Chinook, coho, and steelhead eggs collected in 2006 from Lake Ontario, L. Erie, L. Michigan, and L. Superior were analyzed by gas chromatography for congener-specific polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and select organochlorine pesticides (OCs). Across lake comparisons for total PCB, total DDT, and total PBDE indicated a consistent trend in contaminant concentrations in which Lake Michigan > Lake Ontario > Lake Erie > Lake Superior. The contaminants Mirex and octachlorostyrene (OCS) were found not to be consistent with this trend, but were instead found to exist in higher concentrations in Lake Ontario in relation to the other Great Lakes. PCB chlorination patterns, as indicated by the average number of chlorines per biphenyl (Avg Cl/BP) were compared within species. The PCB chlorination pattern in chinook eggs was found to be similar for both Lake Michigan (5.47) and Lake Ontario (5.43). The PCB chlorination pattern in coho eggs was notably different in Lake Superior (5.78) than in Lake Ontario (5.41).

I. Introduction

The USEPA Great Lakes Fish Monitoring Program (GLFMP) is designed to evaluate contaminant concentrations in Great Lakes sport and top predator fish, evaluate human exposure to contaminants, and obtain new information on persistent and emerging contaminants. As a part of the GLFMP sport fish program, the purpose of this preliminary research was to investigate the utilization of salmon eggs as bioindicators of persistent and emerging contaminants in the Great Lakes.

The utilization of bioindicators in persistent contaminant research is widely discussed in the literature. Species such as snapping turtles and tree swallows have been widely used as reliable bioindicators of persistent contaminants in the environment (Pagano et al., 1999; Nichols et al., 1995). The utilization of fish eggs as biomonitors of persistent contaminants has advantages including: ease and cost effectiveness of collection and analysis; large composite egg batch samples reflecting entire spawning populations; and reduced adverse effects to the general salmon population (Miller, 1993; Miller, 1994; Pagano, 2005; Pagano, 2007).
II. Materials and Methods

The Salmon River Fish Hatchery located in the Village of Altmar, Oswego County is operated by the New York State Department of Environmental Conservation (NYSDEC) to meet the sport fishery stocking needs of Lake Ontario and tributaries. Lake Ontario coho (Oncorhynchus kisutch) and chinook (Oncorhynchus tshawytscha) salmon eggs were randomly sampled by NYSDEC personnel at the Salmon River Fish Hatchery. Sampling locations for Lake Superior, Lake Michigan, and Lake Erie eggs are noted in Fig. 1. Egg samples were collected during the Fall 2006 spawning run. For the Lake Ontario samples, paired egg and muscle fillet (skin off) samples were collected from individual coho and chinook salmon before fertilization as previously described (Pagano, 2005b).

![Fig. 1: Great Lakes 2006 fish egg collection sites. Map obtained from USEPA GLNPO, 2008.](image)

Egg samples were extracted at Clarkson University by Accelerated Solvent Extraction (ASE) using a Dionex ASE 300 and dichloromethane solvent. The sample extract was condensed to 2 mL in a TurboVap II for Gel Permeation Chromatography (GPC) cleanup. Sample cleanup followed EPA method 3640A (GPC cleanup - pesticide option) using a Waters GPC system (binary pump, Envirogel® column, UV detector, and fraction collector) followed by silica gel column for separation of PCBs/OCS/PBDEs from other interferences. Adsorption column chromatography clean-up utilized 5.5 grams of 4% deactivated silica (Sigma-Aldrich, grade 923, 100-200 mesh) placed in a chromatography column and sample extract sequentially eluted with 25 mL of hexane (fraction 1) and 25 mL hexane:dichloromethane (1:1, fraction 2). Each fraction was then concentrated in a TurboVap II to 2 mL for gas chromatographic analysis.

Congener-specific PCB, hexachlorobenzene, p,p' DDE, and Mirex analyses were conducted based on capillary column procedures previously described (Pagano et al., 1995; Pagano, 2005a; Pagano, 2007b). Briefly, analytical instruments were recalibrated every five samples, with a system blank, instrument blank, and mid-level calibration check
solution analyzed during each analytical run. A Hewlett-Packard (HP) Model 5890II GC with an electron capture detector (ECD - Ni\(^{63}\)) and autosampler were used for primary data acquisition. The capillary column utilized was a HP Ultra II, 25 meter with 0.22 mm id and 0.33 \(\mu\)m film thickness. The calibration standard used was a 1:1:1:1 mixture of Aroclors 1221, 1016, 1254, and 1260 each at 200 pg/uL, hexachlorobenzene (HCB) at 5 pg/uL, and p-p' DDE and Mirex each at 10 pg/uL (Custom Mixed Fraction #3, AccuStandard, Inc.), which allows for the analysis of 99 chromatographic zones of 132 congeners/co-eluters. PCB analyses were confirmed with a HP Model 5890 II gas chromatograph with an electron capture detector (Ni\(^{63}\)) and autosampler using a 60 meter DB-XLB capillary column with 0.25 mm id and 0.25 \(\mu\)m film thickness. The calibration standard used was a 1:1:1:1:1 mixture of congener mixture sets C-CSQ-SET 1-5; 10 pg/uL per individual congener (AccuStandard, Inc., New Haven, CT). PCB data was processed using HP ChemStation software and Microsoft Excel spreadsheet procedures such that the mole percent (congener specific and homolog), and average chlorine/biphenyl (Avg Cl/BP) values were generated.

Congener-specific PBDE analyses were conducted with HP Model 5890 II gas chromatograph with an electron capture detector (Ni\(^{63}\)) and autosampler using a 60 meter DB-XLB capillary column with 0.25 mm id and 0.25 \(\mu\)m film thickness. The PBDE calibration standard used was an 800 pg/uL (total PBDE = \(\sum\) 12 components) solution using the original Great Lakes Chemical Corporation (Great Lakes DE-71, CAS 32534-81-9) technical formulation. The DE-71 technical formulation mass fractions and congener identifications were confirmed with pure PBDE congener standards (BDE-MXE) purchased from Wellington Laboratories, Guelph, ON, Canada, and by mass spectrometric confirmation.

II. Results and Discussion

A comparison of select contaminant and percent lipid data for five egg sample groups is presented in Table 1. As noted, total PCB concentrations ranged from 863.1 ng/g in Lake Michigan chinook eggs to 105.3 ng/g in Lake Superior coho eggs. Similar across-lake concentration trends are noted for total PBDE and total DDT. Concentrations in these three contaminants groups were found to be greatest in Lake Michigan, followed by L. Ontario, L. Erie, and L. Superior. In comparison, lake trout whole fish data from the USEPA Great Lakes National Program Office (GLNPO) Great Lakes Environmental Database (GLENDA) is reported in Fig. 2. Similar to the fish egg trend noted in Table 1, total PCB concentrations for 2003 lake trout whole fish were found to be greatest in Lake Michigan, followed by L. Ontario, L. Huron, L. Erie, and L. Superior. The similarity of fish egg and lake trout whole fish trend data appears to indicate that fish eggs have the potential to act as bioindicators of contaminants in Great Lakes fish.
Table 1. Select contaminant data for five egg sample groups (concentrations expressed in ng/g – wet weight).

<table>
<thead>
<tr>
<th></th>
<th>LM Chinook (N=10)</th>
<th>LO Chinook (N=3)</th>
<th>LO Coho (N=3)</th>
<th>LE Steelhead (N=7)</th>
<th>LS Coho (N=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PCB</td>
<td>863.1</td>
<td>663.7</td>
<td>428.8</td>
<td>311.8</td>
<td>105.3</td>
</tr>
<tr>
<td>Ave Cl/BP (PCB)</td>
<td>5.47</td>
<td>5.43</td>
<td>5.41</td>
<td>5.49</td>
<td>5.78</td>
</tr>
<tr>
<td>% Lipid</td>
<td>10.3%</td>
<td>5.2%</td>
<td>3.7%</td>
<td>11.5%</td>
<td>8.9%</td>
</tr>
<tr>
<td>Total PBDE</td>
<td>55.2</td>
<td>40.3</td>
<td>25.6</td>
<td>23.2</td>
<td>23.2</td>
</tr>
<tr>
<td>Total DDT</td>
<td>291.9</td>
<td>267.3</td>
<td>154.9</td>
<td>40.8</td>
<td>34.7</td>
</tr>
<tr>
<td>Mirex</td>
<td>1.2</td>
<td>26.0</td>
<td>19.4</td>
<td>0.6</td>
<td>0.6</td>
</tr>
<tr>
<td>OCS</td>
<td>0.4</td>
<td>4.9</td>
<td>3.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Contrary to total PCB, PBDE, and DDT across-lake trends, Mirex is present in significantly higher concentrations in Lake Ontario than any of the other Great Lakes. For example, L. Ontario chinook egg Mirex concentrations (26.0 ng/g wet) are significantly higher than chinook eggs from L. Michigan (1.2 ng/g wet). Mirex was released into Lake Ontario by the Hooker Chemical company from the Niagara River and, to a lesser extent, the Oswego River (Pagano, 2004). Our fish egg results are consistent with the established fact that Mirex is a contaminant associated with Lake Ontario and not significantly present in the other Great Lakes.

![Fig. 2. Across-lake total PCB concentrations in 2003 lake trout whole fish. Data obtained from EPA GLNPO GLENDNA database. * Lake Erie samples are walleye.](image)

In order to validate use of eggs as environmental biomonitors, it is important to demonstrate if quantitative and qualitative relationships exist between contaminants found in eggs and contaminants found in muscles. In Fig. 3, a linear regression analysis of PCB congener concentrations in 2006 eggs versus muscle tissue was performed. Results indicate ($R^2 = 0.97$ for coho, and $R^2 = 0.98$ for chinook) a very strong quantitative fit.
between egg and muscle PCB congener concentrations for both species. Although the egg to muscle relationships are different for coho and chinook, both demonstrate a very strong quantitative relationship indicating that egg PCB congener concentrations have the potential to be highly predictive of muscle PCB congener concentrations.

PCBs in the environment are a complex mixture of 209 possible congeners, in which the number of chlorines substituted per biphenyl may vary from 1-10. In Fig. 4, PCB assigned congener numbers are displayed by mole percent in order to illustrate PCB chlorination patterns, independent of concentration in each fish-egg group. The lower assigned PCB congener numbers correspond to lower-chlorinated PCB congeners, and the higher assigned PCB congener numbers correspond to higher-chlorinated PCB congeners. In Fig. 4a, visual observation of L. Ontario and L. Michigan chinook egg data demonstrates very similar PCB chlorination patterns. As contrasted in Fig. 4b, a comparison of the L. Ontario and L. Superior coho data demonstrates a markedly different (and higher-chlorinated) PCB chlorination pattern in L. Superior eggs.
Fig. 4. Congener-specific PCB mole percent comparison of L. Ontario versus L. Michigan chinook eggs (4a.) and L. Ontario versus L. Superior coho eggs (4b.).

In Fig. 5, PCB homologs are displayed by mole percent and average number of chlorines per biphenyl in order to represent the PCB chlorination for each sample group. Homologs are subcategories of PCB congeners having equal numbers of chlorine substituents. As noted in Fig. 5a, similar mid-chlorinated homolog patterns and Avg Cl/BP values are observed in both L. Ontario (5.43) and L. Michigan (5.47) chinook eggs. As contrasted in Fig. 5b, notably different homolog chlorination patterns and Avg Cl/BP values are observed for L. Superior (5.78) and L. Ontario (5.41).
Determination of the qualitative relationship between PCB congeners found in fish eggs and muscle tissue is important for evaluating the reliability of fish eggs as biomarkers. In Fig. 6, congener-specific PCB mole percent values for 2006 coho and chinook were submitted to a linear regression analysis for egg and muscle tissue data. A strong qualitative fit between egg and muscle tissue is indicated for both coho ($R^2 = 0.98$) and chinook ($R^2 = 0.98$). These results qualitatively indicate that the contaminants found in salmon eggs are highly predictive of the contaminants found in muscle tissue.
IV. Conclusions

Results from preliminary data indicate an across-lake contaminant concentration trend for total PCB, PBDE, and DDT in which concentrations are highest in Lake Michigan, followed by L. Ontario, L. Erie, and L. Superior. Our data suggests that fish eggs are highly predictive both quantitatively and qualitatively of congener-specific PCBs found in muscle tissue. Although preliminary, our results to date suggest that fish eggs have the potential to be utilized as bioindicators of persistent contaminants in the Great Lakes.

V. Acknowledgements

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VI. References


**Fig. 6.** Comparative linear regression analysis for 2006 coho and chinook average muscle and average egg PCB mole percent. Data adapted from Pagano, 2007a.


Pagano, J. (2005a). Deposition and ambient concentrations of PBTs (PCBs, OCs, PBDEs, and Dioxins/Furans) in support of the Lake Ontario air deposition study (LOADS), Quality Assurance Project Plan (QAPP). Great Lakes Commission-Great Lakes Air Deposition (GLAD) Program, Ann Arbor, MI. June 28, 2005 – Version 01.


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