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THE INFLUENCE OF TEMPERATURE AND CHEMICAL HYDROPHOBICITY ON STEADY AND NON-STEADY STATE POLYCHLORINATED BIPHENYL BIOACCUMULATION IN TEMPERATE FISH.

by

Michael D. Burtnyk

A Thesis
Submitted to the Faculty of Graduate Studies through the Great Lakes Institute for Environmental Research in Partial Fulfillment of the Requirements for the Degree of Master of Science at the University of Windsor

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The influence of temperature and chemical hydrophobicity on steady
and non-steady state polychlorinated biphenyl bioaccumulation in temperate fish.

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DECLARATION OF CO-AUTHORSHIP

This thesis contains material to be published in refereed journals. Co-authors Gordon Paterson, Kenneth G. Drouillard and G. Douglas Haffner all contributed to these works both intellectually and in the field and have given permission for them to be included in this thesis. Chapter 3, “Steady and non-steady state kinetics reveal the biological and ecological scales of polychlorinated biphenyl accumulation in natural populations of bluegill sunfish (Lepomis macrochirus) and cisco (Coregonus artedii), has been submitted to the Canadian Journal of Fisheries and Aquatic Sciences.
ABSTRACT

This thesis investigated the effects of temperature and chemical hydrophobicity on steady and non-steady state accumulation patterns of polychlorinated biphenyls (PCBs) over the lifetime of temperate freshwater fish. In Chapter 2, the elimination of congeners of log\(K_{ow} < 5.7\) by yellow perch (\textit{Perca flavescens}) was regulated by temperature-dependant metabolic rates. Congeners of log\(K_{ow} > 5.7\) however, were not significantly eliminated at any temperature commonly encountered by temperate fishes. It was concluded that the accumulation of congeners of log\(K_{ow} > 5.7\), in natural systems, is therefore regulated by energetically driven consumption rates. This conclusion was confirmed in Chapter 3, where it was determined that the majority of congeners of log\(K_{ow} > 6.8\) did not achieve steady state within the lifetime of either bluegill (\textit{Lepomis macroschirus}) or cisco (\textit{Coregonus artedii}). The bioaccumulation of low \(K_{ow}\) congeners was determined to be a function of temperature-driven physiological processes and the bioaccumulation of high \(K_{ow}\) congeners were related to ecological processes.
This thesis is dedicated to my parents, and biggest supporters, Nick and Lee.
ACKNOWLEDGEMENTS

I have received the assistance of many, both friends and family, in completing this thesis. Without them, this task would have been much more difficult and not as enjoyable it has been. There are a few that deserve special consideration. Without their help, the completion of this project would have been impossible.

I thank Doug Haffner for providing me with the resources and inspiration to explore a fascinating field of research. His patience and ability to keep me on the right path has been invaluable. I also wish to express my gratitude to Mark Cook, Todd Leadley, Gord Paterson and Ken Drouillard for the planning and subsequent problem solving discussions we’ve had at all stages of this thesis. More importantly, however, their determination displayed in the field made this thesis possible. I appreciate their patience as it seemed that things rarely went as planned the first time. Every time, we got it done. Thanks for your help.
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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

Quantifying the effects of temperature on the bioaccumulation of hydrophobic organic compounds (HOCs) such as polychlorinated biphenyls (PCBs) in food webs using traditional steady state models has proven challenging due to the wide range in thermal regimes experienced across species and latitude. For ectotherms, such as freshwater fish species, temperature has the greatest influence on the rates of biological processes, including food consumption, respiration and growth (Brett and Groves 1979). For highly hydrophobic chemicals such as PCBs, consumption of contaminated food remains the primary route of exposure for freshwater fish species (Russell et al. 1999). These observations suggest that the kinetics of PCB bioaccumulation, in fish, is highly regulated by temperature which determines how quickly fish achieve steady state with their environment. This thesis examines if patterns of chemical bioaccumulation are primarily regulated by steady state processes where steady state dynamics are achieved during the lifetime of a fish, or by non-steady state dynamics where steady state is not achieved within the lifetime of a fish.

PCBs are a ubiquitously distributed group of 209 different congeners that vary in a wide range of physical and chemical properties, including a range in hydrophobicity measured as the n-octanol water partition coefficient ($K_{ow}$) that spans over three orders of magnitude (Hawker and Connell 1988; Wania and Mackay 1996). The principal uptake pathway for PCBs is by way of the diet, and the elimination pathways are primarily across respiratory surfaces (i.e. gills) and possibly by fecal elimination (Gobas et al. 1993). Although these compounds can be toxic at
high concentrations (Hattula and Karlog 1972), PCBs are usually found in the environment at sufficiently low levels not to induce toxicity effects (Reiser et al. 2004).

Biomagnification is often used to explain high contaminant concentrations of PCBs commonly observed in top predators of aquatic systems (Oliver and Niimi 1988). Equilibrium partitioning theory, however, predicts that all organisms achieve equal fugacities with their surrounding environment (Mackay 1981). The fugacity (Pa) of a chemical in an organism is calculated as

\[
 f = \frac{C}{Z}
\]

where, \( C \) is wet weight concentration (mol/m\(^3\)) and \( Z \) is the fugacity capacity [(mol · m\(^3\))/Pa]. In biological systems, capacity is proportional to the lipid content (Mackay 1981) such that

\[
 Z = X_L K_{ow} / H
\]

where \( X_L \) is the lipid content of the organism \( H \) is the Henry’s Law constant (Pa · m\(^3\)/mol) of the chemical in question.

Biomagnification is the increase in chemical fugacity in an organism compared with that of its diet (Connolly and Peterson 1988). Biomagnification contradicts equilibrium partitioning theory, which predicts that all organisms achieve equal fugacity (Mackay 1982; LeBlanc 1995). Critics of biomagnification were concerned how chemicals could be transferred from states of low fugacity (i.e. water and/or diet) to higher fugacity states (i.e. predator tissues) by passive uptake and elimination mechanisms. This thermodynamic problem was resolved by the
development of the gastrointestinal magnification model (Gobas et al. 1988, 1993). This model
described the mechanism for biomagnification as the reduction of the volume of gut contents and
lipid content in the diet during digestion, effectively increasing the fugacity of the food as it is
passed through the digestive tract. This increase in chemical pressure during digestion results in
a net inward flux of chemical to the animal, until the animal achieves steady state fugacity with
the digested prey items (Gobas et al. 1993, 1999). Biomagnification is now considered to be the
primary exposure route of many PCBs to biota (Thomann and Connolly 1984; Morrison et al.
1997). The accepted models for describing their accumulation predict that during the lifetime of
an individual these chemicals achieve steady state fugacities between the animal and its
environment (i.e. water and sediment) and/or diet (Mackay and Hughes 1984; Gobas et al. 1988;
Debruyn and Gobas 2006). At steady state, rates of chemical uptake across all exposure routes
equal rates of elimination such that the net flux of chemical in and out of an organism is zero.

The rate of transfer of chemical between media (e.g. water and biota) is dependent on
both the concentration and $K_{ow}$ of the chemical in question. Bioaccumulation occurs when the
rate of uptake from food ($C_f k_f$) and water ($C_w k_1$) exceeds that of elimination across respiratory
surfaces ($C_{org} k_2$):

\[
\frac{dC}{dt} = C_w k_1 + C_f k_f - C_{org} k_2
\]

where $dC/dt$ is the bioaccumulation rate, $C_w$ is the PCB concentration in water, $C_f$ is the PCB
concentration of the diet and $C_{org}$ is the PCB concentration in the organism. The rate constants
$k_1, k_f$ and $k_2$ represent the rate coefficient for the transfer of chemical from water, food and the
organism, respectively.
The negative relationship between the elimination rate constant \( (k_2) \) and \( K_{ow} \) predicts that the time to steady state is an inverse function of \( K_{ow} \) (Clark et al. 1990; LeBlanc et al. 1995). For chemicals with slow elimination rates (Wszolek et al. 1979; Sijm et al. 1992; Buckman et al. 2004), the time required to achieve steady state can be very long, especially for highly hydrophobic congeners, where the length of time required to achieve steady state could be longer than the lifespan of the fish (Fig. 1.1; Paterson et al. 2007a). When \( k_2 \) approaches zero, the concentrations observed in fish increase over the life of the fish and are predicted to be primarily regulated by chemical uptake associated with food consumption rates and efficiencies. As a consequence, the chemical bioaccumulation dynamics of high \( K_{ow} \) compounds is predicted to be a function of the variability in food consumption rates among individuals and species. Variability in food consumption is driven by the demands of metabolic and growth processes, which are the primary energetic demands for fish (Winberg 1956). Paterson et al. (2007b) hypothesized that temperature dependent metabolic rates regulate \( k_2 \) in fish. The aim of this thesis is to quantify the relative importance of temperature with respect to the ability of fish to achieve steady state with HOCs, such as PCBs, within their lifetime (Fig. 1.1).

1.2 Chapter 2 Objectives

In Chapter 2, rates of PCB elimination in yellow perch \( (Perca flavescens) \) were determined, across \( K_{ow} \), in a controlled laboratory environment at two constant temperature regimes of 13°C and 23°C. By studying contaminant elimination between the two temperatures, valuable insight is provided on the relative importance of temperature and \( K_{ow} \) on the time
required for PCBs to reach steady state. This study further tests if high $K_{ow}$ congeners can achieve steady state conditions under different thermal regimes.

The following null hypotheses were tested in this chapter:

1a. $k_2$ values in fish are independent of temperature.

1b. Effects of temperature on $k_2$ do not vary with $K_{ow}$.

2. All PCBs reach steady state within the lifetime of a fish.

### 1.2 Chapter 3 Objectives

The primary objective of this study was to determine the relative importance of steady and non-steady state PCB accumulation dynamics in aquatic ecosystems. Although the effect of temperature on fish bioenergetics is well established (Brett and Groves 1979), temperature effects on PCB bioaccumulation have been assumed to be relatively unimportant. It had been assumed that, under steady state conditions, temperature-driven variability in uptake and elimination negated one another. Chapter 3 investigates differences in congener-specific bioaccumulation between a warm-water species, bluegill sunfish (*Lepomis macrochirus*) and a cold water species, cisco (lake herring; *Coregonus artedii*) inhabiting the same lake.

The following null hypothesis was tested in this chapter:

1. All PCB congeners achieve steady state within the lifetime of bluegill and cisco.

2. The amount of time required to achieve steady state increases with $K_{ow}$.

3. There is no difference in steady state fugacities between bluegill and cisco.

4. Despite having different energetic demands as a consequence of living under different temperature regimes, PCB bioaccumulation rates in warm and cold-water fish does not differ as a function of size and age.
1.3 Literature Cited


Figure 1.1: Theoretical bioaccumulation of PCB congeners across a range of low to high hydrophobicity ($K_{ow}$) where congeners represented by solid lines achieve steady state within the lifetime of the individual and dotted lines represent PCB congeners that also achieve steady state, but do so over a timeframe greater than the lifetime of a fish.
CHAPTER 2: THE INFLUENCE OF TEMPERATURE ON $k_2$ ACROSS $K_{ow}$

2.1 Introduction

Bioaccumulation is a result of higher uptake than elimination rate coefficients of chemicals such as hydrophobic organic contaminants (HOCs) including polychlorinated biphenyls (PCBs). Chemical uptake occurs by way of bioconcentration from water and biomagnification, the uptake of chemical from food. Bioconcentration mechanisms are primarily $K_{ow}$ dependant (Veith et al. 1977; Mackay 1982) and result in the condition where organisms achieve fugacities equal to that of their environment (i.e. water) as the chemical partitions between the lipid phases of the organism and the surrounding environment (i.e. water). Biomagnification occurs as a result of gastrointestinal (GI) magnification where lipids are assimilated as food passes through the GI tract, resulting in an increase in fugacity of the food which may exceed that of the body of the organism (Gobas et al. 1993). All chemicals, independent of $K_{ow}$, have the potential to be equally biomagnified by this fugacity gradient. Rates of elimination, by way of passive diffusion across gill membranes (Clark et al. 1990) and possibly through fecal egestion (Drouillard and Norstrom 2003), dictate the potential for a particular chemical to bioaccumulate. Steady state models assume that organisms, within their lifetime, reach a point where the rates of chemical uptake and loss are balanced and no further net uptake occurs. It has been observed, however, that some aquatic organisms such as fish continue to accumulate chemical throughout all life stages (Jensen et al. 1982; Kiriluk et al. 1995).

The time required to achieve steady state is a function of the elimination rate constant ($k_2$). Some chemicals have the potential to achieve steady state rapidly (i.e. high $k_2$) whereas others (i.e. low $k_2$) take much longer time periods to reach steady state (Clark et al. 1990). All
chemicals, given sufficient time, will achieve steady state. A non-steady state condition exists only when the time required to achieve steady state is greater than the lifespan of the individual (Fig. 1.1). The rate of elimination (- $d[PCB]/dt$) of PCBs from a fish is assumed to follow first order kinetics (Barron et al. 1990), according to eq. (2.1):

\[
(2.1) \quad - \frac{d[PCB]}{dt} = k_2[PCB]
\]

Integrating eq. (2.1) between time 0 and time $t$ produces eq. (2.2)

\[
(2.2) \quad [PCB]_t = [PCB]_0 \cdot e^{-k_2 t}
\]

where $[PCB]_0$ and $[PCB]_t$ is the lipid normalized PCB concentration at time equals zero and time equals $t$, respectively. This equation can be manipulated such that:

\[
(2.3) \quad \ln [PCB]_t = -k_2 t + \ln [PCB]_0
\]

and $k_2$ is the slope of the straight line of $[PCB]$ versus $t$ and is represented as:

\[
(2.4) \quad k_2 = (\ln [PCB]_0 - \ln [PCB]_t) / t
\]

Although several studies have measured $k_2$ in fish, most were conducted at constant, optimal or near-optimal temperature conditions for each particular species, where growth potentials were maximized (Niimi and Oliver 1983; Coristine et al. 1996; Fisk et al. 1998). The
values for a limited number of species have been measured at variable or non-optimal temperatures (Spigarelli et al. 1984; Paterson et al. 2007b). Paterson et al. (2007b) concluded that $k_2$ was strongly influenced by water temperature and physiological adaptations by fish to the variable temperature regimes encountered in temperate lakes. It was determined that $k_2$ was seasonally variable and highest during thermally optimal (i.e. warm) temperature periods while decreasing to near zero, during winter, for all PCB congeners. Paterson et al. (2007b) predicted that $k_2$ was regulated by metabolic rates and that chemical elimination rates could only be predicted by using species specific bioenergetics models.

In this study, the relative importance of temperature-dependant metabolic processes and $K_{ow}$ in regulating chemical elimination rates was tested. By measuring $k_2$ values for PCBs ranging in log$K_{ow}$ from 5.02 to 8.18 in yellow perch (*Perca flavescens*) raised under two constant temperature regimes of 23 and 13°C, the influence of temperature on $k_2$ was determined. An optimal temperature was chosen, 23°C (Kitchell et al. 1977), where feeding rate is high and growth potentials are maximized. A lower temperature of 13°C was selected to reflect a temperature at which metabolic rates are minimized yet feeding still persists. At these temperatures, the bioenergetics model of Enders et al. (2006) predicts that there will be an approximate five fold difference in metabolic rates and consequently, $k_2$ is predicted to differ in the fish at 23 and 13°C. Additionally, it is postulated that because metabolic increases are accompanied by both increases in gill ventilation and fecal egestion rates, increases in temperature will equally affect $k_2$ across a range of $K_{ow}$; that is, the relationship of $k_2$ vs. $K_{ow}$ does not vary as a function of temperature.
2.2 Methods

2.2.1 Experimental Design

Yellow perch (n = 164; mean weight = 30.30 ± 0.78g) were raised in two 5000L fibreglass tanks maintained at 13 and 23°C over a period of 254 days. Dechlorinated City of Windsor water was recirculated through each tank, equipped with a biofilter to remove solid and nitrogenous wastes as well as ultraviolet filters to inhibit the growth of disease-causing microbes. Filters were backwashed biweekly and 50% of the water in each tank was flushed and replaced. To maintain consistency in photoperiod over the course of the experiment, fish were housed in a windowless room under a light regime consisting of a cycle of 12 hours on / 12 hours off. Water temperature was recorded four times each day using in situ temperature loggers (Hoskin Scientific, Burlington, Ontario, Canada) placed in each tank.

Treatment fish (n = 93) were dosed by intraperitoneal (IP) injection of 1.25 μL/g of a 1:1:1 mixture of Aroclor 1248:1254:1260 (4mg/mL total PCBs) suspended in safflower oil (Paterson et al. 2007). Control fish (n=75) were treated with a 1.25 μL/g dose of safflower oil. To account for background PCB levels and potential chemical recycling within each tank, control and treated fish were placed together in each temperature treatment tank. Dosed and control fish were separated in each tank by plastic 6.35mm screening, allowing water but not fish to pass through the barrier. At the same time each day, a measured amount of 3 mm commercial fish food (Martin Mills Inc., Elmira, Ontario, Canada) was given to both dosed and control sides of each tank and excess was removed after 30 minutes. This feeding rate was estimated to be a satiation ration to minimize the effects of food limitation on bioenergetic processes. Food that was not consumed was allowed to dry overnight and weighed to determine consumption rates.
To allow for the absorption and distribution of the IP injected dose throughout the body, 52 days were allowed before the beginning of the experiment. Both dosed and control fish were sampled on days 0, 8, 48, 84, 178, 235 and 254 of the experiment. Control fish however, were not collected in the 23°C tank on days 178 and 235 because the numbers of control fish in this tank were limited. All remaining dosed and control fish were collected on day 254.

Fish were euthanized by immersion in water containing a minimum of 100 μg/mL clove oil (eugenol) for 10 minutes. Samples were then stored at –30°C for dissection and chemical analysis. All experimental procedures were approved by the University of Windsor Animal Care Committee.

2.2.2 PCB Analysis

After gut contents were removed, whole fish samples were homogenized in a hexane-rinsed stainless steel blender. Chemical concentrations were determined for individual fish by gas chromatography (GC). Tissue samples were prepared for GC analysis as according to Daley et al. (In Press). Approximately 0.5g of fish homogenate was ground by mortar and pestle with 15g of Na₂SO₄ (VWR, Mississauga, ON, Canada) and wet packed into 30 mL glass Luer lock microcolumns containing 25 mL dichloromethane (DCM) – hexane solution (50:50 v/v; Fisher Scientific; HPLC grade; Ottawa, ON, Canada) and eluted through a Phenomenex 12 port SPE manifold (Chromatographic Specialties, Brockville, ON, Canada). To determine sample recovery during analysis, 100 ng of a surrogate standard, 1, 3, 5 – tribromobenzene (Chromatographic Specialties, Brockville, ON, Canada) was added to each column at the beginning of the procedure. After 1hr, the initial solvent was collected and the column subsequently eluted with an additional 15 mL of 50:50 DCM – hexane. Two mL of 2, 2, 4 – trimethylpentane (Fisher Scientific; HPLC grade; Ottawa, ON, Canada) was added to the extracts
as a keeper solvent. Subsequently, the final stages of the sample treatment are detailed in Lazar et al. (1992). Extracts were evaporated under reduced pressure to a low volume (approximately 2 mL) and adjusted to a volume of 10 mL. The neutral lipid content of each sample was then determined gravimetrically by removing 10% of the extracts and drying the aliquot in a 110°C oven for 24 h (Drouillard et al. 2004).

Sample extract “clean-up” was performed by Florisil (Fisher Scientific; HPLC grade; Ottawa, ON, Canada) chromatography (Lazar et al. 1992). Sample extracts were added to a 1 cm i.d. glass chromatography column and wet packed with 6 g of activated Florisil in hexane with a Na₂SO₄ cap of approximately 1 cm. PCBs were eluted from the Florisil column with 50 mL of hexane. Florisil eluants were concentrated under reduced pressure, transferred to a 1 mL volumetric flask and brought to a final volume of 1 mL using 2, 2, 4 – trimethylpentane.

A method blank and in-house reference homogenate (Detroit River carp) were extracted along with each batch of six samples. All PCB analyses were performed using a Hewlett Packard 5890 gas chromatograph equipped with a 60m DB-5 capillary column (Chromatographic Specialties, Brockville, ON, Canada), a mass selective detector (Hewlett Packard 5973) and an autosampler (Hewlett Packard 7673). Injection port temperature was 250°C, injection volume was 2μL (spitless), carrier gas was helium (flow rate of 1 mL/min) and detector temperature was 280°C. GC column temperature was programmed from 90 to 280°C. PCBs were quantified against an external PCB standard (Aroclor 1242, 1254 and 1260 at 35μg/mL in 2,2,4-trimethylpentane, diluted to 50mL). Sample recoveries of the internal standard, 1, 3, 5 – tribromobenzene averaged 66.8 ± 1.8 % and therefore, sample concentrations were not recovery corrected. Wet weight concentrations of PCB 31/28, 52, 101, 153 and 180 in reference tissue
homogenates were compared against quality control charts and found to be within two standard deviations of the mean database values. Detection limits were 0.05 ng/g for all PCB congeners.

2.2.3 Data Analysis

PCB concentrations in fish were expressed on a lipid-normalized basis in order to measure changes in chemical fugacity with time. Of the 83 PCB congeners measured, only well defined peaks (i.e. 2x detection limits) were included in the analysis. Paterson et al. (2007a) demonstrated that yellow perch, under ambient temperature conditions, did not exhibit the potential to metabolize PCBs. PCBs 19, 18, 31/28, 52, 95, 101, 110, 149, 153/132, 138, 177, 180, 201, 194 and 206 were selected to provide a broad range of $K_{ow}$ ($\log K_{ow}$ 5.02 – 8.09) to determine the impact of temperature on $k_2$ elimination dynamics. The time required to achieve 90% of the steady state concentration ($t_{90}$) was calculated from each $k_2$; this calculation was derived from eq. (2.3) as:

$$(2.5) \quad t_{90} = \frac{\ln(10)}{k_2}$$

The decrease in PCB concentrations in fish is a function of both elimination and growth rates. PCB concentrations were growth normalized according to the following growth correction:

$$(2.6) \quad [\text{PCB}]_{\text{Growth}} = [\text{PCB}]_{\text{Sacrifice}} \cdot (\text{Mass}_{\text{Sacrifice}} / \text{Mass}_{t=0})$$

where, at the time of sacrifice, $[\text{PCB}]_{\text{Growth}}$ is the growth corrected PCB concentration and $[\text{PCB}]_{\text{Sacrifice}}$ is the lipid corrected concentration. Mass at the beginning of the experiment is denoted as $\text{Mass}_{t=0}$ and mass at the time of sacrifice as $\text{Mass}_{\text{Sacrifice}}$. 

15
To account for potential recycling of PCBs within each tank, all IP injected fish were control corrected by subtracting the mean lipid and growth normalized concentrations of the control fish from those in the dosed fish.

Linear regression analyses were performed to test for temporal changes in animal lipid contents and lipid normalized PCB concentrations. For each congener, $k_2$ was determined as the slopes from the above regressions according to eq. 2.4. Analysis of variance (ANOVA) was performed to test if the linear regressions differed significantly from a value of zero, over the study period. Analyses of covariance (ANCOVA) were performed to test for differences in growth rates and lipid contents between temperature treatments. Paired $t$-tests were performed to test for differences in body mass and $k_2$ values between temperature treatments. A probability value of $P < 0.05$ was considered statistically significant.

2.3 Results

The mean temperature of the 23°C tank was 23.09 ± 0.04°C and the 13°C tank was 12.99 ± 0.01°C. Neither tank departed substantially from their assigned temperatures over the course of the experiment. There was no significant difference in the size of the fish in 23°C (28.6 ± 1.14 g) and 13°C (32.1 ± 8.27 g) tanks at the beginning of the experiment (Paired $t$-test, $P > 0.05$). The sampled 13°C fish did not increase significantly in size, maintaining a mean weight of 33.5 ± 5.30 g over the course of the experiment (Fig. 2.1b; Least squares regression, $P > 0.05$). The fish at 23°C, however, significantly increased in size (Fig. 2.1a; Least squares regression, $P < 0.0001$) and achieved a mean size of 72.1 ± 6.04 g after 230 days. The lipid contents of both 13°C (8.97 ± 0.34 %) and 23°C (6.24 ± 0.36 %) fish remained unchanged (Least squares regression, $P > 0.05$)
but were, however, significantly different from each other over the course of the experiment (ANCOVA, \( P < 0.05 \)).

Control fish maintained similar growth rates and lipid contents as the dosed fish in both the 13°C and 23°C tanks (ANCOVA, \( P > 0.05 \)). Control fish, by the end of the experiment had achieved detectable chemical burdens that averaged, across congeners, two orders of magnitude less than the dosed fish. Dosed fish were subsequently control corrected.

PCB congeners 19, 18, 31/28, 52, 101, and 180 represent a wide range of \( K_{ow} \) (5.02 – 7.36) and were chosen to demonstrate specific elimination patterns over the course of the experiment (Fig. 2.2). At both 23°C and 13°C, congeners with \( \log K_{ow} < 5.7 \) were rapidly eliminated (Least squares regression, \( P < 0.05 \)) while congeners of \( \log K_{ow} > 5.8 \) were not significantly eliminated at either temperature (Least squares regression, \( P > 0.05 \)). The relationship between \( k_2 \) and \( K_{ow} \) was consistent at both temperatures (Fig. 2.3); between \( \log K_{ow} \) 5.02 and 5.67, the \( k_2 \) of the fish at both 23°C and 13°C decreased significantly (Least squares regression, \( P < 0.05 \)) whereas, between \( \log K_{ow} \) 5.84 and 8.09, \( k_2 \) did not change significantly (Least squares regression, \( P > 0.05 \)). Of the significantly eliminated congeners (\( \log K_{ow} < 5.7 \)), \( k_2 \) at 23°C was a mean 2.30 fold higher than \( k_2 \) at 13°C (Paired \( t \)-test, \( P < 0.05 \)) while there was no significant difference in \( k_2 \) between temperature treatments for the congeners that were not significantly eliminated (\( \log K_{ow} > 5.8 \)).

2.4 Discussion

This study confirmed that \( k_2 \) was more rapid at higher temperatures, but that the temperature effect was observed only for congeners of \( \log K_{ow} < 5.7 \). For congeners of \( \log K_{ow} > 5.8 \), however, elimination rates over the course of the experiment were negligible for fish held at
either 23°C and 13°C. As a consequence of the slow eliminations rates of these congeners, it was determined that the time to achieve times to 90% of their steady state concentrations (\(t_{90}\); Table 2.2; eq. 2.5) approach or exceed the average 7 – 10 year lifespan of yellow perch (Scott and Crossman 1973).

These kinetic results are consistent with past research (Buckman et al. 2004; Paterson et al. 2007a,b) that have observed rapid elimination rates for PCBs at low \(K_{ow}\) and very slow elimination rates for PCBs with high \(K_{ow}\). There are, however, a few alternatives to explain both the low \(k_2\) values and the temperature effects observed. The recycling of previously eliminated chemical has been demonstrated in mussels (O’Rourke et al. 2004) and resulted in a reduction of observed elimination rates. Internal controls, in this study, identified that control fish may have experienced small increases in congeners of \(\log K_{ow} > 7.0\); this is a likely a consequence of the ingestion of feces produced by the PCB dosed fish over the course of the experiment. All congeners were nonetheless control corrected. Lipid contents, although they remained unchanged over the course of the experiment, were different between temperature treatments. This difference reflects a potential difference in fugacity capacity between the two temperatures. The 1.4 fold difference in lipid content between the two temperature treatments, however, does not account for the 2.1 fold difference in the \(k_2\) of significantly eliminated congeners. Growth rates were significantly higher in the fish at 23°C than the fish at 13°C, which did not experience significant changes in body mass over the course of the experiment. It was assumed, as all alternatives were corrected for, that observed \(k_2\) values were primarily regulated by \(K_{ow}\) and temperature.

Temperature is the primary influence on metabolic rates for fish of similar body size (Brett and Groves 1979). This study, supported by the work of Paterson et al. (2007b), provides
strong evidence that metabolic rate has a substantial impact only on the $k_2$ of chemicals with low $K_{ow}$ (i.e. $\log K_{ow} < 5.7$). The initial hypothesis, based on the Enders et al. (2006) bioenergetic model, of a 5 fold increase in metabolic rates, is supported by the change in $k_2$ values observed for low $K_{ow}$ compounds. Temperature-driven metabolic rates, however, have no impact on the $k_2$ values of high $K_{ow}$ compounds (i.e. $\log K_{ow} > 5.7$). As the rates of elimination observed for these congeners remained very slow, the influence of temperature-driven metabolic rates on the $k_2$ values for these congeners could not be experimentally determined. For high $K_{ow}$ compounds, $k_2$ values are regulated most strongly by $K_{ow}$.

Relatively rapid elimination rates of low $K_{ow}$ compounds have been demonstrated for warm-water (Jimenez et al. 1987; Paterson et al. 2007b) and cold-water species (Coristine et al. 1996; Fisk et al. 1998) at or near their thermal optimum. At these temperatures, with the exception of the rainbow trout (*Onchorynchus mykiss*) used in Fisk et al. (1998), the elimination of high $K_{ow}$ compounds was slow, similar to the $k_2$ values obtained for yellow perch at 23°C in this study. The small size (2 – 4 g) of fish used by Fisk et al. (1998), relative to the 10 – 45 g fish used in the other studies, was likely the reason behind the discrepancy in the $k_2$ values of high $K_{ow}$ congeners at these optimal or near-optimal temperatures (Paterson et al. 2007a). The influence of temperature on $k_2$ is much less studied at sub-optimal temperatures. Research conducted by Paterson et al. (2007b) in yellow perch and Buckman et al. (2004) in rainbow trout indicate that the elimination of all but the least hydrophobic compounds (i.e. $\log K_{ow} < 5.7$) is negligible under sub-optimal temperature regimes. The results of this study, supported by the above body of literature, indicate that for all but the smallest fish (Fisk et al. 1998), elimination rates of high $K_{ow}$ congeners by freshwater fish are less influenced by temperature-driven metabolic rates. In essence, the long time to achieve steady state means that fish integrate several
seasonal temperature cycles over their lifetime. As such, elimination rates will track the mean
temperature experienced over a lifetime.

The lack of influence by temperature-driven metabolic rates has important implications
for patterns of PCB accumulation in natural systems. Low $K_{ow}$ PCBs achieve steady state as
regulated by temperature and have the potential to be used as indicators of environmental
concentrations in temperate lakes. For high $K_{ow}$ congeners, not in steady state, the observed
bioaccumulation in natural systems will be driven by uptake rates. As uptake rates are regulated
food consumption rates (Morrison et al. 1997), and consequently energetic demands, PCB
bioaccumulation is more a function of bioenergetic processes than background environmental
levels.

This research provides valuable insight into bioaccumulation and high contaminant
burdens carried by fish inhabiting cold-water environments such as those with cold-water
preferences (Madenjian et al. 1994; Paterson et al. 2005) or those living at high latitudes (Kidd et
al. 1998; Gewurtz et al. 2006). For low $K_{ow}$ congeners, the influence of temperature and
metabolism is predicted to have a significant influence on PCB concentrations in natural systems.
More importantly, this study identifies that metabolic rates do not influence the chemical
elimination rates of high $K_{ow}$ congeners in temperate fish and that variability in the accumulation
of these congeners is, therefore, driven primarily by the rate of uptake of chemical from food,
suggesting that high $K_{ow}$ PCBs can be used as environmental indicators of energetic demands of
fish communities.
2.5 Literature Cited


relationship with the octanol/water partition coefficient. Environmental Toxicology and Chemistry 17: 951-961.


Table 2.1: Lipid normalized PCB concentrations (ng \cdot g^{-1} lipid^{-1}) for \textit{in situ} control yellow perch (\textit{Perca flavescens}) at 23\degree C (a) and 13\degree C (b).

a)

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b)

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Table 2.2: PCB elimination rate constants and time to 90% steady state ($t_{90}$) for yellow perch (*Perca flavescens*).

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</tr>
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Figure 2.1: Body mass of individual fish at (a) 23°C and (b) 13°C. Solid lines represent the linear least squares regression lines.
Figure 2.2: Elimination of PCBs (ng/g) across the entire $K_{ow}$ range of tested congeners over a 254 day period at 23°C (◊) and a 180 day period (♦). Each point represents an individual fish and solid lines represent the least squares regression lines.
Figure 2.3: PCB congener $k_2$ values (± 1 standard error) at 23°C (♦) and 13°C (■). $k_2$ values were calculated from Eq. (2.3). Open and solid symbols represent congeners that were significantly and not significantly eliminated, respectively.
3.1 Introduction

There has been considerable debate as to the relative importance of biomagnification as an exposure route in aquatic ecosystems (Oliver and Niimi 1988; Leblanc 1995; Gray 2002; Fisk et al. 2003). Biomagnification is often used to explain high contaminant concentrations of hydrophobic organic chemicals commonly observed in top predators of aquatic systems (Oliver and Niimi 1988). Equilibrium partitioning theory, however, predicts that all organisms achieve thermodynamic equilibrium with their surrounding environment, eventually achieving the same lipid corrected chemical concentration (i.e. fugacity) (Mackay 1981).

The fugacity (Pa) of a chemical in an organism is calculated as

\[
f = \frac{C}{Z}
\]

where, \( C \) is wet weight concentration (mol/m\(^3\)) and \( Z \) is the fugacity capacity ([mol x m\(^3\)]/Pa). In biological systems, capacity is proportional to the lipid content (Mackay 1981) such that

\[
Z = X_L K_{ow} / H
\]

where \( H \) is the Henry’s Law constant of the chemical in question.

Biomagnification is the increase in chemical fugacity in an organism compared with that of its diet (Connolly and Peterson 1988). Biomagnification contradicts equilibrium partitioning theory, which predicts that all organisms achieve equal fugacity, with smaller organisms
achieving this state faster than larger organisms (Mackay 1982; LeBlanc 1995). Critics of biomagnification were originally concerned with the fact that chemicals would need to be transferred from states of low fugacity (i.e. water and/or diet) to higher fugacity states (i.e. predator tissues) by passive uptake and elimination mechanisms, resulting in higher trophic states having higher fugacities than lower trophic states. This thermodynamic problem was resolved by the development of the gastrointestinal magnification model (Gobas et al. 1988, 1993). This model described the mechanism for biomagnification as the reduction of the volume of gut contents and lipid content in the diet during digestion, effectively increasing the fugacity of the food as it is assimilated. This increase in chemical pressure during digestion results in a net inward flux of chemical to the animal, until the animal achieves steady state fugacity with the digested prey items (Gobas et al. 1993, 1999). Biomagnification is now considered to be the primary exposure route of many highly hydrophobic organic contaminants (HOCs), such as highly chlorinated polychlorinated biphenyls (PCBs), to biota (Thomann and Connolly 1984; Morrison et al. 1997).

PCBs are a ubiquitously distributed group of 209 different congeners that vary in hydrophobicity (log $K_{ow}$ 4.09 – 8.18) (Hawker and Connell 1988). Although some congeners are toxic at high concentrations (Hattula and Karlog 1972), PCBs are usually at sufficiently low environmental levels to not induce toxicity effects (Reiser et al. 2004). Exposure to PCBs with log$K_{ow}$ > 6.00, is primarily by uptake from food (Thomann and Connolly 1984; Russell et al. 1999), whereas uptake from water across skin and gill membranes (i.e. bioconcentration) dominates the exposure dynamics of low $K_{ow}$ congeners (Paterson et al. 2006). For PCBs of $K_{ow}$ < 6, an organism can quickly achieve steady state with the surrounding water, due to rapid chemical elimination, where the time to steady state is inversely proportional to the elimination
rate constant ($k_2$) (Clark and Mackay 1990; Leney et al. 2006; Paterson et al. 2007a, b). For these chemicals, animal lipid content, is considered the most important factor determining the bioaccumulation potential of the organism. Biomagnification, on the other hand, is the dominant exposure route for PCBs with log $K_{ow} > 6$, where assimilation efficiencies influence an animal’s exposure and maximum bioaccumulation potential.

For both types of bioaccumulation (i.e. bioconcentration and biomagnification), the accepted models for describing the accumulation of HOCs predict that during the lifetime of an individual these chemicals achieve steady state fugacities between the animal and its environment (i.e. water and sediment) and/or diet (Mackay and Hughes 1984; Gobas et al. 1988; Debruyn and Gobas 2006). At steady state, rates of chemical uptake across all exposure routes equal rates of elimination such that the net flux of chemical to and from the organism is zero. These steady state models assume that kinetic processes controlling both uptake and elimination are coupled to biological processes such as metabolic, feeding and gill ventilation rates and do not alter the steady state fugacity achieved by the animal. By assuming that $k_2$ is constant, steady state models do not address physiological changes in metabolic, feeding or gill ventilation rates that may occur over seasonal cycles in temperate environments (Paterson et al. 2007b).

Contaminant exposure to humans through fish consumption can be significant, especially to people who consume fish as a substantial part of their diet. Given that concentrations of hydrophobic organic contaminants (HOCs) such as PCBs and dichlorodiphenyltrichloroethane (DDT) can vary as much as 100 fold in fish of similar size and age (Kiriluk et al. 1995), contaminant variability, among and within species, must be considered when assessing the risk associated with the consumption of contaminated fish. Despite their general acceptance, steady
state models are unable to explain the large intra- and interspecific variability commonly observed in aquatic ecosystems.

Paterson et al. (2007a, b) measured very slow elimination rates ($k_2$) of PCBs from pond raised yellow perch and concluded that most of these chemicals did not achieve steady state within the lifetime of a fish. This conclusion suggested that net chemical uptake remains positive throughout the lifetime of the animal and the rate of uptake is related to metabolic, feeding and gill ventilation rates. There is a need to evaluate the relative ability of steady state and non-steady state models to predict the bioaccumulation of hydrophobic chemicals observed in aquatic ecosystems. This research investigates, in situ, whether steady state or non-steady state models best describe contaminant bioaccumulation in feral fish populations.

To evaluate the relative importance of steady state and non-steady state dynamics in situ, PCB bioaccumulation was investigated in a warm-water species, bluegill sunfish ($Lepomis macrochirus$) and a cold-water species, cisco ($Coregonus artedii$). Bluegill consistently seek out warm, littoral waters that can approach 30°C during the summer in temperate lakes (Cherry et al. 1977). Cisco are cold water, pelagic species that are confined mainly to the colder (8-15°C) hypolimnetic waters of lakes during the summer months (Rudstam and Magnusson 1985; Aku et al. 1997). These two species are ideal for evaluating steady state versus non-steady state bioaccumulation models because they both feed exclusively on invertebrates and experience minimal ontogenetic diet shifts throughout their life cycle (Keast 1977; Scott and Crossman 1973). Under these considerations, steady state models predict that there will be little variability in fugacity among age-classes within each species, because all chemicals, regardless of their $K_{ow}$ will achieve steady state during the lifespan of a fish. Non-steady state models, however, predict that fugacity will increase among age-classes for both species and that it is possible for these
species, despite having a common trophic level, to exhibit very different bioaccumulation rates based on physiological and ecological differences.

3.2 Materials and methods

3.2.1 Sample collection

Bluegill sunfish and cisco were collected from Sharbot Lake, Ontario (44.46°N, 76.41°W) from May to September 2006. Sharbot Lake is a cold, relatively deep (Z_{max} ~ 34m) lake that supports both warm (littoral areas) and cold-water (pelagic areas) food webs. The cold, deep waters of the lake are well oxygenated throughout the year, as are the warm waters of the littoral zones. Sharbot Lake is an oligo-mesotrophic lake that has no known point sources of PCB contamination and is believed to receive the bulk of its PCB loadings via atmospheric deposition (Hagen 2005).

Bluegill sunfish were collected using both seine (7.6 x 1.8 m with 0.6 cm mesh) and cast (1.8 m diameter with 0.6 cm mesh) type nets. Bluegill collections were completed in littoral zones to depths up to 4 m. Cisco were collected using Fall Walleye Index (FWIN) gill nets consisting of eight, 7.6 x 1.8 m panels ranging in mesh size from 25 - 152 mm. Gill nets were set overnight in waters ranging from 10 to 30 m in depth. Samples were stored in food grade plastic bags and immediately placed on dry ice for transport to the laboratory where they were subsequently stored at –30 °C until analysis. Upon return to the laboratory, morphological data collected from each fish included wet weight (g), total, fork and standard lengths (cm), and sex. Entire gut tracts were dissected from each individual and, where present, diet items were keyed and identified to a genus or species level if possible following the keys outlined in Balcer et al. (1984) and Peckarsky et al. (1990).
Age determination was completed using the sagittal otoliths removed from individual fish. Briefly, once removed from the fish, thin (2 - 3 mm) sections of otolith cores were generated by mounting each otolith on a glass slide and removing extraneous material by sequentially polishing in the anterior and posterior directions of the otolith toward the core using coarse (120 grit, ~115 µm) waterproof sandpaper (Ali Industries Incorporated, Fairborn, Ohio, United States of America). Once the core was approached, the polished surfaces were finished with a fine (3 µm) lapping film until the annuli were clearly visible under a dissecting microscope. In order to avoid bias while assigning ages to fish, all otoliths were analyzed double blind by two individuals experienced in using otoliths for age determination. In the case of discrepancies between the readers, life history tables and literature based length at age ranges generated for the two species were consulted to provide a consensus age estimate (Scott and Crossman 1973; Shuter et al. 2005).

3.2.2 PCB analysis

After gut contents were removed, whole fish samples were homogenized in a hexane-rinsed stainless steel blender. Chemical concentrations were determined for individual fish by gas chromatography (GC). Tissue samples were prepared for GC analysis as according to Lazar et al. (1992). Approximately 3g of fish homogenate were ground by mortar and pestle with 35g of Na$_2$SO$_4$ (VWR, Mississauga, ON, Canada) and wet packed into a glass column containing 50 mL dichloromethane (DCM) – hexane solution (50:50 v/v; Fisher Scientific; HPLC grade; Ottawa, ON, Canada). To determine sample recovery during analysis, 200 ng of a surrogate standard, $^{13}$C labelled PCB mixture ([$^{13}$C] PCB 52 and [$^{13}$C] PCB 153) (Cambridge Isotope Laboratories Inc., Andover, MA, U.S.A.) was added to each column at the beginning of the procedure. After 1hr, the initial solvent was collected and the column subsequently eluted with an
additional 250mL of 50:50 DCM – hexane. Two mL of 2, 2, 4 – trimethylpentane (Fisher Scientific; HPLC grade; Ottawa, ON, Canada) was added to the extracts as a keeper solvent. Extracts were evaporated under reduced pressure to a low volume (approximately 2 mL) and adjusted to a volume of 10 mL. The neutral lipid content of each sample was then determined gravimetrically by removing 10% of the extracts and drying the aliquot in a 110°C oven for 24 h (Drouillard et al. 2004).

Sample extract “clean-up” was performed by Florisil (Fisher Scientific; HPLC grade; Ottawa, ON, Canada) chromatography (Lazar et al. 1992). Sample extracts were added to a 1 cm i.d. glass chromatography column and wet packed with 6 g of activated Florisil in hexane with an Na₂SO₄ cap of approximately 1 cm. PCBs were eluted from the Florisil column with 50 mL of hexane. Florisil eluants were concentrated under reduced pressure, transferred to a 1 mL volumetric flask brought to a final volume of 1 mL using 2, 2, 4 – trimethylpentane.

A method blank and in-house reference homogenate (Detroit River carp) were extracted along with each group of six samples. All PCB analyses were performed using a Hewlett Packard 5890 gas chromatograph equipped with a 60m DB-5 capillary column (Chromatographic Specialties, Brockville, ON, Canada), a mass selective detector (Hewlett Packard 5973) and an autosampler (Hewlett Packard 7673). Injection port temperature was 250°C, injection volume was 2μL (spitless), carrier gas was helium (flow rate of 1 mL/min) and detector temperature was 280°C. GC column temperature was programmed from 90 to 280°C. PCBs were quantified against an external PCB standard (Aroclor 1242, 1254 and 1260 at 35μg/mL in 2,2,4-trimethylpentane, diluted to 50mL). Sample recoveries for internal standards 13C – labelled PCB 52 and 13C – labelled PCB 153 averaged 83.7 ± 34.1% for and 93.6 ± 34.6%, respectively. Sample concentrations were not recovery corrected. Wet weight concentrations of PCB 31/28,
52, 101, 153 and 180 in reference tissue homogenates were compared against quality control charts and found to be within 1 or 2 standard deviations of the mean database values.

### 3.2.3 Stable isotope analysis

Stable isotopes of nitrogen and carbon were performed on all fish as an indirect measure of diet composition. Aliquots of homogenized fish tissue (~1g) were freeze dried for 48 h in a Labconco 4.5 freeze-dryer (Labconco Co., Kansas City, MO, U.S.A.). Freeze dried samples were pulverized in a SPEX CertiPrep 8000-D ball milling unit (SPEX CertiPrep, Metuchen, NJ, U.S.A.). Lipids were removed from freeze dried homogenates because variability in lipid content can cause bias in carbon isotope signatures (Post et al. 2007). Total lipids were extracted from freeze dried homogenates by mixing with 5mL of 2:1 (v/v) chloroform (Fisher Scientific; HPLC grade; Ottawa, ON, Canada)-methanol (Fisher Scientific; ACS grade; Ottawa, ON, Canada) and mixing in a vortex machine for 30 s. Samples were then allowed to sit for 48 h. before being centrifuged for 4 minutes whereupon the remaining chloroform-methanol solution was decanted off. Approximately 500μg of lipid extracted sample was added to a 3.0mm x 5.5mm tin capsule and folded closed for analysis of nitrogen and carbon isotopic signatures. Isotopic signatures of both nitrogen and carbon were determined by gas chromatography on a Thermo Finnigan DeltaPlus mass-spectrometer (Thermo Finnigan, San Jose, CA, U.S.A.). Both $\delta^{15}$N and $\delta^{13}$C signatures were quantified against a reference standard as:

$$\delta X = [\left( \frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1] \times 1000$$

where $\delta X$ (‰) is deviation from the reference standard and R is ratio of the heavy to light isotope.
3.2.4 Data analysis

Growth rates of bluegill and cisco were determined from the relationship between total length and age and modeled using the von Bertalanffy (VBL) growth rate model in eq. 2:

\[
L_t = L_\infty \cdot (1 - e^{-k(t-t_0)})
\]

where \(L_t\) is the total length (cm) of the fish at time \(t\), \(L_\infty\) is the asymptotic length (cm), \(k\) the growth coefficient (year\(^{-1}\)), and \(t\) the age of the fish in years. For \(t_0\), the theoretical age at a total length of 0, the model was iterated using a value of \(t_0 = 0\) (Lumb et al. 2007). The VBL growth models were calculated using the non-linear regression module of SYSTAT (SYSTAT 2004). The regressions were run through multiple iterations to achieve optimal fit to the data. Predicted times to reach 50% and 90% of \(L_\infty\) (\(t_{0.50}\) and \(t_{0.90}\)) were also calculated as outlined in eqs. (3.5) and (3.6) below respectively.

\[
t_{50} = \frac{\ln(2)}{k_2}
\]

\[
t_{90} = \frac{\ln(10)}{k_2}
\]

In investigating chemical bioaccumulation across age, a cohort analysis approach was taken. This approach was taken to investigate PCB bioaccumulation, \(\delta^{15}\text{N}\) and \(\delta^{13}\text{C}\) signatures, age and morphological data for each individual. The collections of bluegill and cisco in this study, as well as those in research of other systems, provide a fair representation of the adult
lifespan of both species (Keast 1977; Paterson et al. 2006). Age two and older fish were included in the study, as these fish would be expected to have achieved steady state as a result of their relatively small size and feeding activity. Both species undergo a major ontogenetic diet shift before age 2, whereby they rely less on zooplankton and begin to incorporate benthic invertebrates into their diet (Scott and Crossman 1973).

A total of 83 PCB congeners were measured by gas chromatography, however, only those congeners that were consistently detected in both species were included in the analysis. A total of 20 individual PCB congeners (31/28, 52, 74, 70/76, 87, 95, 99, 101, 105, 110, 118, 138, 149, 153/132, 170/190, 180, 187/182, 183, 196/203, and 201) were included in this study to provide a broad range of chemical hydrophobicities (log $K_{ow}$ 5.67 – 7.36; Hawker and Connell 1988). All PCB concentrations in fish were expressed on a lipid normalized basis in order to provide a surrogate measure of chemical fugacity. In order to compare age and growth related changes in fugacities among PCB congeners, lipid corrected PCB concentrations for each individual were divided by the mean of the year 2 age-class. This approach normalized differences in baseline environmental concentrations for each congener and allowed for the comparison of bioaccumulation rates across $K_{ow}$. Age 2 normalized PCB congener fugacity ratio was calculated as;

$$f \approx \frac{C_t / X_{L(t)}}{C_2 / X_{L(2)}}$$

where $C_t$ and $X_{L(t)}$ represents the wet weight PCB congener concentration and lipid content of the individual fish at age $t$, and $(C_2 / X_{L(2)})$ represents the mean lipid-normalized PCB congener concentration measured in 2 year old fish. The slope of the age 2 normalized PCB congener
fugacity ratios versus age was called the congener bioaccumulation coefficient and was used as an index of bioaccumulation rate.

Linear regression analyses were performed to examine for age-related differences in animal lipid contents, stable isotope signatures and age-2 normalized PCB concentrations. The regression slope coefficients determined from the relationships between age 2 normalized PCB congener fugacities and age were defined as the congener bioaccumulation coefficient. Analyses of variance (ANOVA) were performed to test if the slope of the above linear regressions differed significantly from a value of zero, as predicted by steady state models. All data were tested for normality using normal probability plots prior to completing any statistical comparisons and a probability ($P$) value of $P < 0.05$ was considered statistically significant.

3.3 Results

A total of 22 bluegill and 34 cisco were analysed for this study, ranging in age from 2 to 5 years old. Between 3 and 10 individuals were collected for each age-class and species. Generally, the younger age-classes in the lake were the most abundant while the older age-classes were the least abundant. There were very few 6+ year old fish captured of either species, suggesting that beyond age 5, both populations suffered high mortality rates. These older fish were excluded from analysis because of the limited numbers of fish captured in these age-classes (i.e. < 3 fish captured per species). Based on the above catch per unit effort observations, age 5 to 6 is considered to be representative of the normal lifespan of bluegill and cisco in this system. Average weights at age for 2 to 5 year old fish ranged from 13.0 to 69.8 g for bluegill, and from 33.1 to 75.6 g for cisco. All age classes of cisco were determined to be significantly larger than similarly aged bluegill in both length (ANOVA; $P < 0.001$) and weight (ANOVA; $P < 0.001$).
The average mass of all bluegill collected was 38.2 ± 4.8 g in comparison to 57.3 ± 5.1 g for the range of cisco used in this study.

Asymptotic lengths ($L_\infty$) estimated from the von Bertalanffy growth model calculations were 20.7 cm and 25.3 cm for bluegill and cisco, respectively (Fig. 3.1). The growth coefficients ($k$), estimated by eq. (3.4), were calculated to be 0.300 and 0.412 yr$^{-1}$ for bluegill and cisco. Times required to grow to 90% of the asymptotic length were determined to be 7.7 years for bluegill in comparison to 5.3 years for the cisco.

As bluegill aged, their lipid content decreased significantly (ANOVA; $P = 0.008$), declining from an average of 2.7 ± 0.1% in 2 year old fish to 1.0 ± 0.4% by 5 years of age. However, lipid contents between years 3-5 did not change significantly (ANOVA; $P = 0.34$). Cisco lipid contents were not significantly correlated with age (ANOVA; $P = 0.16$) and averaged 3.0 ± 0.2 % across the four age classes.

Gut contents analysis revealed that, between years 2-5, both species exhibited a generalist feeding strategy (Table 3.2). However, bluegill consumed a greater diversity of prey items relative to cisco although this is likely a function of the greater diversity of invertebrate prey items available in the littoral zones of the lake. Zooplankton (i.e. cladocerans and copepods) and chironomids (Diptera) were found in both bluegill and cisco stomachs with regularity throughout all age-classes. Bluegill fed on a wide variety of macroinvertebrates across ages 2 to 5 years and did not reveal any evidence of an ontogenetic diet shift. Cisco gut contents consisted primarily of cladoceran and copepod zooplankton in addition to chironomids, regardless of cisco age. Mysid shrimp ($Mysis relictta$) were first identified in the gut tracts of 3 year old cisco and this prey was also observed in the gut tracts of 4 and 5 year old individuals. Amphipods ($Diporeia hoyi$) were observed in the gut tracts of 5 year old cisco only.
Stable isotope signatures of nitrogen (δ\textsuperscript{15}N) signatures did not change significantly in bluegill over the ages 2 to 5 (Table 3.1; Fig. 3.2), supporting the gut content observations (Table 3.2), that bluegill did not change feeding strategies beyond age 2 (ANOVA; \(P = 0.484\)). Cisco δ\textsuperscript{15}N signatures, however, were enriched significantly over the same age range (ANOVA; \(P = 0.01\)) (Table 3.1; Fig. 3.2). The enrichment of δ\textsuperscript{15}N was relatively minor, exhibiting an overall isotopic enrichment of approximately 1.5 ‰ over the four years, the equivalent of 0.44 trophic levels (Minagawa and Wada 1984). This trophic enrichment likely represented the decrease in cladoceran consumption after age 4 in favour of an increased dependence on more energy rich macroinvertebrates such as Mysids (Table 3.2).

Carbon (δ\textsuperscript{13}C) signatures ranged from −25.7 to −30.4‰ in bluegill and from −31.2 to −34.6‰ in cisco (Table 3.1; Fig.3.2), as would be predicted from the littoral and pelagic feeding strategies of these species. For both species, δ\textsuperscript{13}C signatures did not vary significantly with age (ANOVA, bluegill: \(P = 0.46\); cisco: \(P = 0.31\)), indicating that both bluegill and cisco were consistent over their lifespan in the carbon sources they were tracking.

PCB congeners 74, 101, and 180 were chosen to demonstrate the age related changes in chemical fugacities and to also provide examples of the congener bioaccumulation coefficients estimated for these compounds in bluegill and cisco (Fig. 3.3). With the exception of PCBs 31/28 and 74 measured in bluegill, all PCB congener fugacities were positively correlated with age. Additionally, the congener bioaccumulation coefficients determined for PCBs 52, 99, 101, 138, 153/132, 180, 187/182, and 196/203 were statistically significant (linear regression, \(P < 0.05\)) for bluegill. For cisco, all PCB congeners demonstrated increases in fugacity with only congeners 31/28, 70/76, and 74 demonstrating non-significant increases in fugacity from 2-5 years of age (linear regression; \(P > 0.05\)).
The resulting congener bioaccumulation coefficients derived from these relationships were positively correlated with PCB log $K_{ow}$ for both species (Fig. 3.4). For congeners with congener bioaccumulation coefficients < 0.6, fugacities in 5 year old fish were almost double that observed in 2 year old fish for both bluegill and cisco. Additionally, for congener bioaccumulation coefficients > 1.0, fugacities were approximately 4 fold higher in 5 year old bluegill relative to 2 year old individuals, and approximately 5 times higher in 5 year old cisco relative to 2 year old fish.

3.4 Discussion

In this study, significant increases in age normalized fugacities were observed for PCB congeners with log $K_{ow}$ > 6.8 in both bluegill sunfish and cisco. For less hydrophobic PCB congeners (log $K_{ow}$ < 6.2) minimal age related increases in fugacities were observed. These results provide evidence that different mechanisms regulate the accumulation of PCB congeners across the $K_{ow}$ range examined in this study.

Steady state models predict that age related increases in chemical fugacities would be minimal or become independent of age after steady state is achieved. The congener bioaccumulation coefficients determined for PCBs 31/28, 70/76, 74, 95, 105, 110, and 149 represent congeners that did not demonstrate significant age related increases in fugacity in bluegill. These patterns of accumulation in bluegill are more representative of relatively rapid elimination kinetics, and reflect the ability of this warm-water species to achieve steady state with ambient water and diet. For cisco, however, almost all congeners exhibited significant age related increases in fugacity as indicated by congener bioaccumulation coefficients that were consistently > 0.6. These results indicate that the accumulation of these congeners throughout the
age classes of cisco is more typical of non-steady state processes whereby dietary uptake overwhelms elimination mechanisms over the lifetime of the fish. These data predict that only very low log $K_{ow}$ (< 6.0) compounds are able to achieve steady state during the cisco’s lifetime. Of particular importance were the very high (> 1.0) congener bioaccumulation coefficients observed for PCBs 138, 153/132, 180, 187/182, 196/203, and 201 in both bluegill and cisco. These congeners are exceedingly hydrophobic (log $K_{ow}$ > 6.8) and such patterns of bioaccumulation indicate that none of these congeners will achieve steady state during the lifetime of either species.

There are, however, a few possible alternative models to non-steady accumulation: (i) the fugacity capacity of the fish decreased with age as a response to either a decrease in either body weight or lipid content or (ii) the fish consumed more contaminated food as they aged. An increase in the fugacity of a fish could, coupled with slow elimination kinetics, occur if there was either a decrease in body weight and/or lipid content as the fish aged. While the growth rates of both species had slowed considerably by age 5, growth dilution remained a significant attenuating factor to chemical accumulation for all age-classes. Lipid content, between ages 2 – 5, remained relatively stable at approximately 1.53 and 3.03 % for bluegill and cisco, respectively. Therefore, the observed increases in fugacity were not a function of major changes in lipid content. Although cisco demonstrated slight lipid accumulation from age 2 to age 5 (with mean lipid contents of 2.35 and 3.64 % for age 2 and age 5 fish, respectively), this minor change only serves to reduce the observed fugacity increases. Bluegill lipid contents did decrease significantly from age 2-5; however, the change was largely driven by a large decline in lipids between age 2 (2.72%) and age 3 (1.50%), indicating that fugacity increases after year 3 were not influenced by decreases in fugacity capacity due to either declines in lipids or body mass.
The consumption of more contaminated food with age could be responsible for the observed age-related fugacity increases. Gut contents and stable isotopes analyses indicated that any ontogenetic changes that occurred with age in these species were minimal. The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope data generated for bluegill and cisco demonstrate the differences in resource utilisation and trophic status between these two species. Both bluegill and cisco were determined to be generalist feeders within their respective environments, as established by the diversity of prey items within their gut contents (Table 3.1). Based on bluegill gut contents analysis, there was no indication for a substantial difference in dietary items consumed across age-classes. The lack of ontogenetic diet shifts in bluegill was further supported by $\delta^{15}N$ analyses, which showed no differences in mean nitrogen isotopic signatures across age in bluegill (Figure 3.3a) suggesting that they undergo very little change in feeding strategy as they age. Similar results were obtained by Paterson et al. (2006) for Detroit River bluegill, who reported no significant enrichment in $\delta^{15}N$ as this species aged. Cisco were observed to significantly enrich in $\delta^{15}N$ from age 2 – 5, increasing by 0.97‰ over that timeframe. The inclusion of mysids and the benthic amphipod Diporeia hoyi in the diet of older cisco likely contributes to the higher trophic position of these species (Rasmussen et al. 1990). Both of these prey species typically inhabit deeper areas of freshwater lakes, ingest sediment as part of their diets and accumulate higher PCB burdens relative to plankton and other primary consumers (Evans et al. 1982; Kiriluk et al. 1995; Johannsson et al. 2001). The overall increase in trophic status over this age range was, however, less than half a trophic level, assuming that 3.4‰ is representative of one trophic level (Minagawa and Wada 1984). This relatively small increase in cisco $\delta^{15}N$, reinforced by the presence of common diet items across age-classes, support the
conclusion that major diet shifts cannot explain the observed increase in chemical fugacity as fish age.

The more depleted cisco $\delta^{13}C$ signatures demonstrate a primarily pelagic, open water carbon source for this species in contrast to the enriched $\delta^{13}C$ signature associated with the greater incorporation of diet items by bluegill tracking littoral zone primary productivity (Rau 1978; France 1995a, b). In both species, $\delta^{13}C$ signatures did not change between ages 2 to 5 (Fig. 3.2), indicating that habitat differences related to bluegill and cisco foraging activities remained consistent across the different age-classes.

Paterson et al. (2007a) measured PCB elimination in three size classes of yellow perch ($Perca flavescens$) exposed to ambient temperature cycles over a period of one year and concluded that while PCBs of $\log K_{ow}$ less than 5.70 were rapidly eliminated in all size classes (27.4 – 87.7 g), chemical elimination rates of super hydrophobic congeners were not sufficient for even the smallest size class of fish (mean weight = 27.4 g) to achieve steady state fugacities in their lifetime. Wszolek et al. (1979) supported this conclusion, as they demonstrated that lake trout dosed with PCB 153 ($\log K_{ow}$ 6.92) and released into Cayuga Lake, NY, had not eliminated the chemical to any significant degree when captured eight years later. These previous studies demonstrate the limited ability of fish to eliminate high $K_{ow}$ PCBs (Jensen et al. 1982; Kiriluk et al. 1995). In addition to slow elimination rates, these trends have, in part, been attributed to the declines in growth efficiency that typically occur with age in freshwater fish species (Borgmann and Whittle 1992; Paterson et al 2005).

Sharbot Lake bluegill and cisco have a similar ability to accumulate high $K_{ow}$ ($\log K_{ow} > 6.8$) but a different potential to accumulate low $K_{ow}$ ($\log K_{ow} < 5.7$) PCBs. This interspecific difference in bioaccumulation dynamics indicates that different mechanisms regulate the
bioaccumulation of high and low $K_{ow}$ PCBs in these two species of freshwater fish. The differences in the life histories of bluegill and cisco were defined by the difference in their growth rates and the water temperature associated with the habitats they preferred. Reductions in water temperatures have been demonstrated to reduce the capacity of freshwater fish species to eliminate PCBs (Chapter 2; Paterson et al. 2007a, b). Identified in Chapter 2 as being due to temperature-driven metabolic rates, the more rapid bioaccumulation of low $K_{ow}$ congeners in warm-water fish is likely due to the differences in preferred thermal habitats between the two species. Bluegill favour warm-water littoral habitats and will tolerate water temperatures up to 30 ºC (Cherry et al. 1977). Cisco, however, prefer cool - cold water pelagic waters of 8-16 ºC (Rudstam and Magnusson 1985; Aku et al. 1997). This difference in temperature suggests that bluegill have a greater potential to eliminate a broader range of PCBs than cisco and also that low $K_{ow}$ PCBs can achieve steady state more rapidly in bluegill than in cisco.

Growth dilution is often an important process contributing to the dilution of chemical burden (Paterson et al. 2006). The von Bertalanffy (VBL) growth model calculations in this study indicated that differences in growth between bluegill and cisco played an important role in PCB accumulation patterns. The VBL growth coefficients ($k$) predicted that the time to achieve 90% ($t_{90}$) of the asymptotic length ($L_{\infty}$) is approximately 7.7 years for bluegill and 5.3 years for cisco. Such growth kinetics indicate that by 5 years of age, Sharbot Lake cisco are closer to their zero/maintenance growth level than are similarly aged bluegill sunfish. A greater proportion of the lower $K_{ow}$ ($\log K_{ow} < 6.8$) PCB congeners in bluegill demonstrated non-significant bioaccumulation coefficients compared with in cisco. This pattern suggests that growth dilution and chemical elimination are important mechanisms for regulating chemical accumulation in bluegill across the studied age classes.
For high $K_{ow}$ PCBs, the majority of chemical assimilated into the body is absorbed from the diet (Morrison et al. 1997) and uptake rates are directly related to food consumption rates and energetic demands (Paterson et al. 2006). Under these considerations, it is predicted that bluegill, living in an energetically expensive warm-water environment, would be less ecologically efficient and accumulate PCBs at a more rapid rate than cisco to achieve a similar body size.

However, despite occupying different environments, bluegill and cisco exhibited similar congener bioaccumulation coefficients for congeners of $\log K_{ow} > 6.8$, indicating that they have similar energetic demands. Cisco, unlike bluegill which are relatively philopatric and forage along the lake bottom of shallow littoral zones (Table 3.2; Keast 1977), travel extensively as they forage throughout the relatively deep water column (Hrabik et al. 2006; Jensen et al. 2006). The higher energetic costs incurred by bluegill from inhabiting warm water are inferred to be met by the high foraging costs in cisco and results in similar bioaccumulation of highly hydrophobic chemicals in these two species.

This research demonstrated significantly different rates of PCB bioaccumulation between two insectivorous fish species that occupy different thermal habitats within the same aquatic system. The relationships observed between the congener bioaccumulation coefficients and $K_{ow}$ illustrate that a warm water species such as bluegill possess a greater capacity for the elimination of less hydrophobic compounds relative to a cold-water species such as cisco. The greater potential to eliminate low $K_{ow}$ PCBs in a warm-water environment, coupled with a prolonged period over which growth dilution can occur, allows bluegill to maintain low PCB concentrations spanning a wider range of $K_{ow}$ than cisco. In slow growing, cold-water species, the steady state condition occurs for only a limited number of low $K_{ow}$ PCBs while the majority of congeners fail to achieve steady state within the lifetime of the fish. Bluegill and cisco were determined to have
similar energetic demands as indicated by the similar accumulation patterns of high $K_{ow}$ PCBs between the two species. Cisco likely achieve similarly rapid bioaccumulation rates as bluegill as a consequence of their energetically expensive foraging strategy. Importantly, this research demonstrates that high $K_{ow}$ PCBs have the potential to act as tracers of ecological processes associated with bioenergetic demands.
3.5 Literature Cited


Table 3.1. Summarized biological, stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotope, and polychlorinated biphenyl (PCB) data for bluegill sunfish (*Lepomis macrochirus*) and cisco (*Coregonus artedii*) age classes. Values in parentheses indicate 1 standard error.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age (yrs)</th>
<th>N</th>
<th>Average mass (g)</th>
<th>Average length (cm)</th>
<th>Average lipid (%)</th>
<th>Average $\delta^{13}$C (‰)</th>
<th>Average $\delta^{15}$N (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bluegill</td>
<td>2</td>
<td>3</td>
<td>13.0 (0.4)</td>
<td>9.9 (1.2)</td>
<td>2.7 (0.1)</td>
<td>-28.3 (0.3)</td>
<td>8.6 (0.1)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>7</td>
<td>26.1 (4.7)</td>
<td>12.0 (1.1)</td>
<td>1.5 (0.3)</td>
<td>-28.2 (0.4)</td>
<td>8.9 (0.3)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>9</td>
<td>45.4 (5.3)</td>
<td>14.4 (1.3)</td>
<td>1.3 (0.2)</td>
<td>-27.4 (0.5)</td>
<td>9.2 (0.2)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3</td>
<td>69.8 (13.6)</td>
<td>16.3 (2.9)</td>
<td>1.0 (0.4)</td>
<td>-28.7 (0.9)</td>
<td>8.9 (0.3)</td>
</tr>
<tr>
<td>Cisco</td>
<td>2</td>
<td>6</td>
<td>33.1 (6.6)</td>
<td>16.0 (0.8)</td>
<td>2.4 (0.3)</td>
<td>-32.4 (0.4)</td>
<td>10.3 (0.5)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>8</td>
<td>53.4 (12.0)</td>
<td>18.0 (1.1)</td>
<td>3.9 (0.7)</td>
<td>-33.1 (0.5)</td>
<td>10.7 (0.3)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>10</td>
<td>63.8 (6.9)</td>
<td>19.8 (1.3)</td>
<td>2.2 (0.4)</td>
<td>-33.3 (0.4)</td>
<td>11.4 (0.2)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>6</td>
<td>75.6 (10.4)</td>
<td>20.8 (2.0)</td>
<td>3.6 (0.4)</td>
<td>-33.0 (0.4)</td>
<td>11.3 (0.3)</td>
</tr>
</tbody>
</table>
Table 3.2. Mean dietary data for bluegill sunfish (*Lepomis macrochirus*) and cisco (*Coregonus artedii*) age classes. Values indicate the average proportion each prey item represented of the total diet items enumerated in individual fish stomach contents.

<table>
<thead>
<tr>
<th>Age classes</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prey category</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bluegill</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphipoda</td>
<td>-</td>
<td>16.7</td>
<td>11.1</td>
<td>-</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>8.3</td>
<td>0.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Diptera (Chironomidae)</td>
<td>1.7</td>
<td>16.7</td>
<td>11.6</td>
<td>-</td>
</tr>
<tr>
<td>Ephemeroptera</td>
<td>15.0</td>
<td>41.7</td>
<td>14.8</td>
<td>-</td>
</tr>
<tr>
<td>Hymenoptera</td>
<td>29.2</td>
<td>-</td>
<td>12.1</td>
<td>-</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>14.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Odonata</td>
<td>1.4</td>
<td>-</td>
<td>23.1</td>
<td>-</td>
</tr>
<tr>
<td>Trichoptera</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>22.0</td>
</tr>
<tr>
<td>Zooplankton (Cladocera + Copepoda)</td>
<td>16.7</td>
<td>-</td>
<td>22.2</td>
<td>58.0</td>
</tr>
<tr>
<td>Detritus/other</td>
<td>21.5</td>
<td>16.7</td>
<td>2.8</td>
<td>20.0</td>
</tr>
<tr>
<td><strong>Cisco</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amphipoda</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.3</td>
</tr>
<tr>
<td>Diptera (Chironomidae)</td>
<td>40.0</td>
<td>40.0</td>
<td>20.0</td>
<td>33.3</td>
</tr>
<tr>
<td>Bivalvia</td>
<td>-</td>
<td>-</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>Mysidacea</td>
<td>-</td>
<td>20.0</td>
<td>39.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Odonata</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>25.0</td>
</tr>
<tr>
<td>Zooplankton (Cladocera + Copepoda)</td>
<td>60.0</td>
<td>40.0</td>
<td>40.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Detritus/other</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8.3</td>
</tr>
</tbody>
</table>
Figure 3.1. Total lengths for (a) bluegill sunfish (*Lepomis macrochirus*) and (b) cisco (*Coregonus artedii*) age classes. Solid curves in each panel represent the von Bertalanffy growth curves describing the relationships between total length and age for each species. Asymptotic lengths ($L_\infty$; cm; dotted lines) and von Bertalanffy growth coefficient ($k$; year$^{-1}$) estimates are also provided.
Figure 3.2: Average carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures in bluegill sunfish (*Lepomis macrochirus*) and cisco (*Coregonus artedii*) age classes. Bluegill and cisco are indicated by the solid and open symbols, respectively. Error bars for both $\delta^{13}C$ and $\delta^{15}N$ indicate ± 1 standard error.
Figure 3.3: Relationships between age 2 normalized PCB congener fugacities ($f$) and age for (a) bluegill sunfish (*Lepomis macrochirus*) and (b) cisco (*Coregonus artedii*). Solid, dotted and dashed lines represent the best fit least squares regression lines for PCB congeners 74, 101, and 180, respectively. Error bars in both panels represent ± 1 standard error.
Figure 3.4: Relationships between congener bioaccumulation coefficients and log $K_{ow}$ for 17 PCB congeners measured in (a) bluegill sunfish (*Lepomis macrochirus*) and (b) cisco (*Coregonus artedii*) age classes. Open symbols represent those PCB congeners that did not demonstrate significant ($P > 0.10$) increases in fugacity from age 2 to 5. Closed symbols indicate PCB congeners that demonstrated significant increases in fugacity from age 2 to 5. Labels beside each symbol denote the individual PCB congener. Horizontal...
dotted line represents a congener bioaccumulation coefficient value of 0.6. Congener bioaccumulation coefficients > 0.6 indicate those PCBs that exhibit bioaccumulation patterns indicative of non-steady state kinetics. Vertical dotted line represents a log $K_{ow}$ value of 6.8.
CHAPTER 4: GENERAL DISCUSSION

This thesis provides insight into the effects of temperature and chemical hydrophobicity ($K_{ow}$) on polychlorinated biphenyl (PCB) accumulation in three species of freshwater fish. Chapter 2 investigated the influence of temperature on the relationship between elimination rate constants ($k_2$) and hydrophobicity ($K_{ow}$) in yellow perch (*Perca flavescens*). In Chapter 3, the relative importance of steady and non-steady state bioaccumulation models was investigated in natural populations of bluegill sunfish (*Lepomis macrochirus*) and cisco (*Coregonus artedii*). Combined, these studies both provided support for the need to develop non-steady state models, particularly for the most hydrophobic congeners.

It was determined, both experimentally in Chapter 2 and empirically in Chapter 3, that high $K_{ow}$ PCBs do not achieve steady state during the life of fish that experience cold temperatures for a significant portion of the year (i.e. temperate fish). While the body of research that supports this conclusion is substantial (Wszolek et al. 1979; Schrap and Opperhuizen 1988; Sijm et al. 1992; Paterson et al. 2007a,b), the majority of PCB bioaccumulation models assume that all PCB congeners achieve steady state within the lifetime of a fish (e.g. Arnot and Gobas 2004; Debruyne and Gobas 2006; Gewurtz et al. 2006). The assumption that all chemicals achieve steady state was supported by chemical elimination studies that were conducted under thermally optimal conditions and employing small fish (Fisk et al. 1998; Dabrowska et al. 1999). The assumption that the bioaccumulation kinetics of small fish under thermally optimal conditions can be applied to feral fish populations was biased as temperate fish experience sub-optimal temperatures for the majority of the year and undergo physiological changes as they age.
When increases in body size (Sijm et al. 1995; Paterson et al. 2007a) and variable temperature conditions (Chapter 2; Paterson et al. 2007b) are considered, elimination rates, over the course of a lifetime, are considerably slower has been measured in the above studies. The results of both Chapters 2 and 3, support the conclusion that many high $K_{ow}$ congeners do not achieve steady state within the lifetime of temperate fish and these results are consistent with field studies which have examined PCB bioaccumulation with age and $K_{ow}$ (Jensen et al. 1986; Kiriluk et al. 1995).

The results generated from Chapter 2 were critical to understanding PCB bioaccumulation in natural systems. Temperature-mediated metabolic rates were predicted to equally influence $k_2$ across $K_{ow}$, but increases in $k_2$ with temperature were observed only for low $K_{ow}$ congeners while almost negligible for high $K_{ow}$ congeners (Fig. 4.1). For high $K_{ow}$ congeners, $k_2$ is regulated primarily by hydrophobicity, regardless of the ambient temperature. These observations on accumulation dynamics were further confirmed in feral fish populations in Chapter 3. The majority of low $K_{ow}$ congeners (i.e. log$K_{ow} < 6.2$) were observed to achieve steady state in bluegill while the majority of the same congeners in cisco did not achieve steady state within the species’ lifetime. The bioaccumulation of low $K_{ow}$ congeners in these species were significantly associated with temperature.

Cisco maintained higher congener bioaccumulation coefficients of low $K_{ow}$ congeners than bluegill, suggesting that the steady state concentrations of cisco, if achieved, would be greater than those of bluegill. Non-steady state biomagnification in cisco dominated a wider span of $K_{ow}$ than in bluegill where, due to more rapid elimination rates of low $K_{ow}$ congeners, bioaccumulation may be more reflective of
temperature-driven elimination rates. The bioaccumulation of the lowest $K_{ow}$ congeners, which may approach or achieve thermodynamic equilibrium, is regulated by body size, lipid content and fugacities of their surrounding environment. As such, these congeners serve as physiological tracers or indicators of baseline contamination within a system.

The majority of high $K_{ow}$ PCBs, however, measured in bluegill and cisco exhibited PCB bioaccumulation that adhered to the non-steady state condition. This conclusion is supported by the results generated in Chapter 2 whereby elimination mechanisms were inferred to have no influence on bioaccumulation under the various temperatures experienced by temperate fish. Bioaccumulation was, therefore, directly related to chemical uptake, which is determined by food consumption rates (Morrison et. al. 1997; Russell et al. 1999). As such, the observed variability in the bioaccumulation of high $K_{ow}$ PCBs is predicted to be a product of consumption rates, which are reflective of individual specific energetic demands (Fig. 4.2). As bluegill inhabit energetically expensive warm-water environments relative to those of cisco, it was predicted that, due to the higher consumption rates associated with meeting higher, temperature-driven energetic demands, bluegill would accumulate high $K_{ow}$ PCBs at a more rapid rate than cisco. The bioaccumulation rates of high $K_{ow}$ congeners in these species were, however, similar (Fig. 3.4), suggesting that bluegill and cisco were subject to similar energetic demands. Cisco, though their metabolic optima are achieved in energetically inexpensive cold water, may incur similar energetic demands as bluegill due to higher foraging costs. Cisco feed in a more three-dimensionally environment (Hrabik et al. 2006, Jensen et al. 2006) relative to the shallow, littoral environment of bluegill; these higher foraging costs result in energetic demands in cisco which are equal to those of bluegill living in a warm-water
That bluegill and cisco incur similar energetic demands, despite experiencing different environments and employing different foraging strategies, indicates that high $K_{ow}$ PCBs are capable of tracing intra-and interspecific variability in consumption rates and therefore, energetic demands. This variability, being driven by physiological and ecological processes, speaks to the potential of these compounds to act as tracers of these processes, *in situ*, in natural systems.

This thesis demonstrated that, over the lifetime of a fish, low $K_{ow}$ compounds are products of physiological factors, such as growth, temperature preference and diet whereas the accumulation of high $K_{ow}$ compounds are a function of ecological processes associated food consumption efficiencies. In both chapters, it was identified that factors such as temperature and growth have little influence on the bioaccumulation of highly hydrophobic compounds over the lifetime of a fish. That is,

Most importantly, this thesis indicates the potential for PCBs to be used as tracers of bioenergetic processes in the natural environment. Biological and ecological perturbations, such as the introduction of exotic species, overexploitation, pollution, and climate change, all have energetic consequences to the individuals inhabiting affected systems. PCBs will therefore be highly informative in describing the potential impact of these perturbations. Understanding and mitigating the impact of these perturbations will be a major challenge of this century and PCBs, as *in situ* tracers, will prove to be an important tool for not only acknowledging system perturbations, but also quantifying the impact they may have on affected systems.
4.1 Literature Cited


Figure 4.1: Hypothetical relationships of $k_2$ versus $K_{ow}$ at different temperatures. The affect of temperature on $k_2$ may be either (a) equal across $K_{ow}$ or (b) greater as $K_{ow}$ increases.
<table>
<thead>
<tr>
<th>Time</th>
<th>PCB</th>
<th>% lipid</th>
<th>Lifetime</th>
</tr>
</thead>
<tbody>
<tr>
<td>High Consumption</td>
<td>Low Consumption</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low Consumption</td>
<td>High Consumption</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 4.2:** The accumulation of a high (dotted lines) and low (solid lines) $K_{ow}$ PCB at high and low consumption rates is presented. Regardless of food consumption rates, all PCBs eventually achieve steady state. The lower $K_{ow}$ PCB is able to do so within the lifetime of the organism while the higher $K_{ow}$ does not.
<table>
<thead>
<tr>
<th><strong>NAME:</strong></th>
<th>Michael Daniel Burtnyk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PLACE OF BIRTH:</strong></td>
<td>Etobicoke, Ontario, Canada</td>
</tr>
<tr>
<td><strong>YEAR OF BIRTH:</strong></td>
<td>1981</td>
</tr>
<tr>
<td><strong>EDUCATION:</strong></td>
<td>Sacred Heart Catholic High School, Newmarket, Ontario, 1995-1999</td>
</tr>
<tr>
<td></td>
<td>Queen’s University, Kingston, Ontario, 1999-2004</td>
</tr>
<tr>
<td></td>
<td>Great Lakes Institute for Environmental Research, University of Windsor, Windsor, Ontario, 2005-2009</td>
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