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BIOAMPLIFICATION AS A BIOACCUMULATION MECHANISM

by

Jennifer Daley

A Dissertation

Submitted to the Faculty of Graduate Studies

through Great Lakes Institute for Environmental Research

in Partial Fulfillment of the Requirements for

the Degree of Doctor of Philosophy at the

University of Windsor

Windsor, Ontario, Canada

2012

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Bioamplification as a bioaccumulation mechanism

by

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Co-authorship Declaration

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

I was the primary author and person responsible for the experimental implementation and majority of writing for each chapter included in this thesis. Chapter 2 was co-authored by Dr. Ken Drouillard and Dr. Lynda Corkum. Ken Drouillard and Lynda Corkum provided intellectual guidance, editorial and field support and it is published in the journal, Environmental Toxicology and Chemistry. Chapter 3 was co-authored by Dr. Ken Drouillard and Todd Leadley. Todd Leadley provided technical support and design contributions related to animal husbandary and appropriate sampling techniques as well as editorial support. Ken Drouillard provided intellectual guidance and editorial support and it is published in the journal, Chemosphere. Chapter 4 was co-authored with Dr. Ken Drouillard, Dr. Trevor Pitcher and Todd Leadley. Trevor Pitcher contributed to components of the experimental design, provided access to salmon and editorial support. Todd Leadley provided technical support related to animal husbandary and contributed to experimental design components and editorial support. Ken Drouillard provided intellectual guidance and editorial support and it is published in the journal, Environmental Science and Technology. Chapter 5 was co-authored with Dr. Ken Drouillard and Dr. Gordon Paterson. My co-authors provided intellectual guidance and editorial support and it was submitted as a review paper to the journal, Reviews in Environmental Contamination and Toxicology. Chapter 6 was co-authored with Dr. Ken Drouillard, Dr. Trevor Pitcher and Todd Leadley. Trevor Pitcher and Todd Leadley provided access to salmon, contributed to experimental design and editorial support. Ken

Drouillard provided intellectual guidance and editorial support and it was submitted to the journal Chemosphere.

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I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

Declaration of Previous Publication

This thesis includes three original papers that have been previously published for publication in peer reviewed journals, and two original papers that have been submitted for publication, as follows:

Thesis Chapter	Publication title/full citation	Publication status
Chapter 2	Daley J.M., Corkum L.D., Drouillard K.G. 2011. Aquatic to terrestrial transfer of sediment associated persistent organic pollutants is enhanced by bioamplification processes. Environmental Toxicology and Chemistry 30, 2167–2174.	Published
Chapter 3	Daley J.M., Leadley, T.A., Drouillard K.G. 2009. Evidence for bioamplification of nine polychlorinated biphenyl	Published

	(PCB) congeners in yellow perch (*Perca flavescens*) eggs during incubation. Chemosphere. 75, 1500-1505.	
Chapter 4	Daley J.M., Leadley T.A., Pitcher T.E., Drouillard K.G. 2012. Bioamplification and the selective depletion of persistent organic pollutants in Chinook salmon larvae. Environmental Science and Technology. 46, 2420-2426.	Published
Chapter 5	Daley J.M., Paterson G., Drouillard K.G. 2012. Bioamplification as a Non-steady State Bioaccumulation Mechanism for Persistent Organic Pollutants (POPs) in Wildlife. Reviews in Evironmental Contamination and Toxicology.	Submitted
Chapter 6	Daley J.M., Leadley T.A., Pitcher T.E., Drouillard K.G. 2012. The effect of food provisioning on persistent organic pollutant bioamplification in Chinook salmon larvae. Chemosphere. CHEM27504	Submitted

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Abstract

Persistent organic pollutant (POP) bioaccumulation models have been generally formulated to predict two main processes, bioconcentration and biomagnification. A third bioaccumulation process that can mediate chemical potential in an organism is bioamplification. Bioamplification occurs when an organism loses body weight and chemical partitioning capacity faster than it can eliminate contaminants. Bioamplification causes an increase in chemical fugacity in the animal's tissues and results in the redistribution of contaminants from inert storage sites to more toxicologically sensitive tissues. Further, bioamplification generally occurs when an organism experiences major bioenergetic bottlenecks or nutritional stress, frequently associated with critical periods in the animal's life history.

The goal of this dissertation was to characterize bioamplification as a general bioaccumulation process that is additive to bioconcentration and biomagnification mechanisms of chemical exposure. Empirical studies validating bioamplification in three different animal models each undergoing a recognizable bioenergetic bottleneck during their life history were completed. Specifically, bioamplification was validated in emergent aquatic insects, fish embryos during egg development and larval fish. Bioamplification factors in the above studies ranged from 1.9-2.1 in emergent male mayflies, 1.8-5.4 in incubating yellow perch embryos and 1.5-5.3 in larval Chinook salmon (dependent on food resource availability). To complement these studies, a literature review was completed to demonstrate the wide applicability of this concept to different animal species. Examples of bioamplification were presented in invertebrates, fishes, birds and mammals corresponding to bioenergetic bottlenecks related to

migration, reproduction, early life stages, metamorphosis, over wintering weight losses and disease. Bioamplification factors summarized in the literature ranged from 1.1–14 and were similar in magnitude to biomagnification factors typically reported for aquatic and terrestrial organisms.

While most of the descriptions of bioamplification in the literature have treated it as a bioaccumulation curiosity, the results of this dissertation demonstrate that bioamplification is a general bioaccumulation process that contributes to enhanced chemical fugacities of POPs across the animal kingdom. Further, the results of this dissertation showed that bioamplification is producing maximum POP fugacities at critical periods over the animal's life history and as such the consequences of bioamplification may be very important to wildlife hazard and risk assessments.

Dedication

To my parents, Carol and Tom Daley.
For providing me a wonderful life and for whom I owe everything to.

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Chapter 1: General Introduction

General Introduction

Bioaccumulation describes the process by which chemicals are taken up and retained by organisms from their environment and diet (Mackay and Fraser 2000, Arnot and Gobas 2003, Borga et al. 2004). The tendency of a chemical to bioaccumulate depends on a number of factors including its physicochemical properties e.g. hydrophobicity (Fisk et al. 1998, Kelly et al. 2007), and volatility (Komp and McLachlan 1997), the tendency to become associated with tissue components of the organism either through partitioning (Drouillard et al. 2003) or other chemical-tissue interactions (Mason et al. 1995, Lehmler et al. 2006) and ability of the chemical to resist degradation by various metabolic biotransformation pathways possessed by a given species of organism (Klecka 2000). Persistent organic pollutants (POPs), as exemplified by p,p'-DDT and polychlorinated biphenyls (PCBs), exhibit very high bioaccumulation potentials and provide the classic examples of biomagnifying compounds. For the above chemicals, not only are physicochemical properties of importance to bioaccumulation potential, but there is also growing recognition on the roles of physiological and ecological characteristics of organisms which interact with and modify such chemical bioaccumulation potentials.

The field of bioaccumulation within environmental science was arguably developed following the publication of Rachel Carson's *Silent Spring* (1962), whereupon organochlorine pesticides, and DDT in particular, was demonstrated to not only bioaccumulate to high concentrations in animals but also exhibited food web biomagnification. Food web biomagnification is classically defined as the condition where chemical concentrations increase with increasing trophic position of animals within a given food web (Woodwell 1967, Thomann and Connolly 1981, 1984). Since

the development of this field over five decades ago, there have been major changes in bioaccumulation modelling and empirical bioaccumulation assessment approaches.

Following the publication of *Silent Spring*, much of the emphasis in bioaccumulation modelling focused on equilibrium partitioning, such that all organisms were assumed to approach or achieve chemical equilibrium with the major media (water or food) to which they were being exposed (Clayton et al. 1977, Pavlou and Dexter 1979). A seminal paper by Connolly and Pedersen (1988) challenged this paradigm by demonstrating that certain chemicals, including many POP compounds, achieved chemical concentrations within organisms that exceed the expected equilibrium concentration based on water concentrations. Connolly and Pedersen (1988) further demonstrated that food web biomagnification of hydrophobic POPs occurred such that not only chemical concentration, but chemical fugacity (a surrogate measure of chemical potential), increased with increasing trophic status of the organism. This work was influential in shaping the definition of biomagnification as it applies to hydrophobic organic compounds, such that it is now defined to occur when the chemical fugacity in the animal becomes greater than its food or respired media (Connolly and Pederson 1988, Drouillard 2008). Biomagnification of hydrophobic POP compounds has now been widely demonstrated and verified to occur in aquatic (Oliver and Niimi 1988; Russell et al. 1999a) and terrestrial (McLachlan 1996, Kelly and Gobas 2001) organisms and food webs on a global basis.

The most accepted mechanism and modelling approach to explain POP biomagnification was developed by Gobas et al. (1988, 1993ab, 1999) and is referred to as the gastrointestinal magnification (GI-magnification) model. This model recognized

food as a special exposure route that contributes to biomagnification and the basic mechanism was verified through empirical studies performed on fish and humans (Gobas et al. 1993b, 1999; Schlummer et al. 1998, Moser and McLachlan 2001). The model was subsequently expanded into a food web bioaccumulation model (Campfens and Mackay 1997, Thomann et al. 1992, Morrison et al. 1997; Arnot and Gobas 2004; Kelly et al. 2004) and was widely applied to predict trophic transfer bioaccumulation and biomagnification of POPs in aquatic and terrestrial food webs. The model, while undergoing constant improvement in algorithm formulations (Arnot and Gobas 2004), is still considered state of the art today. The GI-magnification based food web model is capable of predicting non-equilibrium, steady state chemical bioaccumulation within complex food webs. These food web models are almost always solved under conditions of steady state, as by assuming steady state, the need for model initiation is omitted and it allows for most toxicokinetic model parameters to become constants. However, a consequence of the above assumption is that steady state GI-magnification models by default attribute all non-equilibrium bioaccumulation for hydrophobic, non-metabolized POPs to be a consequence of GI-magnification and/or the interaction between GI-magnification and trophic transfer via feeding relationships within a simulated food web.

However, growing evidence indicates that non-steady state conditions not only exist but represent a normal condition over the life span of many organisms (Kiriluk et al. 1995, Czub and McLachlan 2007, Paterson et al. 2007, Burtnyk et al. 2009, Volta et al. 2009). Under non-steady state bioaccumulation, animals may still achieve chemical concentrations or chemical fugacity in excess of equilibrium predictions, but do not achieve the maximum biomagnification potential predicted for the species (Paterson et al.

2006). Under these conditions, toxicokinetic parameters governing chemical uptake, elimination and inter-tissue distribution interact with changing environmental conditions as influenced for example by temperature, food resource availability, habitat conditions or the physiological state of the organism that affects animal metabolic rate, foraging behaviour, activity costs and growth rates (Sijm et al. 1992, Goyke and Brandt 1993, Skogland et al. 1996, Roy 1999, Paterson et al. 2006, Paterson et al. 2007ab). Under a range of circumstances, animals may achieve chemical potentials that exceed the maximum biomagnification potential predicted for the animal. This can occur under the situation where an animal at steady state with its environment suddenly looses body weight faster than it can lose chemical mass. The above process is defined here as bioamplification and reflects the central topic of this thesis. Bioamplification is explicitly a non-steady state process that arises from sudden changes in steady state to non-steady state conditions or from perturbation of the non-steady state trajectory (e.g. uptake bioaccumulation curve) as a result of changes in the partition capacity of the animal apart from chemical uptake and elimination processes. Although the phenomena of bioamplification has been recognized in the literature (ie. Drouillard et al. 2003, deBruyn et al. 2004, Leney et al. 2006, Patterson et al. 2007a), there have been few attempts to formalize and model bioamplification as an independent bioaccumulation mechanism.

Bioaccumulation Metrics, Concentration Units and Equilibrium Partitioning Concepts

The bioaccumulation potential of organic chemicals in organisms is typically measured using a number of different empirical bioaccumulation metrics that consist of expressing the concentration ratio of chemical in an organism relative to the

concentration of chemical in a given environmental media or food. Common metrics include the bioconcentration factor (BCF; organism/water concentration ratio during laboratory exposures), bioaccumulation factor (BAF; organism/water concentration ratio during field exposures), biota/sediment accumulation factor (BSAF; organism/sediment ratio concentration) and biomagnification factor (BMF; organism/food concentration ratio) (DiToro et al. 1990; Wong et al. 2001, Burkhard et al. 2006, Borga et al. 2004, Mackay and Fraser 2000). Chemical concentrations in the organism and in some cases, the reference media, may further be expressed in different concentration units including: wet weight, dry weight, lipid weight, lipid equivalents, or on a fugacity basis. The expression of chemical concentration units has important consequences in how a given bioaccumulation metric is interpreted in terms of whether or not the metric is being used to test for equilibrium partitioning (Mackay and Fraser 2000) or to contrast an empirical result against regulatory bioaccumulation criteria (Mackay and Fraser 2000, Borga et al. 2005).

Wet weight concentrations are most commonly used when expressing the bioconcentration factor during the evaluation of new chemicals to test for regulatory criteria of bioaccumulation during the classification of a substance into persistent, bioaccumulative and/or toxic categories. In this case, chemicals exhibiting wet weight bioconcentration factors greater than 5000 are designated as bioaccumulative by regulatory criteria (Kelly et al. 2004, Borga et al. 2005). However, use of wet weight concentrations to express the chemical concentration in the organism's tissues has several problems. Wet weight concentrations are often the most variable means of expressing chemical concentration in an animal's tissues since water makes up a significant portion

of an organism's tissue weight but contains negligible POPs mass relative to organic and lipid phases of the animal. Animals vary widely in water content (84% in mussels to 69% in crayfish from Lake Erie; Gewurtz et al. 2000) and tissue water content can also change during long term storage such as freezing (Szczepanik et al. 2010). Thus, while wet concentrations are typically used in BCF calculations to address regulatory bioaccumulation criteria, the expression of organism concentrations on a wet weight basis provides less information about phase partitioning and equilibrium relationships compared to other concentration expressions described below.

Dry weight concentration provides a means of eliminating the variability associated with water content in animal tissues and is more commonly used when expressing organism concentrations and bioaccumulation metrics as applied to metals (Adrian and Stevens 1979). This is because metal-protein interactions dominate the phase distribution of these compounds in most organisms and protein constitutes one of the chief components of animal dry matter (Einen and Roem 1997). However, for hydrophobic organic chemicals including POPs, dry weight concentrations may still be misleading since these compounds are not as strongly associated with protein but instead tend to be associated predominately with neutral lipids (Drouillard et al. 2004). For the above compounds, the lipid content of the organism is a critical controlling factor of tissue residues of chemicals and therefore is the most commonly recommended means of concentration expression for evaluating phase partitioning and equilibrium relationships (Connolly and Pederson 1988, Kelly et al. 2004).

Expressing the bioconcentration factor using lipid normalized chemical concentrations has direct analogy to solvent/water partition coefficients and through such

comparisons can permit the evaluation of equilibrium partitioning of chemical between an aquatic organism and its respired water. The *n*-octanol/water partitioning coefficient (K_{OW}) is commonly used as a surrogate for lipid/water partitioning (Chiou 1985). *n*-Octanol was originally chosen in the pharmaceutical industry as a surrogate of lipids because it has a similar carbon to oxygen ratio as fatty acids, leading to higher solvation power compared to neutral organic solvents. Octanol is available in pure form and is less susceptible to microbial degradation during partitioning experiments making it easier to work with experimentally then biologically isolated lipids (Leo et al. 1971, Mackay 1982). Finally, as K_{OW} data became more prevalent across different types of compounds in both the pharmaceutical and environmental chemistry fields, this partition coefficient became the standard on which to evaluate chemical behaviour, environmental fate and lipid partitioning relationships. Indeed, the regulatory community uses log K_{OW} values greater than 5 as alternate criteria to evaluate bioaccumulation potential in aquatic organisms (Mackay and Fraser 2000). The basic expression of chemical K_{OW} is as follows:

$$K_{ow} = \frac{C_o}{C_w} \qquad (1)$$

Where, C_O is the equilibrium chemical concentration in octanol (mol/m^3) and C_W is the concentration in water (mol/m^3). Under the assumption that $C_O = C_{Lipid}$, then:

$$K_{ow} = \frac{C_o}{C_w} \approx \frac{C_{Lipid}}{Cw} = BCF_{Lipid} \qquad (2)$$

Thus, when the lipid normalized chemical concentration in an organism is greater than the product of $K_{OW} \cdot C_w$, the organism is considered to have achieved a higher chemical fugacity then the water reflecting a non-equilibrium bioaccumulation condition (Connolly and Pedersen 1988). In addition, because neutral lipids are considered the principle determinant of sample partition capacity for hydrophobic organic chemicals in biological samples (Mackay and Fraser 2000), comparing lipid normalized chemical concentrations between samples of biological origin provides a surrogate measure to contrast chemical fugacity in the samples. Thus, if the ratio of lipid normalized chemical concentration in two biological samples differs from a value of one, a condition of non-equilibrium between the samples can be concluded. Similar to the BCF_{Lipid}, the extent of biomagnification can be quantified by the biomagnification factor (BMF) the ratio of the lipid normalized concentration in the organism compared to its diet according to:

$$BMF = \frac{C_{org(WW)}}{C_{food(WW)}} \cdot \frac{f_{L(food)}}{f_{L(org)}} = \frac{C_{org(Lipid)}}{C_{food(Lipid)}} \quad (3)$$

Where, $C_{org(WW)}$ is the wet weight concentration (ng/g wet weight) in the organism and $C_{food(WW)}$ is the wet weight concentration in the food (ng/g wet weight). $C_{org(Lipid)}$ is the concentration lipid normalized in the organism (ng/g lipid) and $C_{food(Lipid)}$ is the concentration lipid normalized in the food and $f_{L(food)}$ is the fraction of lipid in the food (ng/g food) and $f_{L(org)}$ is the fraction of lipid in the organism (ng/g body weight).

More recently, it has been recognized that neutral lipids are not the only contributors to partition capacity of animal tissues for hydrophobic POPs compounds and the assumption that partition capacity of biological samples can be estimated from lipid content alone has been challenged especially when applied to samples of very low lipid

content (lipid < 1%). Gobas et al. (1999) defined non-lipid organic matter (NLOM), essentially referring to lean dry protein (LDP) content, as having a partition capacity approximately 3% of that provided by neutral lipids. deBruyn and Gobas (2007) provided an in depth literature review of the partition capacity of lean dry matter and concluded that LDP is more appropriately modelled to have an approximate 5% partition capacity relative to lipid. Thus, when dry weight lipid content approaches a value of 5%, the LDP of tissues provide an approximate equal contribution to the partition capacity as lipids. When dry weight lipid content drops below this value, LDP begins to dominate the sample partitioning capacity for hydrophobic organic compounds. For organisms or tissue samples having low lipid content, the lipid equivalent concentration therefore provides a better representation of chemical concentration and chemical fugacity than lipid normalized concentrations do. The lipid equivalent concentration is therefore interpreted in the identical manner as lipid normalized concentrations described above. The lipid equivalent concentration is calculated as:

$$C_{Lip(eq)} = \frac{C_{org(WW)}}{f_{Lipid} + 0.05 f_{LDP}} \qquad (4)$$

Where $C_{Lip(eq)}$ is the concentration lipid equivalents (ng/ g lipid equivalents) in the organism and $0.05f_{LDP}$ is five percent of the fraction of lean dry protein in the organism. Similarly, the lipid equivalents BMFs are calculated according to:

$$BMF = \frac{C_{org(WW)}}{\left(f_{L(org)} + 0.05 \cdot f_{LDP(org)}\right)} \cdot \frac{\left(f_{L(food)} + 0.05 \cdot f_{LDP(food)}\right)}{C_{food(WW)}} = \frac{C_{org(Lip.Eq)}}{C_{food(Lip.Eq)}} \qquad (5)$$

Where, the $C_{org(Lip.Eq)}$ is the concentration lipid equivalents in the organism and $C_{food(Lip.Eq}$ is the concentration lipid equivalents in the food.

Another alternative metric that is strongly related to the chemical potential is chemical fugacity. Fugacity describes the escaping tendency of a chemical from a given phase and is provided in units of pressure (Pa; Mackay 1979). When interacting compartments are in thermodynamic equilibrium with one another, the net diffusive flux of chemical approaches zero and the chemical fugacities become equal. Comparing chemical fugacities between samples enables direct evaluation of equilibrium between the samples much like comparing lipid normalized and lipid equivalent concentrations between samples of biological origin. However, fugacity has greater utility across different types of media since chemical fugacity can be established for abiotic and biotic media whereas lipid normalized and lipid equivalent concentrations can only be legitimately estimated for some biological media. Another advantage of fugacity as a system of concentration expression is that it standardizes units related to concentration, partition capacity and transfer rates whereas concentration based models often conflict in basic units of measurement across model parameters (Mackay 1979). Chemical fugacity in biological phases is calculated according to:

$$f \cong \frac{C_{org}}{Z_{org}} \tag{6}$$

Where, C_{org} is the wet weight chemical concentration in the biological sample (mol/m^3) and Z_{org} is the fugacity capacity (mol/m^3/Pa) of a given sample type and chemical (Mackay and Paterson 1981). The fugacity capacity of a phase depends on the nature of the chemical as well as the composition of the sample. The fugacity capacity (Z) for biological tissues is approximated in a fashion similar to that described for lipid

equivalents concentration but also considers the relative partition capacity of lipid equivalents compared to air:

$$Z_{org} = \frac{\left(f_L + 0.05 \cdot f_{LDP}\right) \cdot K_{OW} \cdot \rho_{org}}{H} \qquad (7)$$

where f_L and f_{LDP} are the fractions of neutral lipids and lean dry protein in the sample (ng/g body weight), Kow is the octanol water coefficient, ρ_{org} is the density, H is Henry's Law constant. It can be demonstrated that the biomagnification factor when expressed on a lipid equivalents basis becomes equal to the biomagnification factor when expressed in fugacity notation. Exceptions to this case may occur if there are large density differences between the organism and its diet items. In most cases, both fugacity and lipid equivalents are expected to provide similar metrics to evaluate biomagnification potentials in biological samples. However, the fugacity method is preferred when comparing chemical potentials in biological samples relative to abiotic organic media such as soils and sediments (Czub and McLachlan 2004).

Bioaccumulation models

POPs bioaccumulation is largely considered to be contributed by two main processes, bioconcentration and biomagnification (Russell et al. 1999a). Bioconcentration is inherently an equilibrium partitioning process that describes the diffusive transport of chemicals across respiratory surfaces (Neely et al., 1974; Leblanc et al., 1999). Through bioconcentration, the organism equilibrates with its respired media via respiratory exchange and can approach or achieve similar chemical fugacities with its respired media (Di Toro et al. 1991, Landrum et al. 2001). A simple one compartment mass balance bioconcentration model describing the change in chemical mass in the organism per unit time can be derived as follows:

$$\frac{\Delta X_{org(Chem)}}{\Delta t} = Q_v \cdot E_w \cdot C_{w(t-1)} - \left(\left(\frac{Q_v \cdot E_w}{K_{biota,\,w}} \right) \cdot C_{org_{(t-1)}} + K_m \cdot C_{org_{(t-1)}} \right) \tag{8}$$

Where $X_{ORG(Chem)}$ is the chemical mass in the animal, Q_v is the total ventilation rate ($mL \cdot d^{-1}$), E_w is the organism/water exchange efficiency coefficient for the POP (unitless), C_w is the concentration in water ($ng \cdot mL^{-1}$), C_{org} is the concentration in the organism ($ng \cdot g^{-1}$ lipid equivalents), $K_{biota,w}$ is the biota to water partition coefficient and K_m is the metabolic biotransformation coefficient. Under steady state, equilibrium conditions and assuming no growth or metabolic biotransformation, the BCF_{LIPID} reduces to the concentration ratio between the lipid equivalent concentration in the organism relative to water:

$$BCF = \frac{Q_v \cdot E_w \cdot C_{org_{(t-1)}}}{Q_v \cdot E_w \cdot C_{w(t-1)}} = \frac{C_{org_{(t-1)}}}{C_{w(t-1)}} \tag{9}$$

Notably, there is no provision in Equation 9 to allow for a condition where the chemical fugacity of the animal exceeds that of water.

Biomagnification is a non-equilibrium process, commonly modeled under steady state conditions. Under biomagnification, additional chemical exposures occur via ingested diet items in the gastro-intestinal tract that result from reductions in feces production relative to feeding rates (i.e. mass balance failure of material flows in the GI-tract) in conjunction with reductions in partition capacity of feces relative to food due to assimilation of lipids and LDP. The reduced partitioning capacity of feces as well as reductions in total fecal production compared to ingested food collectively lowers the potential for fecal elimination of contaminants relative to dietary intake realized by food consumption (Gobas et al. 1993, 1999). Biomagnification can result in the chemical fugacity of an animal's tissues exceeding that of its ingested food when elimination of

chemical across respiratory surfaces is slow. This can translate into elevated chemical fugacities in the organism compared to the surrounding environmental media (Connolly and Pederson 1988, Morrison et al. 1996). The increase in chemical fugacity can also propagate through successive trophic levels in a food web resulting in non-equilibrium food web biomagnification.

The mass balance model modified to incorporate exposure and chemical elimination via food and feces (GI-magnification model) is outlined as follows:

$$\frac{\Delta X_{org(PCB)}}{\Delta t} = Q_v \cdot E_w \cdot C_{w(t-1)} + Q_{feed} \cdot E_f \cdot C_{food(t-1)} - \left(\frac{Q_v \cdot E_w}{K_{biota,\,w}} + \frac{(1-AE_{diet})Q_{feed} \cdot E_f}{K_{biota,eg}} + K_m \right) \cdot C_{org_{(t-1)}} \quad (10)$$

Where C_{food} is the concentration in the food (ng·g^{-1} dry weight or wet weight), Q_{feed} (g·d^{-1}), E_f (unitless), AE is the diet digestability (unitless), $K_{biota,eg}$ is the animal to feces equilibrium partition coefficient (g feces *g^{-1} animal lipid equivalents).

Assuming steady state conditions, the BMF becomes:

$$BMF = \frac{C_{org}}{C_{food}} = \frac{Q_v \cdot E_w \cdot C_{w(t-1)} + Q_{feed} \cdot E_f}{\left(\frac{Q_v \cdot E_w}{K_{biota,\,w}} + \frac{(1-AE_{diet})Q_{feed} \cdot E_f}{K_{biota,eg}} + K_m \right)}$$

(11)

Equation 11 accounts for steady state, non-equilibrium bioaccumulation but does not explicitly consider growth and weight loss. In more general bioaccumulation model formulations, growth is added to the GI-magnification model as a pseudo-elimination process affecting the concentration balance (Kelly et al. 2004). However, this assumes that the growth rate remains a constant over the animal's life. Such an assumption is unlikely to be correct and it is well known that individual growth rates are highly influenced by ecological and physiological factors (MacDonald et al. 2002, deBruyn and

Gobas 2006). In this thesis, the basic chemical mass balance model is modified to consider both growth (growth dilution) as well as weight loss (bioamplification) (MacDonald et al. 2002, Kelly et al. 2004, Drouillard, 2008).

Growth dilution (or biodilution) becomes a non-steady state process when the growth rate of an organism changes over a period of time that is shorter than the time required for the animal to re-achieve steady state with its environment (McLachlan 1996). In natural systems, organism growth rates can be highly variable and influenced by factors including temperature and latitude, changes to foraging costs, alterations in diet quantity and quality characteristics and ontogenetic factors (Blais et al. 2003, Chiuchiolo et al. 2004, Czub and McLachlan 2004). Juvenile life stages of fishes and many animals generally exhibit far higher growth potential than adults after which growth slows (Olsson et al. 2000, Magnusson et al. 2003, Paterson et al. 2006). Fish such as Chinook salmon and birds such as herring gulls also demonstrate pronounced seasonal variation in growth rates in adult life stages (Norstrom et al. 1986, Goyke and Brandt 1993, Roy 1999). Thus growth dilution as a bioaccumulation process can be punctuated throughout an organism's life history, being a very rapid process under some conditions that results in biodilution of contaminant residues below that of equilibrium partitioning (Skogland et al. 1996) or it can lead to residues below the maximum biomagnification potential of the animal but still greater than the equilibrium concentration prediction (Sijm et al. 1992, Paterson et al. 2007).

The second closely related process to growth is weight loss which can result in bioamplification. Bioamplification occurs when an organism loses body weight and chemical partitioning capacity at a faster rate than it can eliminate contaminants. This

causes an elevation of both the chemical concentration and chemical potential in the animal's tissues independent of contaminant uptake mechanisms. The bioamplification factor (BAmF) can subsequently be defined in analogous fashion to the biomagnification factor. Here, the BAmF is the lipid equivalent concentration ratio, or the fugacity ratio, between earlier and later stages of an organism's life cycle. It should be noted that under non-steady state bioaccumulation conditions during the uptake phase of the chemical bioaccumulation curve, the BAmF will always yield a ratio greater than 1. However, in such cases both the chemical mass balance and BAmF will increase between the two different life stage sampling periods. Thus, when the BAmF exceeds a value of 1, and the change in chemical potential occurs predominately through alteration in partition capacity of the animal rather than changes in chemical mass balance, bioamplification has occurred. Bioamplification is rarely included in most POPs bioaccumulation models in use today. This is because a steady state weight loss cannot be sustained over long periods of time in an animal's life without resulting in death. Thus, bioamplification is ignored under steady state bioaccumulation frameworks and as a result has not been examined in detail within the bioaccumulation literature which has focused on calibration of steady state models.

The mass balance model describing the change in chemical mass in the organism is modified to consider growth and weight loss as follows:

$$\frac{\Delta X_{org(PCB)}}{\Delta t} = \frac{C_{org(t)} \cdot X_{Lip.Eq(t)} - C_{org(t-1)} \cdot X_{Lip.Eq(t-1)}}{\Delta t} = Q_v \cdot E_w \cdot C_{w(t-1)} + Q_{feed} \cdot E_f \cdot C_{food(t-1)} - \left(\frac{Q_v \cdot E_w}{BCF} + \frac{(1 - AE_{diet}) Q_{feed} \cdot E_f}{K_{biota,eg}} + K_m \right) \cdot C_{org(t-1)}$$

(12)

From Eq. 12, the bioamplification factor is solved as:

$$BaMF = \frac{C_{org(t)}}{C_{org(t-1)}} = \frac{X_{Lip.Eq(t-1)}}{X_{Lip.Eq(t)}} + \frac{Q_v \cdot E_w \cdot C_{w(t-1)}}{C_{org(t-1)} \cdot X_{Lip.Eq(t)}} \cdot \Delta t + \frac{Q_{feed} \cdot E_f \cdot C_{food(t-1)}}{C_{org(t-1)} \cdot X_{Lip.Eq(t)}} \cdot \Delta t - \left(\frac{Q_v \cdot E_w}{BCF} + \frac{(1-AE_{diet})Q_{feed} \cdot E_{eg}}{K_{biota,eg}} + K_m \right) \cdot \frac{\Delta t}{X_{Lip.Eq(t)}}$$

(13)

Where, $X_{Lip.Eq}$ is the lipid equivalent tissue weight (g, based on neutral lipid and lean dry protein). From Eq. 13, bioamplification is shown to be additive to chemical uptake processes (bioconcentration and biomagnification) and can predict chemical potentials in an organism that exceed the maximum biomagnification potential predicted by the GI-magnification model during rapid weight loss. Furthermore, Eq. 13 predicts that the maximum BaMF will approach the amount of lipid equivalent weight loss when the total chemical elimination losses (k_{tot}) approach zero. It is therefore expected that organisms exhibiting high chemical elimination rates (ie. zooplankton and phytoplankton) will not undergo bioamplification. As well, chemicals with low K_{ow}'s (contributing to reduced BCF_{Lipid}) , and/or that have high biotransformation rates are not expected to exhibit bioamplification.

Although bioamplification is not generally recognized as a bioaccumulation process independent of biomagnification, the consequences of bioamplification can be important. Further, any process that increases the chemical fugacity in the animal will result in the redistribution of the contaminants from inert storage sites such as fat to blood and other tissues, where toxicity is more likely to be realized. For example, birds have been shown to exhibit enhanced POP toxicity following periods of dramatic weight loss (Stickel et al. 1973, DeFreitas and Norstrom 1976, Gabrielsen 1995). Bioamplification generally occurs under specific life history stages when an organism experiences major bioenergetic bottlenecks. This process has been recognized and verified in a variety of species including bird embryos during egg development (Drouillard et al. 2003),

spawning salmon (deBruyn et al. 2004), overwintering yellow perch (Paterson et al. 2007) and in amphibians during tadpole-frog metamorphosis (Leney et al. 2006).

Thesis Objectives and Hypotheses

POP bioaccumulation models have been generally formulated to predict two main processes, bioconcentration and biomagnification under a steady-state framework. Transitioning steady-state models to non-steady state models allows for the development of bioaccumulation models that further consider variable growth and weight loss rates and how these non-steady state processes approach and possibly surpass the steady state predicted bioconcentration and biomagnification factors. Between growth and weight loss, only weight loss can potentially contribute to chemical potentials in the animal that are above those predicted under combined bioconcentration and biomagnification models. As such, this research focuses on weight loss leading to bioamplification and characterizing the relative influence of bioamplification processes as contrasted against biomagnification and bioconcentration.

This dissertation will demonstrate that bioamplification is a general bioaccumulation process that contributes to enhanced chemical fugacities of POPs in a wide variety of model organisms. Empirical studies were performed to validate bioamplification in three different animal models (mayflies, yellow perch and Chinook salmon), each undergoing a recognizable bioenergetic bottleneck during their life history. Additionally, a manipulation study using larval salmon was performed to control the magnitude of the bioenergetic bottleneck experienced by larval salmon and explore the potential scope for bioamplification that can take place in this species and life stage. The null and alternative hypotheses of this thesis are:

H_0 : The loss of chemical partitioning capacity will result in significant elimination of POP residues without effect on chemical potential in the animal.

H_1 : The loss of chemical partitioning capacity does not result in the equivalent loss of POP residues resulting in bioamplification.

With regards to the null and alternate hypotheses for this dissertation, it is expected that bioamplification will occur during bioenergetic bottlenecks in an animal's life history. Based on this prediction, the hypotheses are as follows for each data chapter:

Chapter 2: The second chapter of this thesis investigates PCB bioamplification in emerged forms of aquatic insects using mayflies, *Hexagenia* spp., as a case study. *Hexagenia* in both its larvae and emerged forms are commonly used as biomonitors of POPs and provide a vector for aquatic-terrestrial contaminant transfer, as they are an important source of food to a variety of terrestrial animals including birds, bats, amphibians and other insectivores during mass emergence events (Sweeney and Vannote 1982, Nichols et al. 2004, Smits et al. 2005) . Mayfly larvae achieve steady state with their associated sediments for POP compounds such as PCBs and OCs (Drouillard et al. 1996) and exhibit biomagnification as exemplified by BSAFs > 2 for PCBs and OCs (Drouillard et al. 1996, Gewurtz et al. 2000). Adult stages of mayflies do not feed after they emerge and deplete accumulated lipid reserves due to bioenergetically expensive flight costs associated with mating swarms (Sartori et al. 1992) making this life stage an ideal model on which to test bioamplification.

Specifically, the testable hypotheses in Chapter 2 include:

1) Adult mayflies lose lipid between the sub-imago to imago life stage

(Further test: are there differences between sexes in the loss of lipid content between life stages?).

2) Elimination of POPs by imago staged mayflies relative to sub-imago stages approximates zero.
3) POPs bioamplification occurs in adult mayflies.

(Further test: are there differences beween sexes in the degree of POPs bioamplification?)

4) POPs bioamplification is independent of chemical K_{OW}.

Chapter 3: The main objective of this chapter was to evaluate embryo bioamplification in an aquatic species using yellow perch as a model fish species. Bioamplification has been hypothesized to be a general phenomenon applicable to all oviparous species during the critical stage of embryo development within the egg (Kleinow et al. 1999). Prior to this thesis, the process had only been demonstrated in birds and there was debate as to whether chemical elimination would attenuate embryo bioamplification when eggs are incubated in an aqueous medium (Kleinow et al. 1999). Specifically, the testable hypotheses in Chapter 4 included:

1) POPs bioamplification will occur within yellow perch eggs during the egg incubation period when yolk resorption is maximized.
2) POPs bioamplification will be independent of K_{OW} in the egg stage.
3) POPs elimination will occur in the early larval stages and will attenuate and/or nullify bioamplification.

(Further test: POPs elimination and attenuation of bioamplification in early larvae states will be dependent on chemical K_{OW}.)

Chapter 4: The objective of this study was to investigate bioamplification of POPs in a fall spawning fish, using Chinook salmon eggs and larvae as a case study. Fall spawning fish such as salmon provide significantly more maternal resources to eggs compared to spring spawning fish in order to allow over-wintering incubation and survival of larvae during the critical period which reflects the transition from endogenous (yolk reserves) to exogenous feeding (Wiegand 1996). It was anticipated that bioamplification in Chinook salmon, with greater yolk provisioning and longer egg incubation periods will experience higher POP bioamplification than observed for yellow perch eggs. The study was also extended in order to characterize changes in growth, partition capacity, chemical mass balance and attenuation of POPs in larvae salmon after hatching.

Specifically, the hypotheses tested in Chapter 4 included:

1) POP bioamplification occurs in Chinook salmon eggs during egg incubation.
2) POPs bioamplification will be independent of K_{OW} in the egg stage.
3) K_{OW} dependent elimination of POPs occurs in Chinook salmon larvae following hatching attenuating PCB bioamplification.
4) Bioamplification of POPs in larvae Chinook salmon is nullified prior to completion of yolk sac resorption.

Chapter 5: Chapter 5 presents a comprehensive literature review of bioamplification as a general phenomenon in natural ecosystems and under experimental conditions. The

review incorporates the work from Chapters 2 to 4 as well as literature examples of POPs bioamplification documented during bioenergetic bottlenecks associated with reproduction, early life stages, migration, metamorphosis, over-wintering/hibernation and due to weight loss related to disease.

Specifically, the hypotheses tested in Chapter 5 included:

1) POPs bioamplification is a general phenomenon and occurs in several animal species and life stages where bioenergetic bottlenecks are common to the life history of the animal in question.

2) The magnitude of POPs bioamplification during selected time points in an animal's life history can approach or exceed the magnitude of biomagnification experienced by organisms during adult life stages.

Chapter 6: The main objective of this chapter was to perform a manipulation study and quantify the scope for bioamplification in Chinook salmon larvae under experimental conditions of no food provisioning versus a high food provisioning environment. Specifically, this study sought to determine when larvae salmon begin using external food resources post hatching and whether growth dilution in a high food resource environment attenuates or nullifies POPs bioamplification. Specifically, the testable hypotheses tested in Chapter 6 included:

1) Post critical period fed larvae have a slower rate of weight loss (or show positive growth) compared to non-fed larvae.

2) Chemical bioamplification is higher for non-fed larvae compared to post-critical period fed larvae.

3) Although low food resource environments are likely to result in POP bioamplification in larvae Chinook salmon, no bioamplification during this life stage will occur under a high food resource environment.

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Chapter 2: Aquatic to terrestrial transfer of sediment associated persistent organic pollutants is enhanced by bioamplification processes.

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Introduction

Aquatic insect larvae readily bioaccumulate sediment associated contaminants, such as metals and persistent organic pollutants (Di Toro et al. 1991, Kiffney and Clement 1993). As an important component of their respective food webs (Hobson et al. 2002), aquatic insects act as vectors for contaminant transfer of in-place pollutants to upper food web components through benthic to pelagic coupling (Vander Zanden and Rasmussen 1996). This process can be extended to terrestrial wildlife when considering aquatic-terrestrial food web coupling, which commonly occurs for emergent aquatic insects during mass mating swarms (Nichols et al. 2004, Smits et al. 2005).

The bioaccumulation of persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and organochlorine pesticides, from sediments to larval stages of aquatic invertebrates has received considerable attention (Di Toro et al.1991, Morrison et al. 1996). Hydrophobic organic contaminants bioaccumulate in benthic invertebrates through bioconcentration and biomagnification processes. Through bioconcentration, the benthic invertebrate equilibrates with pore water by respiratory exchange and can approach or achieve chemical potentials similar to the sediment it inhabits and/or the water that it respires (Di Toro et al. 1991, Landrum et al. 2001). Under biomagnification, additional chemical exposures occur by ingested diet items in the gastro-intestinal tract (Gobas et al. 1993, Gobas et al. 1999), causing the chemical potential of an animal's tissues to exceed that of its ingested food; for benthic invertebrates, this can translate into elevated chemical potentials in the organism compared to the surrounding sediments and overlying waters (Connolly and Pedersen 1988, Morrison et al. 1996). Biota-sediment accumulation factors greater than one are

considered indicative of biomagnification and have been reported to range from one to >10 for PCBs and organochlorines in a variety of aquatic invertebrates (Drouillard et al. 1996, Gewurtz et al. 2000).

A third bioaccumulation process that can mediate chemical potential in an organism is bioamplification. Here, the term bioamplification is defined as a physiological process whereby an organism loses body weight and chemical partitioning capacity at a faster rate than it can eliminate contaminants (Daley et al. 2009). This causes an elevation of both the chemical concentration and chemical potential in the animal's tissues independent of contaminant uptake mechanisms. Bioamplification generally occurs under specific life history stages when an organism experiences major bioenergetic bottlenecks. This process has been recognized and verified in a variety of species, including bird and fish embryos during egg development (Drouillard et al. 2003, Daley et al. 2009), spawning salmon and silver eels (Duursma et al. 1991, deBruyn et al. 2004, Kelly et al. 2011), overwintering yellow perch (Paterson et al. 2007), and amphibians during tadpole-frog metamorphosis (Leney et al. 2006). Bioamplification has the potential to occur in emergent aquatic insect adults that rely on accumulated energy reserves obtained during the larval stages to satisfy reproductive costs, such as flight activity, following emergence. Adult mayfly species within the order Ephemeroptera do not feed following emergence from the water; their gut fills with air, aiding in flight (Sartori et al. 1992). In addition, emergent insects revert to air breathing, which is a much less efficient POP elimination pathway compared to respiratory losses to water (Kelly et al. 2007), so the elimination of sediment-accumulated POPs during the emerged life stages is likely to be negligible. Bioamplification in the emerged life stage is therefore

expected to occur as an additive factor above bioaccumulation processes contributed by bioconcentration and biomagnification experienced during the aquatic larval stages.

Bioamplification of POPs by emerged aquatic insects can also have significant consequences, from the perspective of trophic transfer of sediment-associated contaminants, to terrestrial predators that consume them. Bioamplification raises the chemical potential of food items above that of the earlier life stage, resulting in a higher potential for gastrointestinal magnification to be experienced by the predator while consuming emerged aquatic invertebrates over sustained periods of time (Gobas et al. 1993, Gobas et al. 1999). Even when the predator is in non-steady state bioaccumulation with an ephemeral food source, bioamplification occurs in conjunction with decreases in the energy density of the ingested food items. Thus, a predator consuming organisms that have undergone bioamplification will need to consume a larger number of insects and higher biomass to satisfy daily metabolic requirements (Drouillard and Norstrom 2003). This will result in higher total daily influx of contaminants and greater overall exposures to contaminated sediments than risk-based models of aquatic-terrestrial transfer typically assume.

The objective of the present study was to evaluate bioamplification in emerged forms of aquatic insects using mayflies (*Hexagenia spp.)* as a case study. *Hexagenia* in both its larval and emerged forms are commonly used as biomonitors of sediment contamination (Corkum et al. 1997, Gewurtz et al. 2000) and as a routine laboratory bioassay organism to assess contaminant bioavailability from field-collected sediments (Van Geest et al. 2010). Burrowing mayflies are considered ideal biomonitors of sediment contamination for several reasons: they inhabit organic-enriched sediments of

shallow lakes and large rivers that are likely to contain high POP concentrations (de Haas et al. 2002); they are relatively large in body size and achieve high biomass (especially on emergence), facilitating ease of collection (Corkum et al. 1997); and they are an important source of food to a variety of terrestrial animals, including birds, bats, amphibians, and other insectivores (Sweeney and Vannote 1982, Nichols et al. 2004, Smits et al. 2005). Mayfly larvae achieve steady state with their associated sediments for POP compounds, such as PCBs, within approximately 30 d (Drouillard et al. 1996) and exhibit biomagnification, as exemplified by biota-sediment accumulation factors ranging from 1.4 to 9.4 for PCB and organochlorines (Drouillard et al. 1996, Gewurtz et al. 2000). Finally, adult stages of mayflies do not feed after they emerge and deplete accumulated lipids due to bioenergetically expensive flight costs associated with mating swarms (Sartori et al. 1992). To our knowledge, bioamplification as a bioaccumulation process for POPs compounds has not been described for invertebrates, nor has the potential impact of this process on the trophic transfer of sediment associated contaminants to terrestrial insectivores been evaluated. In the present study, pre-emergent and two emerged life stages (subimago and reproductively active imago life stages) of mayflies were collected from three study locations in the Huron-Erie corridor to verify and quantify bioamplification across life stages. The present study also examined sex-based differences in bioamplification of emerged mayflies and tested whether or not bioamplification could be specifically attributed to flight costs as evaluated using a caging study.

Materials and Methods

Life history of Hexagenia spp.

Great Lakes populations of burrowing mayflies are largely of two species, *Hexagenia limbata* and *Hexagenia rigidia* (Corkum et al. 1997). Both species have similar morphology and life history characteristics and co-occur during nymphal and emergence periods. In the present study, no efforts were made to distinguish between the two species. Depending on mean seasonal temperatures, the aquatic life stage of *Hexagenia spp.* lasts from one to three years (Edmunds et al. 1976, Giberson and Rosenberg 1994), but lasts two years in Lake Erie (Corkum 2010). Approximately two to three weeks prior to emergence, the wing pad of the mayfly nymph thickens and turns black, representing the final instar before emergence. Past research has shown that male mayfly larvae accumulate more lipids than females (Cavaletto et al. 2003), which can potentially influence bioconcentration/ biomagnification processes and sex-differences in the wet chemical concentration of animals at emergence. On emergence, mayflies exist as the subimago molt stage and are not yet reproductively active. The subimagos fly or are carried by wind to shore (seldom more than 5 km; (Kovats et al. 1996), where they rest for a day before molting into a sexually mature adult, known as an imago. The combined emerged stages seldom last for more than 3 d, after which the animal dies (Sartori et al. 1992, Corkum et al. 1997). Mass emergence activity of mayflies occurs over extended periods of two to four weeks through June and early July at the study sites (Corkum et al. 2006). Subimagos and imagos are active and collected at different times. Male imagos establish mating swarms approximately 1 h before sunset. Individual females fly into the swarm, and males and females drop to the ground to mate. These swarms continue after

dusk for several hours. Imagos, especially females, are attracted to lights approximately 30 to 60 min after sunset, while subimagos arrive later (90–120 min after sunset).

Sampling strategy

Emerged mayflies (subimagos and imagos, n=5 per life stage, sex and location) were collected from three locations in Ontario, Canada: Middle Sister Island (MSI; 41º85'N 83º00'W) in western Lake Erie; Colchester Harbour (CH; 41º59'N 82º56'W), a marina on the north shore of the western basin of Lake Erie, located approximately 15 km away from Middle Sister Island; and Lakeview Marina (LVM; 42º20'W 82º55'W) on the Detroit River, where Lake St. Clair enters the river (Fig. 1). Subimagos and imagos were collected using light traps or near street lamps in late June of 2006 and 2007 during their period of greatest emergence activity (Corkum et al. 1997). Adults were separated and placed into solvent-rinsed glass jars according to their life stage (subimago or imago) and by sex (males and females). Black wing pad–staged nymphs (n=5 per sex and site) were collected from two of the three sampling locations in early June of 2006 and 2007 prior to peak emergence activity. The sites included adjacent waters to Middle Sister Island and at Peche Island (42º20'N 82º56'W), located in the upper Detroit River within 1 km of Lakeview Marina (Fig. 1). Mature nymphs were identified by their black wing pads and sorted by sex. The nymphs were sampled using a petite Ponar grab sampler, washed through a 500-µm sieve, and transported to the lab for analysis in solvent rinsed jars on ice. Black wing pad nymphs were not collected from Colchester Harbour due to difficulties finding samples in the heterogeneous substrates surrounding the harbor.

To control for reproductive flight, a caging study was performed in which male and female subimagos were collected from each location and placed in cages (14.5 cm X

10.5 cm X 10 cm) to allow molting but not flight. After sampling a portion of the collected subimagos for analysis, the remaining animals were placed in cages for 48 h, consistent with the normal emergence period. At the end of the caging period, all subimagos had completed the final molt into the imago stage. The imagos were then removed from cages, sorted by sex, and stored frozen until chemical analysis (n=3 per sex and site).

Chemical analysis

Sampled organisms were analyzed to determine dry weight, neutral lipid weight (dry wt basis), lean dry protein (LDP), and congener-specific PCB concentrations. Polychlorinated biphenyls and lipids were analyzed using a micro-extraction technique described in Daley et al. (2009), with modifications described below. Mayflies were pooled and kept as close to 0.2 g (wet wt) as possible. The extraction used a 0.45-μm glass fiber syringe filter in place of a 1-μm filter between extraction columns and the vacuum manifold. The final volume of the extraction solvent (dichloromethane:hexane, 1:1 v/v; VWR International) was adjusted to 40 ml rather than 30 ml. The extracts were concentrated by rotary evaporator and diluted to 10 ml in hexane. One milliliter of sample was removed for the gravimetric determination of neutral lipids using a microbalance (Drouillard et al. 2004). The remaining extract was concentrated to 2 ml and sample cleanup was performed by florisil chromatography as described by Lazar et al., (1992). After florisil chromatography, extracts were concentrated to 1 ml by rotary evaporator. Samples were analyzed for individual PCBs by gas chromatography-electron capture detection (Lazar et al. 1992). For each batch of six samples, a reference homogenate, method blank, and an external PCB standard (Quebec Ministry of Environment Congener

Mix; AccuStandard) were analyzed. Although forty PCB congeners present in the external standard were examined for, only PCB congeners 49, 95, 101, 118, 138, 149, 153, 158, 170, 180, 194, and 201 (International Union of Pure and Applied Chemistry) were detected with sufficient frequency to be included in the data analysis. Sum PCBs therefore refers to the sum of the above 12 congeners measured in the different samples. Recoveries of individual PCB congeners in the in-house reference tissue extracted with each batch of samples were within two standard deviations of the mean laboratory control charts values for the Great Lakes Institute for Environmental Research accredited organic analytical laboratory. Mean recovery for the sum of 32 PCB congeners in the reference tissue compared to control charts was 96.7±7.2%. The mean recoveries for four individual congeners compared to control charts were as follows: PCB 52 was 108.0±7.7, PCB 101 was 100.3±7.3, PCB 153 was 96.1±6.5, and PCB 180 was 97.4±6.9.

Total nitrogen was determined for pooled mayflies from each life stage and sex as a surrogate measure of tissue protein content. Total nitrogen was determined using an ECS 4010 CHNSO Analyzer. The lean dry protein (%) was calculated by multiplying the percentage of nitrogen in the sample above by a factor of 6.25, determined by the Kjeldahl method.

Data analysis

Polychlorinated biphenyl concentrations were expressed on a lipid equivalents basis to provide an appropriate metric for changes in chemical potential of PCBs. The lipid equivalents concentration was calculated for each PCB congener according to the following equation:

$$C_{org(lipid)} = C_{org(dry)} / (X_{lipid} + 0.05*(X_{LDP})) \qquad (1)$$

Where $C_{org(lipid)}$ is the PCB concentration normalized for lipid equivalents (ng/g), $C_{org(dry)}$ is the dry weight PCB congener concentration (ng/g) in the pooled sample and X_{lipid} and X_{LDP} are the mass fractions (unitless) of neutral lipids and lean dry protein (LDP), respectively, in the sample. The constant of this equation specifies the partition capacity difference of LDP relative to neutral lipids (deBruyn and Gobas 2007).

Unless otherwise stated, all data presented in the text and figures are reported as mean ± standard error (SE). Statistical differences between mayfly life stages, sex, and site for measured variables, including dry weight, percent lipids, LDP, and lipid-equivalent sum PCB concentrations, were evaluated using analysis of variance (ANOVA). Differences among groups were determined using Tukey's multiple comparison. The normality assumption was tested using the Shapiro-Wilk's test. All statistics were performed using SPSS version 18 (IL, USA). For all statistical tests, a probability value of 0.05 or less was considered a significant difference.

Bioamplification factors (BAmF) were calculated as the ratio of mean lipid-equivalent PCB concentration in imago to subimagos collected from a given site and experimental treatment. For the two locations where black wing pad nymphs were collected, BAmFs were also evaluated between the subimago/nymph and imago/nymph life stages, respectively. Bioamplification ratios in mayfly life stages were evaluated for sum PCBs, but also compared on a congener-specific basis to examine for trends in BAmF with chemical hydrophobicity.

Results

Proximate composition changes across sexes, life stages

Females showed no significant change in dry mass between the subimago and imago life stages. Males showed evidence of significant weight loss ($p < 0.05$; ANOVA) between the subimago and imago life stage at all sites. For the emerged life stages, lipid equivalents differed significantly between sex and life stage ($p < 0.05$; two-way ANOVA). Male subimagos had lower total lipid equivalents compared to the larger female subimagos but generally higher lipid percentages (Fig. 2). Females did not show a major depletion in lipid equivalents between the subimagos and imago life stages across sites or any changes in caged female imagos relative to subimagos (Fig. 2). Males lost a significant ($p < 0.05$; ANOVA) amount of lipid equivalents between the two emergent life stages at all sites (31–61%). The exception was for caged males, which did not undergo significant weight loss or lipid equivalents loss between the subimago to imago stages.

PCB mass and concentration changes

For the locations in which black wing pad nymphs were collected, sum PCB concentrations in nymphs were generally similar to those measured in subimagos, with the exception of female subimagos collected at Lakeview Marina (Fig. 3). At this location, female subimagos had higher sum PCB concentrations compared with values measured in the other female life stages. Generally, lipid-equivalent sum PCB concentrations in male and female nymphs and male and female subimagos were similar (Fig. 3). This observation held for the two immature life stages at Colchester Harbour and Middle Sister Island, and for the black wing pad nymph stages at Lakeview Marina.

However, differences were observed between nymphs and subimagos at Lakeview Marina because of the high lipid-equivalent sum PCB concentration noted in subimago females measured at this site.

Lipid-equivalent sum PCB concentrations in male imagos were significantly higher ($p < 0.05$; ANOVA) compared to black wing pad nymphs and subimagos at each of the sites (Fig. 3). Lipid-equivalent sum PCB concentrations between female subimago and imago were not significantly different at Middle Sister Island but did show differences at the other two sites. The PCB concentrations in caged male imagos were not significantly different from field subimagos at any site. The concentrations in caged female imagos compared to subimagos were not different at Middle Sister Island but were at the other two sites. Despite the above-noted differences in lipid- equivalent sum PCB concentrations for males, no significant changes in the sum PCB mass of animals were noted between the subimago to imago life stage within a given sex and site ($p > 0.5$; ANOVA among all tests between life stages by site and sex). This indicates that PCB elimination did not occur from emerged mayflies during reproductive activity.

Linear regression analysis indicated no significant trends in bioamplification factors with chemical K_{OW} ($p > 0.05$) for the individual sex and site data sets, except for male mayflies from Lakeview Marina, which exhibited an increasing trend of BAmFs with chemical K_{OW} (Fig. 4). The across-site mean BAmF for all congeners for male imago/subimago was 1.91±0.18 and ranged from 1.58±0.05 to 2.43±0.20 between individual sites. For females, the mean BAmF for the imago/subimago was close to a value of unity at 0.97±0.09 and ranged from 0.72±0.03 to 1.22±0.13 between sites. For

imago/nymph life stages, the mean BAmFs were 2.05±0.38 for males and 1.17±0.05 for females.

Discussion

Field-collected male mayflies demonstrated an overall weight loss (apart from water) between subimagos and imagos, whereas females did not. Sartori et al. (1991) found a similar relationship between male and female mayflies (*Siphlonurus spp.).* Male imagos lost as much as half their lipid equivalents mass between the subimago and the sexually mature imago life stage. A much weaker but nonsignificant decline in lipid equivalents was observed for females. It should be noted that female imagos were not checked to identify if egg deposition had occurred, which could alter the proximate composition. Also, neither male nor female imagos were completely exhausted when they were collected, and it is anticipated that greater declines in lipids in the late imago life stages could occur. The different patterns of lipid utilization between field collected males and females are consistent with other mayfly studies (Sartori et al. 1991, Cavaletto et al. 2003). The large difference in lipid content between males and females on emergence has been hypothesized to be related to reproductive flight costs required by males during mating swarms (Sartori et al. 1991).

The caging studies allowed the testing of reproductive flight costs as a causal mechanism of energy utilization and lipid depletion observed for males. Caged male and female imagos exhibited nonsignificant dry weight and lipid equivalents loss over a period of time equivalent to the time required for field males to complete their adult life cycle. This supports the conclusion that the lipid losses experienced in field-collected

males were largely due to activity, most likely related to flight costs, rather than differences in basal metabolism between the sexes.

The major objective of the present study was to quantify bioamplification in emerged mayflies and compare the magnitude of bioamplification for POPs between sexes and across study locations. The term bioamplification was defined as the process by which an organism loses body weight and chemical partitioning capacity faster than it can eliminate contaminants. Although several studies have provided empirical observations of bioamplification of POP compounds (Duursma et al. 1991, Drouillard et al. 2003, deBruyn et al. 2004, Leney et al. 2006, Paterson et al. 2007, Daley et al. 2009, Kelly et al. 2011), few attempts have been made to formalize a descriptive term and definition for this bioaccumulation mechanism to distinguish it from bioconcentration and biomagnification (Daley et al. 2009). Macdonald et al. (2002) referred to this process as ‘‘solvent depletion,’’ to describe the physical mechanism of concentration when lipids are lost from an animal. However, solvent depletion as a descriptive term does not portray the major causative physiological (e.g., bioenergetic constraints) and physiological/ecological interactions (e.g., food resource limitations) that result in solvent depletion, nor does it provide a unique and meaningful keyword term for use in ecotoxicology. Alternatively, the word bioamplification has close associative ties with the words bioconcentration and biomagnification, which have very specific meaning in ecotoxicology, particularly when applied to hydrophobic, nonionic organic chemicals. However, it is acknowledged that the word bioamplification has been used in other contexts within the field and was first published by Potter et al. (1975) to describe the increase of mercury up a food chain. It is specifically noted that the above definition is

now more widely accepted under the term biomagnification and, to the authors' knowledge, the word bioamplification has yet to be applied to POP compounds outside the proposed definition presented above.

In the present study, congener-specific PCB bioamplification was observed in field-collected male imagos. Bioamplification of PCBs was mostly independent of chemical K_{OW} because the elimination of PCBs by emergent mayflies approximated zero. Consequently, the magnitude of bioamplification approached the magnitude of the difference in PCB-partitioning capacity of organisms between life stages. Although significant bioamplification of PCBs was demonstrated for males, the estimates of bioamplification factors established in the present study may not reflect maximum bioamplification factors achieved in spent male or female imagos, because it was impossible to verify and collect mature animals at the very end of their life span.

As expected, caged male mayflies, prevented from flight, exhibited lower to negligible bioamplification compared to field-collected mayflies. One exception was female subimagos from Lakeview Marina, which had unusually high PCB concentrations compared to other female samples, including caged imagos, collected from this site. Given that there were no exceptional differences in lipid or other proximate contents of subimago females from this sample set, the high subimago sum PCB concentration observed in females at this site was likely a result of sampling artefacts at this location due to high spatial heterogeneity of sediment contamination. Drouillard et al. (2006) reported spatial variation in PCB concentrations greater than sixfold between the U.S. and Canadian sites of the Detroit River. Due to the constriction of the river channel at the Lakeview Marina location, it is possible that field collected emergent mayflies came from

both sides of the Detroit River and thus integrated the high spatial variability of sediment contamination in this region of the Huron-Erie corridor.

Emerged mayfly life stages have been used as biomonitors to detect spatial gradients of sediment contamination (Corkum et al. 1997). However, differences in the lipid content of male emergent mayflies compared to females can result in different wet-weight PCB concentrations between the sexes. The differences in wet weight PCB concentration between female and male subimagos occurs as a result of lipid differences accumulated by mayfly nymphs. However, site-specific sex-based differences in PCB concentrations are reduced when concentrations are expressed on a lipid-normalized or lipid-equivalent basis. Last-instar nymphs showed no sex differences in lipid-equivalent PCBs at either site. Drouillard et al. (1996) demonstrated that time to steady state for mayflies exposed to sediment-associated POPs was approximately 30 d, a short period of time compared to the aquatic nymph duration of two years. Given the above, and lack of sex-based differences in lipid-equivalent PCB concentrations of black-wing pad nymphs, it is concluded that mayflies, regardless of sex, achieve steady state with the sediments at the time of emergence (Drouillard et al. 1996).

If the goal of interpreting contaminants in adult mayflies is to establish spatial gradients in sediment contamination (e.g., use as biomonitors), then it is recommended that mayflies be separated by life stage and sex to insure greater precision in spatially resolved contaminant levels across sites. Subimago males may prove to be the best life stage to monitor because their higher wet-weight concentration means a greater likelihood of detecting POP residues. Alternatively, if subimagos are not to be sorted by sex, then lipid-normalized data are recommended because there is less bias between the

sexes compared to wet-weight POP concentrations. Imago life stages will be more strongly influenced by bioamplification processes that will contribute additional variability in samples, particularly when sexes are mixed into sample pools. When using the imago life stage for contaminant biomonitoring purposes, it may be best to utilize female samples to reduce variation of sample concentrations related to individual differences in bioamplification factors.

The risk of sediment contaminant exposure to wildlife consumers of emergent mayflies will likely be underestimated if bioamplification is not considered as part of the exposure assessment. Using the highest BSAF reported by Drouillard et al. (1996) of 9.4 for mayfly nymphs and an average bioamplification factor of two in male adults, the combined BSAF * BAmF can be as high as 18.8 above that of sediments for animals that feed predominantly on male imagos. However, the time required to achieve steady state for wildlife that consume emergent insects likely exceeds the approximate two- to four-week period over which mass emergence occurs. Thus, it is difficult to quantify additional risk to wildlife consumers on the basis of potential change in steady-state biomagnification factors because emergent insects reflect only a small component to the annual integrated exposures of wildlife to seasonally changing diet items. A more realistic assessment may involve model scenarios that estimate the change in total daily intake (TDI) of PCBs that occur pre- and postemergence among wildlife species that exhibit sex- and life stage– biased feeding during such emergence events.

Here, it is demonstrated that animals that preferentially feed on male imagos would achieve higher TDIs compared to animals that feed on subimago life stages (see Supplemental Data for sample calculations). For example, a predator at Colchester

Harbour that requires 100 calories to support its daily energy needs and feeds primarily on male imagos would need to consume 69.1% more mayflies and 13.1% greater biomass compared to wildlife having the same energy demands but that feed on subimagos. In this scenario, the predators feeding on Colchester Harbour male imagos would increase their PCB TDIs during the emergence period by 27.0%. Across the three sites, the average increase in wildlife PCB TDIs for animals feeding exclusively on male imagos during the emergence period was estimated to be 36.1±18.7% compared to wildlife feeding exclusively on male subimagos. Given that imago and subimago life stages show distinct differences in the timing of their availability to predators, it is likely that the above TDI scenarios have some basis in reality. Imagos commence their mating swarms at dusk and are more likely to be consumed by avian consumers such as tree swallows, which are most active at this time of day and capture prey in flight. If male imagos do in fact spend a greater amount of time in active flight defending aerial territories in the mating swarms, then it is possible that aerial feeders, such as tree swallows, will exhibit both stageand sex-biased feeding and include more male imagos within their diets. Subimagos generally arrive from offshore sites approximately two hours after sunset and remain suspended on vegetation during the next day. These animals are more likely to be consumed by late night feeders (e.g., bats) or a wide variety of diurnal birds including gleaning house wrens, robins, starlings, and other insectivores. These animals will ultimately be exposed to lower mass transfer of PCBs compared to aerial dusk-feeders.

It should be noted that the above simulations demonstrate the potential impacts of bioamplification in emergent insects to wildlife TDIs but do not directly quantify the overall contribution of sediment-terrestrial transfer related to such diet shifts. To estimate

the latter, further research to quantify seasonal diet selectivity and diet-specific PCB concentrations would be needed to calculate the overall contribution of emergent insects relative to other diet items to the annual PCB load and bioaccumulation rate in an indicator wildlife species. While such calculations are beyond the scope of the present study, past research on tree swallows has indicated that emergent insects reflect an important contribution to the overall PCB load of these animals (Nichols et al. 2004, Smits et al. 2005). Therefore, further research documenting the role of emergent insects to sediment-terrestrial transfer and the effect of bioamplification to this transfer process is warranted.

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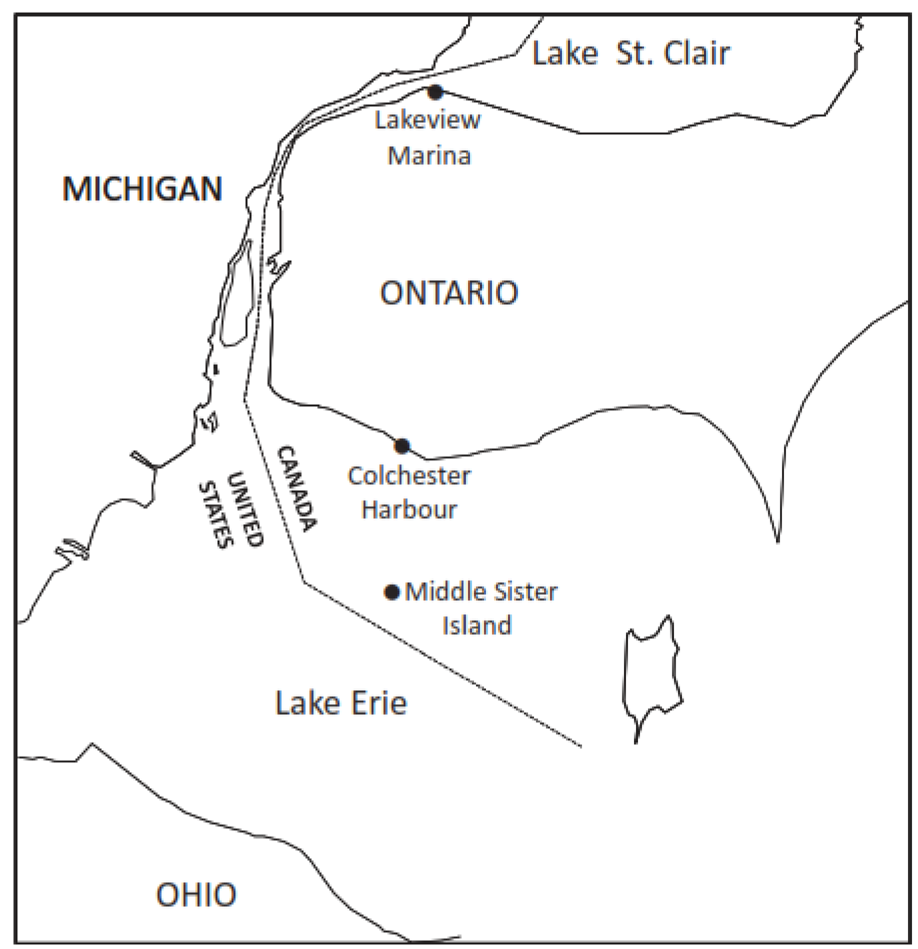

Figure 2.1. Collection locations for *Hexagenia spp*. The study sites included Lakeview Marina (**42º20'N 82º55'W**) on the Detroit River, Colchester Harbour **(41º59'N 82º56'W)** on the western basin of Lake Erie, and Middle Sister Island **(41º51'N 83º00'W)** in western Lake Erie.

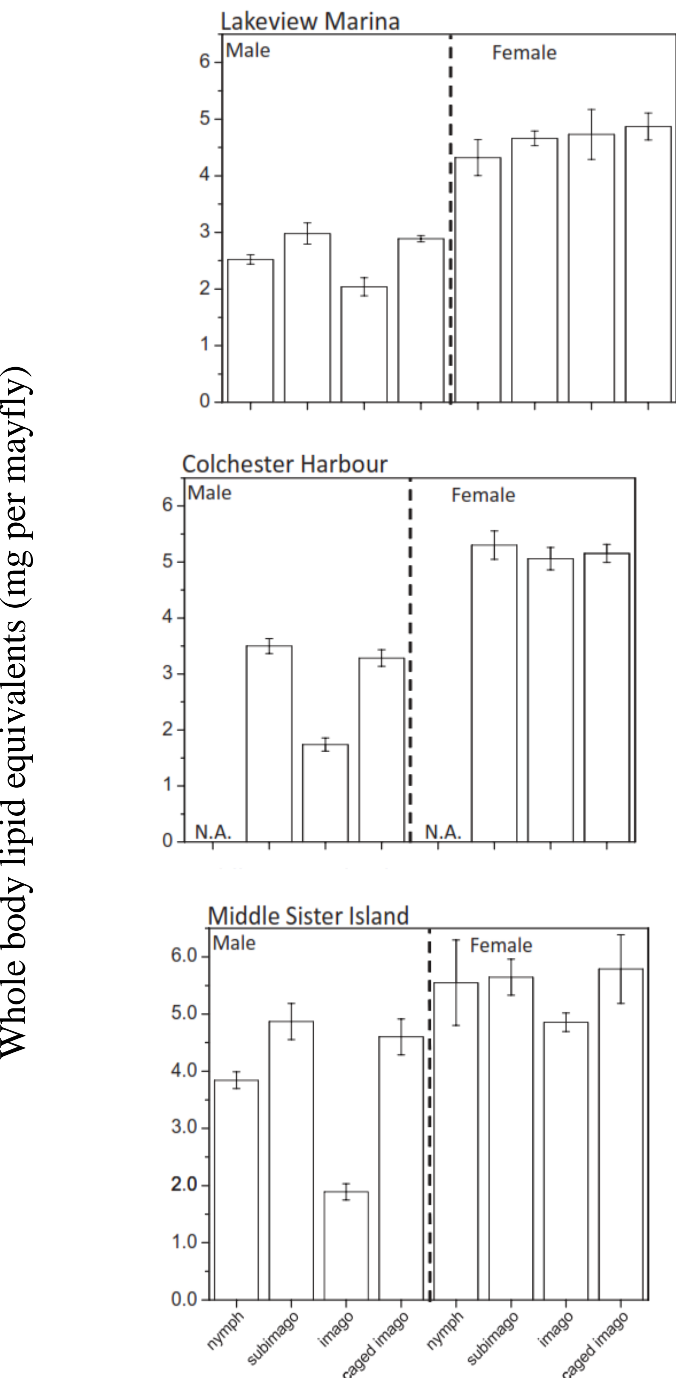

Fig. 2.2. Mean±standard error lipid-equivalent mass (mg neutral lipid + 5% lean dry protein) in *Hexagenia spp*. across different life stages and by sex from three collection locations. Nymphs from Colchester Harbour (ON, Canada) were not collected.

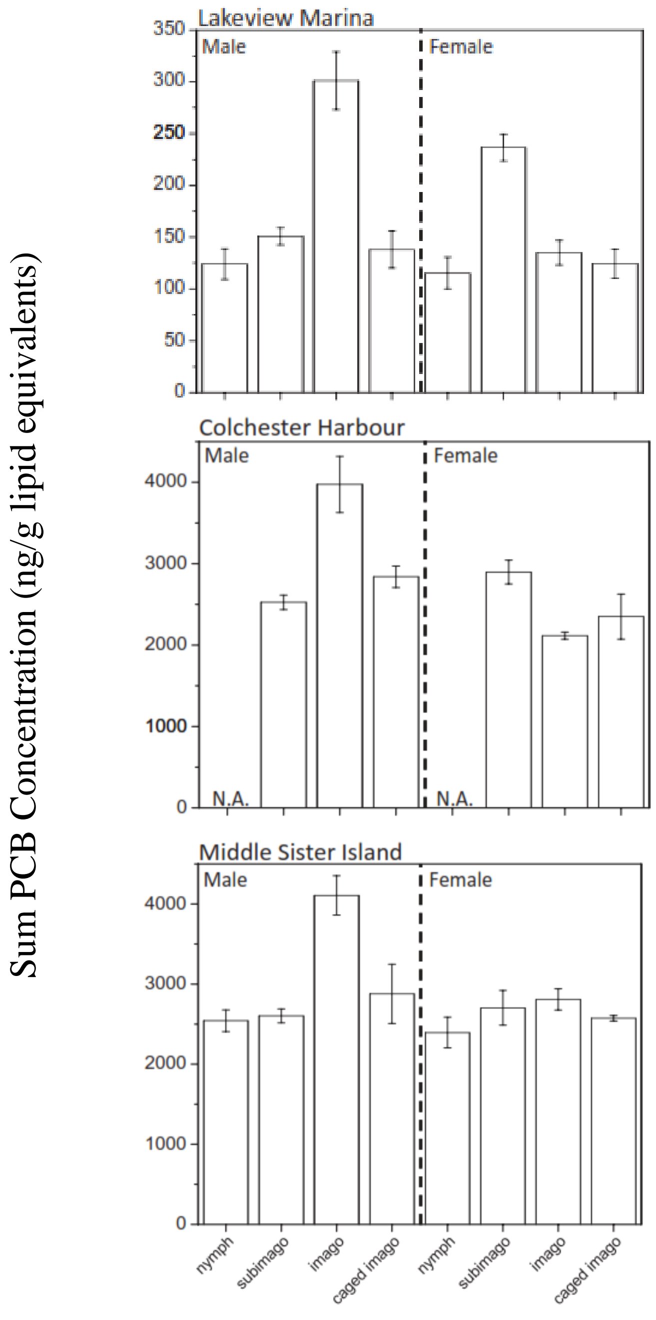

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Fig. 2.3. Mean±standard error sum polychlorinated biphenyls (ng/g lipid equivalents) in *Hexagenia spp.* across different life stages and by sex from three collection locations. Nymphs from Colchester Harbour (ON, Canada) were not collected.

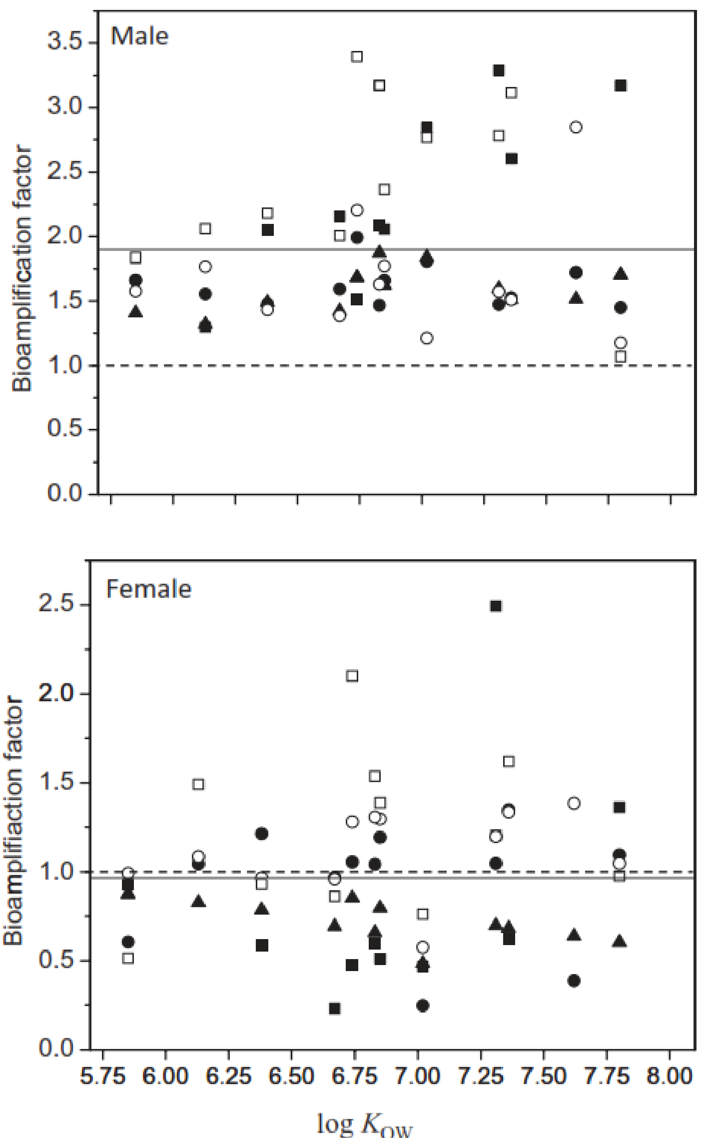

Figure 2.4. Bioamplification factors determined for individual polychlorinated biphenyl congeners for Lakeview Marina imago/subimago (■) and imago/ nymph (□), Colchester Harbour imago/subimago (▲), and Middle Sister Island imago/subimago (●) and imago/nymph (○). Solid line represents mean bioamplification factor across sites and congeners for males (1.90±0.18) and females (0.97±0.13). Dashed line represents theoretical equilibrium bioamplification factor of 1.0. All sites are located in Ontario, Canada.

Supporting Information

Table S2.1. Mean (standard error) proximate composition and PCB concentrations (ng/g lipid equivalents) in mayflies collected from the Huron-Erie Corridor, Canada.

Lakeview Marina	Male Nymph	Male Subimago	Male Imago	Male Caged	Female Nymph	Female Subimago	Female Imago	Female Caged
Dry Weight (mg)	13.9 (0.6)	13.3 (0.2)	11.2 (0.1)	13.2 (0.3)	28.0 (0.8)	28.3 (0.4)	28.5 (2.0)	27.9 (0.5)
% Lipid	15.1 (0.8)	19.00 (1.31)	14.08 (1.36)	18.81 (0.30)	12.82 (.97)	13.04 (0.57)	12.94 (0.71)	13.20 (0.15)
% LDP	60.9 (2.4)	72.0 (1.2)	78.6 (2.4)	74.0 (8.2)	65.7 (1.6)	67.7 (3.7)	72.5 (1.7)	69.7 (0.9)
Sum [PCB] lipid equiv	124.1 (14.8)	150.9 (8.1)	301.1 (28.1)	138.0 (18.0)	115.3 (15.1)	236.6 (13.0)	135.0 (11.7)	124.6 (14.2)
PCB49	11.1 (0.6)	11.2 (1.8)	20.2 (2.7)	12.0 (2.0)	12.2 (1.8)	6.8 (1.6)	6.1 (1.0)	14.0 (2.2)
PCB95	17.1 (1.4)	27.2 (0.9)	35.2 (2.9)	5.3 (0.9)	17.9 (3.4)	23.8 (3.0)	25.2 (2.5)	13.5 (3.8)
PCB101	18.4 (1.5)	19.6 (1.8)	40.1 (4.4)	21.4 (1.7)	21.8 (3.7)	34.5 (6.4)	20.2 (2.8)	16.5 (1.6)
PCB 118	7.3 (1.1)	16.3 (0.8)	24.7 (1.9)	19.9 (5.2)	6.9 (1.1)	26.1 (1.8)	12.4 (1.0)	11.6 (2.7)
PCB 138	13.8 (1.3)	21.0 (1.0)	43.9 (4.8)	20.0 (2.0)	10.6 (2.0)	27.4 (0.9)	16.4 (1.3)	16.7 (0.8)
PCB149	15.7 (4.0)	14.6 (1.4)	31.5 (3.2)	16.7 (3.8)	12.4 (1.4)	46.3 (4.4)	10.7 (0.7)	11.1 (0.8)
PCB153	20.1 (3.9)	23.2 (1.1)	47.6 (5.8)	22.3 (3.1)	16.6 (1.4)	45.0 (6.9)	23.0 (1.7)	19.7 (0.8)
PCB158	1.2 (0.1)	1.2 (0.1)	3.3 (0.7)	1.1 (0.1)	1.4 (0.2)	2.3 (0.2)	1.1 (0.1)	1.3 (0.3)
PCB170	3.9 (0.7)	4.1 (1.1)	13.6 (1.5)	1.3 (0.6)	4.0 (0.3)	1.9 (0.4)	4.8 (0.5)	2.4 (0.9)
PCB 180	8.9 (1.2)	10.6 (1.2)	27.7 (4.4)	12.8 (1.8)	6.6 (0.5)	17.2 (0.9)	10.7 (0.9)	12.7 (0.9)
PCB 194	5.3(2.5)	2.1 (0.2)	6.8 (0.9)	3.9 (0.8)	3.1 (0.5)	2.2 (0.5)	3.0 (0.3)	3.8 (0.8)
PCB201	1.3(0.3)	1.9 (0.4)	6.6 (1.1)	1.9 (0.2)	1.9 (0.2)	5.6 (0.9)	3.9 (0.8)	1.4 (0.2)

Colchester Harbour	Male Subimago	Male Imago	Male Caged	Female Subimago	Female Imago	Female Caged
Dry Weight (mg)	16.0 (0.2)	10.7 (0.2)	15.7 (0.2)	35.9 (1.0)	37.1 (0.9)	35.0 (1.5)
% Lipid	18.4 (0.5)	11.7 (0.8)	17.5 (0.8)	10.9 (0.3)	9.5 (0.3)	11.0 (0.3)
% LDP	74.8 (2.3)	81.9 (4.3)	74.2 (2.6)	78.0 (1.1)	84.2 (3.5)	75.6 (4.0)
Sum [PCB] lipid equiv	2525.1 (89.0)	3974.0 (344.1)	2839.2 (133.0)	2896.3 (147.2)	2114.9 (43.8)	2349.7 (276.6)
PCB49	60.8 (11.7)	85.7 (5.1)	54.1 (1.6)	58.6 (3.6)	51.3 (2.3)	54.6 (2.2)
PCB95	234.9 (25.4)	310.2 (25.9)	246.6 (10.9)	227.4 (11.9)	188.4 (10.3)	205.7 (10.6)
PCB101	300.9 (7.8)	448.7 (33.5)	311.3 (16.0)	331.7 (16.9)	260.5 (7.2)	270.6 (27.3)
PCB 118	113.9 (3.7)	191.8 (17.6)	125.3 (2.5)	128.2 (9.6)	109.4 (3.6)	108.3 (10.3)
PCB 138	354.8 (8.8)	664.4 (59.6)	392.2 (17.1)	462.5 (22.1)	304.6 (6.2)	315.7 (39.8)
PCB149	284.2 (9.0)	404.8 (32.8)	312.5 (10.7)	307.3 (14.7)	213.3 (5.2)	259.1 (27.3)
PCB153	480.1 (11.4)	779.5 (77.0)	558.1 (13.7)	538.4 (25.0)	428.4 (9.8)	474.1 (63.4)
PCB158	24.3(0.9)	44.8 (4.7)	30.7 (4.4)	37.0 (1.4)	18.0 (0.7)	20.6 (2.6)
PCB170	153.8 (4.4)	245.7 (21.7)	180.0 (9.3)	180.1 (10.4)	125.8 (1.6)	143.8 (21.1)
PCB 180	392.3 (11.4)	592.8 (53.3)	477.9 (39.9)	453.9 (28.6)	310.0 (5.5)	271.8 (56.8)
PCB 194	84.3 (2.5)	143.4 (13.6)	102.6 (5.1)	123.1 (6.4)	74.2 (1.2)	90.4 (14.7)
PCB201	40.8 (1.6)	61.8 (5.4)	48.2 (4.9)	47.9 (3.1)	30.6 (0.7)	34.7 (5.0)

Middle Sister Island	Male Nymph	Male Subimago	Male Imago	Male Caged	Female Nymph	Female Subimago	Female Imago	Female Caged
Dry Weight (mg)	23.5 (2.2)	22.4 (0.6)	15.0 (1.5)	22.1 (0.4)	35.7 (2.8)	25.6 (3.0)	34.4 (1.0)	36.0 (0.3)
% Lipid	14.6 (1.6)	18.4 (1.5)	8.4 (1.1)	17.1 (1.1)	10.6 (1.4)	11.9 (0.4)	9.7 (0.3)	12.40 (1.6)
% LDP	63.2 (2.6)	68.8 (2.1)	78.8 (2.2)	70.0 (2.0)	65.7 (.6)	69.9 (1.4)	78.8 (1.4)	73.0 (3.1)
Sum [PCB] lipid equiv	2543.0 (136.1)	2604.0 (88.0)	4106.2 (246.4)	2878.0 (369.1)	2395.1 (191.5)	2703.5 (217.5)	2808.5 (132.8)	2574.1 (35.2)
PCB49	71.6 (8.9)	70.6 (3.6)	117.5 (14.4)	56.2 (8.1)	63.6 (9.6)	104.2 (14.2)	63.1 (5.8)	50.4 (3.4)
PCB95	181.7 (12.9)	210.1 (23.5)	326.7 (23.5)	250.7 (39.7)	195.3 (14.0)	202.9 (29.9)	211.8 (24.3)	206.9 (5.3)
PCB101	257.2 (5.8)	249.6 (22.4)	368.5 (37.7)	345.7 (54.0)	275.7 (11.3)	219.3 (11.3)	266.3 (31.8)	269.1 (21.6)
PCB 118	114.7 (12.5)	127.0 (6.3)	252.9 (9.5)	128.3 (20.5)	109.9 (12.9)	133.5 (9.2)	140.8 (6.7)	112.7 (2.0)
PCB 138	372.6 (19.4)	414.4 (16.0)	607.4 (34.5)	393.8 (46.0)	337.4 (33.7)	423.1 (39.0)	440.8 (18.))	358.5 (4.8)
PCB149	288.1 (18.1)	250.5 (7.4)	399.2 (26.7)	308.0 (37.6)	264.4 (37.6)	261.5 (16.2)	253.5 (9.8)	266.8 (5.5)
PCB153	475.9 (28.0)	507.8 (28.7)	843.3 (48.4)	545.6 (63.4)	454.7 (35.3)	493.4 (42.1)	589.2 (22.5)	520.9 (6.1)
PCB158	59.3 (8.0)	39.8 (6.9)	71.8 (15.8)	32.6 (2.2)	47.3 (7.1)	109.9 (5.5)	27.2 (1.1)	30.5 (1.2)
PCB170	158.6 (9.1)	169.1 (4.2)	249.2 (19.6)	179.6 (21.9)	143.4 (11.7)	164.1 (18.0)	171.9 (7.5)	168.4 (7.1)
PCB 180	397.5 (23.9)	393.6 (11.6)	600.4 (31.5)	489.0 (58.3)	359.3 (34.0)	355.7 (49.6)	480.0 (23.5)	441.6 (7.0)
PCB 194	121.8 (5.8)	98.8 (2.6)	143.1 (9.2)	102.8 (13.1)	106.6 (10.7)	102.0 (9.3)	111.6 (5.)	105.6 (2.2)
PCB201	44.2 (2.2)	73.0 (10.9)	125.8 (27.4)	46.5 (6.0)	37.6 (3.4)	133.9 (4.4)	52.0 (5.5)	42.7 (1.7)

Table S2.2 Example TDI calculation demonstrating the change in daily PCB exposures of a hypothetical animal which feeds exclusively on Colchester Harbour male imagos compared to one feeding on Colchester Harbour subimago stages during the mass emergence period. The hypothetical animal is assumed to require 100 calories per day to satisfy its daily energetic demands.

Parameter	Male subimago	Male imago
Dry weight of individual mayfly (mg)	16.0	10.7
Lean dry protein (LDP) content (%)	74.8	81.9
Lipid equivalents content (%)	18.4	11.7
Sum PCB concentration (ng/g dry weight)	559.1	627.7
Assimilated LDP by Predator (mg/mayfly)[*]	7.18	5.26
Assimilated Lipid by Predator (mg/mayfly)[**]	2.71	1.15
Assimilated calories per mayfly consumed (cal)[***]	0.05	0.03
# Mayflies consumed to achieve 100 calories (#)	1883.3	3185.0
Mass of mayflies consumed to achieve 100 calories (g)	30.1	34.1
Mass of PCBs consumed by predator per day (ug/d)	16.8	21.4

[*] Using Arnot and Gobas (2004) recommendation of 60% assimilation for lean dry protein
[**]Using Arnot and Gobas (2004) recommendation for 92% assimilation for lipid

Chapter 3: Evidence for bioamplification of nine polychlorinated biphenyl (PCB) congeners in yellow perch (*Perca flavascens*) eggs during incubation

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Introduction

Persistent organic pollutants (POPs), as exemplified by organochlorine pesticides such as p,p-DDT and polychlorinated biphenyls (PCBs) exhibit high bioaccumulation potentials within organisms and through food webs. Both bioconcentration, which describes chemical exposures through respiratory surfaces (Neely et al. 1974, Leblanc 1995) and biomagnification, reflective of chemical exposure through food (Connolly and Pedersen, 1988, Gobas et al. 1993), have been described as the main exposure mechanisms for POP bioaccumulation. Biomagnification is of particular interest because it can lead to the condition where the chemical fugacity (i.e. the ‘‘escaping tendency” of a chemical to move from one phase to another), in the organism achieves a higher level than the animal’s food (Mackay 1979, Connolly and Pedersen 1988). This phenomena, described by the gastrointestinal magnification mechanism (Gobas et al. 1993, 1999) can subsequently propagate through the food web resulting in food web biomagnification, whereby chemical fugacities increase through successive trophic steps in the ecosystem (Connolly and Pedersen 1988, Russell et al. 1999).

Apart from chemical uptake and elimination kinetics to and from the organism, other factors including ecological and physiological attributes of the animal can greatly affect tissue concentrations and the chemical fugacity of POPs (MacDonald et al. 2002). The chemical fugacity may be reduced by growth (growth dilution) or magnified as a result of weight loss, known as bioamplification (MacDonald et al. 2002, Kelly et al. 2004, Drouillard 2008). Bioamplification can be defined as the special case where the partition capacity (or fugacity capacity) of an animal’s tissues decrease faster than chemical can be eliminated. This condition typically occurs when the animal loses body

mass, lipids and/or lean dry protein at rates that exceed chemical elimination (MacDonald et al. 2002, Drouillard 2008). Under this condition, chemical fugacity increases in the animal and will result in the redistribution of the contaminant from inert storage sites such as fat to blood and other tissues, where toxicity is more likely to be realized. For example, birds have been shown to exhibit enhanced POP toxicity following periods of dramatic weight loss (deFreitas and Norstrom 1974, Stickel et al. 1984, Gabrielsen 1995).

Bioamplification is expected to occur during bioenergetic bottlenecks and can reflect a marker of ecological stress due to nutritional constraints and/or physiological changes related to metamorphosis, diet shifts or reproduction (Drouillard 2008). PCB bioamplification was verified in several organisms including overwintering yellow perch (Paterson et al. 2007), migrating salmon and silver eels (Duursma et al. 1991, deBruyn et al., 2004) metaphorizing frogs (Leney et al. 2006), and herring gull embryos (Drouillard et al. 2003).

Bioamplification has been hypothesized to be a general phenomenon applicable to all oviparous species during the critical stage of embryo development within the egg (Kleinow et al. 1999). To date, the process has only been demonstrated in birds and there is debate as to whether chemical elimination would attenuate embryo bioamplification when eggs are incubated in an aqueous medium (Kleinow et al. 1999). This information is significant in that the embryo larval life stages of fish are sensitive to toxicological impacts (Petersen and Kristensen 1998) and bioamplification processes have the potential to raise the chemical fugacity in the egg above that contributed to fresh eggs via maternal deposition (Miller 1994, Fisk and Johnston 1998). The objective of this study was to

evaluate embryo bioamplification in an aquatic species using yellow perch as a model fish species.

Materials and Methods

Experimental

Yellow perch egg masses (12–24 h following spawning) were obtained from a local aquaculture pond (Leadley Environmental Inc., Essex Ontario). The yellow perch in the aquaculture pond were reared in the same pond their entire life (6–8 years) and contained small traces of PCBs in their tissues as a result of being fed a commercial pellet food ration. The fish were allowed to spawn naturally in the ponds, thus maternal deposition of contaminants to eggs occurred by natural processes. The egg masses were collected by hand, placed over horizontal racks and incubated in a near-by flow through system receiving the same pond water at in situ temperatures. Replicate egg masses were placed in solvent rinsed glass scintillation vials at days 0 (newly fertilized eggs), 4, 8, 10 and 12 (n=6 yellow perch egg pools except on day 12 where n=5). Eggs were observed to hatch between days 12–13 and additional samples of free swimming larvae were collected on day 14. Fresh egg samples were brought back to the laboratory on the day of collection and examined under a dissecting microscope to establish viability.

In addition to the egg samples described above, five replicate freshwater mussels (*Elliptio complanata*), which were placed in the main aquaculture pond 8 mo prior to their sampling, were collected from the same ponds the egg masses were laid in to analyze for the background water contamination (n=5 fresh water mussels). In addition, dorsal mussel samples from three adult female perch captured from the same aquaculture

pond were analyzed (n=3 adult yellow perch dorsal muscle samples). All samples were frozen at -20ºC until analysis.

Laboratory processing

Samples were analyzed to determine % moisture, % lipid, lean dry protein (LDW) and congener specific PCB concentrations. PCB and lipids were measured using a micro-extraction technique. This technique was developed in order to use a small sample mass (0.2– 0.5 g) for each replicate and reflects a miniaturized version of the cold column extraction described in Lazar et al. (1992). The extraction set up consisted of 20 mL glass syringes with luer lock connectors connected in line to a 1μm glass fibre syringe filter. The syringe (plunger removed) and filter were fitted to a 12 port solid phase extraction manifold (PTFE valves and glass manifold; Phenomenex, Torrance, CA, USA). Custom glass reservoirs were placed inside the vacuum manifold and secured inside a customized rack to receive the eluents from the syringes. Eggs and larvae were too small for individual egg measurements and required pooling to facilitate chemical and proximate analysis. All sample pools were kept as close to 0.5 g wet weight as possible (Table 1) to ensure equivalent numbers of eggs were included in each sample. Pooled egg or larvae samples were weighed and ground in a mortar and pestle with 15 g of activated sodium sulphate (ACS-grade, 10– 60 mesh; activated by muffle furnace at 450ºC overnight; Fisher Scientific; Ottawa, ON, Canada), and wet packed into a 20 mL glass syringe containing 15 mL of dichloromethane (DCM):hexane (1:1; pesticide grade; VWR, St. Catherines, ON, Canada). The syringe was spiked with 50 ng mL^{-1} of PCB #30 (neat 2,4,6-trichlorobiphenyl, AccuStandard, New Haven, CT, USA) for use as a recovery standard. Another 15 mL of DCM: hexane was used to rinse the mortar and pestle and

added to the syringe. The manifold valves were closed and the sample was allowed to extract in solvent for 1 h, after which the valve was opened and the column was eluted by gravity. Occasionally a small amount of vacuum was required to initiate column flow and the valves were adjusted to achieve a flow rate of approximately three drips per second until elution was completed. Additional vacuum was used to draw any remaining solvent from the syringes and bedding into the reservoirs.

The extracts were concentrated by rotary-evaporator and then made to a volume of 10 mL in volumetric flasks. One milliliter of sample was removed for the gravimetric determination of neutral lipids (Drouillard et al. 2004). The remaining extract was concentrated to approximately 2 mL and sample clean up was performed by florisil chromatography as described by Lazar et al. (1992). Since only PCBs were examined in the present study, only the first fraction of florisil (50 mL hexane) was collected. After florisil chromatography, extracts were concentrated to 1 mL by rotaryevaporator, further concentrated to 0.4 mL under nitrogen gas stream, and added to gas chromatography vials. Samples were analyzed for individual PCBs by gas chromatography electron capture detection (GC-ECD), described elsewhere (Lazar et al. 1992). For each batch of six samples, a reference homogenate, method blank, external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard, New Haven, CT, USA) and PCB 30 recovery standard were analyzed. Although 40 PCB congeners present in the external standard were examined for, only PCB congeners (IUPAC #'s): 52, 70, 74, 99, 110, 149, 118, 153, 187 and 180 were detected with sufficient frequency in samples to include in subsequent data analysis. Recoveries of PCB #30 in samples averaged (±SE) 85 ± 2.0%. Recoveries of individual PCB congeners in the in-house reference tissue extracted with

each batch of samples were within two standard deviations of the mean laboratory database value derived from laboratory control charts from the Great Lakes Institute for Environmental Research accredited organic analytical laboratory (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified) established by standard cold column extraction techniques.

Data analysis

Polychlorinated biphenyl fugacity was calculated using Eqs. (1) and (2). Fugacities (Pa) were calculated for each congener as follows:

$$f = C/Z \quad (1)$$

Where f is the PCB fugacity (Pa), C is the PCB congener concentration (mol m^{-3}) in the pooled sample and Z is the fugacity capacity (mol m^{-3} Pa) of the sample. Concentrations (mol g^{-1}) of PCBs measured in each sample were converted to a volume basis assuming a specific gravity of 1 g mL^{-1} for all samples. The fugacity capacity (Z) of the sample considers the partitioning capacity contributed by water, lipids and lean dry protein in the sample and is estimated by:

$$Z= (X_W + (X_L * K_{OW})+(X_{LDW} + 0.05\ K_{OW)}))/H \quad (2)$$

Where, X_W, X_L, X_{LDW} are the mass fractions of moisture, neutral lipids and lean dry protein, respectively in the sample. The lean dry protein was determined by subtracting the total lipid weight from the total dry weight of each sample and dividing by the wet sample weight. The n-octanol/water partition coefficients (K_{OW}) for individual PCB congeners were obtained from Hawker and Connell (1988). The constant, 0.05 indicates that lean dry protein has a partitioning capacity that is equivalent to 5% of lipids (deBruyn and Gobas 2007). Henry's Law Constants (H; $Pa*m^3*mol^{-1}$) were obtained

from Brunner et al. (1990) and converted to appropriate units. Values for H were not provided for PCB 110, 118 and 187 in the above citation and it was assumed that the H for PCB 110 = PCB 99, the H for PCB 118 = PCB 153 and the H for PCB 187 = 180. The latter assumptions influence the absolute fugacity estimates for these three congeners, but does not influence the fugacity ratio calculations.

Percent dry weight, percent lipids, percent lean dry weight and PCB fugacities in the yellow perch eggs and post-hatch larvae, at different time intervals were compared using an analysis of variance (ANOVA) and Tukey's post hoc test. A p value of 0.05 or less was considered a significant difference. Fugacity ratios in matured eggs and larvae were calculated by expressing the ratio of PCB fugacity in the incubated egg or larvae to the mean fresh egg value. Linear regression analysis was used to test for relationships between the magnitude of the fugacity ratio observed in mature eggs or larvae and physical properties (chemical log K_{OW}) of individual PCBs. Unless otherwise stated, all data presented in the text and figures are reported as mean ± standard error (SE).

Results

The mean % dry weight for yellow perch eggs, both pre-hatch and post-hatch, are summarized in Fig. 1A (mean ± SE) and in Table 1. The total dry weight content showed a distinct decreasing trend for eggs with incubation time but subsequently increased again in larvae after hatching. There were significant differences between % dry weight of newly hatched larvae and both 10-d and 12-d old incubated eggs (Tukeys; $p < 0.05$). The mean percent lipid and percent lean dry protein weight expressed on a wet sample weight basis for yellow perch are summarized in Fig. 1B. There were significant differences (ANOVA; $p < 0.05$) between percent lipid content of eggs across different incubation

times, but no significant differences (ANOVA; $p > 0.05$) between the percent lipid content of fresh eggs and post-hatched larvae. There were also significant differences between percent lean protein weight of eggs across different incubation times but no significant differences (ANOVA; $p < 0.05$) between the percent lean protein weight of fresh eggs and post-hatched larvae. When analyzing the mean percent lipid expressed on a dry sample weight basis, the trend was no longer significant although a slight decreasing trend was apparent.

Table 2 summarizes proximate composition and PCB fugacities in freshwater mussels ($n = 5$), adult perch dorsal muscle samples ($n = 3$ fish) and fresh yellow perch eggs harvested from the aquaculture pond. PCB congeners 52, 74 and 118 were not detected in mussel samples and an interference in the chromatogram prevented quantitation of PCB 70/76 in mussel and adult yellow perch dorsal muscle samples. Similarly, for the adult yellow perch no detectable peaks were apparent for PCBs 74 and 118. For all comparable compounds analyzed, yellow perch exhibited higher PCB fugacities compared to freshwater mussels inhabiting the same pond. The mean yellow perch/mussel fugacity ratio for adult perch samples was 5.4 and for fresh eggs, the egg/mussel fugacity ratio was 8.2.

Table 1 summarizes changes in PCB fugacities in egg pools during the various incubation time points and in newly hatched embryos. Fig. 2 also presents time changes in the PCB fugacity for selected congeners (PCB #52, 149 and 180) at each life stage. Each PCB congener exhibited a similar increasing trend in fugacity (average of 2.7-fold across the congeners) between fresh eggs and 10-d, and 12-d incubated eggs (Tukey's; $p < 0.05$). After hatching, 2-d post-hatch larvae showed significant ($p < 0.05$; t-test)

reductions in PCB fugacities relative to day 12 incubated eggs for congener 52 ($p < 0.05$; t-test) but not for congeners 149 and 180 ($p > 0.05$; t-test). Individual PCB congeners exhibited differences in the degree to which post-hatch larvae lost PCB residues relative to day 12 incubated eggs (Table 1; Fig. 2). The fugacity for congener 52 decreased by approximately 2.2-fold to about the same concentration as measured in fresh eggs (t-test; $p > 0.05$). For more hydrophobic congeners such as PCB 149 and 180, the fugacity for 2-d old post-hatch larvae, were still significantly higher ($p < 0.05$; t-test) than fugacities determined in fresh eggs.

The fugacity ratios between fresh eggs and 12-d old incubated eggs are compared in Fig. 3A for PCB congeners 52, 70, 74, 99, 110, 149, 118, 153, 187, and 180 as a function of chemical K_{OW}. There was no significant trend in incubated/fresh egg fugacity ratio across K_{OW} ($p > 0.05$; ANOVA). The exceptions appeared to be PCB congeners 70/76 and 74 which exhibited higher incubated egg/ fresh egg fugacity ratios then the rest of the congeners. Across the congeners, the average incubated/fresh egg fugacity ratio was 2.7. Fig. 3B also summarizes 2-d larvae/fresh egg fugacity ratios for each of the measured PCB congeners. In the case of larvae, an increasing trend in larvae/fresh egg fugacity ratio with increasing K_{OW} was observed and the slope of the regression line was significantly different from zero ($p < 0.05$). The maximum larvae/fresh egg fugacity ratio was 1.58 observed for PCB 180 and was attenuated relative to the mean fugacity ratio determined for incubated/ fresh eggs (upper dashed line of Fig. 3B). Fig. 3B further identifies congeners (PCB 70, 99, 149, 118, 153, 187, and 180) where 2-d old larvae retained significantly higher ($p < 0.05$; t-test) fugacity values compared to fresh eggs.

Discussion

Variable patterns of dry weight mass for embryos and hatched larvae were observed over the egg incubation period (Table 1). Although egg numbers were not counted as part of the sample pooling procedure, efforts were made to standardize the pooled weight of eggs and larvae samples. It was noted that as incubation progressed, the activity level of embryos within the eggs increased. This internal motion of embryos within eggs weakened the egg– egg strand associations and could have resulted in inclusion of fewer numbers of eggs in sample pools towards the later stages of incubation. The differences in dry weight mass between the incubation stages thus can be attributed to inclusion of fewer numbers of eggs in sample pools at the later stages of incubation and/or the absorption of water by eggs over time. At hatching, the egg sac was lost as well as the accumulated moisture by individual eggs and the number of larvae contributing to the sample pool was likely higher compared to the number of eggs contributed to late-incubated egg sample pools. The observations noted above were consistent with the findings of others. De Silva et al. (1998) reported a similar increase in dry weight content between fertilized eggs and newly hatched larvae in the macquarie perch (*Macquaria australasica*) (De Silva et al., 1998).

A significant decreasing trend was apparent when lipids were analyzed on a wet sample weight basis whereby the lipid percentage decreased by a factor of 4.3 from days 0 to 12 incubated eggs (Fig. 1A). Similarly, the percentage of lean dry weight decreased by 3.2-fold from days 0 to 12. However, when expressed on a dry weight percentage basis, the lipid content did not show significant change throughout the embryo or larvae development period. This trend has also been identified with Eurasian perch (*Perca*

fluviatilis) (Abi-Ayad et al. 2000) and turbot (*Scophthalmus maximus*), and was interpreted by the above authors to indicate that these species of fish rely primarily on amino acids and/or carbohydrates rather than lipids to support embryo energy demands (Planas et al. 1993). Different fish species vary in their utilization of nutrients during their early developmental stages (Abi-Ayad et al. 2000) and many species rely on free amino acids as their primary energy source (Wiegand 1996). Unfortunately, the need to pool egg samples for chemical and proximate analysis and the inability to ensure a constant egg number in each pool prevents mass balance calculations necessary to determine bioenergetic demands and the source of nutrients utilized by yellow perch embryos through time.

The present study demonstrated an increase in PCB fugacity of eggs as a function of incubation time until hatching. The increase in chemical fugacity occurred because of decreases in the wet weight proximate content of pooled samples (neutral lipid and lean dry protein content) which contributed to decreases in the sample partitioning capacity of late-incubated eggs relative to fresh eggs. While it is possible that fewer eggs were present in pooled egg samples at late-incubation periods, it is assumed that only the interior of the eggs contained maternally deposited PCBs. Thus inclusion of fewer eggs in late-incubated egg sample pools would have resulted in lower PCB mass and would not have affected the PCB fugacity estimates.

Alternatively, bioconcentration of PCBs into eggs could explain the relative fugacity increase if the fugacity of PCB congeners in perch eggs were below those of water during incubation. This seems unlikely given that aquaculture fish attain most of their PCBs from contaminated feed rather than water (Pinto et al. 2008) and given that

the perch eggs were incubated in the same flow through water from which they were collected. To address this issue, fresh water mussels and adult yellow perch were collected from the same pond as eggs. The data from Table 2 demonstrate that PCB fugacities in yellow perch eggs and in adult dorsal muscle samples were elevated relative to freshwater mussels. Freshwater mussels are widely considered to accumulate PCBs primarily through water (O'Rourke et al. 2004). Thus the higher PCB fugacities in yellow perch relative to mussels suggests that yellow perch achieved higher PCB fugacity than water. Finally, hatched larvae would not have shown a decrease in PCB fugacity following hatching if bioconcentration was the source of PCB bioaccumulation in eggs during incubation. These observations indicate that the increases in PCB fugacities in incubated eggs through time cannot be attributed to bioconcentration and support the conclusion of bioamplification.

Just prior to hatching, PCB fugacities were an average of 2.7 (range of 1.8–5.4-fold) higher than values measured in fresh eggs. The incubated/fresh egg fugacity ratio was also found to be independent of chemical hydrophobicity. This indicates that PCB diffusion rates from the egg were slow throughout the incubation process and proceeded at a slower rate than nutrient catabolism. Two congeners, PCB 74 and 70/76, did not follow the same trend as the others and this may be due to an analytical interference, particularly for congeners 70/76 which could not be quantified in mussel or adult yellow perch samples due to a major interfering peak in the chromatograms. PCB 74 was not detected in mussel or adult perch dorsal muscle samples and it is possible the interferences were present in the eggs for this congener as well. However, a re-examination of the egg chromatograms could not conclusively establish interferences for

these two compounds. Unfortunately, the concentrations of both congeners were too low to be detected and confirmed by gas chromatography-mass selective detector. Since interferences for PCB 70/76 and 74 could not be excluded, the congeners were not censored from the dataset. However, they are indicated as separate symbols in Fig. 3B. Despite the above noted problems, both PCBs 74 and 70/76 showed fugacity decreases to values approaching the fresh eggs following hatching.

Several other studies have demonstrated PCB bioamplification in different life stages of animals including frogs (Leney et al. 2006), birds (Clark et al. 1987, Drouillard et al. 2003) and adult fish (Paterson et al. 2007). In all of the above cases, the biomagnification observations were driven primarily by rapid depletion of lipids in animals over time. In the present study, lipid and lean dry weight content decreased with incubation time when data were expressed on a wet weight basis. Closer examination of lipids show that the dry weight lipid content remained constant through time and that bioenergetic driven depletion of lipids was potentially less important than would be apparent from comparisons of the wet weight-based measurements. Indeed in other fish species, nutrients including amino acids contribute prominently to developing embryo bioenergetics (Wiegand 1996). Unfortunately potential changes in egg numbers included in egg sample pools prevents definitive conclusions from being made about the relative roles of lipids and lean dry protein utilization rates in individual eggs towards the generation of fugacity gradients measured during egg incubation.

Assuming equilibrium partitioning continues to occur within the egg itself, this study suggests that yellow perch embryos are exposed to higher PCB fugacity at the point of hatching than would be predicted from maternal deposition. The empirical dataset also

suggests that the risk of embryo exposure to high PCB fugacity increases, and hence risk of toxicity, will increase with increasing incubation time. Given that late embryonic stages (days 10 and 12 embryos) are being exposed to the highest fugacities of chemical, it is predicted that egg toxicity will occur more frequently in later incubation stages. Similar observations were made for herring gull embryos. Drouillard et al. (2003) reported a maximum PCB fugacity gradient of 3.1-fold in pipping herring gull embryos compared to fresh eggs.

After hatching, PCB residues were lost from the larvae, attenuating the maximum chemical fugacity achieved in incubating eggs. However, the rate of PCB elimination in the early larvae stages was K_{OW} dependent resulting in retention of elevated fugacity gradients for the most hydrophobic compounds. This observation is significant in that it validated the previous assumption that yellow perch embryo's (as opposed to whole eggs) were exposed to higher PCB fugacities at the time of hatching. It also re-affirms rapid, K_{OW} dependent elimination of PCBs in these small animals as has been described elsewhere such as in the guppy (Sijm et al. 1992) and goldfish (Mackay and Hughes 1984). It is anticipated that even super hydrophobic congeners, including PCB 180, will re-achieve equilibrium with the water in larvae after a few more days as a consequence of additional PCB elimination (Sijm et al. 1992) and growth dilution (Sijm et al. 1992; deBruyn and Gobas 2006). This study provides the first indication of PCB bioamplification occurring in incubating fish eggs. It is predicted that bioamplification in fish species with larger eggs, greater yolk provisioning and longer incubation periods will experience higher PCB fugacity gradients over the incubation period than observed for yellow perch. Further studies to measure PCB fugacity increases in developing embryos

as opposed to whole eggs that can be coupled with bioenergetic models of embryo development would be useful to establish the boundaries of bioamplification that occurs during fish embryonic development.

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Table 3.1. Mean ± standard error for the wet weight of the sample, % moisture, % lipid wet weight, % lean dry weight (LDW), and the fugacities across congeners with time (n = the number of replicate samples).

Day	0	4	8	10	12	14
Proximate composition of pooled egg or larvae samples						
Wet weight (g)	0.49 ± 0.01	0.51 ± 0.01	0.49 ± 0.01	0.46 ± 0.03	0.49 ± 0.04	0.49 ± 0.03
% Moisture	93.14 ± 0.38	94.19 ± 0.28	96.18 ± 0.40	97.53 ± 0.27	98.32 ± 0.05	92.93 ± 0.15
% Lipid[a]	1.52 ± 0.10	1.57 ± 0.13	0.75 ± 0.09	0.58 ± 0.14	0.35 ± 0.04	1.45 ± 0.11
% LDW[b]	4.24 ± 0.34	5.34 ± 0.42	3.08 ± 0.32	1.89 ± 0.19	1.33 ± 0.06	5.61 ± 0.23
PCB fugacity in pooled egg or larvae samples ($\times 10^{-13}$ Pa)						
PCB 52	5.35 ± 0.44	4.23 ± 0.34	10.22 ± 1.05	14.77 ± 2.54	14.64 ± 4.03	6.06 ± 0.55
PCB 70/76	2.70 ± 0.19	4.25 ± 0.41	5.70 ± 0.75	11.23 ± 2.98	14.68 ± 3.11	2.78 ± 0.68
PCB 74	1.82 ± 0.22	2.26 ± 0.15	3.89 ± 0.45	5.03 ± 1.33	7.27 ± 1.26	2.48 ± 0.10
PCB 99	1.53 ± 0.26	1.31 ± 0.11	1.97 ± 0.19	2.88 ± 0.51	3.24 ± 0.63	2.33 ± 0.13
PCB 110	2.19 ± 0.37	1.97 ± 0.13	2.55 ± 0.33	3.85 ± 0.95	5.00 ± 1.38	2.81 ± 0.18
PCB 149	0.55 ± 0.09	0.48 ± 0.07	0.63 ± 0.04	1.03 ± 0.20	0.99 ± 0.21	0.76 ± .05
PCB 118	0.38 ± 0.06	0.28 ± 0.04	0.48 ± .02	0.57 ± .07	0.69 ± 0.10	0.53 ± 0.03
PCB 153	1.34 ± 0.22	1.17 ± 0.11	1.74 ± 0.12	23.24 ± 0.53	3.14 ± 0.65	1.94 ± 0.11
PCB 187	0.14 ± 0.03	0.10 ± 0.01	0.17 ± 0.03	0.20 ± 0.04	0.26 ± 0.06	0.22 ± 0.01
PCB 180	0.17 ± 0.04	0.11 ± 0.01	0.16 ± 0.01	0.38 ± 0.04	0.40 ± 0.09	0.27 ± 0.02

Table 3.2. Mean ± standard error proximate composition and PCB fugacities for congeners in freshwater mussels (*Elliptio complanata*), adult yellow perch and yellow perch eggs collected from the aquaculture pond.

Parameter	Freshwater Mussels	Adult yellow perch Dorsal muscle	Yellow perch Fresh egg pool
Proximate composition			
% Moisture	91.00 ± 0.85	81.21 ± 0.41	93.14 ± 0.38
% Lipid[a]	1.10 ± 0.20	0.62 ± 0.07	1.52±0.10
% LDW[b]	7.95 ± 0.68	18.16 ± 0.47	4.24 ± 0.34
PCB fugacity ($\times 10^{-13}$ *Pa*)			
PCB 52	ND	18.02 ± 2.54	5.35±0.44
PCB 70/76	INT	INT	2.70 ± 0.19
PCB 74	ND	ND	1.82 ± 0.22
PCB 99	0.40 ± 0.10	0.75 ± 0.25	1.53 ± 0.26
PCB 110	0.32 ± 0.02	1.50 ± 0.45	2.19 ± 0.37
PCB 149	0.05 ± 0.01	0.38 ± 0.15	0.55 ± 0.09
PCB 118	ND	ND	0.38 ± 0.06
PCB 153	0.11 ± 0.01	0.97 ± 0.29	1.34 ± 0.22
PCB 187	0.03 ± 0.01	0.12 ± 0.05	0.14 ± 0.03
PCB 180	0.015 ± 0.003	0.09 ± 0.05	0.17 ± 0.04

ND = non-detected peak; INT = matrix interference in chromatogram.

[a] % Lipid weight and % lead dry weight expressed on a wet sample weight basis.

[b] % LDW is percent lean dry weight and is expressed on a wet sample weight basis. $n = 5$ fresh water mussels, 3 adult yellow perch dorsal muscle samples, 6 yellow perch fresh egg pools.

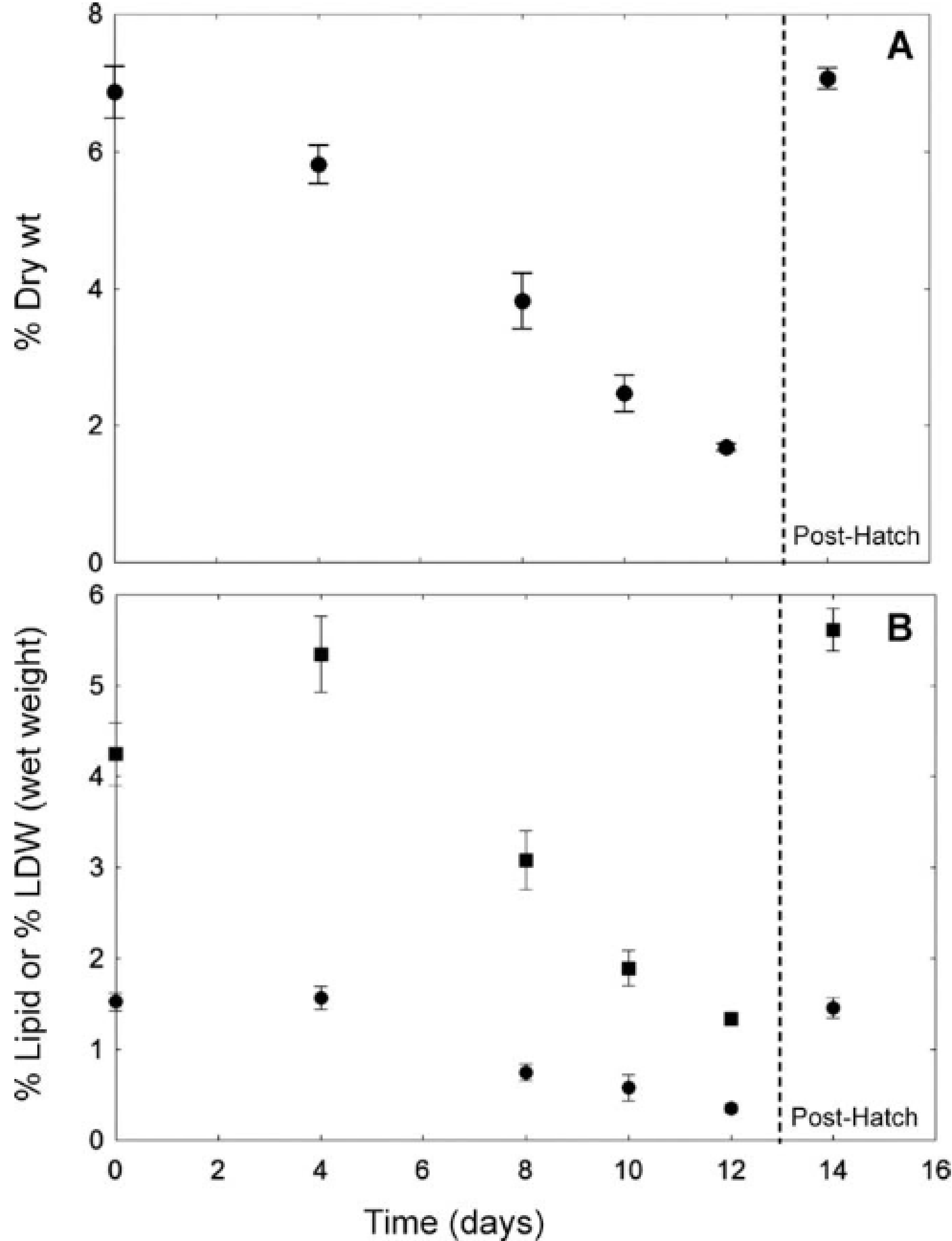

Figure 3.1. Changes in proximate composition of yellow perch eggs and post-hatch larvae during incubation. (A) Mean ± standard error dry weights (%) of egg and larvae samples over time. (B) Mean ± standard error lipid content (%) provided by (●) and lean protein weight (%) provided by (■) of egg and larvae samples expressed on a wet weight basis over time. Time refers to incubation date relative to newly collected fertilized eggs. Vertical line indicates the day of hatching.

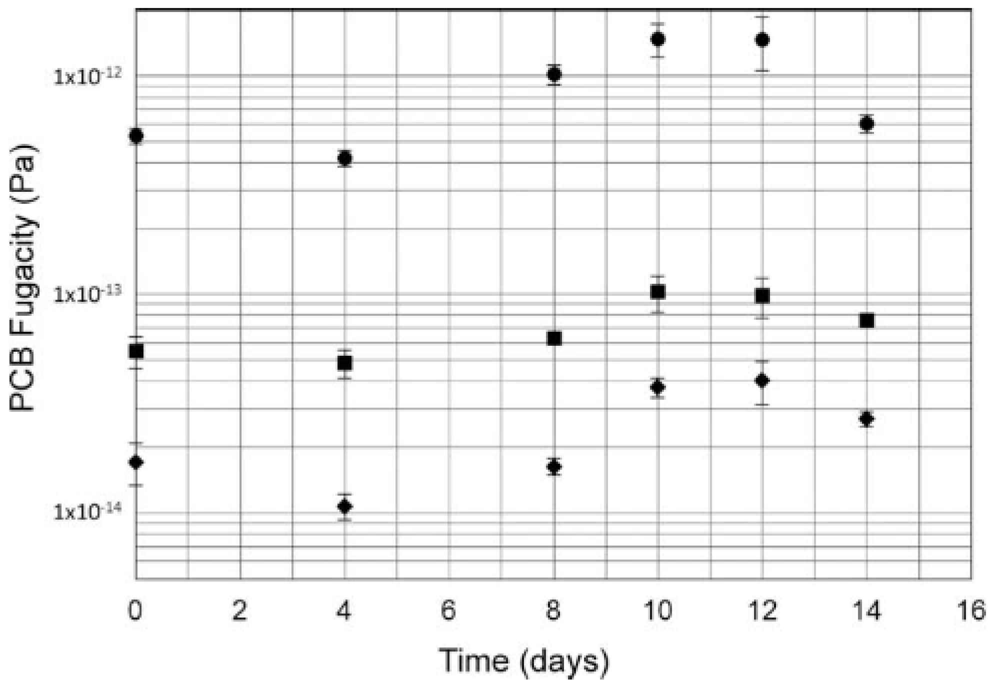

Figure 3.2. Mean fugacity of selected polychlorinated biphenyl (PCB) congeners in yellow perch eggs and post-hatch larvae over time. PCB 52 designated by (●), PCB 149 designated by (■) and PCB 180 designated by (♦). Error bars represent standard errors generated around mean estimates (n = 6 observations per time point, except day 12 where n = 5 observations).

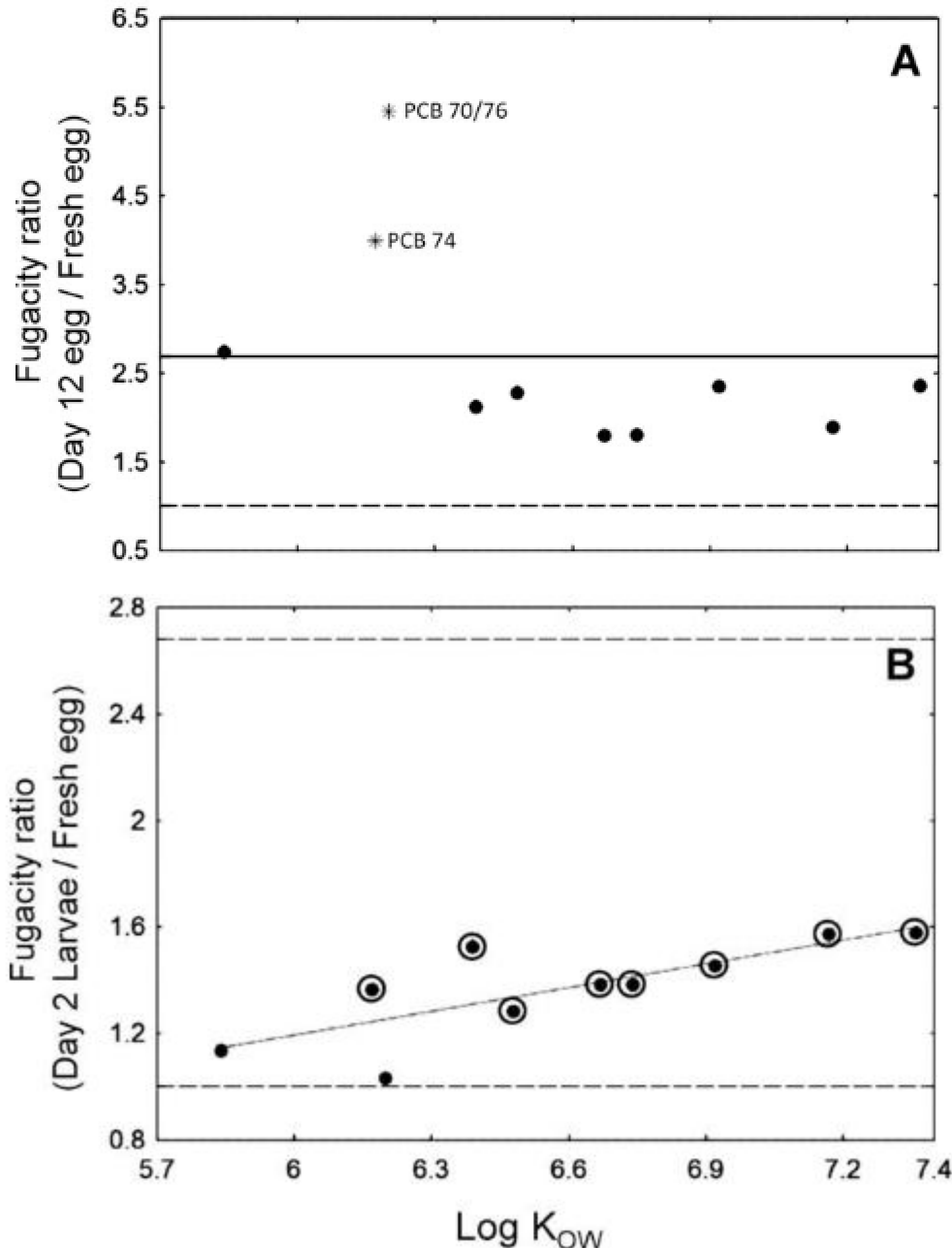

Figure 3.3. Polychlorinated biphenyl (PCB) fugacity ratios generated in incubated yellow perch eggs (A) and post-hatch larvae (B) at selected time points. (A) Day 12 incubated egg to fresh egg fugacity ratio determined for individual PCB congeners as a function of chemical Kow. Upper dashed line represents the mean (2.7) fugacity ratio of all PCB congeners. Lower dashed line refers to the equilibrium fugacity ratio. Data points indicated by (*) are congeners that may be different due to a small analytical interference. (B) Post-hatch larvae/fresh egg fugacity ratio as a function of chemical Kow. Solid line is the linear regression fit to the data. Circled data points are congeners with fugacity ratios significantly different (t-test; $p < 0.05$) than a value of 1.

Chapter 4: Bioamplification and the selective depletion of persistent organic pollutants in Chinook salmon larvae

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Introduction

Salmon are an important economic resource as they support both commercial and sport fisheries as well as form the bulk of aquaculture fish production in Canada (Rand et al. 1994, Arkoosh and Collier 2002, Kelly et al. 2011). Persistent organic pollutants (POPs) including dioxins and polychlorinated biphenyls (PCBs) have been demonstrated to elicit toxic effects on salmon, including growth inhibition, immune dysfunction, and reproductive impairments (Arkoosh and Collier 2002, Meador et al. 2002, deBruyn et al. 2004, Kelly et al. 2011). Toxicity studies have shown that the early life stages of fish (egg and larval stages) demonstrate greater toxicological sensitivity to POPs than do the juvenile and adult life stages (Walker et al. 1994, Petersen and Kristensen 1998, Russell et al. 1999). Furthermore, life stage specific toxicity studies on salmonids have shown that the yolk sac and early fry stages appear to be a critical period in the life history of salmonids where toxicity-induced mortality is highest (Van Leeuwen et al. 1985, Walker et al. 1991, Petersen and Kristensen 1998). This has been interpreted to be due to the yolk sac acting as a "toxicant sink" for hydrophobic compounds, where consequently the fry receives a greater dose of POPs near the end of yolk resorption (Atchison 1976, Van Leeuwen et al. 1985, Giesy et al. 1986, Petersen and Kristensen 1998).

Chinook salmon (*Oncorhynchus tshawytscha*) have a complex life history that often involves the exposure to POP compounds by bioconcentration and biomagnification processes in the adult life stages that integrate wide spatial gradients of contamination, due to its andromonous life history and its upper trophic level piscivorous feeding ecology. Pacific salmon such as Chinook and sockeye salmon (*Oncorhynchus nerka*), which are semelparous species that return to their native spawning rivers and streams and

die postreproduction, have been shown to be significant biological vectors of both nutrients as well as POPs to streams and tributaries (Krummel et al. 2003, O'Toole et al. 2006, Gregory-Eaves et al. 2007). This transfer of contaminants from ocean/lake habitats to rivers/streams through decomposing somatic tissues may be mirrored in reproductive products via maternal transfer of POPs to eggs and their subsequent transfer to embryo and larvae (Miller 1993, Fisk and Johnston 1998).

Fish eggs are considered to achieve equilibrium with somatic tissues at the time of egg formation resulting in similar lipid normalized POP concentrations between eggs and maternal tissues (Fisk and Johnston 1998, Russell et al. 1999). Furthermore, fall spawning fishes (as exemplified by many populations of Chinook salmon) are required to provide significant maternal resources to eggs to enable overwintering incubation and survival of larvae during a prolonged critical period, which reflects the transition from endogenous (use of yolk reserves) to exogenous feeding (Kaitaranta and Ackman 1981, Wiegand 1996, Russell et al. 1999). This increased transfer of maternal resources to the egg results in the transfer of a greater POPs mass to eggs and larvae. The matter is further complicated by fish migrations during spawning, exemplified in dramatic fashion by salmon where some populations are known to swim distances of over 3000 km (range: 1 to >3200 km; Kinnison et al. 2001) and utilize as much as 95% of their somatic lipid contents to support the bioenergetic costs of spawning and reproduction (deBruyn et al 2004). Under conditions where an organism loses body weight and chemical partitioning capacity faster than it can eliminate contaminants, lipid normalized POP concentrations become enriched. This process was referred to as solvent depletion by Macdonald et al. (2002) and was later coined bioamplification (Daley et al. 2009, 2011). deBruyn et al.

(2004) found that migrating female sockeye tissues exhibited bioamplification factors, characterized as the ratio of lipid normalized tissue concentrations in post- relative to prespawning individuals, of 3.4-5.6 for POP compounds following migration. They also reported bioamplified lipid normalized POP concentrations in the roe of fish post migration. Kelly et al. (2011) observed similar trends between eggs and muscle of spawning Pacific salmon and further demonstrated how migration distance of a given population influences the magnitude of POPs bioamplification. Although enrichment of POP concentrations in gonads was found to occur to a lesser extent (1.9-2.5) than measured in somatic tissues, the above studies highlight how migration and bioenergetic costs serve to increase POPs exposure in eggs and larvae.

Our laboratory recently demonstrated that POP residues can be further bioamplified within the eggs of yellow perch (*Perca flavascens*) as a result of yolk utilization by fish embryos during incubation (Daley et al. 2009). This process, should it occur in incubating Chinook salmon eggs, would be in addition to POPs bioamplification in eggs occurring from maternal spawning activities described above. It is predicted that bioamplification in fish species such as Chinook salmon, with larger eggs, greater yolk provisioning, and longer incubation periods, will experience higher POP bioamplification than observed for yellow perch. The objectives of this study were to examine whether bioamplification of POP residues occurs in incubating eggs and/or larvae of Chinook salmon and to place this into the context of potential POPs exposures contributed by bioamplification during parental spawning, egg incubation, and in larvae, up to the critical period when larvae rely primarily on the yolk-sac to satisfy bioenergetic costs.

Materials and Methods

Experimental Section

Chinook salmon eggs were collected from a single spawning female in the Credit River, Mississauga, ON, Canada (43°34'39.24″ N, 79°42'8.19″ W). The female would have achieved its exposures to POP compounds within Lake Ontario, known for relatively high concentrations of POP compounds including PCBs and mirex (Oliver and Niimi 1988). The eggs were brought back to an aquaculture facility (Great Lakes Institute for Environmental Research) in Windsor, ON, Canada, and manually fertilized using the combined milt from three males. The eggs were then incubated in Heath trays using a flow through system receiving filtered water from the Detroit River at in situ temperatures. Water temperatures were recorded daily throughout the experiment. Replicate eggs (n = 10 at each time point) were collected postfertilization at day 1 and at a later egg developmental stage on day 25. The majority of eggs hatched between days 30-34. Similar to natural, under the ice conditions, larvae were not provisioned with food during the experimental trials. Larval Chinook salmon (n = 10 at each time point) were collected on days 35, 61, 91, 105, 134, 155, and when larvae had fully absorbed their yolk on day 168 corresponding to the onset of ice-out and spring temperature increases. Following sampling, the eggs and larvae were kept frozen at -20 °C until chemical and proximate analysis.

Laboratory Processing

Individual eggs or larvae were analyzed to determine neutral lipid content, moisture content, and POP concentrations. POPs and lipids were analyzed using a micro extraction technique described in Daley et al. (2009, 2011) using PCB 30 as a recovery

standard. One milliliter of sample was removed for the gravimetric determination of neutral lipids using a microbalance (Drouillard et al. 2004). The remaining extract was concentrated to 2 mL, and sample cleanup was performed by florisil chromatography as described by Lazar et al. (1992). In the present study, the first (50 mL hexane; ACP, Montreal, Quebec, Canada) and second fractions (50 mL; hexane/dichloromethane 85/15 v/v; Fisher Scientific, Fair Lawn, NJ) were collected. After florisil chromatography, extracts were concentrated to 1 mL by rotary-evaporator. Samples were analyzed for individual POPs by gas chromatography electron capture detection (GC-ECD). For each batch of six samples, a reference homogenate, method blank, an external PCB standard (Quebec Ministry of Environment Congener Mix; AccuStandard, New Haven, CT), external certified organochlorine pesticide standard (AccuStandard, New Haven, CT), and PCB 30 recovery standard were analyzed. Only PCB congeners (IUPAC no.) 18/17, 31/28, 44, 49, 52, 70, 99, 101, 110, 118, 138, 149, 151/82, 153, 158, 170, 177, 180, 183, 187, 194, and 201, as well as QCB, HCB, OCS, and Mirex, were detected with sufficient frequency to be included in the data analysis. Recoveries of individual PCB congeners in the inhouse reference tissue extracted with each batch of samples were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts from the Great Lakes Institute for Environmental Research accredited organic analytical laboratory (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified). The recovery of the PCB 30 standard was 87 ± 5.0% across samples.

Data Analysis

Persistent organic pollutant concentrations were expressed on a lipid equivalents basis (deBruyn et al. 2007). The lipid equivalents concentration for each POP congener was calculated as follows:

$$C_{org(lipidequiv)} = C_{org\ (wet)} / (X_{lipid} + 0.05 * X_{LDP}) \quad (1)$$

where $C_{org(lipidequiv)}$ is the POP concentration normalized for lipid equivalents (ng/g lipid equivalents), $C_{org\ (wet)}$ is the wet weight POP congener concentration (ng/g) in the pooled sample, and X_{lipid} and X_{LDP} are the fractions (ng/g body weight) of neutral lipids and lean dry protein (LDP), respectively, in the sample. The lean dry protein was determined by subtracting the total lipid weight from the total dry weight of each sample and dividing by the wet sample weight. The constant 0.05 indicates that lean dry protein has a partitioning capacity that is equivalent to 5% of lipids (deBruyn and Gobas 2007). Percent lipids and lipid equivalents POP concentrations in the salmon eggs and posthatched larvae at different time intervals were compared using a t test or an analysis of variance (ANOVA) and Tukey's post hoc test where applicable.

POP mass balance estimates were derived by multiplying the wet weight POP concentration (ng/g wet wt) by the mass (g) of individual eggs and larvae at the time of sampling. The bioamplification factor (BAmF) is the lipid equivalents concentration ratio between two stages of an organism's life cycle.

$$BaMF = C_{org(t)} / C_{org(t-1)} \quad (2)$$

where C_{org} is the concentration in the organism (ng/g lipid equivalents). Bioamplification factors in eggs and larvae were calculated by expressing the ratio of POP lipid equivalents concentrations in the 168 d larvae to the mean fresh (newly

fertilized) egg value. Bioamplification factors were contrasted against chemical hydrophobicity (log K_{OW}) values to examine the relationship with chemical physical properties. Log K_{OW} values for PCBs were obtained from Hawker and Connell (1988). The OCs were obtained from Mackay et al. (2006).

Unless otherwise stated, all data presented in the text and figures are reported as mean ± standard deviation (SD).

Results and Discussion

There was no change in lipid content (t test, $p = 0.58$) or total lipid mass per egg (t test, $p = 0.52$) during the egg incubation period. This trend of no lipid loss in the embryo has also been observed with Eurasian perch (*Perca fluviatilis*) and turbot (*Scophthalmus maximus*) and may indicate that these species rely primarily on amino acids and/or carbohydrates rather than lipids to support bioenergetic demands during egg incubation (Planas et al. 1993, Abi-Ayad et al. 2000).

Alternatively, a significant decreasing linear trend in lipid contents was observed in posthatched Chinook larvae ($r = 0.94$; $p < 0.0001$). The mean lipid contents (±SD) for individual Chinook salmon eggs and larvae across time are presented in Figure 1 and demonstrate linear lipid decreases in larvae through time throughout the experiment. Throughout the egg and larval period, lipid contents of eggs decreased from 8.6 ± 0.21% (range: 7.7-9.0%) in fresh eggs to 1.2 ± 0.21% (range: 0.9-1.5) in 168 d old larvae with 168 d larvae containing only 14% of the total lipid mass originally deposited to the fresh egg. These declines in lipid content are consistent with what has been reported for overwintering larval and juvenile populations of salmon (Srivastava and Brown 1991, Bull et al. 1996, Beckman et al. 2000). For example, juvenile Chinook salmon from the

Yakima River (tributary of Columbia River in Washington, USA) were reported to exhibit lipid declines from approximately 7-8% in early fall to under 2% in early spring (Beckman et al. 2000).

The experimental design that utilized eggs from a single female to reduce potential interegg variation of maternally deposited POPs, as well as the ability to sample individual eggs/larvae for chemical analysis without pooling, enabled precise mass balances to be performed to test for chemical elimination during egg incubation and in larvae following hatching. Figure 2 presents the change in chemical mass within eggs/larvae over time for three select POP compounds, a representative from the low log K_{OW} (below 6), moderate log K_{OW} (6-7), and high log K_{OW} (above 7) groups: PCB congeners 18/17 (log $K_{OW} = 5.24$), 99 (log $K_{OW} = 6.39$), and 180 (log $K_{OW} = 7.36$). For all chemicals of study, there were no significant changes ($p > 0.05$; ANOVA) in chemical mass between eggs over the period of fertilization to hatch-out.

Following hatch-out, mass balance estimates indicate that POP depuration from Chinook larvae occurred for several compounds, but different trends occurred for chemicals with different hydrophobicities. For the compounds in the low log K_{OW} group, 50-100% of chemical mass was eliminated by day 168 as compared to fresh eggs, whereas for the moderate range log K_{OW} compounds, 19-62% was eliminated, and only 11-24% of chemical was eliminated for those compounds with the highest log K_{OW}'s (Supporting Information 2).

The above observations of slow to negligible chemical elimination in post hatched larvae were unexpected, as generally with small animals chemical elimination is considered to be rapid due to their high surface area to volume ratio (Sijm et al. 1991,

Leblanc 1995). There are two hypotheses that could possibly explain the lack of chemical elimination, one being related to temperature and the other to physiological mechanisms. In the present study, the increase in the rate of chemical depuration during days 145-168 coincided with increases in water temperatures related to spring warming (see Figure 2). Water temperature directly regulates the metabolic rate of fish and has been demonstrated to reduce depuration of POPs from larger more mature fish under cold water conditions (Paterson et al. 2007, Drouillard et al. 2009). For example, slow elimination rates of PCBs were observed in overwintering yellow perch where significant elimination occurred only for the least hydrophobic PCB congeners (log K_{OW} = 5.2) under cold water conditions (<10 °C) as compared to the spring period when elimination of 71 congeners was observed during a much shorter time frame (Paterson et al. 2007).

However, water temperature/metabolic relationships were not likely the only factors regulating chemical depuration rates observed in larvae from the present study. An examination of the rate of lipid reduction with time (Figure 1) indicates that larvae salmon exhibited steady declines in lipid content and total lipid mass per individual, suggesting that metabolic activity continued to proceed at a relatively high rate over much of the experimental period. Indeed, over the days 0-135 period, larvae lost 78% of their fresh egg lipid mass, representing a majority of lipid declines observed over the total study duration. Thus, while higher water temperatures have been shown to increase yolk absorption rate of larvae fish (Beachman et al. 1990, Kamler 2008), other factors in addition to increased metabolic rate appear to be important. Developmental studies on Atlantic salmon (*Salmo salar*) demonstrate that gill development increases rapidly by the end of yolk absorption, whereas cutaneous respiration dominates in newly hatched larvae

(Rombough and Moroz 1990, Wells and Pinder 1996). Thus, elevated water temperatures as observed toward the end of the study not only correspond with increased metabolic rate and faster yolk resorption, but also with the development of gills, which can act as a more efficient method of chemical exchange (Paterson et al. 2010). Given that yolk is the major repository of maternally deposited lipids as well as POPs (Atchison 1976, Guiney et al. 1980, Van Leeuwen et al. 1985, Petersen and Kristensen 1998), it is hypothesized that the combined onset of gill development along with final resorption of yolk sac acted in concert to increase the rate of POP elimination. Because yolk sac resorption rate and developmental cues for gill formation are related to water temperature, further studies to determine how seasonal water temperature profiles interact with these variables and influence POPs loss, and bioamplification would be useful.

As indicated from the mass balance estimates, the lipid equivalents POP concentrations did not change in eggs from fertilization to hatch-out for any of the compounds studied (see Figure 3). This differs with trends reported for yellow perch where PCBs exhibited enriched lipid normalized chemical concentrations in eggs just prior to hatching relative to that measure in fresh eggs (Daley et al. 2009). These differences are attributed to the fact that yellow perch eggs showed declines in lipid content of eggs during the egg incubation period, but salmon did not show such declines, whereas both species showed no chemical elimination.

Post hatching, changes in lipid equivalents POP concentrations occurred for all chemicals, but different patterns of concentration trends were evident depending on chemical hydrophobicity. The lowest log K_{OW} congener presented in Figure 3, PCB18/17 (log K_{OW} = 5.24), showed a significant increase in concentration from days 60-100

(ANOVA; $p < 0.0001$). This was a period of rapid decreases in animal lipid content but little or no change in chemical mass. After day 100, chemical depuration for less hydrophobic compounds caused an attenuation of bioamplification trends such that by day 155, chemical loss rates exceeded lipid utilization rates, causing a drop in lipid normalized chemical concentrations to values well below those present in the fresh egg. Compounds having log K_{OW} values exceeding 6 demonstrated a different pattern of lipid equivalents concentrations with time. For these compounds, the slow chemical elimination across time caused an increasing trend in concentration, with the highest concentrations occurring between days 155 and 168.

Bioamplification factors between fresh eggs and 168 d larvae are compared in Figure 4 and plotted as a function of chemical log K_{OW} for all POPs studied except for two compounds. The latter chemicals, QCB and PCB 18/17, had concentrations below detection limits at day 168, and the bioamplification factor would therefore be much less than 1 for these compounds. There was an increasing trend in larvae/egg bioamplification factor with increasing log K_{OW} until it reached an asymptote at log K_{OW} values exceeding 7. The magnitude of bioamplification for the highest log K_{OW} POPs showed an approximate 5-fold increase in chemical lipid equivalent concentrations in larvae as compared to fresh eggs (Figure 4). This magnitude of the maximum bioamplification factor approached the degree of decrease in partitioning capacity (~6 fold) jointly contributed by lipids and lean dry protein.

As noted earlier, the trend of POPs bioamplification in Chinook salmon early life stages differed from that of yellow perch eggs and larvae. Yellow perch showed clear evidence of POPs bioamplification in the eggs, whereas posthatched perch larvae began

to eliminate chemical within 2 days of hatching (Daley et al. 2009). In Chinook salmon, there was little chemical elimination either in eggs or in larvae for over 100 days post hatching until water temperatures began to increase during spring warming. The differences between these species early life stage bioamplification trends are likely related to life history differences in reproductive strategies. In the present study, Chinook salmon represents a fall spawning, semelparous life history strategy that provisions significantly more lipids to the eggs to survive prolonged under ice periods during egg and larvae development where food resources may be limiting post hatch. Newly hatched salmon typically remain in the gravel until their yolksac is nearly depleted (Bull et al. 1996). Heming (1982) demonstrated at a constant water temperature of 6 °C the time to 50% emergence for Chinook salmon larvae was 192 days, which is similar to the 168 days it took for complete yolk absorption in the present study. Alternatively, yellow perch represent iteroparous spring spawners whose eggs typically hatch in 10-20 days following spawning and emerge as free-swimming larvae capable of feeding immediately following hatching (Post and McQueen 1988).

This study demonstrates that late stage Chinook salmon larvae are exposed to higher POP concentrations than predicted by maternal deposition, which could lead to underestimates in the toxicity experienced by this species if toxicity studies are confined to the maternal tissues and/or egg life stages. While the early life stages of fish are generally considered more sensitive to toxicological effects than adults (Russell et al. 1999) some studies have demonstrated that later larval stages of salmonids experience the greatest sensitivity to maternally deposited POPs evidenced by increased mortality (Walker and Peterson 1994, Petersen and Kristensen 1998). One example is 2,3,7,8-

TCDD in Lake Trout (*Salvelinus namaycush*) that at concentrations of 400 pg/g generated deleterious effects on eggs but produced the greatest mortality in yolk-sac larvae (Spitsberg et al. 1991). Such trends would be expected given both the concentration increase in tissues and the mobilization of yolk-sac associated POPs to tissues following yolk-sac resorption. It is widely recognized that fish mortalities are increased during the critical period, representing the transition from endogenous maternally deposited yolk lipids to exogenous food resources (Broyles and Noveck 1979, Van Leeuwen et al. 1985, Walker et al. 1991, Petersen and Kristensen 1998). This research suggests that POPs-based toxicity can interact with, and augment, critical-period associated mortality, potentially resulting in underestimates of the true toxic consequences of POP compounds in the environment. In comparing an estimated threshold for PCB concentrations that protects juvenile salmonids from experiencing adverse health effects of 2400 ng/g lipid, (Meador et al. 2002, Johnson et al. 2007) to the sum of the 22 PCB congeners (21 600 ± 1100 ng/g lipid or 13 800 ± 1800 ng/g lipid equivalents) evaluated in this study at day 168, the concentrations in the present study were observed to be approximately 5-9 fold greater than this threshold. Although this PCB threshold is suggested for juvenile salmon, studies have demonstrated that newly hatched rainbow trout are 20-40 times more sensitive to the lethality of TCDD than 1 year old juvenile rainbow trout (Walker and Peterson 1994). As such, documenting the critical tissue residues in larval fish that have completed bioamplification would be a better reference point then using the fresh egg concentration as they may have both heightened bioamplification factors and toxicological sensitivities. Further research to identify the relative sensitivity of late stage Chinook salmon larvae as compared to

embryos using lipid normalized tissues concentrations is needed. Studies verifying bioamplification of POPs in lake trout fry would also be useful to extend the concentration enrichment mechanism posthatch to this more sensitive species. Notable in the present research is that the larvae bioamplification mechanism occurs only for highly hydrophobic substances (log $K_{OW} > 6$ and maximized for chemicals with log $K_{OW} > 7$) narrowing the range of chemicals that can elicit enhanced toxicity by concentration enrichment in the larvae life stage.

Finally, it is worth noting that bioamplification processes occur in Chinook salmon at multiple life stages that include both reproducing adults and larvae life stages, which can serve to increase the vulnerability of this species to hydrophobic toxics. deBruyn et al. (2004) found that migrating female sockeye tissues showed bioamplification factors ranging from 3.4 to 5.6 between pre- and postmigration areas as a result of lipid utilization to satisfy migration energy costs. These bioamplification factors vary by environment and migration distance experienced by a given population (Kelly et al. 2011). Although gonads were observed to have exhibited lower bioamplification factors (1.9-2.5) as compared to somatic tissues, gonads did exhibit significant enrichment of POPs residues and are also dependent on population specific migration costs. Using the highest bioamplification factors reported by deBruyn et al. (2004) of 2.5 for the postmigration adult female gonads as compared to gonads premigration and the highest bioamplification factor of 4.9 in larval Chinook salmon observed from the present study, the combined bioamplification factor could be as high as 12 in larval Chinook salmon as compared to prespawning maternal tissue concentrations. Arguably the coupling of bioamplification processes across these life

stages contributes to POPs exposures that equal, and may exceed, the magnitude of biomagnification processes experienced by adult fish. On the basis of these joint observations, it is hypothesized that the sensitivity of a given salmon population to POPs toxicity can be influenced by a large number of factors including distance/duration and bioenergetics of spawning migration, maternal provisioning of yolk lipids to eggs, environmental temperature trends, and the availability of food resources to larvae during the critical period.

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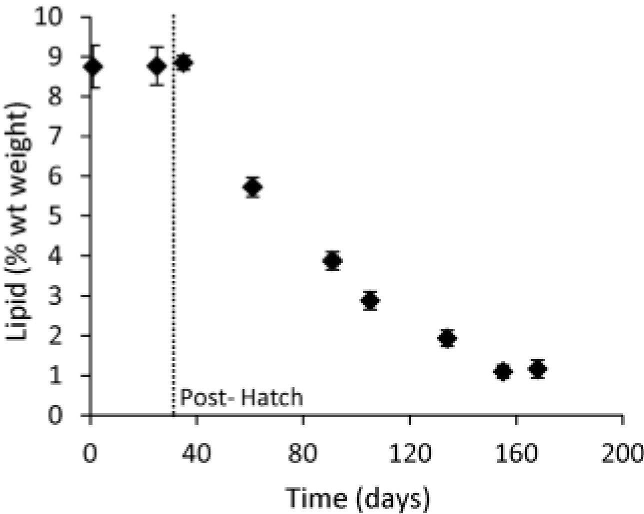

Figure 4.1. Mean ± standard deviation lipid contents (%) of Chinook eggs and larvae samples over time. Time refers to incubation date relative to newly collected fertilized eggs. Vertical line indicates the day of hatching (n = 6 observations per time point).

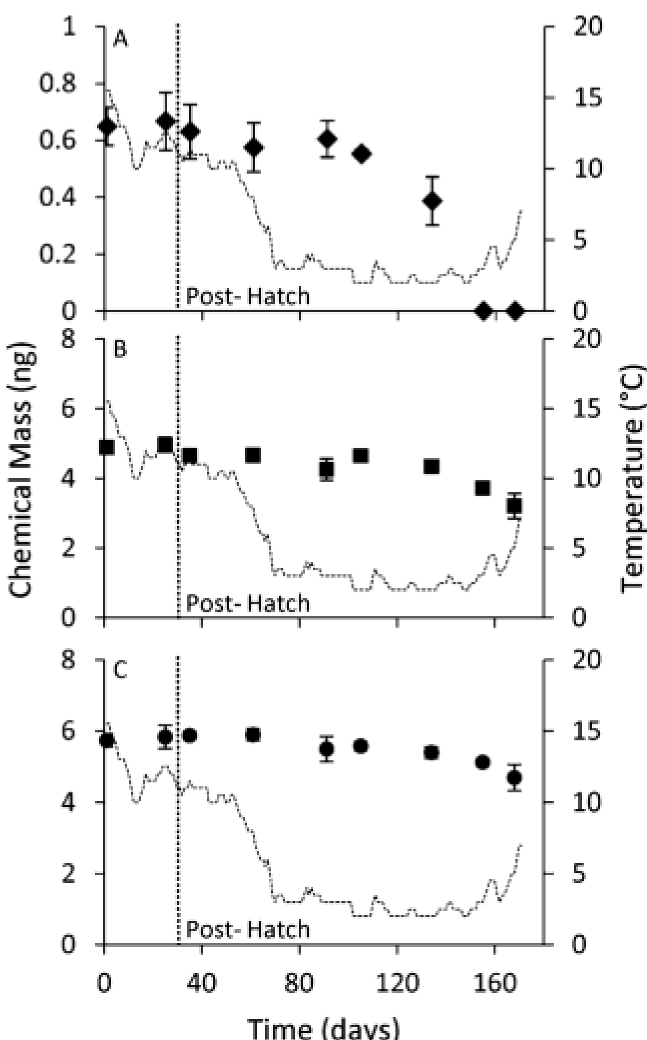

Figure 4.2. Mean ± standard deviation of chemical mass of selected polychlorinated biphenyl (PCB) congeners in individual Chinook eggs and larvae over time. PCB 18/17 designated by (◆), PCB 99 designated by (■), and PCB 180 designated by (●). Vertical line indicates the day of hatching (n = 6 observations per time point). The secondary axis represented by hatching dots is the temperature profile over the experimental duration.

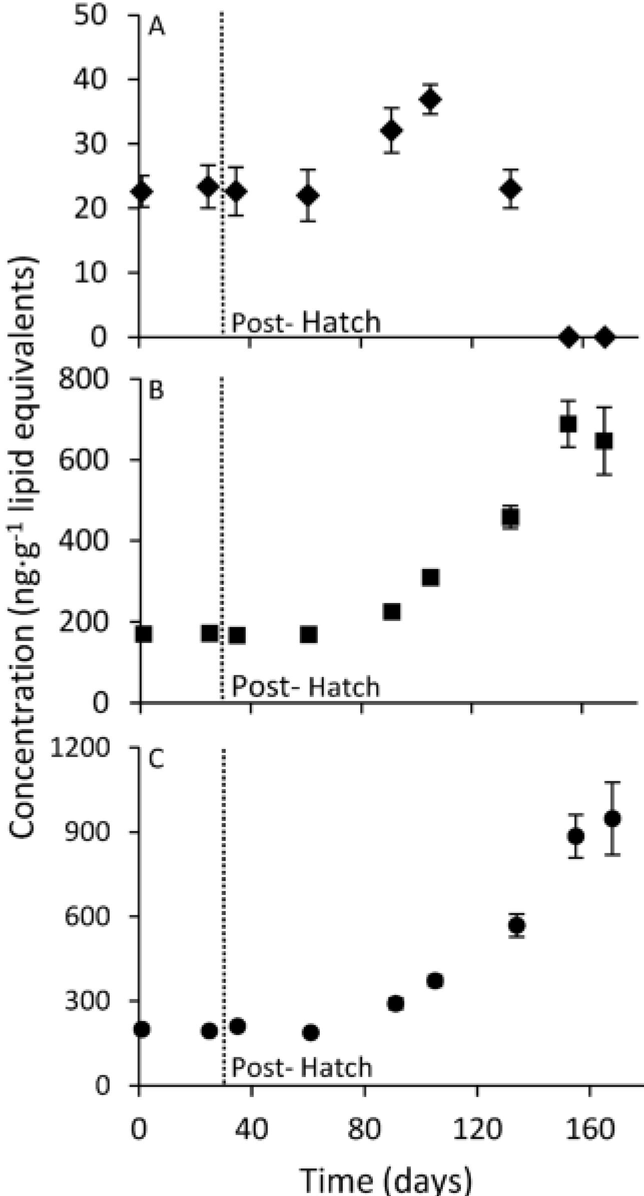

Figure 4.3. Mean ± standard deviation of chemical concentration (ng/g lipid equivalents) of selected polychlorinated biphenyl (PCB) congeners in Chinook eggs and larvae over time. PCB 18/17 designated by (◆), PCB 99 designated by (■), and PCB 180 designated by (●). Vertical line indicates the day of hatching (n =6 observations per time point).

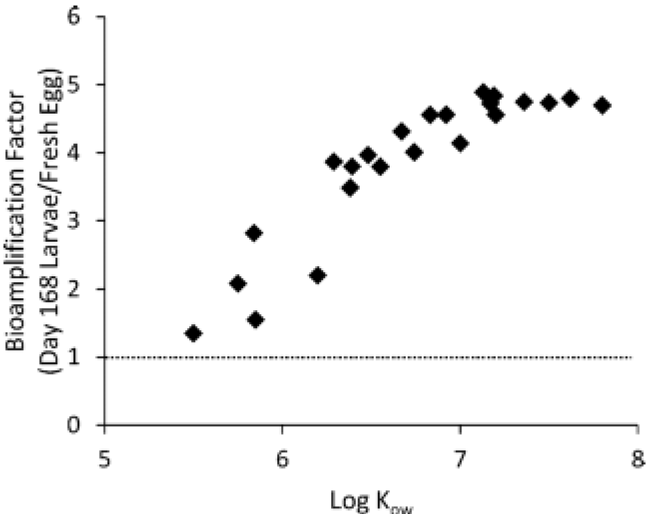

Figure 4.4. Persistent organic pollutant (POP) bioamplification factors comparing 168 d larvae/fresh egg as a function of chemical KOW. Lower horizontal dashed line refers to the bioamplification factor of 1.

Supporting Information:

Table S4.1. The mean ± SD of individual POP congener concentrations (ng/g lipid equivalents) are summarized over time. A value of zero is in place of any non-detect congeners across time.

	Time (days)	POP congeners (ng/g lipid equivalents)						
		QCB	HCB	OCS	Mirex	PCB18/17	PCB31/28	PCB52
Mean	1	4.1	43.0	39.5	152.6	22.6	72.7	120.9
SD	1	1.4	3.2	1.3	5.5	2.4	2.2	5.6
Mean	25	4.0	43.3	37.3	146.6	23.4	64.7	130.0
SD	25	0.2	3.0	2.4	9.2	3.4	19.5	7.8
Mean	35	5.1	50.4	38.8	148.1	22.6	61.3	106.4
SD	35	1.9	6.9	1.6	5.6	3.7	25.7	9.3
Mean	61	3.8	35.1	39.2	131.5	22.3	60.7	136.9
SD	61	0.4	6.4	1.4	3.1	4.0	7.9	19.9
Mean	91	4.0	41.5	51.4	207.2	32.1	80.0	167.2
SD	91	1.3	5.6	2.8	12.8	3.5	4.8	28.5
Mean	105	3.3	49.7	67.9	270.1	36.9	100.4	251.7
SD	105	3.1	3.0	2.7	13.3	2.3	3.9	11.8
Mean	134	0.0	37.4	99.9	411.5	26.3	101.7	389.7
SD	134	0.0	7.3	8.0	26.6	21.1	13.0	94.5
Mean	155	0.0	22.4	143.7	666.6	0.0	63.4	336.3
SD	155	0.0	1.9	12.7	62.4	0.0	9.8	34.0
Mean	168	0.0	19.9	152.7	746.2	0.0	0.0	340.6
SD	168	0.0	4.3	19.0	103.1	0.0	0.0	61.8
		PCB49	PCB44	PCB70	PCB101	PCB99	PCB110	PCB151/82
Mean	1	76.8	64.5	147.5	337.2	170.3	234.6	107.8
SD	1	4.6	31.8	3.8	14.0	4.6	33.6	8.0
Mean	25	72.3	79.1	146.6	332.9	171.7	247.2	106.0
SD	25	4.5	5.6	9.2	24.8	6.2	11.2	8.6
Mean	35	66.5	76.6	146.6	361.9	166.7	247.2	115.3
SD	35	3.5	3.5	6.1	29.3	6.8	10.5	5.2
Mean	61	68.5	72.8	141.4	321.1	169.8	228.5	100.5
SD	61	1.2	2.2	2.4	7.1	1.9	10.8	3.2
Mean	91	88.6	93.7	190.4	446.2	225.4	300.8	140.5
SD	91	6.8	12.2	7.6	23.6	14.4	32.9	12.9
Mean	105	119.7	129.0	239.6	642.2	309.9	457.0	211.2
SD	105	5.2	5.0	10.0	35.3	13.7	20.4	9.7
Mean	134	156.5	156.6	322.8	881.9	458.1	657.3	293.5
SD	134	11.5	15.2	19.0	49.0	27.8	47.8	20.7
Mean	155	155.7	171.3	395.6	1267.0	681.2	967.9	423.2
SD	155	21.6	19.4	38.9	106.6	54.7	84.7	38.1
Mean	168	119.0	134.2	324.9	1175.3	646.9	930.9	409.0

SD	168	25.8	25.0	47.8	147.1	82.9	114.5	49.0
		PCB149	PCB118	PCB153	PCB138	PCB158	PCB187	PCB183
Mean	1	259.9	279.6	453.5	471.6	33.6	183.5	67.7
SD	1	7.7	8.5	13.3	16.3	1.6	12.0	3.0
Mean	25	263.2	272.5	449.8	468.0	32.6	179.5	65.1
SD	25	10.6	20.4	18.8	19.0	2.5	12.6	4.6
Mean	35	256.7	286.7	457.7	475.9	38.4	210.5	74.9
SD	35	9.6	10.6	17.4	17.1	2.4	7.3	2.6
Mean	61	262.1	268.6	445.8	455.1	30.2	176.4	68.1
SD	61	2.9	4.3	6.0	7.8	2.6	11.4	3.0
Mean	91	351.0	378.5	623.5	644.6	56.4	270.2	103.9
SD	91	22.6	26.3	41.5	45.9	4.1	30.1	8.7
Mean	105	477.9	432.3	845.3	883.9	61.7	359.1	124.9
SD	105	22.4	212.7	39.3	41.7	4.8	17.2	7.7
Mean	134	713.3	763.5	1278.5	1341.2	96.4	540.4	195.8
SD	134	46.6	54.4	92.5	97.5	8.7	41.0	14.9
Mean	155	1112.3	1146.8	1989.4	2011.4	120.0	817.3	297.7
SD	155	90.1	97.8	170.9	171.6	10.9	76.8	28.9
Mean	168	1121.4	1120.7	2068.2	2149.0	139.0	867.4	308.7
SD	168	137.1	146.2	271.4	281.9	19.7	120.4	46.1
		PCB177	PCB180	PCB170	PCB201	PCB194		
Mean	1	61.2	199.6	80.2	42.0	25.8		
SD	1	2.4	7.0	2.8	1.5	2.1		
Mean	25	58.6	193.8	75.1	40.4	24.5		
SD	25	3.5	8.5	5.1	1.7	1.4		
Mean	35	63.1	210.5	81.8	45.7	30.0		
SD	35	2.9	8.7	3.5	2.0	3.0		
Mean	61	57.1	188.7	73.9	39.0	20.9		
SD	61	0.8	3.5	1.4	0.7	0.7		
Mean	91	87.2	290.9	112.0	63.1	34.5		
SD	91	7.8	19.6	7.7	4.2	3.3		
Mean	105	116.8	371.8	150.0	78.2	53.6		
SD	105	5.5	19.1	7.3	4.0	5.4		
Mean	134	177.2	568.6	226.6	118.6	72.1		
SD	134	13.3	40.4	17.0	8.8	7.5		
Mean	155	273.2	885.3	352.2	185.1	104.6		
SD	155	25.2	76.0	31.7	11.2	8.4		
Mean	168	296.3	947.8	379.7	201.8	121.1		
SD	168	39.5	129.3	52.6	25.1	13.8		

Table S4.2. The mean ± SD of the mass of individual POP congeners (ng) for fresh eggs and day 168 larvae, the fraction eliminated, and the p-value for the regression of chemical mass over time are presented.

	Mean Fresh Egg	SD Fresh Egg	Mean day 168	SD day 168	Eliminated (%)	Probability p-value	
QCB	0.12	0.01	0.00	0.00	100	0.000	***
HCB	1.24	0.04	0.10	0.03	92	0.011	**
OCS	1.13	0.03	0.76	0.08	33	0.002	**
Mirex	4.38	0.08	3.61	0.31	18	0.034	*
PCB18/17	0.65	0.07	0.00	0.00	100	0.001	***
PCB31/28	2.09	0.05	0.00	0.00	100	0.002	**
PCB52	3.47	0.16	1.66	0.45	52	0.082	
PCB49	2.21	0.11	0.58	0.14	74	0.001	***
PCB44	1.87	0.11	0.66	0.19	65	0.005	**
PCB70	4.23	0.08	1.60	0.34	62	0.001	***
PCB101	9.68	0.24	5.81	0.83	40	0.013	*
PCB99	4.89	0.08	3.21	0.39	34	0.012	*
PCB110	6.75	0.15	4.64	0.63	31	0.037	*
PCB151/82	3.10	0.08	2.05	0.20	34	0.028	*
PCB149	7.46	0.12	5.49	0.59	26	0.019	*
PCB118	8.03	0.11	5.79	0.73	28	0.005	**
PCB153	13.02	0.17	10.54	1.02	19	0.047	*
PCB138	13.55	0.17	11.00	1.10	19	0.058	
PCB158	0.97	0.02	0.74	0.06	24	0.026	*
PCB187	5.27	0.22	4.48	0.38	15	0.095	
PCB183	1.94	0.03	1.59	0.13	18	0.025	*
PCB177	1.76	0.03	1.53	0.12	13	0.201	
PCB180	5.73	0.07	4.97	0.42	13	0.043	*
PCB170	2.30	0.02	2.01	0.17	12	0.121	
PCB201	1.21	0.02	1.06	0.06	12	0.045	*
PCB194	0.74	0.04	0.66	0.10	11	0.045	*

Probability levels:

*	<0.05
**	<0.01
***	<0.001

a value of zero is in place of any non-detect congeners across time

Chapter 5: Bioamplification as a non-steady state bioaccumulation mechanism for persistent organic pollutants (POPs) in wildlife.

General Introduction

Bioaccumulation describes the process by which anthropogenic chemicals are consumed by organisms from their environment and diet and subsequent assimilation into somatic tissues (Table 5.1) (Mackay and Fraser 2000, Arnot and Gobas 2003, Borga et al. 2004). Chemical toxicokinetics dictate that bioaccumulation is primarily regulated by the imbalance between chemical uptake and elimination for an individual. Thus, this process becomes central to ecotoxicology as it helps define the maximum concentration that can be achieved by an organism relative to the exposure media and also to determine the potential dose/ toxicity of the chemical to an individual. Understanding the dynamic processes that regulate the magnitude of bioaccumulation achieved by animals is therefore essential for the protection of species, ecosystems and ultimately human health (Arnot and Gobas 2004, Kelly et al. 2004).

Persistent organic pollutants (POPs) such as dichloro-diphenyl-trichloroethane (DDT) and polychlorinated biphenyls (PCBs) exhibit very high bioaccumulation potentials (Fisk et al. 1998, Kelly et al. 2007) and exemplify the range of chemicals that demonstrate food web biomagnification (Woodwell 1967, Thomann and Connolly 1981, Oliver and Niimi 1988). The development of bioaccumulation models explaining the behavior of POPs in organisms, food webs and ecosystems evolved as new bioaccumulation mechanisms became uncovered. For example, bioaccumulation models of classic POP compounds now incorporate many diverse concepts including; hydrophobicity-driven equilibrium partitioning (Neely et al. 1974, Hawker and Connell 1988), bioavailability constraints related to chemical sequestering in abiotic organic and inorganic carbon matrices (Black and McCarthy 1988, Cornelissen et al. 2005, Lohmann

et al. 2005), biomagnification related to chemical exposure from food, and complex food web feeding relationships (Campfens et al. 1997, Morrison et al. 1997, Fisk et al. 2001, Alonso et al. 2008) and biological vectors as sources of POPs to and from ecosystems (Ewald et al. 1998, Krummel et al. 2003, Gregory-Eaves et al. 2007). More recently, biological mechanisms associated with species' life histories have also been demonstrated to have a substantial impact on the extent of bioaccumulation achieved by animal species. However, these mechanisms are rarely accounted for within the mathematical framework of current bioaccumulation models.

The focus of this review is to draw attention to a non-steady state, non-equilibrium mechanism of bioaccumulation herein described as bioamplification and applied to POPs. The term bioamplification was coined to define the condition when an organism loses body weight and chemical partitioning capacity at a faster rate than it can eliminate contaminants (Daley et al. 2009, 2011). Like biomagnification, bioamplification describes a non-equilibrium condition where the chemical potential (fugacity) in an animal exceeds that of its environment. However, bioamplification is not associated with chemical uptake but rather the increases in chemical potential that occur within an organism's tissues are due to changes in the partitioning capacity of the animal. Specifically, bioamplification is a consequence of weight and lipid loss similar to the mechanism of solvent depletion described by MacDonald et al. (2002). This review provides a brief introduction to basic bioaccumulation concepts such as bioconcentration and biomagnification in order to contextualize bioamplification, followed by an examination of a series of case studies demonstrating the generality of bioamplification under laboratory and field conditions in a variety of animal species.

Bioaccumulation Concepts and Models

The inception of the field of POP bioaccumulation and ecotoxicology primarily followed the publication of Rachel Carson's *Silent Spring* (1962) whereupon organochlorine pesticides, specifically DDT, were demonstrated to bioaccumulate to high concentrations in non-target animals at upper trophic levels. Although *Silent Spring* focused on food web biomagnification of organochlorine pesticides and the wildlife toxicity implications as a result of such exposures, the mechanism associated with POP bioaccumulation was at the time not well understood. Following the publication of *Silent Spring*, the first bioaccumulation models and validation datasets focused on food web transfer of POP compounds (Harrison et al. 1970). Harrison et al. (1970) described 'magnification' of DDT residues in food webs to be a consequence of low trophic transfer efficiencies of ingested food coupled with high chemical assimilation and low chemical elimination. Thus, while only a fraction of ingested food consumed by organisms is converted to biomass and most of the ingested food is lost by excretion and respiration, chemical assimilation and retention remains high leading to a concentration effect in the organism's tissues. However, these observations were not considered coherent with equilibrium partitioning theory commonly applied in environmental fate and distribution models of POPs and which formed the basis for the first theoretically-based bioaccumulation models (Hamelink et al. 1971, Neely et al. 1974).

Under equilibrium partitioning theory, all organisms inhabiting a given environment are assumed to approach or potentially achieve equilibrium with the chemical concentration in the major exposure media (Clayton et al. 1977, Pavlou and Dexter 1979). Movement of hydrophobic chemicals across biological membranes such as

respiratory surfaces or the gastrointestinal tract is hypothesized to occur by passive diffusion (Gobas et al. 1986) and therefore chemical flux into the organism should proceed up until equilibrium, but not exceed equilibrium concentrations with the exposure media (Veith et al. 1979). As such, the biomagnification observations described by Woodwell et al. 1967 and others were considered an artifact of differences in partitioning capacity of higher trophic level organisms compared to lower trophic ones (Hansch 1969, Hamelink et al. 1971, Neely et al. 1974, Leblanc et al. 1995).

Differences in the partitioning capacity of animals and their respective tissues are commonly calculated on the basis of the neutral lipid content of the sample (Mackay and Paterson 1981, Drouillard et al. 2004). Correcting or normalizing for lipid content provides a surrogate measure of chemical potential (fugacity) in the sample and also the ability to contrast the extent of chemical bioaccumulation among biological samples (Clark et al. 1988, Clark et al. 1990, Mackay 1991). Similarly, the quotient of the lipid normalized chemical concentration in an organism relative to that in water is defined as the lipid normalized bioconcentration factor (BCF) and in theory is equivalent in magnitude to the *n*-octanol/water partition coefficient of the chemical for persistent chemicals and non-growing organisms (Chiou 1985, Mackay and Fraser 2000). Equilibrium partitioning models have shown a high degree of success for predicting POP bioaccumulation under laboratory bioconcentration tests where water is the predominant exposure route (Mackay 1982, Di Toro et al. 1991, Meylan et al. 1999, Landrum et al. 2001). Under field conditions, the equilibrium partitioning bioconcentration model appears to be best suited to negligibly metabolized hydrophobic organic chemicals over a log K_{OW} range of approximately 3 to 5 (Meylan et al. 1999, Gobas and Morrison 2000b,

Barber et al. 2008). However, for increasingly hydrophobic POPs, equilibrium partitioning theory typically underestimates the extent of bioaccumulation that can be achieved by an animal (Connolly and Pedersen 1988).

In their seminal paper, Connolly and Pedersen (1988) challenged the equilibrium partitioning paradigm by demonstrating that certain POPs achieve chemical concentrations within organisms that exceed the expected equilibrium concentration based on chemical concentrations measured in water. Furthermore, Connolly and Pedersen (1988) demonstrated that food web biomagnification of these POPs occurs such that chemical potential increases with increasing trophic status of the organism. This led to a re-definition of the term biomagnification as it applies to hydrophobic organic compounds, whereby biomagnification is defined to occur when the chemical potential in the animal becomes greater than its food and respired media (Connolly and Pederson 1988; Drouillard 2008). Significantly, food-web biomagnification of POP compounds has been widely demonstrated for multiple animal species from both aquatic (Oliver and Niimi 1988; Russell et al. 1999a) and terrestrial (McLachlan 1996, Kelly et al. 2001, Kelly and Gobas 2003, Czub and McLachlan 2004) food webs.

In an effort to predict and understand patterns of POP biomagnification, one of the most widely accepted theoretical models is described by the gastrointestinal magnification (GI) model initially developed by Gobas et al. (1988). This model recognizes food as the primary exposure route for most animals to POPs and also defines intestinal absorption of ingested food as one of the key components contributing to their biomagnification (Gobas et al. 1988, 1993ab, 1999). This model has subsequently been expanded into a food web bioaccumulation model (Thomann et al. 1992, Campfens and

Mackay 1997, Morrison et al. 1997, Arnot and Gobas 2004, Kelly et al. 2004) and has been widely applied to predict trophic transfer and biomagnification of POPs in aquatic and terrestrial food webs. The GI model, while undergoing constant improvement in algorithm formulations (Arnot and Gobas 2004), is one of the most widely applied predictive models for understanding biomagnification patterns in aquatic and terrestrial food webs. In food web applications, the GI- model has the capacity to predict non-equilibrium, steady state chemical bioaccumulation across multiple trophic levels. Food web models of this type are almost always solved under steady state conditions. Under this consideration, the requirement for model initiation is omitted and the steady state assumption forces many toxicokinetic parameters included within the model to become constants. This latter constraint also reduces model complexity and the need for additional calibration. A consequence of the above assumption is that steady state GI-magnification models by default attribute non-equilibrium bioaccumulation for hydrophobic, non-metabolized POPs to be GI-magnification, and/or the interaction between GI-magnification and trophic transfer via feeding relationships within a simulated food web.

However, growing evidence indicates that non-steady state bioaccumulation not only exists, but represents a normal condition for highly hydrophobic POPs bioaccumulation over the life span of many organisms (Kiriluk et al. 1995, Paterson et al. 2007, Czub and McLachlan 2007, Burtnyk et al. 2009, Volta et al. 2009). Under non-steady state bioaccumulation, animals may still achieve chemical potentials in excess of equilibrium predictions, but are not likely to achieve the maximum biomagnification potential predicted for the species under a constant diet within their lifetimes (Paterson

2006, Drouillard et al. 2012). Under these conditions, the toxicokinetic parameters governing chemical uptake, elimination and inter-tissue distribution interact with changing environmental conditions. Specifically, environmental parameters such as temperature, habitat and food resource availability, and foraging behaviours and associated changes in activity costs that can negatively impact growth and metabolic rates have the potential to remove the animal from the steady state condition (Sijm et al. 1992, Goyke and Brandt 1993, Skogland et al. 1996, Roy 1999, Paterson et al. 2006, Paterson et al. 2007ab). From this perspective, GI-model predictions completed under the assumption of steady state can either under- or over predict the maximum extent of chemical biomagnification possible for an animal during its life history.

Here, it is postulated that under a wide range of circumstances animals may achieve chemical potentials that exceed the maximum biomagnification predicted for an animal via gastrointestinal magnification. This can occur under the situation when an animal, at steady state with its environment, experiences a reduction in partitioning capacity faster than it can lose chemical mass. This condition results in an increase in chemical potential such that the magnitude of the predicted biomagnification is amplified by the loss of partitioning capacity and is herein defined as bioamplification. Therefore, bioamplification can result in a sudden shift from steady state to non-steady state conditions especially when coupled with slow chemical elimination kinetics that prevents rapid re-establishment of steady state (Clark et al. 1988). Bioamplification may also occur during the uptake portion of the non-steady state bioaccumulation curve. In this case, bioamplification increases the chemical potential of the animal over what would be the normal non-steady state uptake trajectory that would be experienced without a

partitioning change experienced by the animal. Bioamplification is more difficult to distinguish during the uptake phase of non-steady state bioaccumulation and requires high resolution sampling along with simultaneous determination of animal partitioning capacities as well as characterizing changes in the mass balance of chemical in the animal.

A characteristic of bioamplification is that it is a consequence of weight loss, specifically negative growth and reduction in animal partitioning capacity for the chemical of study. Although growth dilution has long been adopted within bioaccumulation models, growth is commonly assumed to be constant (Clark et al. 1990, Gobas et al. 1993). Yet growth is well known to be highly dynamic over the life history of animals and highly influenced by ecological and physiological factors (Hickie et al. 1999, MacDonald et al. 2002, Blais et al. 2003, Chiuchiolo et al. 2004, Czub and McLachlan 2004, deBruyn and Gobas 2006, Norstrom et al. 2007, Ng and Gray 2009). Weight loss by organisms is less well studied in ecosystems but is known to occur during restricted periods in the life history of many animal species. These weight loss events are typically the driving mechanism of bioamplification, which thus represents the opposite of growth dilution (Clark et al. 1990). Unless terminating in the death of the animal, weight loss is always a temporary condition and is therefore often ignored or negated in models by reducing the long term growth rate of the organism under study. Thus, bioamplification is typically not considered during the development of steady state bioaccumulation models. Arguably, this exclusion of bioamplification within bioaccumulation model framework has contributed to the lack of study of this

phenomenon in natural systems and its relegation to anecdotal observations (Gabrielsen et al. 1995, MacDonald et al. 2002).

Bioamplification, in common with other bioaccumulation processes, will be attenuated by chemical elimination. This places restrictions on the types of chemicals and organism for which the degree of bioamplification is likely to occur. While bioamplification will always occur during weight loss, the extent is determined by the rate of chemical elimination compared with the rate of loss of lipid equivalents. From the perspective of anthropogenic pollutants, in aquatic food webs, bioamplification will be maximized for chemicals that exhibit high hydrophobicity, given that hydrophobicity inversely correlates with chemical elimination (Paterson et al. 2007, Kelly et al. 2007) and for chemicals that undergo little to no metabolic biotransformation (Rasmussen et al. 1990, Safe 1994, Boon et al. 1994). Consequently, bioamplification will be most prominent for those chemicals of high log K_{OW} (> 6.5) that also exhibit food web biomagnification (Clark et al. 1988). While, for the terrestrial food webs, chemicals with a log K_{OW} >2 and a log K_{OA} (octanol-air partition coefficient) >6 should also be considered for high bioamplification potential, because of their slow rate of respiratory elimination (Kelly et al. 2007). From the organismal perspective, bioamplification is expected to occur in those species that 1) exhibit pronounced weight or lipid loss at specific times during their life cycle and 2) exhibit slow elimination kinetics of chemicals relative to the time frame where weight loss occurs. In the latter case, the extent of bioamplification achieved by an individual is expected to be correlated with animal body size, inversely related to the metabolic biotransformation capacity of the animal and in

most cases, be greater for terrestrial compared to aquatic organisms (Fisk et al. 1998, Drouillard and Norstrom 2000, Paterson et al. 2007; Kelly et al. 2001, 2003, 2007).

Bioamplification simulations

Similar to the ecotoxicological formulations for quantifying chemical bioconcentration (BCF), bioaccumulation (BAF) and biomagnification (BMF) factors, the degree of bioamplification can be characterized by expressing the ratio of chemical potential in an animal ($C_{org(t)}$) relative to a reference state (Table 5.2). Specifically, the reference state refers to the chemical potential of the animal prior to the weight loss event ($C_{org(t-1)}$). Thus, the bioamplification factor (BAmF) is herein defined as the ratio of lipid equivalents concentrations between two life stages according to:

$$BAmF = \frac{C_{org(t)}}{C_{org(t-1)}} \quad (1)$$

If the animal has achieved steady state with its environment prior to the initial sampling event, a BAmF > 1 provides evidence of bioamplification. However, under non-steady state bioaccumulation conditions, such as during the uptake phase of the chemical bioaccumulation curve, the interpretation of BAmF's requires some caution. Under non-steady state uptake conditions, the BAmF will always exceed 1 even in situations where bioamplification does not occur. Here bioamplification can be distinguished from bioconcentration and biomagnification processes by considering both the chemical mass balance and the BAmF. In cases where the magnitude of the BAmF >1 and also exceeds the change in chemical mass balance in the organism following and prior to weight loss, bioamplification will have occurred. For example, such a condition may occur when an

organism's feeding rate declines to the extent that it is insufficient to meet the basal metabolic demands associated with the weight loss event. Finally, under conditions of net chemical elimination which may occur for an animal following a diet switch to a less contaminated diet, BAmFs > 1 are always indictative of bioamplification.

To demonstrate conditions in an animal's life history when bioamplification may be observed, Figure 1 provides a set of bioaccumulation simulations under differing weight loss scenarios. In Fig. 1a, the base bioaccumulation simulation for a 100 g fish approaching steady state with chemical present at constant levels in its water and diet over a four year period at constant and optimal temperatures is depicted. This simulation applies to a chemical of log K_{OW} = 6.5 that is negligibly biotransformed by the animal. The fish is exposed to a constant dissolved water concentration of 0.05 ng/L and fed a diet containing 5% lipids and chemical concentration of 7.6 ng/g wet weight that is in equilibrium with water. Bioenergetic terms including gill ventilation, feeding and fecal egestion rates were obtained from Drouillard et al. (2009). Toxicokinetic parameters used to estimate daily uptake and elimination rates were derived from recommended values presented in Arnot and Gobas (2004). In the base simulation, the animal maintains a steady lipid content (9%) and also a consistent body weight over time such that weight loss does not occur. Under this scenario the animal is predicted to achieve the 95% steady state concentration with the exposure media after 854 days (d) with chemical mass balance closely following this profile over the simulation. Maximum lipid normalized BCF and BMF values of 6.91 and 2.55, respectively, were reached by the animal after 1500 d.

The second simulation (Fig. 1b) included occurrences of weight loss for the animal. Specifically, bioaccumulation simulations included the loss of 29% of total body lipids over a 2 week period followed by a 2 week growth event when the animal subsequently regains the lost lipid mass. Weight loss events were included at 3 time periods in the simulation commencing on days 50, 300 and 1400 and growth events were only simulated to occur following these incidences. Elimination rate coefficients quantifying chemical elimination to water and feces were also allowed to vary as a function of the changing partition capacity of the animal as dictated by lipid content. Model simulations predicted three spikes in lipid normalized chemical concentration during the 1500 d time frame. During the first simulated weight loss event at day 50, the maximum BaMF achieved was 1.74 within this two week period and the chemical mass balance ratio in the animal post- vs. pre-weight loss event was 1.24. For the second weight loss event, the animal achieved a maximum BaMF of 1.43 while the ratio of chemical mass in the animal over the 28 day simulation interval was 1.02. During the last weight loss event, the maximum BaMF was 1.39 and exceeded the steady state biomagnification factor. The ratio of chemical mass in the animal was 0.99 over the same time period. Although the change in partitioning capacity of 1.41 for the animal was greatest during the third weight loss event, the apparent BaMF was greatest during the first weight loss event owing to high net chemical uptake into the animal at this early stage of bioaccumulation. Importantly, since the BaMF for each weight loss event exceeded the ratio of chemical mass in the animal over the same time interval, bioamplification is interpreted to occur during each event. Notably, the BaMF determined for the third weight loss scenario was slightly less than the change in animal partition

capacity. This is due to additional chemical elimination that occurs when chemical potentials (fugacities) become maximized under high bioamplification. This is evidenced by the slight drop in chemical mass outlined by the mass balance curve. Regardless, by the end of this bioaccumulation scenario which included the three weight loss events, lipid normalized chemical concentrations in the animal approached the steady state concentration predicted by the baseline model simulation (Fig. 1a) completed in the absence of weight loss episodes.

The last simulation presented in Fig. 1c represents a more complex scenario. For this simulation, fish gill ventilation and feeding rates were allowed to vary over an annual temperature cycle as experienced by larger individuals such as yellow perch inhabiting north temperate latitude waters (Drouillard et al. 2009). These changes cause daily variations in the chemical uptake and elimination flux from the animal in addition to seasonal changes in animal lipid contents as observed for yellow perch held in outdoor mesocosms (Paterson et al. 2007; Drouillard et al. 2009). Under such natural temperature cycles, animal lipid contents achieved a minimum of 4.3 g during the late winter and were maximized at 9.1 g by the end of the fall. For yellow perch, the predicted time to steady state under this bioaccumulation scenario is estimated to be approximately 13 years and thus exceed the average life span of this species (~6 years; Scott and Crossman 1973). The mass balance curve demonstrates that much of chemical uptake occurs during the restricted period when water temperatures are at or near the optimal temperature of the species. However, by day 1500 of the simulation, the chemical mass in fish only reaches approximately 73.5% of the steady state chemical mass established by the base simulation depicted in Fig. 1a. Such protracted bioaccumulation kinetics occur due to

very slow rates of chemical elimination that occur during cold water periods and the relatively small proportion of the annual period when animal metabolic rates are at their maxima (Paterson et al. 2007ab). The bioamplification peaks in Fig. 1c are also predicted to be much broader relative to the punctuated events predicted under the second weight loss simulation (Fig. 1b). These kinetics result from the slower time frames over which weight loss and weight gain occur under more natural growth conditions. Additionally, weight loss is maximized in the late winter at a time when uptake and elimination rates are also minimized (Drouillard et al. 2009). As a consequence, negligible loss of chemical mass occurs in contrast to that predicted in the second simulation (Fig. 1b) following the weight loss events. The amplitude of the concentration peaks for the third simulation were also greater compared to those predicted in Fig. 1b primarily due to the larger degree of lipid loss (2.1 fold difference in lipid over the year). Significantly, such substantial changes in animal partitioning capacity are predicted to facilitate lipid normalized chemical concentrations in the animal by year three of the simulation that exceed the steady state baseline concentration. This is predicted to occur even though the animal is several years from achieving steady state with its water and food.

The above simulations represent increasingly complex bioaccumulation scenarios that use established bioaccumulation model algorithms under non-steady state conditions. These simulations by no means bracket the potential complexity of bioamplification events and the myriad of interactions between bioamplification with bioconcentration and biomagnification that occur in nature. However, the above simulations do serve to illustrate that bioamplification can contribute to peaks in lipid normalized chemical concentrations under realistic weight loss events as well as substantial variation in

residues of animals over their life history. Importantly, the simulations show that steady state can easily be falsely interpreted to occur for a species if samples are collected at coarse time scales and without regard to the timing of sample collections for different cohorts. Unfortunately, because fish and many animals are routinely sampled destructively, it becomes difficult to distinguish individual variability from bioamplification and also steady state from non-steady steady state bioaccumulation under natural conditions. From these perspectives, the application of higher resolution sampling frequencies to include temporal cycles of bioaccumulation and multiple cohort studies would provide powerful insights into the mechanisms contributing to the variation observed in chemical bioaccumulation in natural populations. Lastly, expressing chemical bioaccumulation using both mass balance and lipid normalized metrics would greatly improve the ability to capture such fluctuations in chemical bioaccumulation kinetics.

Animal Energetics

Animal energetics encompasses the processes through which individuals acquire, assimilate and allocate their food resources (McNab 2001, Humphries et al. 2004). It is widely recognized that there are direct links between animal energetics and the survival of individual animals (Hobbs 1989, Golet et al. 1998, 2000, Fort et al. 2009). For example, in young fish, winter mortality events are often the result of starvation that occurs during this period of generally lower food availability (Steinhart and Wurtsbaugh 2003, Biro et al. 2004). However, changes in body proximate composition such as lipid and/or protein losses that occur during the winter months not only affect the survival to spring but can also affect future life history events. For salmonid species including

brown trout (*Salmo trutta*) and Kokanee salmon *(Oncorhynchus nerka*), individuals failing to reach a threshold lipid or body size may not be able to meet the energetic demands of smolting or migration events (Jonsson and Jonsson 1998, Steinhart and Wurtsbaugh 2003). Winter can also have direct consequences for adult fishes. For example, in salmonid species such as brook trout (*Salvelinus alpinus*), individuals are nutritionally compromised entering the winter season owing to intensive fall spawning activities (Webster and Hartman 2007). The lack of food availability can also impact future reproductive success in fall spawning salmonids such as lake trout (*Salvelinus namaycush*; Henderson and Wong 1998). For homeotherms, winter periods represent a unique set of challenges owing to the generally higher metabolic costs associated with endothermy and thus novel approaches to enduring such events must be evolved (Speakman and Rowland 1999).

Overwintering is a wide-ranging term that defines an animal's response for survival during the prolonged sub-optimal cold temperature exposures that are associated with winter months. These responses can include specific strategies such as migration and hibernation (Yahner 2012). A common strategy among birds is to undergo long distance migrations to more productive climates with more widely available food resources in order to avoid the energetic challenges associated with winter in the home range. Another strategy is to enter hibernation, which greatly reduces the metabolic functioning and thus energetic requirement of an animal during winter. However, each of these overwintering strategies employed by animals still incurs energetic demands. Some animal species inhabit these highly variable environments year round, where nutrient abundance and quality are inconsistent and periods of starvation are common (Kirk

1997). This becomes especially true for species inhabiting north temperate or Arctic climatic zones where cold temperature periods can extend for several months and dominate the annual temperature cycle (Webster and Hartman 2007, Fort et al. 2009). The combinations of low temperatures, decreased food and water availability and potential ice cover conditions all represent instances of resource shortages to animals experiencing overwinter events. Accordingly, animals need to evolve strategies that permit the acquisition and assimilation of sufficient food energy resources to not only meet current demands but to also sustain metabolic requirements during such energetic bottlenecks.

Energetic bottlenecks are a widespread occurrence in natural ecosystems and populations as both physiological and ecological stressors commonly lead to instances of negative energy balance for many taxonomic groups. While winter represents a common energetic bottleneck for animals inhabiting north temperate and extreme latitude environments, there exists a number of energetic bottlenecks that are driven by a range of physiological events in various animal species' life histories. Reproduction is often a period when an animal must increase the extent of energy investment in order to achieve a higher degree of reproductive success and also for rearing of young (Golet et al. 2000, Lambert and Dutil 2000, Bech et al. 2002). Frequently, these energetic costs can increase the risk of mortality for mature individuals and may also compromise the success of future reproductive activities (Golet et al. 2000). For anuran species with complex life cycles, metamorphosis represents a critical life history stage when the animal transitions from an aquatic larval to a terrestrial adult form (Orlofske and Hopkins 2009). During this transition, the physiological reorganization of feeding structures and the digestive

tract represents substantial metabolic expense and are also accompanied by a significant reduction in feeding activities and nutrient assimilation efficiency (Gutleb et al. 2000, Leney et al. 2006). In addition to the need to meet the energetic demands of metamorphosis, transformed individuals will also be entering new habitats and facing challenges with respect to predation and competition for food resources (Alvarez and Nicieza 2002). Fasting is also common to a range of life history events including migration, metamorphosis and reproduction (deBruyn et al. 2004, Leney et al. 2006, Golet et al. 2000). Examples of fasting exist in nearly all major taxonomic groups and can often be extended for prolonged periods of time (Wang et al. 2006).

To compensate for the metabolic demands posed by such energetic imbalances, animals are frequently required to rely on accumulated somatic energy reserves. Lipids represent the most calorically rich tissue reserves providing up to eight times more total energy relative to protein and carbohydrate stores while also generating water during catabolism (McWilliams et al. 2004). Consequently, animals that maximize their lipid reserves prior to periods of nutritional stress are those most likely to successfully endure the event. The rates at which lipids are mobilized during an energetic bottleneck will depend on key factors including an animal's basal metabolic rate, as influenced by thermoregulation, and also the duration of the bottleneck itself (Humphries et al. 2004). Although lipids are a critical component for surviving energetically demanding events, large protein stores are required for muscle repair and, for species undergoing periods of intense exercise (i.e. migrations) (Golet and Irons 1999). Body size also becomes important as larger bodied individuals can often withstand prolonged starvation events due to their higher ratio of somatic energy reserves to metabolic rate (Bystrom et al.

2006). From these perspectives, the bioamplification of hydrophobic organic chemicals is predicted to occur frequently in nature as the mobilization of somatic lipid stores represents the primary response of animals under conditions of energetic imbalance.

Bioamplification Case Studies

Embryo and juvenile development

In addition to representing a highly dynamic period with respect to tissue differentiation and animal development, embryo development for oviparous species is characterized by a dependence on maternally provided food resources. Therefore, during this period, the mobilization of endogenous lipid and protein reserves by the developing embryo has the potential to generate conditions suitable for the bioamplification of POPs (Kleinow et al. 1999). Over the past 10 years, evidence for such embryonically derived bioamplification has been observed among avian, fishes and mammalian species. For example, Drouillard et al. (2003) developed a bioenergetic model for herring gull (*Larus argentatus*) embryos during the egg incubation period which predicted a steady decline in egg lipid mass over the incubation period. During this life history stage, lipid normalized PCB concentrations quantified in developing chicks generally increased as a function of incubation date (Drouillard et al. 2003). Significantly, for pipping chicks, lipid normalized PCB concentrations were higher than those quantified in the egg laying females (Drouillard et al. 2003). However, following hatching and the initiation of exogenous feeding, growth dilution quickly attenuated the extent of derived bioamplification (Drouillard et al. 2003).

For fish species such as yellow perch (*Perca flavescens*) and Chinook salmon (*Oncorhynchus tshawytscha*), the duration of the egg incubation period can range from only a few days in yellow perch to over 100 days (depending on water temperature) in some Pacific salmon, with life history characteristics differing substantially between these species during embryo development (Scott and Crossman 1973). Yellow perch spawn in the spring and are iteroparous with the larval stage emerging as free swimming individuals that are capable of external feeding (Post and McQueen 1988). In contrast, Chinook salmon are semelparous and spawn in the fall and allocate substantial maternal resources into eggs in order to survive the prolonged ice cover period (Kaitaranta and Ackman 1981, Bull et al. 1996, Wiegand 1996, Russell et al. 1999). Following hatching, larval Chinook salmon tend to remain on the gravel substrates and are nourished primarily by endogenous yolk reserves as external food resource availability remains low. Daley et al. (2009) quantified changes in yellow perch egg proximate composition and PCB concentrations during embryo development and demonstrated loss of egg dry mass over time in addition to a decline in lipid content. Significantly, PCB concentrations quantified in yellow perch eggs at later stages of incubation were an average 2.7 (range: 1.8-5.4) fold higher than measured in newly fertilized eggs (Daley et al. 2009). For Chinook salmon eggs, no declines in lipid content were observed during the incubation period with no concomitant bioamplification determined (Daley et al. 2012). However, during the free swimming larval stage, juvenile Chinook salmon fry exhibited steady decreases in lipid content over time which resulted in significant increases in the extent of PCB bioamplification with a maximum BaMF of 4.9 determined for fry by the end of yolk resorption (Daley et al. 2012). Bioamplification generated PCB concentrations in

Chinook salmon eggs were 5 - 9 fold greater than the predicted threshold for juvenile salmon at the no adverse effect level (Meador et al. 2002, Johnson et al. 2007, Daley et al. 2012). A similar magnitude of bioamplification has been observed in developing sole (*Solea solea*) larvae whereby late yolk sac stage larvae had approximately 8 fold higher lipid normalized PCB concentrations relative to newly laid eggs (Foekma et al. 2012).

Bioamplification related increases in POP concentrations have also been observed during the development of juvenile grey (*Halichoerus grypus*) and northern elephant (*Mirounga angustirostris*) seals (Addison and Stobo 1993, Debier et al. 2006). Northern elephant seal pups are generally abruptly weaned from the mother but can fast for up to 2.5 months and potentially lose up to 30% of their post-weaning body mass while fasting (Debier et al. 2006). During this period, lipid normalized PCB concentrations quantified in blubber samples from elephant seal pups exhibit bioamplification in addition to elevated blood serum PCB levels (Debier et al. 2006). Bioamplification related increases in PCB concentrations have also been demonstrated during the post weaning fast period in the blubber tissues of juvenile grey seals ranging from 0 - 13 months of age (Addison and Stobo 1993). Similar to the growth dilution observed for herring gull chicks on initiation of feeding, decreases in POP concentrations were observed for grey seal pups with the resumption of feeding (Drouillard et al. 2003, Addison and Stobo 1993).

These studies demonstrate that bioamplification typically generates chemical concentrations that increase during embryo development until independent external feeding is initiated by the animal. Such bioamplification trends can also be log K_{OW} dependent whereby more hydrophobic chemicals achieve the greatest BaMFs during

larval development (Daley et al. 2012). These highly hydrophobic chemicals are those that typically exhibit food web biomagnification and thus have the potential to induce toxicological effects (Connolly and Pedersen 1988). Embryonic and larval development also represents one of the most sensitive periods in an animal's life history with respect to the potential toxicological effects of chemical exposure. As such, bioamplification likely represents an important mechanism mediating chemical toxicity during this critical developmental period.

Metamorphosis

Metamorphosis represents a complexity of changes in the biochemical, metabolic and physiological functioning of the animal species that experience such transformations. For amphibians, this critical life stage transition also represents a period when bioamplification mechanisms play an important role in chemical kinetics within animal tissues. In an experimental study using green frog (*Rana clamitans*) tadpoles, Leney et al. (2006) dosed individuals with a PCB Aroclor mixture approximately three weeks prior to the onset of metamorphosis. Owing to the lack of tadpole feeding during metamorphosis, the mobilization of endogenous lipids was observed during this period, along with bioamplification of the PCB dose (Leney et al. 2006). Bioamplification factors were maximized during tadpole metamorphosis by approximately 4.5 fold for the most hydrophobic PCB congeners (Leney et al. 2006). Aquatic insects have also been observed to demonstrate bioamplification during metamorphosis from pupae to larval life stages. Bartrons et al. (2007) demonstrated an approximate 3 fold increase when comparing mean dry weight POP concentrations between pupae and larvae stages across four species of aquatic invertebrates. It was concluded that the increases in POP

concentrations were due to the lack of feeding and subsequent loss of body weight during metamorphosis (Bartrons et al. 2007). During metamorphosis from juvenile instar to adult life stages, non-feeding emergent chironomids have been observed to bioamplify PCB concentrations approximately 4.6 fold (Larsson 1984). Similarly, Harkey et al. (1992) documented increased concentrations of the pesticide chlordane in adult *Chironomus decorus* following metamorphosis. Given the wide range of invertebrate species that undergo multi-stage metamorphosis, it is likely that this phenomenon is commonly observed across this taxonomic group. For amphibians, there also exist substantial differences with respect to the duration of the tadpole life stage across species (Leney et al. 2006). Therefore, the magnitude of bioamplification that can occur during this critical life stage is likely correlated with the duration and timing of metamorphosis (Leney et al. 2006).

Reproduction

Reproduction represents a substantial energy investment for most taxonomic groups and often encompasses substantial periods of feeding, fasting, and, for mammalian species including phocid seals, a lactational period. For example, female elephant seals follow a prolonged feeding period with a 3 month period that includes fasting, competition for mates and breeding (Schneider 2004). In some phocid species, up to 40% loss of body mass can occur during reproductive activities in comparison to the non-breeding season (Lydersen et al. 2002). These species have a unique life history in that females undergo an extensive lactation period which is consequently followed in time by moulting and both of these characteristics incur substantial energetic costs (Nilssen et al. 1995). For these individuals, the highest body condition indices often

occur prior to lactation and breeding (Lydersen et al. 2002). Individual females with poor condition during moulting were determined to have blood sum PCB concentrations that were 7.2 fold higher than measured from individuals having higher body fat content (Lydersen et al. 2002). Increases in serum and milk PCB concentrations have also been determined in blood and milk samples collected from lactating female grey seals (Debier et al. 2003, Sormo et al. 2003). Tissue specific bioamplification has also been documented in lactating female grey seals, whereby PCB concentrations in inner blubber layers were twice those quantified in outer blubber samples (Debier et al. 2003). Critically, the trends in POP concentrations that occur during such reproductive fasting periods are consistent with respect to the characteristics of bioamplification. Specifically, adult harp seals (*Phoca groenlandica*) fasted over a 28-day period were observed to lose up to 24 kg of body mass and to also have exhibited significant increases in blood POP concentrations by the termination of the fasting period (Lydersen et al. 2002). Of particular importance, the weight losses that occur while fasting are often compounded when the animal moults which could lead to even higher BaMFs in nature (Lydersen et al. 2002).

Several avian species including common eiders (*Somateria mollissima*), kittiwakes (*Rissa tridactyla*), and Adelie penguins (*Pygoscelis adeliae*) experience lengthy fasting periods during reproductive activities (Subramanian et al. 1986, Henrikson et al. 1996, Bustnes et al. 2010). Female common eiders in Arctic regions have been demonstrated to lose approximately 25% of their body mass and 35% of lipid reserves during the egg incubation period (Bustnes et al. 2010). Similarly, female kittiwakes can lose up to 20% of their body mass from the pre-breeding to late chick

rearing stages of reproduction (Henrikson et al. 1996). For these species, the bioamplification related changes in lipid normalized POP concentrations ranged from 1.7 - 8.2 fold across tissues including blood, liver, brain, and adipose lipid reserves (Henriksen et al. 1996, Bustnes et al. 2010). The data of Subramanian et al. (1986) indicate that maximum BaMFs of 3.5 and 2.8 can be achieved by male and female Adelie penguins, respectively, during the reproductive fasting period. Subramanian et al. (1986) also observed similar bioamplification related changes in POP concentrations among liver, muscle and brain tissues in Adelie penguins during reproduction. Many bird species occupy a wide range of habitats which may increase the extent of chemical exposure to the animal and potentially increase the extent of bioamplification achieved during fasting events (Bustnes et al. 2010). Similarly, the duration of reproductive stages including egg incubation can range greatly among avian species which is also hypothesized to increase the magnitude of bioamplification achieved by the species (Bustnes et al. 2010).

Reproductive activities also often include courtship rituals and rigorous competition between male individuals in efforts to ensure mating success. In the mayfly genera *Hexagenia* spp. male individuals engage in intensive mating swarms that lead to significant reductions in lipid reserves between the sub-imago and imago life stages (Daley et al. 2011). Mayflies have a unique life history in that the majority of their lifespan in spent burrowed in lake and river sediments from where they emerge as sub-imagos and rapidly moult into their final reproductive stage as adult imagos (Edmunds et al. 1976). Adult male Ephemeropteran mayfly species including *H. limbata* and *H. rigidia* also do not feed following their emergence and rely exclusively on accumulated

lipid reserves during the search for females. Daley et al. (2011) demonstrated that during this reproductive flight, male individuals lose up to 50% of their lipid reserves in contrast to females which may lose < 10%. Further, lipid normalized PCB congener concentrations quantified in male mayflies were observed to bioamplify by roughly a factor 2 during reproductive swarm events. This contrasts BaMFs of approximately 1 determined for PCB concentrations in female mayflies which do not participate in the reproductive swarms (Daley et al. 2011).

Overwintering

In addition to the metabolic challenges associated with thermoregulation for both poikilo- and homeotherms, reduced food availability often accompanies the extended cool – cold temperatures associated with winter months. For many animal species, metabolism is reduced to basal maintenance levels during overwintering and, consequently, seasonal lipid depletion is a common characteristic for animal survival, especially at extreme polar latitudes (Jorgensen et al. 2002). Bioamplification is therefore predicted to occur in most animals in the winter as the general reductions in metabolic rate by animals during overwinter survival are often accompanied by a lack of capacity for chemical elimination (Buckman et al. 2007ab, Paterson et al. 2007).

For extreme latitude fish species such as Arctic charr (*Salvelinus alpinus*), lipid reserves can be reduced up to 80% during the overwinter period (Jobling et al. 1998). Jorgensen et al. (1999) demonstrated experimentally that PCB concentrations in the tissues of starved Arctic charr demonstrate bioamplification. Further, redistribution of the chemical dose into liver and brain tissues also occurs during overwintering by Arctic charr (Jorgensen et al. 2002). Similar observations have been made for sole (*Solea solea*)

whereby up to 4 fold bioamplification of PCB concentrations in liver and muscle tissues occurred during experimental starvation trials and in wild collected overwintering fish (Boon and Duinker 1985). Overwintering mesocosm studies have confirmed the bioamplification of PCBs by overwintering yellow perch (Paterson et al. 2007). Specifically, PCB congener bioamplification factors ranging from 1.7 – 2.3 were generated during the overwintering months consistent with a lack of chemical elimination and depletion of lipid reserves that occurred during the cold water period (Paterson et al. 2007).

Avian and mammalian species including the greater scaup (*Aythya mariya*), bald eagle (*Haliaeetus leucocephalus*) and Arctic fox (*Vulpes lagopus*) have also demonstrated POP bioamplification during the overwinter season. Seasonal lipid mobilization by greater scaup and bald eagles have been demonstrated to bioamplify POPs in muscle and adipose fat tissues up to 5.5 fold during the overwinter periods (Elliot et al. 1996, Perkins and Barclay 1997). Arctic fox rely on sea bird forage during the spring and summer however as these prey items migrate from Arctic regions during the winter, individual foxes often face starvation (Fuglei and Oritsland 1999). During this period, a significant negative relationship between animal lipid content and POP concentrations has been observed which is consistent with POP bioamplification (Fuglei et al. 2007). Similar observations have also been made when comparing POP patterns quantified in winter collected older lean foxes vs. younger fatter individuals (Wang-Andersen et al. 1993).

Hibernation

Hibernation can be generally characterized as a behavioural and/or physiological mechanism invoked by species for enduring prolonged overwinter events and periods of low food availability. While bioamplification is likely to occur to the greatest extent for hibernating species inhabiting high temperate and polar latitude regions, recently it has been recognized that hibernation is not solely confined to these more remote climates and can occur throughout the world as a species response to reduce energy expenditure (Schmid et al. 2000, Dausmann et al. 2009). Current examples of hibernation induced bioamplification include bat (Clark and Prouty 1977, Clark and Krynitsky 1983), amphibian (Angell et al. 2010) and bear species (Christensen et al. 2007), but is also likely to occur for the majority of animal taxa that undergo a state of hibernation, torpor or estivation.

For bear species such as the grizzly (*Ursus arctos horribilis*), hibernation represents a relatively unique state such that animals maintain a body temperature within a few degrees (4-5°C) of normal and the potential for chemical elimination via defecation and urination becomes completely minimized (Svihla and Bowman 1954, Hock 1960, Nelson 1978, Lyman et al 1982). In a study of hibernating grizzly bears, there was an average 2.21 concentration effect for sum PCBs in post-hibernation compared to pre-hibernation bears (Christensen et al. 2007). For POPs such as DDT, which can be metabolized, no significant increases in chemical residue were demonstrated (Christensen et al. 2007).

Unlike other bear species, polar bears (*Ursus maritimus*) remain active over the winter months while searching for prey during the ice cover season. However, polar bears are considered to endure a "walking hibernation" which occurs during the summer months when animals experience lengthy periods of low food availability owing to the lack of ice cover. Polischuk et al. (2002) sampled polar bears in the months of July and August and demonstrated a BaMF of approximately 1.33 fold for lipid normalized sum PCBs during this fasting period relative to animals sampled in the winter from September to November. The highest BaMFs of 1.52 were determined for female bears with nursing cubs (Polischuk et al. 2002). Elevated levels of POPs have also been quantified in blood samples collected from polar bears during the summer months indicating the potential mobilisation of these chemicals into the circulatory system (Polischuk et al. 1995, 2002, Knott et al. 2011). The increase in blood level POPs for females with nursing cubs is of particular concern as the cubs will receive higher contaminant loads while being weaned (Peterson et al. 1993, Polischuk et al. 1995, 2002, Knott et al. 2011).

Some of the earliest observations of POP bioamplification during hibernation were reported for a range of bat species (Clark and Prouty 1977, Clark and Krysnitsky 1983). Hibernation in many bat species commonly occurs from early autumn to spring and the animals typically rely on a combination of fat reserves to survive and recover during this period (Fenton and Barclay 1980). During this hibernation, concentrations of POPs such as the DDT metabolite dichloro-diphenyl-dichloroethylene (DDE) have been demonstrated to bioamplify significantly with the depletion of lipid reserves in hibernating big brown (*Eptesicus fuscus*) little brown (*Myotis lucifugus*) and eastern pipistrelle (*Pipistrellus flavus*) bats (Clark and Prouty 1977, Clark and Krynitsky 1983).

Of particular significance, mortalities in bat populations have been observed coincident with animal arousal from hibernation when lipid reserves are at their lowest and thus the potential for bioamplification maximized (Clark 1981). Northern latitude amphibian species also often respond to harsh and wintering conditions by entering a hibernation state. In a recent study, Angell and Haffner (2010) sampled hibernating green frogs during a hibernation period from the months of October – January in order to quantify changes in PCB dose kinetics and animal lipid contents during this timeframe. Lipid content of frogs declined through the sampling period with maximum BaMFs of 1.4 determined for more hydrophobic (Log $K_{OW} > 6.5$) PCB congeners included in the PCB dose (Angell and Haffner 2010).

Migration

Migration is a ubiquitous life strategy that is demonstrated in nearly all major taxonomic groups including birds, mammals, fishes, reptiles, amphibians and insects. Among these species, fish and birds provide primary examples of extensive long distance migrations that require energetic demands from animals that are sufficient to potentially bioamplify POP concentrations.

Anadromous fishes such as salmonid species often exhibit lengthy migrations in order to return to natal streams and rivers to spawn. For example, the Yukon River Chinook salmon population is known to swim distances over 3000 km during migration (Beacham et al. 1989). Such extensive migrations are demonstrated to be highly costly with up to 90% depletion of somatic lipid reserves occurring for some species during migration to spawning sites (Hendry and Berg 1999). This extensive loss of somatic

energy reserves has been demonstrated to bioamplify POPs including PCBs up to 10 fold in migrating sockeye salmon (*Oncorhynchus nerka*) over the combined duration of the migration and spawning events (Ewald et al. 1998, deBruyn et al. 2004, Kelly et al. 2007). Similar examples of POP bioamplification have also been demonstrated for migrating Chinook and Atlantic (*Salmo salar*) salmon populations (Hansson et al. 2009, Kelly et al. 2011). Of particular significance, the magnitude of PCB bioamplification by salmonid species is positively correlated with both migration distance and chemical log K_{OW} (deBruyn et al. 2004, Kelly et al. 2011). From this perspective, bioamplification may be a contributing factor for understanding the health and decline of North American Pacific salmonid populations (deBruyn et al. 2004).

Numerous bird populations also provide primary examples of lengthy migrations. However, less is known of the extent to which this behaviour influences bioamplification in this group owing to difficulties associated with measuring POP concentrations and body condition during migration events. Migration patterns among bird species also differ substantially resulting in widely different energy demands. For many bird species, migration involves flight over relatively large food limited areas thus providing minimal opportunity for refueling (Klaasen et al. 1996). Additionally, in larger non-gliding migratory species, active flight rather than wind assisted gliding will also serve to more rapidly deplete energy reserves (Klaasen et al. 1996). During such long distance flights, wind direction and climate also play crucial roles in the metabolic costs associated with migratory flight with considerations such as headwinds leading to more rapid use of lipid stores (Piersma 2002, Colabuono et al. 2012). Such rapid depletion of lipid stores during migration has been observed to result in the redistribution of POPs among lipid, liver and

muscle tissues in antarctic migratory species (Colabuono et al. 2012). Critically, overall animal body condition as indicated by lipid content and starvation have been determined to be important factors resulting in the redistribution of POPs in animal tissues during bird migration (Sodergren and Ulfstrand 1972, Colabuono et al. 2012).

Body condition represents a key factor for animal survival during migration events. Similar to Pacific salmonids, the catadromous European eel (*Anguilla anguilla*) often returns to spawning grounds that are thousands of kilometres from feeding areas and the animal tends to fast during the migration (Olivereau and Olivereau 1997). This species can spend between 5 – 18 years in river and coastal feeding grounds prior to migrating to oceanic spawning grounds (Belpaire and Goemans 2007). Lipid normalized concentrations of the organochlorine insecticide lindane have been quantified at 9255 ng/g in pre-spawning European eel populations (Belpaire and Goemans 2007). An experimental study on Euopean eels compared PCB concentrations in resting eels to those swmming along an 800km swim tunnel over a period of 27 days (van Ginneken et al. 2009). van Ginneken et al. (2009) demonstrated that the depletion of lipid reserves in more actively swimming eels resulted in the bioamplification of PCB congeners up to 14 fold higher relative to concentrations quantified in resting individuals. Eels also demonstrate little capacity for the elimination of chemicals during such non-feeding events (Duursma et al 1991). The European eel is an at risk species for conservation and the exceedingly high POP concentrations and low lipid reserves have been identified as factors contributing to stock declines (Belpaire and Goemas 2007).

Other

Fasting and starvation represent relatively consistent biological mechanisms associated with the bioamplification of POPs that occurs with migration, hibernation, and reproduction (Bustnes et al. 2010). For some animal species, aphagia often occurs with the onset of disease and this condition can generate the negative energy imbalance that is consistent with energetic bottlenecks and weight loss. For example, California sea lions (*Zalophus californianus*) exposed to the domoic acid neurotoxin cease feeding and can lose substantial body mass and blubber content (Hall et al. 2008). During the 12 day aphagia period associated with neurotoxin exposure, individuals lost approximately 16% of their body mass and exhibited bioamplification of PCBs, DDT and brominated flame retardant and other POP compounds in blubber tissues (Hall et al. 2008). Other studies on diseased and starved mammals have also demonstrated that the capacity for POP bioamplification exists during this biological state. Stranded sea otters (*Enhydra lutris nereis*) which died from disease and emaciation have been found to have higher lipid normalized DDT concentrations relative to animals that died from acute trauma (Nakata et al. 1998). Similar patterns of POP bioamplification have also been observed in stranded dolphins relative to those caught as by-catch (Chou et al. 2004). The patterns of PCB bioamplification were consistent with higher levels of lipid mobilisation in the diseased and starving animals that were found stranded (Chou et al. 2004). Evidence for POP bioamplification has also been observed in diseased black-backed gulls (*Larus fuscus fuscus*) and little brown bats (Hario et al. 2004, Kannan et al. 2010). In addition, weight loss and starvation experiments have demonstrated POP bioamplification in humans (Walford et al. 1999, Chevrier et al. 2002, Pelletier et al. 2002, 2003, Tremblay

et al. 2004, Jandacek et al. 2008), birds (Ecobichon and Saschenbrecker 1969, Defreitas and Norstrom 1974, Stickel et al. 1984), fishes (Antunes et al. 2007) and rodents (Dale et al. 1962, Ohmiya and Nakai 1977).

Implications of bioamplification

The above studies have been consistent in conveying a central point of bioamplification: POP mobilization from inert lipid reserves results in elevated POP levels in more sensitive animal tissues. Although most bioaccumulation models have been generally formulated to predict bioconcentration and biomagnification, the consequences of bioamplification can be important to hazard and risk assessments of wildlife exposures to POPs. Specifically, any increase in chemical potential within the animals tissues will pose a greater risk to the animal, since critical tissue concentrations are more likely to be exceeded during these time periods (Mackay 1982). Loss of animal fat reserves is typically associated with proportional increases in tissue concentrations such as blood, liver, muscle and brain (Subramanian et al. 1986, Henriksen et al. 1996, Perkins and Baclay 1997, Jorgensen et al. 1999, Lydersen et al. 2002, Debier et al. 2003, 2006, Bustnes et al. 2010). This occurs because the time required to achieve inter-tissue equilibrium is typically short (approximately 1 week; Braune and Norstrom 1989) even though the time to achieve steady state between the organism and its environment may be quite long. Thus equilibrium partitioning often provides a strong predictive framework to describe the between tissue differences in chemical concentrations within a given organism, even if equilibrium partitioning fails to describe differences in chemical potential between individual animals inhabiting a given environment (Drouillard et al. 2004).

The fact that POPs mobilized from tissues may enhance toxicity is well known. Studies involving insects, fishes, and mammals have shown a significant positive relationship between the lipid content of an animal and the magnitude of the LD_{50}, indicating that toxicity increases with a decreasing lipid content of the animal (Geyer et al. 1990, Geyer et al. 1993). Birds have been shown to exhibit enhanced POP toxicity following periods of dramatic weight loss (Ecobichon and Saschenbrecker 1969, Stickel and Stickel 1969, deFreitas and Norstrom, 1976, Gabrielsen, 1995). Van Velzen et al. (1972) observed that exposing birds to a DDT dosage (100, 200 or 300 µg/g) or weight loss due to reduced food availability produced no mortality. However, the combined effects from weight loss and DDT exposure caused significant mortality in brown-headed cowbirds (*Molothrus ater*). PCB exposed fish (low dosage: 25µg/kg food/day and high dosage 2,500 µg/kg food/day) with poor nutritional condition, experienced enhanced negative effects (Quabius et al. 2000). deFrietas and Norstrom (1974) starved pigeons for a period of seven days and observed a net movement of PCBs from the adipocytes into brain, liver and muscle cells. In a study of breeding kittiwakes, Henriksen et al. (1996) showed a steady decrease in body mass and lipids which resulted in a quadrupling PCB concentrations in brain tissues.

Lastly, many energetic bottlenecks occur at critical and highly sensitive periods during an animal's life history and POP bioamplification may ultimately lead to increased chemical toxicity during these susceptible periods. Bioenergetic stress and weight loss effects are expected to correspond to increased stress and enhance possibilities of chemical/stress interactions (Fuglei et al. 2007, Knott et al. 2011). Several POPs are also known to interfere with behavioral responses such as predator avoidance (Weis and Weis

1987, Schulz and Dabrowski 2001). For example, POP bioamplification occurring during larval and/or fry development may serve to reduce the capacity for predator avoidance when these small animals become free swimming individuals and are more susceptible to predation. Further, the switch point from endogenous to exogenous food resources is often associated with elevated mortality and bioamplification during this critical period for larval fish can potentially augment mortality at this time. While stranded cetaceans often have higher total POP burdens relative to healthy animals, it is unknown as to what role bioamplification plays toward mortality (Chou et al. 2004). Amphibian metamorphosis represents a period of substantive changes in gene transcription and translational activities in addition to significant physiological and metabolic reorganization (Kawahara et al. 1991). Subsequently, the potential for chemical/gene expression interference is much greater during metamorphosis relative to other life stages.

Conclusions

This review has demonstrated that energetic bottlenecks and environmental stressors influence POP exposure dynamics over many taxonomic life history strategies. Bioamplification is driven by kinetic mechanisms specifically in that the rate of loss of partition capacity experienced by an animal frequently exceeds the rate of chemical elimination. Bioamplification is therefore a non-equilibrium non-steady state process whereby chemical residues in an animal become concentrated as a result of reductions in chemical partitioning capacity without commensurate loss of chemical mass. As outlined in section 3, bioamplification provides the potential for animals to achieve and/or exceed the magnitude of the steady state concentration prior to the animal actually achieving this

condition with the exposure media (Fig. 5.1c). Consequently, it is highly likely that bioamplification will result in chemical potentials that are above those predicted by current steady state GI- and food web biomagnification models. This review demonstrated that bioamplification factors can approach or surpass BMF's reported in the literature (Table 5.1). For example observed maximum BMFs for POPs in fish species often range from 5-10, whereas migrating salmon and eels have been shown to achieve BAmF's over 10. Additionally larval salmon had an approximate BAmF of 5 for the highest log K_{OW} POPs (Debruyn et al. 2004, Kelly et al. 2004, Kelly et al. 2008, Daley et al. 2012). Christensen et al. (2009) reported maximum BMF's of approximately 5 for PCBs based off the diet of a projected fall grizzly bear and the same author reported similar BaMF's during winter hibernation (Christensen et al. 2007). Even in marine mammals, for which maximum BMFs of greater than 20 have been documented for a range of POPs, bioamplification has the potential to augment these concentrations by a further factor of 7 (Fraser et al. 2002, Lydersen et al. 2002, Debier et al. 2003, 2006, deBruyn and Gobas 2006).

Common life history periods where bioamplification occurs in animal species include embryo development, juvenile life stages, metamorphosis, reproduction, migration, overwintering, hibernation, and disease. This review has summarized a series of such cases in a range of animal taxa including invertebrates, amphibians, fishes, birds and mammals with corresponding bioamplification of POP residues due to the range of energetic bottlenecks experienced by these species. Where most POP bioaccumulation models fail to fully consider the full scope of ecological, climatic and physiological variables regulating POP kinetics and bioaccumulation, several authors have recently

reported the need for both acquiring more robust datasets and integration of more thorough life history information for model calibration and validation (deBruyn and Gobas 2006, Norstrom et al. 2007, Ng and Gray 2009, Muir and de Wit 2010). These needs are not only critical for understanding and predicting POP bioaccumulation and biomagnification in food webs but also for protecting wildlife, ecosystem and human health.

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Table 5.1 Definitions related to the main processes by which there is an increase in chemical concentration or thermodynamic activity in the organism.

Bioaccumulation[1]	The process by which chemicals are taken up and accumulated in the organism and it's tissues by all routes of exposure.
Bioconcentration[1]	The process which describes the organism's chemical exposure through respiratory and dermal surfaces.
Biomagnification[1]	The process by which the chemical concenration in the organism exceeds that of it's diet, as a result of dietary absorption.
Bioamplification	The process by which an organism loses body weight and chemical partitioning capacity at a faster rate than it can eliminate contaminants.

Definitions are modified from Gobas and Morrison 2000[1]

Table 5.2. Common bioaccumulation metrics used to express the concentration ratio of chemical in an organism relative to the concentration of chemical in a given environmental media or food.

Bioaccumulation Factor (BAF)[1]	The ratio of the chemical concentration in the organism to the water concentration ratio during field exposures (often expressed on a wet weight or lipid basis)
Bioconcentration Factor (BCF)[1]	The ratio of the chemical concentration in the organism to the water concentration during laboratory exposures (often expressed on a wet weight basis)
Biomagnification Factor (BMF)[1]	The ratio of the chemical concentration in the organism to that of the concentration in its food (often expressed on a lipids basis)
Bioamplification Factor (BAmF)	The ratio of the concentration between two life stages of the organism (often expressed on a lipid equivalents, lipid or fugacity basis).

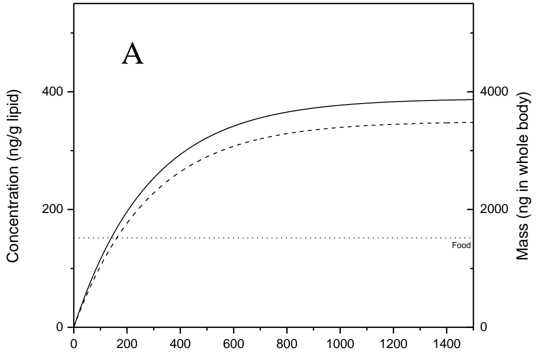
Definitions are modified from Mackay and Fraser 2000[1]

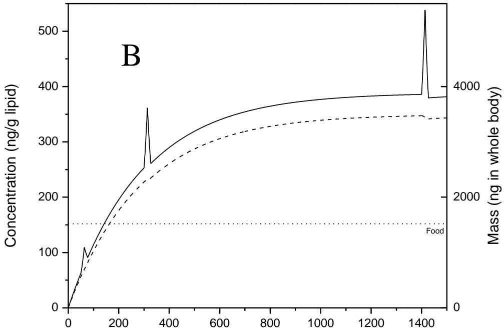
Table 5.3. A list of case studies demonstrating bioamplification across taxonomic groups. [a] BaMFs were calculated or reported using a lipid equivalents ratio from the available data found in the corresponding citation. [b] BaMFs were calculated or reported using a fugacity ratio from the available data found in the corresponding citation. [c] BaMFs were calculated or reported using a lipid normalized ratio from the available data found in the corresponding citation. [e] Chemical acronyms: PCBs (polychlorinated biphenyls), OCs (organochlorines), PCDD/Fs (polychlorinated dibenzodioxins/ furans), PBDEs (polybrominated diphenyl ethers)

Species	Life history	Sex	Field/Experiment	Chemicals[e]	Tissues	$BaMF_{max}$	Reference
Invertebrates							
Mayflies (*Hexagenia* spp.)	Reproductive Flight	M	Field	PCBs	Whole	3.4	Daley et al. 2011[a]
Amphibians							
Green frogs *(Rana clamitans)*	Metamorphosis	M/F	Experiment	PCBs	Whole	4.3	Leney et al. 2006[b]
Green frogs *(Rana clamitans)*	Hibernation	M/F	Experiment	PCBs	Whole	1.4	Angell and Haffner 2010[b]
Fish							
Sockeye salmon *(Oncorhynchus nerka)*	Migration	M/F	Field	PCBs, PCDD/Fs	Muscle, Gonad, Liver	9.7	deBruyn et al. 2004[a]
Europeans eel *(Anguilla anguilla L)*	Migration	F	Experiment	PCBs	Muscle	14.0	van Ginneken et al. 2009[a]
Chinook salmon *(Oncorhynchus tshawytscha)*	Larval development	M/F	Experiment	PCBs, OCs	Whole	4.9	Daley et al. 2012[c]
Arctic Char *(Salvelinus alpinus)*	Overwintering	M/F	Experiment	PCBs	Liver, Kidney	6.8	Jorgensen et al. 1999[c]
Yellow perch *(Perca flavascens)*	Incubating eggs	M/F	Experiment	PCBs	Whole	5.4	Daley et al. 2009[b]

Common sole *(Solea solea)*	Larval development	M/F	Experiment	PCBs	Whole	10.1	Foekema et al. 2012[c]
Yellow perch *(Perca flavascens)*	Overwintering	M/F	Experiment	PCBs	Whole	2.3	Paterson et al. 2007[c]
Pacific salmon *(Oncorhynchus spp)*	Migration	M/F	Field	PCBs, OCs PCDD/Fs	Muscle, Gonad Liver	10	Kelly et al. 2011[c]
Birds							
Kittiwakes *(Rissa tridactyla)*	Reproduction	F	Field	PCBs	Liver, Brain, Fat	4.8	Henriksen et al. 1996[c]
Herring Gull *(Larus argetatus)*	Incubating eggs	M/F	Experiment	PCBs	Embryo	3.3	Drouillard et al. 2003[c]
Greater scaup *(Aythya marila)*	Overwintering	M	Field	PCBs, OCs	Muscle, Fat Pad	5.5	Perkins et al. 1997[c]
Common eiders *(Somateria mollisima)*	Incubation fast	F	Field	PCBs, OCs	Blood	6.1	Bustnes et al. 2010[c]
White Carneau pigeons *(Columba livia)*	Starvation	M/F	Experiment	PCBs	Blood, Liver Kidney Brain, Muscle, Whole	5.7	DeFreitas and Norstrom 1974[c]
Mammals							
Harp seals *(Phoca groenlandica)*	Reproductive fast	F	Field/Experiment	PCBs, OCs	Blubber	7.2	Lydersen et al. 2002[c]
Sea lions *(Zalophus californianus)*	Domoic acid fast	M/F	Field/Experiment	PCBs, OCs PBDEs	Blubber	2.5	Hall et al. 2008[c]
Polar bears	Summer fast	M/F	Field	PCBs, OCs	Fat	1.5	Polischuk et al.

(Ursus maritimus)							2002[c]
Grizzly bears *(Ursus arctos horribilis)*	Hibernation	M/F	Field	PCBs, OCs PBDEs	Fat	3.8	Christensen et al. 2007[c]
Elephant seals *(Mirounga angustirostris)*	Juvenile development	M/F	Field	PCBs	Blubber, Blood	1.9	Debier et al. 2006[c]
Sea otters *(Enhydra lutris nereis)*	Infectious disease	M/F	Field	PCBs, OCs	Liver, Kidney, Brain	6.3	Nakata et al. 1998[c]
Humans *(Homo sapiens)*	Restricted diet	M/F	Field/Experiment	PCBs, OCs	Blood	1.3	Pelletier et al. 2002[c]
Humans *(Homo sapiens)*	Restricted diet	M/F	Field/Experiment	PCBs, OCs	Blood, Fat	1.4	Chevrier et al. 2000[c]
Grey seals *(Halichoerus grypus)*	Lactation	F	Field	PCBs	Blood, Blubber	2.5	Debier et al. 2003[c]
Grey seals *(Halichoerus grypus)*	Lactation	F	Field	PCBs, OCs	Blood, Blubber	2.7	Sormo et al. 2003[c]





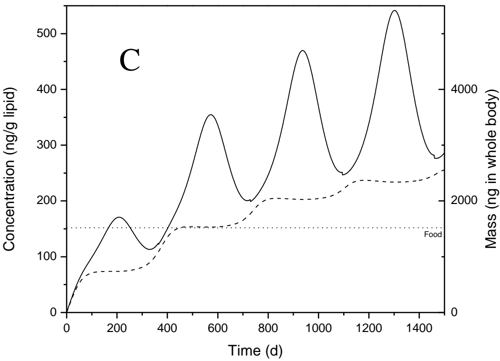


Figure 5.1. Bioaccumulation simulations under differing weight loss scenarios A) Base simulation of bioconcentration and biomagnification in a 100 g fish experiencing no growth or weight loss for a negligible metabolized chemical of log K_{OW} 6.5. B) Bioaccumulation simulation allowing for bioamplification due to 31% weight loss over a 2 week period and subsequent regain of weight after 50, 300 and 1400 d of simulation. C) Bioaccumulation simulation of a fish under a temperate seasonal temperature cycle and allowing for normal seasonal lipid loss and lipid gain. Solid line presents lipid normalized concentration. Dashed line presents chemical mass in the animal. Dotted line is the concentration of chemical in food on a lipid basis.

Chapter 6: The effect of food provisioning on persistent organic pollutant bioamplification in Chinook salmon larvae

Introduction

Fall spawning fish such as Chinook salmon (*Oncorhynchus tshawytscha*), provision large amounts of lipids (yolk) to eggs to allow incubation of eggs and the subsequent survival of larvae post hatching (Wiegand 1996, Urho 2002, Kunz 2004, Teletchea and Fontaine 2010). These endogenous resources are critical for many northern fall spawners to ensure their offspring survive the extended winter period until food becomes available (Kaitaranta et al. 1981, Wiegand 1996, Russell et al. 1999). The maternal provisioning of yolk to eggs also deposits considerable amounts of persistent organic pollutants (POPs) to the eggs. As yolk utilization progresses POP concentrations in the animal increase, because lipids are being depleted faster than POPS, a process known as bioamplification (Daley et al. 2012).

Daley et al. (2012) recently demonstrated bioamplification of POPs in Chinook salmon larvae relative to fresh eggs. Although bioamplification was not observed during egg incubation, bioamplification in larvae became pronounced after day 100 following significant depletion of lipid stores and limited chemical elimination. These observations lead to an increase in lipid equivalent POP concentrations up to 500 percent higher for the most hydrophobic chemicals in the late yolk-sac stages compared to the newly fertilized eggs. Furthermore, these increases in POPs concentration occurred at a critical time in the salmonid's life history, as it is a period of time that is generally accompanied by an increase in mortality (Elliott 1984, Jensen et al. 1991). It has previously been reported that later larval stages of yolk-sac salmonids often experience the greatest sensitivity to toxicological effects (Van Leeuwen et al. 1985, Walker et al. 1994, Petersen et al. 1998),

so a 5-fold increase in concentrations could potentially interact and augment these heightened sensitivities.

One limitation of the Daley et al. (2012) study was that it was performed without provisioning food to the larvae post hatching. This maximized the potential for bioamplification to occur but may reflect an unrealistic condition compared to the wild where some, albeit variable, food resources are potentially available to over wintering and early spring fish. This raises an important question regarding the interactions between food resource availability and POPs bioamplification in larvae Chinook salmon and whether or not food provisioning causes an attenuation of the maximum bioamplification potential reported in larvae of this species by Daley et al. (2012). Although fasting is considered a normal process for Chinook salmon larvae which utilize yolk lipids post hatch (Wiegand 1996, Urho 2002), relatively little information is available about the timing and abundance of food resources associated with wild populations of this species and how this varies across different environments. Field-Dodgson (1988), studied the diet of emergent Chinook salmon in a New Zealand stream and saw that while yolk quantity was generally low for early emergent fry, up to 90% of fry sampled at the start of emergence (day 144) had empty stomachs and intestines, indicating very little feeding had occurred prior to emergence. Emergence is generally characterized in the literature as a critical period for fry, which occurs when their yolk-sacs are nearly depleted and they must leave their gravel beds to begin feeding exogenously (Jones et al. 2003, Skoglund and Barlaup 2006, Gilbey et al. 2009). A laboratory study on Chinook larvae by Heming et al. (1982) indicated that while some "precocious feeding' had been initiated prior to the emergence phase, it appeared to have no effect on the growth rate.

However, delaying exogenous food resources beyond a certain point reduced size and the survival of the fish. Studies involving other salmonid species such as Atlantic salmon (*Salmo salar*), and Brook trout (*Salmo trutta*) have linked first feeding in larval salmon to a number of possible factors, including water flow, predator cues, substrate, and temperature (Hansen and Moller 1985, Jensen et al. 1991, Metcalfe and Thorpe 1992, Jones et al. 2003, Skoglund and Barlaup 2006, Gilbey et al. 2009). A field study in Scotland sampled Atlantic salmon from the earliest dispersal in late April until it finished in late June (Garcia De Leaniz et al. 2000). The authors reported that although complete re-absorption of the yolk sac generally leads to exogenous feeding, up to 35% of fry with no visible yolk also had empty stomachs. It is important to understand aspects such as the timing of feeding and the normal scope for weight loss and/or growth, to place maximum bioamplification potentials into context of what occurs in nature.

The growth of larval salmon, and the body condition of newly emerged fish, has important consequences on their survival and future life history events, such as smoltification, and their migration back to the sea or lake (Metcalfe and Thrope et al. 1992). Growth may have significant impacts on the toxicological consequences of POPs in these young salmonids by attenuating bioamplification and even reducing chemical concentrations below those of fresh eggs via biodilution; the condition where an increase in lipid content occurs to a greater extent than an the increase in chemical content in the organism.

In the present study, the scope for bioamplification and growth in Chinook salmon larvae was characterized over a period of 184 days post hatching. The scope for bioamplification and growth was determined by comparing larval size, lipid content and

chemical bioamplification through time across two treatments consisting of no-food provisioning and maximum food provisioning environments. The study also sought to characterize when salmon begin using exogenous food resources and whether biodilution during the over-wintering period of larval salmon can attenuate or eliminate POPs bioamplification observed under prolonged starvation conditions.

Methods

Experiment

Eggs were collected from a single spawning Chinook salmon female from the Credit River, Mississauga, ON, Canada (43°34′39.24″ N, 79°42′8.19″ W). The eggs were kept separate and brought back to two aquaculture facilities (Great Lakes Institute for Environmental Research (GLIER) in Windsor and Leadley Inc. in Essex) in Ontario, Canada. The milt from three males was collected and mixed together and was used to manually fertilize all of the eggs at each site. At the GLIER site, each group of eggs was incubated in separate Heath trays, with a flow through system, receiving filtered water from the Detroit River at *in situ* temperatures (Daley et al. 2012). The same set up was used at Leadley Inc. but with eggs receiving filtered water from a large aquaculture pond at *in situ* temperatures. The temperature profiles were within 2 standard deviations of each other each day. Six replicate eggs were collected postfertilization on day 1 and at a later egg developmental stage on day 21. The majority of eggs were observed to hatch between days 30-38. On day 75, the larvae were split between fed and non-fed groups at GLIER and Leadley Inc. Similar to under the ice conditions, the non-fed larvae groups were not provisioned with any food throughout the course of the study. The fed treatments were placed into three replicate flow-through aquariums (receiving the same

Detroit River or aquaculture pond water at in site temperatures). Black plastic mesh was attached halfway down the aquarium, in order to allow undigested food to fall to the bottom of the aquarium to maintain high water quality in the upper portion of the tank where flow through conditions were maximized. The fed larvae were rationed salmon starter (Martin Mills, Almira, Ontario) for the first 60 days and were then introduced to a mixture of salmon starter and 1mm food (Martin Mills, Almira Ontario). To account for potential food POP sources, a mass balance between starved and fed fish was performed. The fish were generally fed at a high feeding rate of approximately 5-10% of the average body weight per day. Six replicate larvae were sampled from fed and non-fed groups, on days 104, 129, 155, 164 and 184 after which the experiment was terminated.

Chemical Analysis

Neutral lipid content, moisture content and POP concentrations were analyzed in individual eggs and larvae. A micro extraction technique described in Daley et al. (2009, 2011) was used to analyze for POPs and lipids. Columns were spiked with PCB 34, to be used as a recovery standard. Neutral lipids were determined by gravimetric analysis according to the methods used by Drouillard et al. 2004. Sample cleanup was performed by florisil chromatography using the methods described by Lazar et al (1992). To analyze the sample for POPS, the first (50 mL hexane; ACP, Montreal, Quebec, Canada) and second fractions (50 mL; hexane/dichloromethane 85/15 v/v; Fisher Scientific, Fair Lawn, NJ) were collected. Following florisil chromatography, the remaining extracts were concentrated to 1 mL by rotary-evaporator. Gas chromatography electron capture detection (GC-ECD), was used to analyze the individual POPs. Analysis of each batch of six samples, included a reference homogenate, method blank, an external PCB standard

(Quebec Ministry of Environment Congener Mix; AccuStandard, New Haven, CT), external certified organochlorine pesticide standard (AccuStandard, New Haven, CT), and the PCB 34 recovery standard. The following POPs were detected with sufficient frequency and were included in this analysis: PCB congeners (IUPAC no.) 18/17, 31/28, 44, 49, 52, 70, 99, 101, 110, 118, 138, 149, 151/82, 153, 158, 170, 177, 180, 183, 187, 194, and 201, as well as QCB, HCB, OCS, and Mirex. The recoveries of individual PCB congeners from the in-house reference tissue, were within 2 standard deviations from the mean laboratory database value derived from laboratory control charts from the Great Lakes Institute for Environmental Research accredited organic analytical laboratory (Canadian Association for Environmental Analytical Laboratories Accreditation and ISO17025 certified) for each batch of samples. The recovery of the PCB 34 standard was 87 ± 5.0% and samples were not recovery corrected for PCB 34.

Data Analysis

Persistent organic pollutant concentrations were expressed on a lipid equivalents basis, using the methods described by Daley et al. (2012). The lipid equivalents concentration for each POP congener was calculated as follows:

$$C_{org(lipid\ equiv)} = C_{org(wet)}/(X_{lipid} + 0.05 \cdot X_{LDP}) \qquad (1)$$

where $C_{org(lipid\ equiv)}$ is the POP concentration normalized for lipid equivalents (ng/g lipid equivalents), $C_{org(wet)}$ is the wet weight POP congener concentration (ng/g) in the organism, and X_{lipid} and X_{LDP} are the fractions of neutral lipids and lean dry protein (LDP), respectively, in the sample. LDP was determined as the dry weight minus the lipid weight in the sample.

Dry weight and lipid equivalents in individual animals were compared by factorial ANOVA between treatment groups (feeding and nonfeeding) and between the two study locations (Glier, Leadley). A one-way ANOVA or t-test was used to compare lipid equivalents POP concentrations or POP mass at different time intervals in the salmon eggs and post-hatched larvae. Normality probability plots or Levene's homogeneity tests were used to test for normality in each group over time.

Elimination rate constants (k_{total}) for individual POPs were calculated using POP mass values (ng) derived from multiplying the wet weight POP concentration (ng/g wet weight) by the mass (g) of the individual larvae from days 104-184. They were calculated using a one-compartment first order rate constant model, where the k_{total} value of each chemical was calculated as the slope of the linear regression performed on a plot of the natural logarithm of chemical mass in the organism beginning on day 104 through day 184.

Bioamplification factors in eggs and larvae were calculated by expressing the ratio of POP lipid equivalents concentrations in the larvae/egg to the mean fresh (newly fertilized) egg value. Unless otherwise noted, all values are reported as mean ± standard deviation and there were three samples per location and treatment group per time point (n=3). Standard deviations for PCB 180 biomagnification factors were generated using Monte Carlo simulations (Crystal Ball Software) to provide a measure of variability for the ratio which considered error in measured concentrations from both eggs and larvae. The mean and standard deviation values of larvae and egg PCB concentrations were used as inputs for Monte Carlo simulations. Simulations assumed concentrations followed a

normal distribution (negative BMFs were not permited) and were run for 1000 iterations. Standard deviations were derived from the simulation output.

Results and Discussion

There were no significant differences between locations (GLIER versus Leadley Inc.) for dry weight (p=0.51) or whole body lipid equivalent contents (p=0.37) within a given treatment group. The interaction terms (treatment X location) were also non significant (p=0.36; p=0.98 respectively). There was however significant differences between treatments (feeding versus nonfeeding) for both dry weight (p=0.003) and whole body lipid equivalent contents (p=0.006). Given that all eggs were taken from the same female and that there were no significant differences between grow out locations for dry weight and lipid equivalent contents, data for the two locations were combined for the remainder of the analysis (n=6). The combined location mean dry weight of individual eggs and larvae across different time points is summarized in Figure 1. Egg weights, measured on a later day in egg development (day 29) exhibited no significant differences from fresh eggs (t-test, t=-0.05; p = 0.48). There was however a significant decline in dry weight over the larval period in the nonfeeding group (day 39-184) (R^2=0.98; n=6; p<0.000). Significant declines in dry total body weight over time were also observed in Chinook larvae from New Zealand (Kinnison et al. 1998). There were no significant differences in dry mass between treatment groups, until day 164 (t-test; t=5.4; p=0.001) where fed larvae were an average 2.3 fold higher than the non-fed group, suggesting feeding did not begin until after day 129.

The mean percent lipid equivalents (expressed on a wet weight basis) in eggs and larvae over time are summarized in Fig. 2. Similar to dry weight, there was no change in

lipid equivalents content (t-test, $p=0.12$) during the egg incubation period. There was a strong negative correlation between % lipid equivalents in larval salmon through time for the nonfeeding group ($R^2=0.97$; $p<0.000$). The above trend in lipid equivalents is comparable to the trend observed in Daley et al. (2012). While, there were no differences in % lipid equivalents between the fed and non fed groups on days 104 ($p=0.15$) or 129 ($p=0.08$), a significant ($p<0.05$) increase in % lipid equivalents in the feeding group was observed on days 164 and 184. Although food was available to the post-hatched larvae after day 75 in the fed group, both the lipid equivalents and dry weight data suggests that feeding did not commence until sometime after day 129.

The onset of exogenous feeding in Chinook salmon has been related to environmental factors such as available territory and migration distance (Beachman and Murray 1993), temperature (Fleming and Gross 1990, Jonsson and Jonsson 1999), gravel size (Quinn et al. 1995), as well as physiological factors such as when the larvae reach their maximum wet body weight (Heming 1982), when yolk constitutes less than 5% of the total larval wet weight (Thorpe et al. 1984, Jensen et al. 1991), as well as possible genetic factors (Heath et al. 1999). The field study by Field-Dodgson (1988) examining emergent Chinook salmon in New Zealand observed that emergence occurred 144-201 days after fertilization. A lab study by Heming et al. (1982) examined the effects of temperature on the initial feeding of salmon, where fish were incubated at 4 constant temperatures throughout the experiment. They observed that temperature had no effect on the stage of development where Chinook were first able to feed, but as expected, the development of larvae was faster for the warmer temperatures. The time when 50% of the larvae showed the presence of some ingested material was 905 ± 14 thermal units

among the four treatments, approximately 150 days for the 6ºC treatment and 75 days at the warmest temperature (12ºC). The results of the above studies along with the present study suggest that Chinook salmon won't commence feeding until a certain threshold has been reached whether it's related to the remaining yolk resources, temperature or other factors.

Elimination rate constants were calculated for both treatment groups (feeding and nonfeeding) and a regression was performed to test if the slope was significantly different from zero. There was no significant loss of chemical mass from days 1-69 across all POPs. Elimination was first evident for the lowest hydrophobic POPs on day 104. As a result, elimination rate constants were calculated between days 104-184 when elimination was observed to occur. Examination of these elimination rate coefficients showed that elimination was slow to negligible for most POPs, and only the lowest K_{OW} POPs (18/17, 31/28, QCB) had slopes that were significantly different from zero ($p<0.05$). For all POPs in both treatment groups, with a log K_{OW} greater than 5.6, there was no significant elimination of chemical from days 104-184. There were no consistent differences between k_{tot} values across feeding groups among chemicals where significant elimination was observed. This was counter to expectations, since the lower lipid content of the non-fed group was expected to contribute to higher PCB elimination by this group. Elimination rates are a function of temperature and physiological condition and since both temperature and lipid equivalents were changing throughout the study, variable patterns in elimination rates were expected over time. This contributed to additional variation that precluded detection of differences between the groups. However, quantifying k_{tot} parameter is still important to understand the extent of bioamplification in

larval Chinook salmon as the lack of elimination for the higher K_{OW} chemicals is expected to lead to maximum bioamplification potentials.

The change in lipid equivalent PCB 180 concentration with time for both treatment groups is presented in Figure 3. PCB 180 was selected as a representative highly hydrophobic chemical which exhibited negligible elimination from larvae over the experimental duration. There was no significant change in PCB 180 concentrations from the fresh egg value until day 104 at which point there was a significant increase ($p<0.05$; t-test) in concentration in both the feeding and nonfeeding groups (days 104: 1.4 ± 0.06 fold higher and 129: 1.6 ± 0.09 higher than fresh eggs). Following day 129, there were key differences between treatment groups. The nonfeeding group closely resembled the trend presented for PCB 180 lipid equivalents in Daley et al. 2012. The fed group showed considerable biodilution as a result of feeding and by day 184 biodilution had attenuated the concentrations back to the original levels observed for fresh eggs.

The scope for bioamplification and biodilution was observed to change as a function of time and is presented for PCB 180 in Figure 4. In the case of the non-fed group, bioamplification increased with time whereas bioamplification in the fed group was maximized on day 129 and declined to a value approaching 1 by day 184. Assuming that the non-fed and fed groups present the boundaries of bioamplification potential for this species (i.e. animals given excess food exhibited the maximum growth rates possible, contrasted against starved larvae), the difference between PCB 180 BaMFs between the two treatments present the range over which natural BaMFs for this chemical may be expected to occur depending on resource availability and other factors determining the onset of feeding in natural populations of Chinook salmon.

BaMFs for all chemicals of study are presented for day 129 (upper graphic) and day 184 (lower graphic) as a function of chemical K_{OW} in Figure 4. Similar to the data presented for PCB 180, BaMFs were similar for the two treatment groups across the different chemicals on day 129. At this time, BaMFs were observed to be a function of chemical hydrophobicity reaching asymptotic values for chemicals with log K_{OW} values > 5.5. The mean±SD BaMF for chemicals having log $K_{OW} > 5.5$ for the non-fed and fed groups were 1.5±0.2 and 1.6±0.2, respectively. The equivalence of BaMFs between the two groups on day 129 was a result of the delay in feeding which resulted in bioamplification occurring regardless of food provisioning. Delayed feeding has been linked to a number of variables including yolk utilization, where studies have shown that salmonids commonly delay feeding until the majority of their yolk is utilized (Jensen et al 1991, Garcia De Leaniz 2000, Skoglund and Barlaup 2006). This is a key finding as it demonstrates across a wide range of POPs that even in an ideal situation, with unlimited food resources and no predation, bioamplification will still occur. Though this seems unlikely to be the case in natural northern climates, the feeding treatment in this study may more closely resemble a hatchery environment, where fish are often fed maximum food rations throughout the year (Larsen et al. 2001).

By day 184, differences in BaMFs between the groups were maximized across the chemicals. For the non-fed group, BaMFs were positively associated with chemical hydrophobicity and ranged between values of 2.4 to 5.3 over the log K_{OW} range of 5.8 to 7.8. In the fed group, day 184 BaMFs were also dependent on hydrophobicity, but showed BaMFs values of <1 for chemicals having log $K_{OW} < 6.5$ and approached an asymptotic value of 1 for more hydrophobic chemicals.

The magnitude and hydrophobicity trends of BaMFs for the day 184 time point was comparable to the results of Daley et al. (2012) who reported BaMFs above 4.9 for the most hydrophobic POPs after 168 days of starvation. These trends of no food provisioning likely represent the maximum bioamplification potential in Chinook larvae. Although most animals would likely begin feeding before day 184, it could represent a potential realistic scenario in nature, especially in the most northern areas where food is frequently limited. It is common for overwintering animals in these areas to rely on their stored energy reserves when they are unable to match intake demands due to unpredictable and prolonged periods of food shortage. Studies on natural Chinook juveniles have shown that some fish experience extremely variable temperature profiles and in general stomach contents in these fish declined to near empty in the winter (Gardiner and Geddes 1980, Beckman et al. 2000, Larsen et al. 2001). Higher bioamplification factors could therefore be expected to occur in these temperate and arctic conditions where it is unrealistic to assume that the fish are able to feed to their maximum satiation even in early March as many northern rivers are still frozen over (Cunjak 1988).

The Credit River, where eggs were collected in the present study has extensive periods of ice accumulation up until March and this would seemingly alter food availability. A natural study on the Credit River of 1+ year old wild brook and brown trout (*Salmo trutta*) showed lipids were rapidly depleted in early winter and suffered a second depletion in February-March (Cunjak 1988). Similar results were observed in the Yakima River on naturally reared juvenile Chinook salmon, where lipid levels fell below 2% in the winter (Beckman et al. 2000). The above studies demonstrate that even in the

more advanced 1+ year old juveniles, feeding is extremely limited in the winter period due to unpredictable environmental conditions.

Bioamplification processes have been verified in other stages in the salmonid life history. While Lake Ontario salmon only migrate a few kilometers to the Credit River, many salmonid populations swim distances of over 3000 km in order to return to their native rivers and streams to spawn (Kinnison et al. 2001). This arduous migration may result in the loss of over 90% of their somatic lipid contents in order to support the bioenergetic costs of migration. Studies on spawning pacific salmon have demonstrated that bioamplification is a general process occurring in migrating fish (Ewald et al. 1998, deBruyn et al. 2004, Hansson et al. 2009, Kelly et al. 2011). deBruyn et al. (2004) found that migrating female sockeye tissues exhibited bioamplification factors as high as 10, in the soma tissues for POP compounds (PCBs, PCDDs, and PCDFs) following migration. The authors also investigated the change in lipid normalized concentration in the gonad tissues of females. While the bioamplification factors in the gonads were lower than that of the soma tissues they still exhibited significant bioamplification with factors up to 2.5 (range: 1.9-2.5). Coupling bioamplification factors from the gonads of the ratio between post-spawning to pre-spawning adult females with the average BaMF from day 129 (~1.6), the combined BaMF's would be as high as 4 in larval salmon compared to the pre-spawning maternal concentrations. Bioamplification would be significantly higher when using the highest factor in the nonfeeding group on day 184 (~5.3) combined with the post-spawning gonads, and could result in bioamplification factors as high 13 in emergent salmon. Therefore, depending on food availability, the risk of contaminant exposure to larval salmon could be 2.8 to13 fold above that of pre-spawning salmon.

Finally, this significant increase in POP exposure to larval salmon is occurring at a critical period in their life history; during the transition from endogenous to exogenous resources, where mortalities are often heightened (Van Leeuwen et al. 1985, Shuter and Post 1990, Walker et al. 1991, Petersen et al. 1998). For young fish, winter mortality is often the result of starvation, as they frequently reach the point-of-no return in which a critical lipid level is attained and mortality due to starvation is possible (Heming et al. 1982, Steinhart and Wurstbuagh 2003, Biro et al. 2004). Steinhart and Wurtsbaugh studied age-0 kokanee (*Oncorhynchus nerka*) and found this critical lipid threshold level was approximately 2%, in which animals often died from starvation. Winter body changes and lipid losses not only affect the survival to spring but can also affect future life history events. Often times, if winter conditions do not allow the fish to reach a threshold lipid or size level they may then not be able to reach the demands of smelting or migration events (Jonsson and Jonsson 1998, Steinhart and Wurtsbaugh 2003).

In the present study on day 129, both feeding and nonfeeding treatment groups had lipid levels between 2-4%. Therefore even in the fed treatment group, the combination of approaching lipid levels near the point of no return and the enhanced toxicological stress due to bioamplification, could lead to augmented mortalities. These toxicological consequences would likely be highest in the most nutritionally compromised larvae, such as the nonfed larvae on day 184 of the present study (~1% lipid). Daley et al. (2012) reported that the elevated sum POP concentrations in the late yolk-sac stages of Credit River Chinook salmon due to bioamplification were approximately 5 to 9 fold higher than the estimated threshold level used to protect juvenile salmonids from adverse health effects (Meador et al. 2002, Johnson et al. 2007).

The authors also noted that this protection benchmark was placed on juvenile salmonids, but a study on newly hatched rainbow trout estimated they are 20-40 times more sensitive to POP mortalities than one year old juveniles (Walker et al. 1994, Daley et al. 2012).

While bioamplification in a real population would likely be captured between the two extremes of the high food provisioning and the non-feeding groups in the present experiment, other stressors could interact with the toxicokinetics of POPs. Based on the observations from the current study, it is predicted that multiple stressors such as resource limitation, habitat quality and climate change could potentially push bioamplification factors closer to the non-feeding state.

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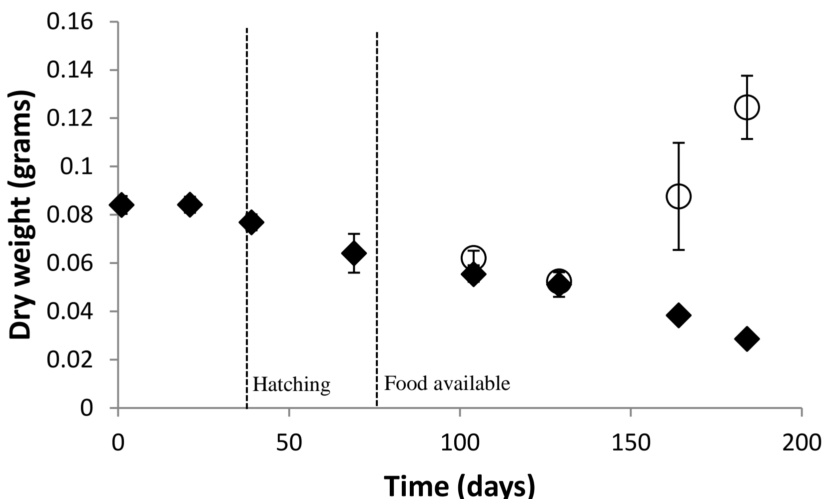


Figure 6.1. The mean (±STD) of the whole body dry weight (grams) of individual eggs and larvae across time for both and nonfeeding (♦) and (○) salmon, (n=6 for each point). Dashed horizontal lines represent the approximate day of hatching, and the day when larvae were provisioned with food.

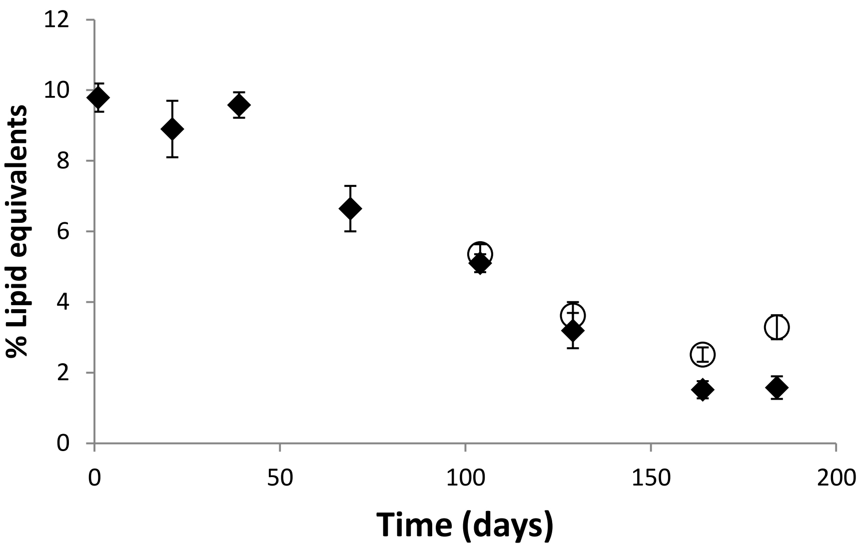
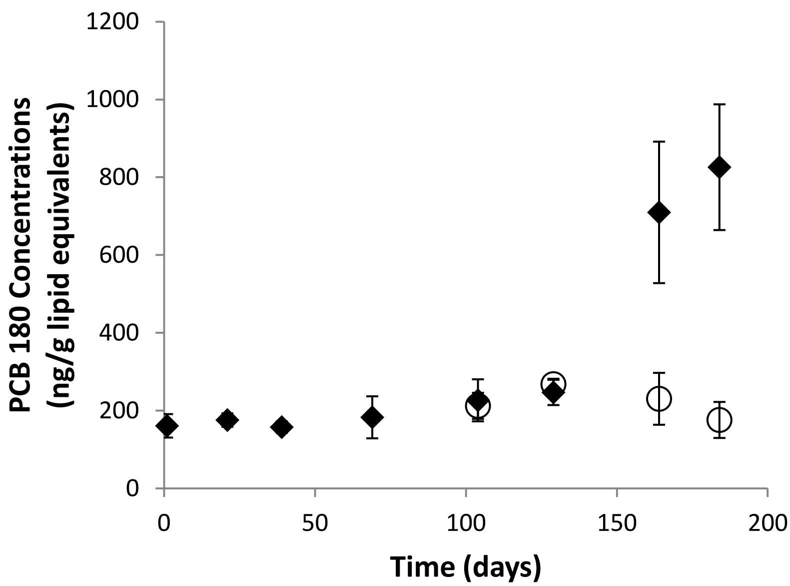


Figure 6.2. The mean (±STD) of the % lipid equivalents individual eggs and larvae across time for both feeding (○) and nonfeeding (♦) salmon (n=6 for each point).



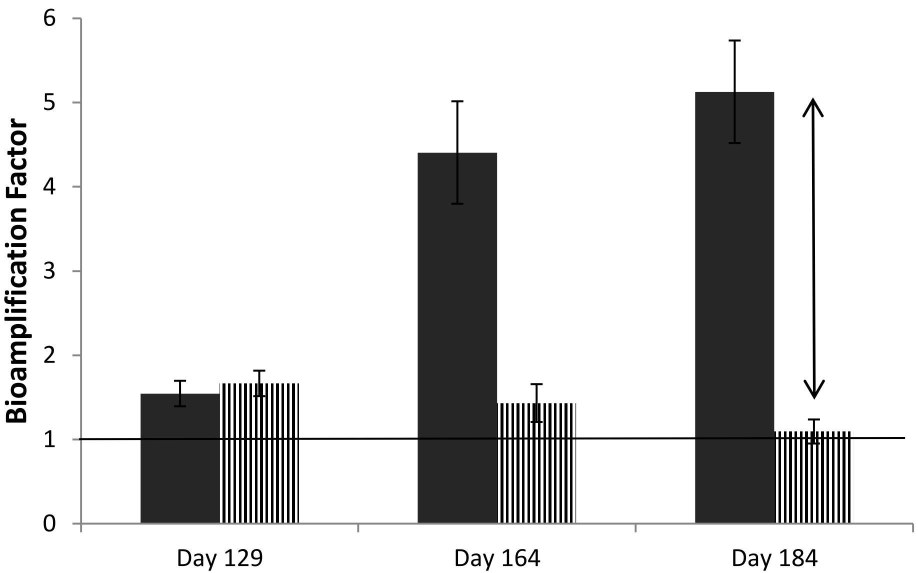
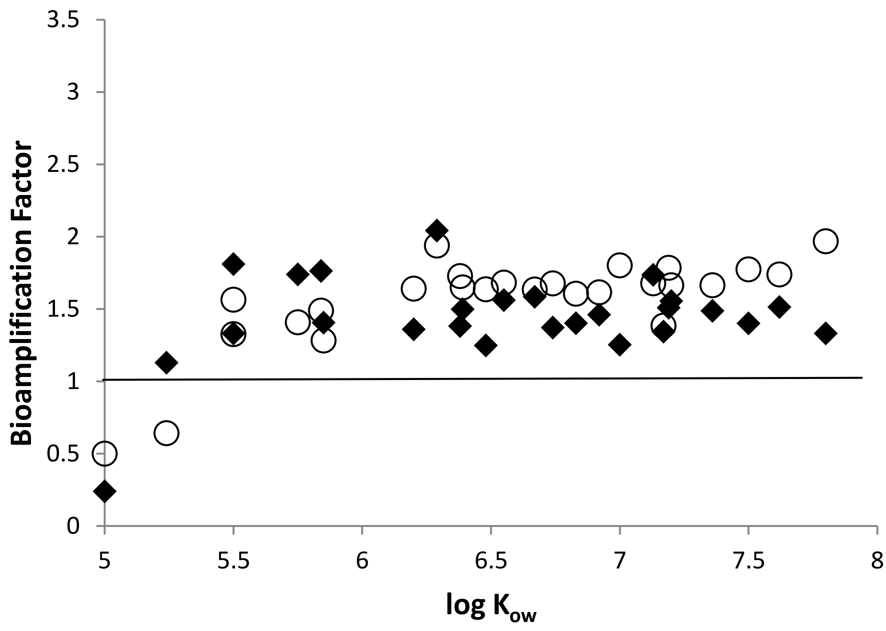


Figure 6.3. Mean ± standard deviation of panel A) chemical concentration (ng/g lipid equivalents) of PCB 180 in eggs and larvae over time for both feeding (○) and nonfeeding (♦) salmon and panel B) mean ± standard error of bioamplification factors across three time points (day 129, 164 and 184) for PCB 180. Bioamplification factors are presented across time. Lower horizontal line refers to the bioamplification factor of 1. The double arrow represents the scope of bioamplification at day 184 for PCB 180. (n = 6 observations per point).



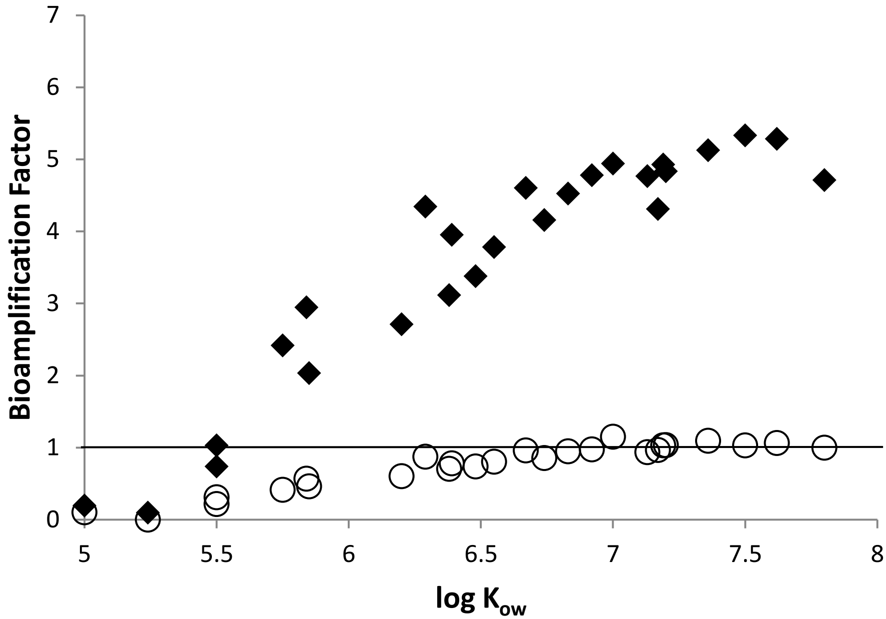


Figure 6.4. Persistent organic pollutant (POP) bioamplification factors comparing both nonfeeding and feeding salmon for panel A) day 129 d larvae/fresh egg, as a function of chemical K_{ow} where (♦) represents nonfed salmon and (○) is the fed group and for panel B) day 184 d larvae/fresh egg, as a function of chemical K_{ow} where (♦) represents nonfed salmon and (○) is the fed group. Lower horizontal line refers to the bioamplification factor of 1.

Chapter 7: General Discussion

Summary

Bioamplification occurs when an animal loses body mass and/or chemical partitioning capacity at a faster rate than it can eliminate contaminants. This causes an increase in chemical fugacity in the animal's tissues and will result in the redistribution of the contaminants from inert storage sites to other more sensitive tissues. As a bioaccumulation process, bioamplification is additive to bioconcentration and biomagnification mechanisms of chemical exposure. Consequently, understanding the dynamics of bioamplification and how life history can alter critical tissues residues under different scenarios is important to risk assessment studies but has rarely been considered explicitly in the bioaccumulation assessment and risk assessment literature.

The goal of this dissertation was to demonstrate that bioamplification is a general bioaccumulation process that contributes to enhanced chemical fugacities of POP compounds in a variety of animals undergoing recognizable bioenergetic bottlenecks during their life history. In Chapter 2, bioamplification was verified in male emergent mayflies (*Hexagenia* spp.) due to bioenergetically expensive flight costs associated with reproduction. Bioamplification factors of 1.91 and 2.05 were observed between male imago to sub-imago and male imago to pre-emergent nymphs, respectively. Unlike males, females did not undergo bioamplification post emergence indicating between sex differences in reproductive bioenergetics and bioenergetic bottlenecks experienced by this species. Chapter 3 provided the first example of *in-ovo* bioamplification occurring in an aquatic species, the yellow perch (*Perca flavascens*). Just prior to hatching, the late stage egg PCB fugacities were 2.7 fold higher than measured in newly fertilized eggs, demonstrating that incubating yellow perch embryos are exposed to higher chemical

fugacities in-ovo than predicted by maternal deposition alone. Chapter 4 tested *in ovo* bioamplification in a fall spawning fish species, Chinook salmon (*Oncorhynchus tshawytscha*). In this case, salmon had different early life history trends in lipid and POP concentrations compared to yellow perch likely related to differences in their life history strategies (Chapter 4). While bioamplification did not occur in the Chinook salmon eggs (no change in lipid content occurred during the egg incubation period), bioamplification was observed in the yolk-sac larvae post hatching. Following hatching, there was a steady decrease in lipid equivalents in the larval stage through time, resulting in lipid equivalent POP concentrations up to 5 fold higher for the most hydrophobic compounds at the latest larval time points. To complement the above studies, Chapter 5 was completed in order to both formalize bioamplification as a bioaccumulation process and bring together the first three chapters in context with other examples of bioamplification of POPs from the literature. Chapter 5 demonstrated the wide applicability of this concept to different animal species under natural and experimental settings. Examples of bioamplification were presented in invertebrates, fishes, birds and mammals corresponding to bioenergetic bottlenecks related to migration, reproduction, early life stages, metamorphosis, over wintering weight losses (including hibernation) and diseases. Bioamplification factors summarized in the literature ranged from 1.1–14 and were similar in magnitude to biomagnification factors typically reported for aquatic and terrestrial organisms. Chapter 6 provided a manipulation study using larvae Chinook salmon to establish realistic boundaries of bioamplification during larval development in this species. Chinook larvae were separated into fed and non fed groups in order to determine the scope for bioamplification for this species (i.e. animals given excess food exhibited the maximum

growth rates possible, contrasted against starved larvae contributing to maximum weight loss). The chapter demonstrated that even in an ideal situation, with unlimited food resources and no predation, bioamplification will still occur albeit to a lesser extent. Bioamplification factors peaked in the fed group after 129 days whereas bioamplification continued to increase in non-fed groups up to the termination of the study where animals were pushed to the point of no return in terms of lipid condition and unlikely to survive beyond such a point. Given this study, the scope for bioamplification in larval stages of Chinook salmon can range from 1.5 to 5.3 under different scenarios of food resource partitioning in natural systems.

General Discussion

This dissertation verified over a diversity of life history traits and animal species, how multiple stressors and energetic constraints influence POPs exposure in animals. Although several case studies have provided empirical observations of bioamplification in the literature (Chapter 5), this dissertation represents the first attempt to compile and characterize bioamplification as a general bioaccumulation process in heterotrophic organisms.

Bioamplification frequently occurs during critical periods in an animal's life history, and as such the consequences of bioamplification can be important to hazard and risk assessments of wildlife exposures to POPs. This was evident in chapters 3, 4 and 6, where bioamplification was verified in early critical life stages of yellow perch and Chinook salmon. These early life stages (egg and larval stages) have been shown to elicit greater toxicological sensitivities to POPs compared to juvenile and adult life stages (Walker et al. 1994, Petersen et al. 1998, Russel et al. 1999). In chapter 4 and 6,

maximum bioamplification factors of ~5 were reported in the late yolk-sac stage, a period of time linked to the transition from endogenous (yolk lipids) to exogenous food resources (Jones et al. 2003, Skoglund and Barlaup 2006, Gilbey et al. 2009). As this life stage is already associated with elevated mortality (Elliott 1984, Jensen et al. 1991), bioamplification could significantly augment mortality at this time contributing to an ecological/toxicity interaction not previously identified. The above chapters provide support for adopting a life-history approach to risk assessments for hydrophobic chemicals that considers bioenergetic bottlenecks as critical stages for evaluation of chemical bioaccumulation, toxicity and epizootic outbreaks under realistic dosing and natural conditions of interacting multiple stressors.

The above chapters also highlighted the importance of different life history strategies on bioamplification. The trend of POPs bioamplification in Chinook salmon early life stages differed from that of yellow perch eggs and larvae. Yellow perch are a spring spawning, iteroparous fish compared to the Chinook salmon which are a fall spawning, semelparous fish that provision larger amounts of maternal resources to their eggs to allow survival of eggs and larvae for lengthy periods of under the ice conditions where food is limited. Yellow perch eggs displayed bioamplification during the egg stage that corresponded with a drop in the dry and lipid weight. However, the high water temperatures at egg laying, rapid yolk-sac resorption, reduced developmental time and greater probability of available food resources on hatching appeared to attenuate bioamplification post hatching, limiting this process largely to the within egg period. The colder temperatures experienced by Chinook larvae reduced metabolic rates, delayed developmental time and are believed to have resulted in significant bioamplification in

post-hatched larvae. A key difference in this species is that Chinook salmon larvae often remain in the gravel post hatch and are nourished primarily by endogenous (yolk sac) resources whereas yellow perch generally have rapid yolk-resorption and hatch as free-swimming larvae capable of immediate feeding (Post and McQueen 1988). Even when food was provisioned to the larval Chinook salmon, they did not engage in exogenous feeding until significantly later in their development (Chapter 6). Thus, yellow perch experienced biodilution associated with growth immediately following hatching (Chapter 3), while Chinook salmon did not exhibit biodilution until 164 days following hatching (Chapter 6). In both species, there was no loss of POPs from eggs during the egg stage. An unexpected observation from the Chinook salmon studies was that larvae did not eliminate POP compounds to a significant extend during the prolonged over wintering period. POPs elimination only became prominent for lower hydrophobic compounds after 164 days (Chapter 6). The reasons why POPs elimination was so slow in these small organisms during this time frame is not fully understood, although it was hypothesized that warming water temperatures coupled with physiological development (formation of gills at later stages of larvae development) are necessary to achieve more rapid chemical depuration rates evident in the later stages of larvae development. Overall, these studies highlight how environmental (temperature), ecological (feeding capabilities) and physiological (yolk utilization rates and development of respiratory structure) differences alter the timing and magnitude of bioamplification experienced by early life stages of fish.

Bioamplification can have important consequences to the trophic transfer of sediment associated contaminants, evident in Chapter 2. Mayflies are an important food

item, contributing to the trophic transfer of sediment accumulated contaminants to both aquatic (fishes), and terrestrial predators (ie. birds, bats, spiders) (Flecker 1992, Vander Zanden and Rasmussen 1996, Nichols et al. 2004, Smits et al. 2005). Mayflies are commonly used as biomonitors because of their intimate and long term contact with sediment associated contaminants during the nymphal stages (Drouillard et al. 1996; Corkum et al. 1997). As biomonitors, mayflies can therefore provide useful data to address: 1) information about spatial patterns of sediment contamination and/or 2) information about POPs exposure risk to wildlife consumers of emergent aquatic insects. The data from Chapter 2 indicate that sampling strategies for mayfly based biomonitoring programs should be optimized depending on which of the above two objectives best reflect the study goals. In the case of sediment contamination assessment, differences in lipid content and bioamplification between sexes following emergence provide confounding variables that contributes additional variation and complicate chemical concentration interpretation. For example, biomonitoring program designs that selectively collect sub-imago life stages would avoid bioamplification as a confounding factor. However, given the differences in lipid accumulation by sex, and our understanding of how this interacts with POPs bioaccumulation from sediments, interpretation of sub-imago chemical concentrations would best be performed following lipid normalization of the data. Alternatively, if only imago life stages are available for collection, separation of samples by sex and selective collection of female imagos would substantially reduce variability of contaminant residues contributed by bioamplification. The results from Chapter 2 further demonstrate that the risk of sediment contaminant exposure to wildlife consumers of emergent mayflies will be underestimated if

bioamplification is not considered as part of the exposure assessment. Furthermore, the temporal and spatial availability of different emergent life stages (subimago versus imago) and sexes to terrestrial predators can potentially contribute to variation in accumulated residues not previously identified. To better address wildlife risks related to sediment-terrestrial transfer of POPs, diet studies in candidate wildlife predators to determine relative contributions of different mayfly life stages and sex would be necessary to apportion additional risk associated with bioamplification.

Collectively this dissertation showed that bioamplification occurs under specific life history stages when an organism experiences major bioenergetic bottlenecks. While the empirical studies in this dissertation verified bioamplification during emergence, larval and embryonic development, the review (Chapter 5) further showed that bioenergetic bottlenecks common in other life stages (ie. migration, hibernation, reproductive activities, metamorphosis) are a widespread occurrence in different ecosystems and animal populations. Many animals live in environments with fluctuating food availability and experience lengthy periods of fasting but as this dissertation has demonstrated, fasting is also common in a wide variety of life history events which preclude feeding, such as migration, moulting or reproductive activities (Golet and Irons 1999, Golet et al. 2000, Humphries 2004, Wang et al. 2006). Based on the generality of these periods of negative energy balance, and the results of this dissertation, bioamplification is expected to be a general occurrence across taxonomic groups.

Future directions

Empirical studies

While the review chapter demonstrated the wide applicability of bioamplification across taxonomic groups, additional studies capturing bioamplification in at risk animals are required. More specifically, while limited research exists on bioamplification in amphibians, it may be critical in setting contaminant guidelines, as Leney et al. (2006) found bioamplification factors up to 5 fold in a lab experiment with metamorphosing green frogs. Research suggests that while no single overarching cause is linked to global declines of amphibians, several multiple stressors are likely interacting such as climate change (Carey and Alexander 2003), habitat destruction (Marsh and Trenham 2001), infectious disease (Dascak et al. 2003) and contaminants (Gutleb et al. 2000, Hayes et al 2002). Studies testing the effects of the above stressors and their implications on bioamplification in amphibians during critical periods in their life history (ie. metamorphosis, hibernation) are necessary. Bioamplification could also be impacting endangered sea turtles as studies have shown that their lengthy reproductive migration to shore and natal dispersal have significant energetic costs (Kwan 1994, Hamann et al. 2007). Research suggests that even the accumulation of low POP concentrations in sea turtles may have negative effects on their health, so enhanced concentrations from bioamplification could be detrimental (Keller et al. 2004).

A terrestrial example could involve caribou, where it has become increasingly apparent that the caribou in the Canadian boreal forest are largely at risk of extinction due to significant habitat degradation and loss (Vors et al. 2007, Sorenson et al. 2008). A study by Chan-McLeod et al. (1999) showed that fat stores were highest in November-

December, but overwintering led to a fat depletion of over 4 fold in March-April. Bioamplification was partially verified by Kelly and Gobas (2001) who analyzed POP fugacities in caribou sampled in July and September, and observed 7 fold higher fugacities in the July caribou. Consequently, studies testing the effects of overwintering and habitat degradation on POPs bioamplification may be beneficial in this at risk species. Other examples could be related to the disappearing ephemeral wetlands which are a key stopover for migratory birds, increased precipitation leading to deeper snow in mountain regions further limiting foraging abilities, decreased arctic sea ice impacting seals, polar bears, walruses, and seabirds and how these factors interact with and potentially augment bioamplification. In general, studies that characterize the interactions between bioamplification and other environmental stressors would be beneficial.

Bioaccumulation modeling

Assessing exposure and bioaccumulation potential of animals forms the basis of most risk assessments which are critical to developing suitable guidelines for the protection of wildlife (Hickie et al. 2007). Despite empirical evidence demonstrating the generality of POPs bioamplification, this process has rarely been incorporated in bioaccumulation models used for toxicological risk assessments. This is mostly because current paradigms in bioaccumulation modeling are generally formulated under a steady state framework (deBruyn and Gobas 2006, Hickie et al. 2007). However, steady state assumptions fail to incorporate many environmental, ecological and physiological stressors (both anthropogenic and natural) over the lifespan of the animal (Norstrom et al. 2007, Muir and de Wit 2010). This dissertation demonstrated that these stressors are

intimately linked to chemical dynamics and can have broad effects on the bioaccumulation potential, tissue distribution and the toxicokinetics of contaminants (Klanjscek et al. 2007, Muir and de Wit 2010). While there are several studies that have implemented life history variation and/or bioenergetic parameters in modeling bioaccumulation of indicator species (ie. Hickie et al. 1999, 2000, Klanjscek et al. 2007, Norstrom et al. 2007, Ng and Gray 2009), most modeling efforts have focused on a steady state framework, ignoring restricted periods of weight loss in their bioenergetic sub-models (Norstrom et al. 2007).

The shift towards non-steady state, life-history bioaccumulation models requires considerable effort to calibrate life-stage specific toxicokinetic parameters and to understand how bioenergetic surpluses (growth) and deficits (transient weight loss) occur during critical life history events, how this interacts with variable environmental characteristics and habitats, and how it interacts with multiple stressors (e.g. climate change, harvesting pressures, invasive species or habitat loss). This represents a significant departure from past approaches employed to characterize chemical toxicokinetics in selected species under constant temperature (often conducted at the species' optimum temperature), feeding (ad libitum) and at specific life stages (often juvenile for fish). However, such models would enable more realistic exposure assessments to characterize time points where tissue residues become maximized in an animal's life history and to establish sensitive life stages for toxicity evaluation, characterize processes driving variability in bioaccumulated residues in the field, risks related to consumers of contaminated animals and address chemical/multiple stressor interactions.

Using the data collected throughout this dissertation, and supplementary literature information, full life cycle bioaccumulation models can be developed and calibrated for a representative insect (mayflies) and fish (salmon). Information collected in this thesis, on weight and POP changes of emergent life stages, can be used to parameterize the model for emergent mayfly life stages and to validate assumptions such as the elimination of hydrophobic POPs (K_{total}) approaches zero in these stages. Significant information on the specific toxicokinetics of mayflies at late larval stages was reported by Drouillard et al. (1996). Additional supplementary information is required to complete the model for mayflies related to factors regulating the dietary bioavailability of PCBs, and bioenergetic parameters such as growth rates from egg to pre-emergent mayfly larvae (Giberson and Rosenberg 1992).

While information on the toxicokinetics of POPs in salmon can be found across many critical life stages (ie. eggs, larval salmon, smoltification, and migration) these parameters are for a range of salmonid species, including Pacific salmon, Atlantic salmon, charr and trout, for which there is large variability in life histories (Groot and Margolis 1991). It is therefore essential to find toxicokinetic parameters for a specific species (ie. Chinook), and later determine whether the model can be applied across all salmonids. A thorough literature search is required to find adequate species specific values on seasonal changes in growth rate/lipid accumulation, ventilation rates, and the necessary toxicokinetic parameters, including life stage specific rates of uptake and elimination, absorption efficiencies, and bioconcentration factors (Gobas et al. 1999, Lundebye et al. 2004, Jorgensen et al. 2006). Standard metabolic rates have been widely reported across salmon species and life stages (ie. Rombough and Ure 1991, Stewart and

Ibarra 1991, Trudel et al. 2004) as well as specific growth rates (ie. Bull et al. 1996, Hutching and Jones 1998, Nordgarden et al. 2003). Some difficulties in confirming salmonid models may include diet matrix and chemical concentrations in the diet matrix during grow-out in open oceans and large lakes. These full life history bioaccumulation models over the life cycle of salmon and mayflies can be evaluated against the predicted timing and magnitude of POP fugacity gradients and would be immediately applicable to toxicological risk assessments and characterizing periods of enhanced toxicological stress from bioamplification peaks over the animal's lifetime.

Lastly, bioamplification often occurs during the most sensitive life stages in an animal's life history and the results of this dissertation have demonstrated the risk of contaminant exposure is underestimated if bioamplification is not accounted for. Yet there is often a paucity of life-stage specific information about species sensitivity to individual toxicants and there is a need for future studies to characterize organism sensitivities to toxic effects across life stages with a focus on those life stages where biomagnification and bioamplification are likely to be maximized. Furthermore, much of the toxicology literature expresses chemical dose in reference to the exposure media (air, water or food chemical concentrations) rather than toxicant concentrations achieved in organism tissues confounding interpretation of organism sensitivity to a given dose. Utilization of non-steady state life history models to aid in the design of toxicity tests would provide benefits in guiding life stages and life stage/environment interactions that result in environmentally realistic exposures and maximized tissue residues. Thus the non-steady state, life history modeling approach would provide benefits both towards understanding bioaccumulation and variability of residues realized in field collected

animals and also to the refinement of toxicity risk assessments to wildlife and sensitive bioindicator species.

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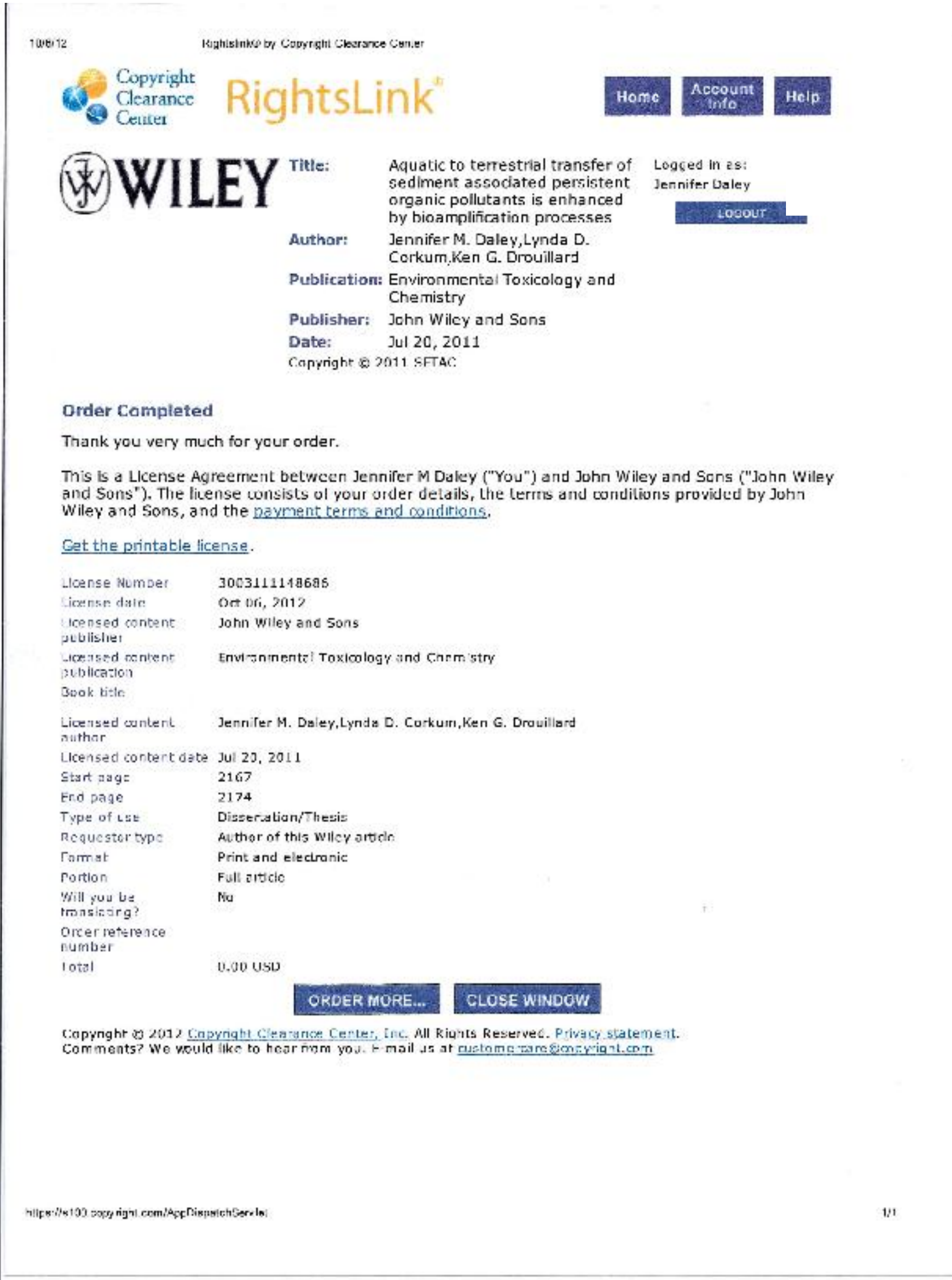

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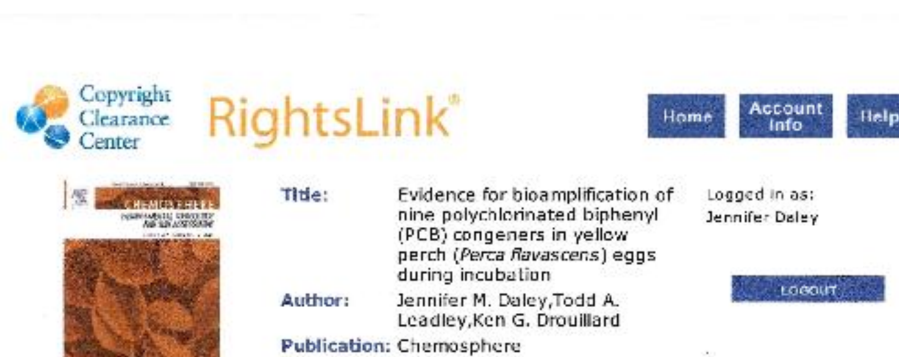

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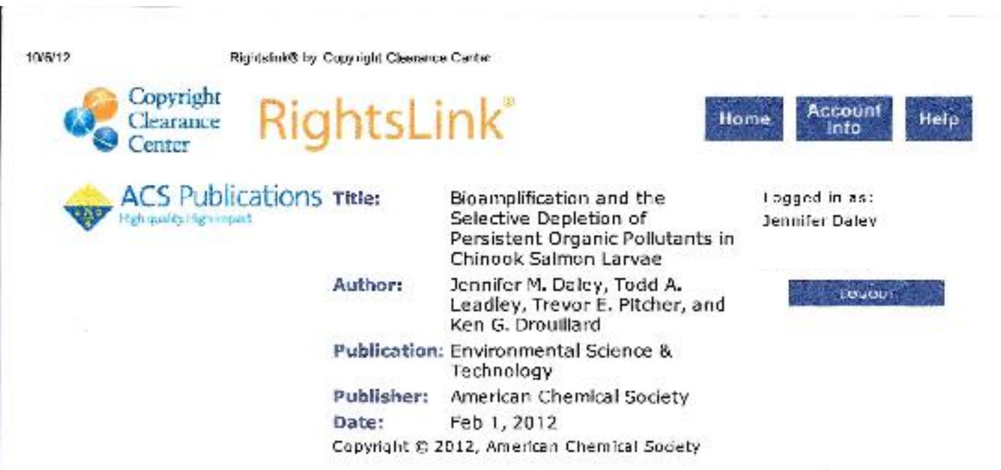

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