (*Alosa pseudoharengus*) while also helping to rehabilitate predator populations, namely
lake trout (*Salvelinus namaycush*), that had been decimated by the invasive sea lamprey (*Petromyzon marinus*) (Dettmers et al., 2012). Overall, this introduction was successful in terms of controlling alewife and also for building an economically important sport fishery. However, the negative impacts of this introduction with respect to native species and bioaccumulation of contaminants are now evident (Madenjian et al., 2008).

Currently, whole-lake ecosystem changes due to non-native dreissenid mussels has shifted the trophic base of production away from the open water pelagic zone to the nearshore benthic zone, altering the available prey base for salmon in the Great Lakes (Bunnell et al., 2012). Lake Huron underwent similar changes beginning in 2004, and salmon are largely absent from the lake at this time (Dettmers et al., 2012). These changing lake dynamics have altered how salmon are managed, primarily through reductions in fish stocking (Tsehaye et al., 2014).

Changes in ecosystem dynamics, salmon populations, and fish stocking have implications for salmon-mediated contaminant biotransport in the Great Lakes. Reductions in overall population size and stocking rate will reduce the number of salmon spawners in streams, and consequently the overall flux of pollutants delivered by salmon. However, uncertainty remains in predicting contaminant biotransport impacts by salmon under these scenarios. For instance, more than half of the Chinook populations in Lake Michigan are believed to be of ‘wild’ origin, that is naturalized to tributaries that lack hatcheries (Williams 2012). At present, it is unclear whether lower spawner densities will improve juvenile survival (i.e., compensatory response), allowing wild recruitment to increase, and in turn increasing the number of wild spawning salmon (Walters and Martell, 2004). Such changes could potentially lead to streams with the highest quality of
spawning habitat becoming ‘hotspots’ for contaminant biotransport relative to stream reaches with degraded or sub-optimal habitat that are often used as stocking locations for salmon. Thus, predicting whether changing recruitment dynamics will result in a net reduction or increase in salmon-mediated movement of contaminants is a challenge facing environmental managers.

Salmon-mediated contaminant biotransport is not monitored or managed by state or federal regulatory agencies. However, our data from both our basin-wide survey and salmon-addition experiment show that 74% of trout from our survey and 73% of trout from the treatment zone of our experiment exceed the EPA advisory threshold for ‘no consumption’ (Fig 7.2).

![Figure 7.2. Empirical cumulative frequency distribution of resident trout PCB concentrations in the (A.) basin-wide survey and (B.) Hunt Creek salmon addition treatment zone. Dashed line represents the EPA advisory threshold for no consumption.](image)

Many of the streams sampled for my work are considered near-pristine, lacking point sources of pollution, exhibit intact riparian corridors, little human development, and were often located on state or federal lands. Yet, even in these streams, my research
clearly demonstrates that spawning salmon are a predictable point source of organic contaminants to streams with consequences for ecosystems and human consumers. To better quantify this problem, monitoring agencies could leverage the large quantity of data they already collect to assess the spatial extent of biotransport in streams receiving migratory fish across the Great Lakes. For instance, a relatively simple analysis using existing data on stream fish PCB concentrations combined with locations open to migratory fish could allow an assessment of the potential magnitude of this problem at a regional level. In addition, future monitoring should develop rapid assessments of salmon spawner abundances in tributaries. We found, in general, that streams with larger salmon runs had higher contaminant burdens but we lacked data on salmon run size integrated over time. A sampling regime that monitors both the timing and size of salmon runs while also evaluating the extent of salmon egg consumption would be a simple and effective way to predict the transfer and uptake of salmon-derived contaminants using our model.

The monitoring and management of biotransported contaminants present a new challenge for managers because they represent a diffuse source of pollutants that requires different mitigation techniques then have been traditionally used to manage environmental contaminants (cf. Qi et al., 2014). Traditional techniques rely on engineering approaches to stop physicochemical non-point sources, such as removal of contaminated sediments or prevention of contaminated leachate loss. By contrast, contaminant biotransport represents a biological non-point source of pollution to streams that actually works against the usual physical movement of material (e.g., downstream transport). Literature concerned with managing non-point sources of nitrogen or
phosphorus (Carpenter et al., 1998) and preventing the spread of invasive species while maintaining connectivity (Rahel, 2013) is relevant to potential approaches to managing contaminant biotransport by migratory fish. Techniques for managing non-point sources of pollution have been effective at reducing the transport of fertilizers to streams when threshold levels of nutrient levels are defined, source locations or “hot-spots” are delineated, and best management practices are implemented (Carpenter et al., 1998). Future management could minimize contaminant biotransport through selective stocking to minimize contamination of systems prioritized for native fish conservation or implement seasonal barriers that limit contaminant influx to streams with spawning salmon.

Implementation of such management approaches has significant implications because many fish species in the Great Lakes exhibit a migratory life history, and deposit eggs in the process. Moreover, these species differ in many ways, including their spawning mode (e.g., broadcast versus redd construction), body mass [e.g., lake sturgeon (Acipenser fulvescens) versus salmon versus sucker (Catastomus spp.)], run size [e.g., very small (lake sturgeon)], intermediate (salmon, steelhead), very large (suckers)], seasonal run timing, and fecundity (e.g., egg size, gonadosomatic index). In addition, expanding research to quantify the extent to which other resident fish (e.g., non-salmonid species) utilize resources supplied by migratory fish would provide a detailed trait matrix to establish the configuration of spawners and resident species composition that magnifies or diminishes contaminant biouptake.

The effects of legacy contaminants (Murphy et al., 2012) and man-made dams (Stanley and Doyle, 2003) are persistent ecological stressors to the Great Lakes and their
fisheries. Removal of obsolete dams has increasingly become a preferred management action to improve stream connectivity and restore ecosystem function. The benefits of dam removal include increasing sediment transport, restoring natural thermal and flow regimes, and extending migration corridors for fish (Poff et al., 1997). However, dam removal also has unintended consequences for Great Lakes tributaries by allowing colonization by invasive species as well as contaminant biotransport by migratory fish (Lanse et al., 2014, McLaughlin et al., 2013). Although dam removal can provide clear ecological benefits, careful consideration and prioritization should be employed to minimize the risk of contaminant transport given the results of my dissertation research. Mitigating the impacts of salmon contaminant biotransport due to dam removal may be possible by using a combination of both modeling and on-the-ground approaches. For instance, a joint project between the Nature Conservancy and the University of Wisconsin-Madison has developed a barrier optimization tool where potential conservation gains (as indicated by miles opened up) can be balanced against the risk of Sea lamprey colonization (greatlakesconnectivity.org/). This approach could be linked to my work on contaminant biotransport to provide insight into potential areas where the benefits of leaving barriers in place outweighs the benefits of restoring ecosystem connectivity. Similarly, an optimization approach may help establish allowable levels of contaminant transport to streams; Rahel (2013) argues that while the reestablishing connectivity can have lasting ecological benefits for mobile organisms, those benefits must be prioritized in ways that balance against the risks of restored connectivity, be it for non-native species spread or contaminant biotransport. For instance, approaches used to control Sea lamprey, such as temporary, seasonal barriers may be effective tools for
minimizing upstream contaminant biotransport by salmon (Siefkes, 2014). Temporary barriers could be erected at locations lower in watersheds to prevent salmon from obtaining access to areas upstream that have been identified as being important with respect to intact native fish communities, low background contaminant levels, or high quality inland fisheries. This approach could be piloted using existing Sea lamprey barriers and the success of the program directly evaluated by managers. Another potential approach is the installation of resistance board weirs, which are commonly used to assess salmon run dynamics in Alaska (Tobin, 1994). The weir consists of a series of PVC pipes that create an impassable, floating fence across the river. Each individual PVC pipe is spaced a sufficient distance to allow movement of smaller resident trout, and non-game species, such as sculpin, while preventing movement of larger migratory species, such as salmon. This technique could be piloted in Great Lakes tributaries to reduce biotransport risks associated with Pacific salmon while minimizing the impact on smaller, native fish species.

7.4 Dissertation conclusions

My dissertation research increases our understanding of the mechanisms and variation surrounding the process of contaminant biotransport by Pacific salmon, an issue of high importance given widespread introductions of salmon on several continents. Overall, my dissertation research used differing scales and levels of control to shown convincingly that salmon have a marked impact on stream-resident fish contaminant burdens. However, salmon do not uniformly impact the stream-resident fish community and the magnitude of their effect appears tightly linked to the biological context. My dissertation demonstrates that the contaminant type, species identity, and trophic pathway
to contamination interact to determine the magnitude of salmon biotransport and uptake. Consequently, consideration of the recipient food web and route of exposure is critical to understanding the fate of biotransported contaminants in ecosystems.

7.5 Literature Cited


Williams M. C. 2012. Spatial, temporal, and cohort-related patterns in the contribution of wild Chinook salmon (Onchorynchus tshawtscha) to total Chinook harvest in Lake Michigan. Thesis Michigan State University, East Lansing, MI.

Figure A.1. PBDE congener pattern (mean percentage ± standard error) of salmon eggs and whole fish from lakes Michigan (eggs N=16, tissue N=28), Huron (eggs N=4, tissue N=9), and Superior (eggs N=7, tissue N=12). PBDE congeners quantified were BDE28, BDE47, BDE99, BDE100, BDE153, and BDE154.
Figure A.2. Non-metric, multidimensional scaling (NMDS) plots with 95% confidence ellipses of the mean for PCBs (A.) and PBDEs (B.) in salmon whole fish and eggs from lakes Michigan, Huron, and Superior. A convergent solution for PCBs was found after two iterations (stress=7.9) and for PBDEs after three iterations (stress=17.54). Tissue samples are represented by triangles and egg samples by circles. Ellipses are the same color as corresponding data points.
TABLE A.1.
PAIRWISE BONFERRONI COMPARISONS FOR PCB AND PBDE PERMANOVA MODELS IN SALMON FROM LAKES MICHIGAN, HURON, AND SUPERIOR.

<table>
<thead>
<tr>
<th>POP</th>
<th>Source</th>
<th>Comparison</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCB</td>
<td>Tissue</td>
<td>Michigan-Huron</td>
<td>4.37</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Michigan-Superior</td>
<td>42.72</td>
<td>0.001</td>
</tr>
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<td></td>
<td></td>
<td>Superior-Huron</td>
<td>24.20</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Eggs</td>
<td>Michigan-Huron</td>
<td>3.46</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Michigan-Superior</td>
<td>79.62</td>
<td>0.001</td>
</tr>
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<td></td>
<td></td>
<td>Superior-Huron</td>
<td>18.17</td>
<td>0.003</td>
</tr>
<tr>
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<td>Tissue-Eggs</td>
<td>Michigan</td>
<td>15.35</td>
<td>0.001</td>
</tr>
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<td></td>
<td></td>
<td>Huron</td>
<td>6.88</td>
<td>0.001</td>
</tr>
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<td></td>
<td></td>
<td>Superior</td>
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<td>0.010</td>
</tr>
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<td>Tissue</td>
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<td>Michigan-Superior</td>
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<td>1.24</td>
<td>0.266</td>
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<td>Eggs</td>
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<td></td>
<td>Michigan-Superior</td>
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<td>0.004</td>
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<td>Tissue-Eggs</td>
<td>Michigan</td>
<td>3.10</td>
<td>0.040</td>
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<td></td>
<td></td>
<td>Huron</td>
<td>0.54</td>
<td>0.590</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Superior</td>
<td>4.20</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Note: The alpha value for each pairwise comparison was 0.006 (0.05/9 comparisons per POP class). Significant comparisons are in bold. For each contaminant we compared differences in tissue and pattern among basins. In addition, we also compared tissue and egg patterns within a given basin.
TABLE A.2.

PAIRWISE BONFERRONI COMPARISONS FOR PCB MODELS IN PERMANOVA BROOK TROUT AND MOTTLED SCULPIN IN REACHES WITH SALMON PRESENT AND SALMON ABSENT FROM LAKES MICHIGAN, HURON, AND SUPERIOR.

<table>
<thead>
<tr>
<th>Basin</th>
<th>Comparison</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Michigan</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brook Trout-Salmon Present: Brook Trout-Salmon Absent</td>
<td>5.61</td>
<td><strong>0.001</strong></td>
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<tr>
<td></td>
<td>Brook Trout-Salmon Present: Salmon Spawners</td>
<td>1.86</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>Brook Trout-Salmon Absent: Salmon Spawners</td>
<td>18.35</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Mottled Sculpin-Salmon Present: Mottled Sculpin-Salmon Absent</td>
<td>4.96</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Mottled Sculpin-Salmon Present: Salmon Spawners</td>
<td>50.17</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
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<td>Mottled Sculpin-Salmon Absent: Salmon Spawners</td>
<td>39.15</td>
<td><strong>0.001</strong></td>
</tr>
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<td>Brook Trout-Salmon Present: Mottled Sculpin-Salmon Present</td>
<td>13.20</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Brook Trout-Salmon Absent: Mottled Sculpin-Salmon Absent</td>
<td>8.95</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Huron</strong></td>
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<td></td>
</tr>
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<td>Brook Trout-Salmon Present: Brook Trout-Salmon Absent</td>
<td>3.40</td>
<td>0.003</td>
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<td></td>
<td>Brook Trout-Salmon Present: Salmon Spawners</td>
<td>2.23</td>
<td>0.039</td>
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<tr>
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<td>Brook Trout-Salmon Absent: Salmon Spawners</td>
<td>8.90</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Mottled Sculpin-Salmon Present: Mottled Sculpin-Salmon Absent</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Mottled Sculpin-Salmon Present: Salmon Spawners</td>
<td>12.17</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Mottled Sculpin-Salmon Absent: Salmon Spawners</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Brook Trout-Salmon Present: Mottled Sculpin-Salmon Present</td>
<td>4.50</td>
<td>0.004</td>
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<tr>
<td></td>
<td>Brook Trout-Salmon Absent: Mottled Sculpin-Salmon Absent</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td><strong>Superior</strong></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Brook Trout-Salmon Present: Brook Trout-Salmon Absent</td>
<td>14.52</td>
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<td><strong>0.001</strong></td>
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<td>Brook Trout-Salmon Absent: Salmon Spawners</td>
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<td>33.38</td>
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<td>Brook Trout-Salmon Absent: Mottled Sculpin-Salmon Absent</td>
<td>2.14</td>
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Note: The alpha value for each pairwise comparison was 0.00625 (0.05/8 comparisons per basin). Significant comparisons are in bold.
TABLE A.3.
PAIRWISE BONFERRONI COMPARISONS FOR PBDE PERMANOVA MODELS
FOR BROOK TROUT AND MOTTLED SCULPIN IN REACHES WITH SALMON
PRESENT AND SALMON ABSENT FROM LAKES MICHIGAN, HURON, AND
SUPERIOR.

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Note: The alpha value for each pairwise comparison was 0.00625 (0.05/8 comparisons per basin). Significant comparisons are in bold.
**APPENDIX B:**

SUPPLEMENTAL INFORMATION FOR CHAPTER 3

TABLE B.1.

**TABLE OF SITE CHARACTERISTICS FROM 13 WATERSHEDS SAMPLED TO ASSESS HOW ENVIRONMENTAL CONTEXT MEDIATES CONTAMINANT BIOTRANSPORT BY PACIFIC SALMON IN THE LAURENTIAN GREAT LAKES.**

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<th>Watershed</th>
<th>Basin</th>
<th>Salmon Presence</th>
<th>Watershed area (km²)</th>
<th>Forested % total landcover</th>
<th>Agriculture % total landcover</th>
<th>Wetland % total landcover</th>
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<td>PCB Flux (ng/g/m²)</td>
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<td>Octachlorobiphenyl</td>
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*Note: Cl=Chlorine*
### TABLE B.3.

MODEL-AVERAGED COEFFICIENT ESTIMATES FOR TOP PCB AND Hg MIXED-MODELS ASSESSING THE EFFECT OF ENVIRONMENTAL CONTEXT ON UPTAKE OF SALMON-DERIVED CONTAMINANTS BY STREAM-RESIDENT FISH.

<table>
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<tr>
<th>Contaminant</th>
<th>Variable</th>
<th>Estimate</th>
<th>SE</th>
<th>P-value</th>
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<tbody>
<tr>
<td>PCB</td>
<td>(Intercept)</td>
<td>4.46961</td>
<td>1.4534</td>
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<td></td>
<td>δ¹³C</td>
<td>0.09582</td>
<td>0.0247</td>
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<td></td>
<td>PCB flux</td>
<td>0.65287</td>
<td>0.0475</td>
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<tr>
<td></td>
<td>Species Brown trout</td>
<td>-0.64782</td>
<td>0.1964</td>
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<tr>
<td></td>
<td>Species Mottled sculpin</td>
<td>-0.64848</td>
<td>0.1796</td>
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<tr>
<td></td>
<td>Species Rainbow trout</td>
<td>0.03095</td>
<td>0.3504</td>
<td>0.930</td>
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<td>PCB flux x Species Brown trout</td>
<td>0.20143</td>
<td>0.0719</td>
<td>0.005</td>
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<td>PCB flux x Species Mottled sculpin</td>
<td>-0.24194</td>
<td>0.0667</td>
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<td>PCB flux x Species Rainbow trout</td>
<td>0.17553</td>
<td>0.1268</td>
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<td>Large wood volume</td>
<td>0.09535</td>
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<td>Watershed area</td>
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<td>Stream temperature</td>
<td>0.01189</td>
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<td>% Forested</td>
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<td>0.0022</td>
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<td>Stream substrate</td>
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<td>0.0015</td>
<td>0.906</td>
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<td></td>
<td>(Intercept)</td>
<td>3.94700</td>
<td>0.1500</td>
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<tr>
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<td>Hg flux</td>
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<td>0.0473</td>
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<td></td>
<td>Length</td>
<td>0.00326</td>
<td>0.0007</td>
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<td>Hg flux x Length</td>
<td>-0.00086</td>
<td>0.0002</td>
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</table>
Figure C.1. Weekly individual brook trout growth in the mesocosm experiment. Line represents the optimized growth trajectory from maximum likelihood that minimized the error between the observed data and model fit by varying the proportion of maximum consumption realized. The optimized p-value used for all additional modeling was 0.53.
Figure C.2. Brook trout growth rates with respect to mass (g/day) and tissue $\delta^{15}$N (‰) among size classes across treatments. Brook trout from the large size class grew at a higher rate (Tukey HSD, $p=0.002$) and had higher $\delta^{15}$N (Tukey HSD, $p=0.01$) than fish from the small size class. Values reported are medians with upper and lower quartiles to illustrate variability.
Figure C.3. Relationship between proportion of diet derived from salmon and mercury level for brook trout from salmon treatments. Salmon dietary proportion estimated from a Bayesian stable isotope mixing model. Dashed line represents the standard error around proportion estimate.