University of Windsor

Scholarship at UWindsor

2009

The Influence of Temperature and Chemical Hydrophobicity on Steady and Non-Steady State Polychlorinated Biphenyl Bioaccumulation in Temperate Fish

Michael Burtnyk
University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

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

Windsor, Ontario, Canada
2009
© 2009 Michael D. Burtnyk

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

by
Michael D. Burtnyk

APPROVED BY:

_____
S.J. Loeb
Chemistry and Biochemistry

_____
K.G. Drouillard
Great Lakes Institute for Environmental Research

_____
G.D. Haffner, Advisor
Great Lakes Institute for Environmental Research

_____
B.J. Fryer, Chair of Defence
Great Lakes Institute for Environmental Research

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 (*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 (*Lepomis macrochirus*) or cisco (*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.

TABLE OF CONTENTS

LIST OF TABLES

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 age classes 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.

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.

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.

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

1

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

$$(1.1) \qquad f = C / Z$$

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

$$(1.2) \qquad Z = X_L K_{ow} / H$$

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 ($C_{org} k_2$):

(1.3) $$dC/dt = C_w k_1 + C_f k_f - C_{org} k_2$$

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. 2007*a*). 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. (2007*b*) 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 13ºC and 23ºC. 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

Brett J.R. and T.D.D. Groves. 1979. Physiological Energetics. In Fish Physiology. Edited by W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. Pp 279 – 352.

Buckman A.H., Brown S.B., Hoekstra P.F., Soloman K.R. and A.T. Fisk. 2004. Toxicokinetics of three polychlorinated biphenyl technical mixtures in rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry 23: 1725-1736.

Clark K.E., Gobas F.A.P.C. and D. Mackay. 1990. Model of organic chemical uptake and clearance by fish from food and water. Environmental Science and Technology 24: 1203-1213.

Connolly J.P. and C.J. Pederson. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. Environmental Science and Technology 22: 99-103.

Debruyn A.M.H. and F.A.P.C. Gobas. 2006. A bioenergetic biomagnification model for the animal kingdom. Environmental Science and Technology 40: 1581-1587.

Gobas F.A.P.C., Muir D.C.G. and D. Mackay. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. Chemosphere 17: 943-962.

Gobas F.A.P.C., Zhang X. and R. Wells. 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environmental Science and Technology 27: 2855-2863.

Gobas F.A.P.C., Wilcockson J.B., Russell R.W. and G.D. Haffner. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. Environmental Science and Technology 33: 133-141.

Hattula M.L. and O. Karlog. 1972. Toxicity of polychlorinated biphenyls (PCB) to Goldfish. Acta Pharmacologica et Toxicologica 31: 238-240.

Hawker D.W. and D.W. Connell. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. Environmental Science and Technology 22: 382-387.

Kitchell J.F., Stewart D.J. and D. Weininger. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). Journal of the Fisheries Research Board of Canada 34: 1922-1935.

Leblanc G.A. 1995. Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. Environmental Science and Techology 29: 154-160.

Mackay D. 1981. Calculating fugacity. Environmental Science and Technology 15: 1006-1014.

Mackay D. 1982. Correlation of bioconcentration factors. Environmental Science and Technology 16: 274-278.

Mackay D. and A.I. Hughes. 1984. Three-parameter equation describing the uptake of organic compounds by fish. Environmental Science and Technology 18: 439-444.

Morrison H.A., Gobas F.A.P.C., Lazar R., Whittle D.M. and G.D. Haffner. 1997. Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in western Lake Erie. Environmental Science and Technology 31: 3267-3273.

Oliver B.G. and A.J. Niimi. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environmental Science and Technology 22: 388-397.

Paterson G., Drouillard K.G. and G.D. Haffner. 2007*a*. Long-term polychlorinated biphenyl elimination by three size classes of yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences 64: 1222-1233.

Paterson G., Drouillard K.G. and G.D. Haffner. 2007*b*. PCB elimination by yellow perch (*Perca flavescens*) during an annual temperature cycle. Environmental Science & Technology 41: 824-829.

Reiser D.W., Greenberg E.S., Helser T.E., Branton M. and K.D. Jenkins. 1994. In situ reproduction, abundance and growth of young-of-the-year and adult largemouth bass in a population exposed to polychlorinated biphenyls. Environmental Toxicology and Chemistry 23: 1762-1773.

Russell R.W., Gobas F.A.P.C. and Haffner, G.D. 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in food webs. Environ. Toxicol. Chem. 18: 1250-1257.

Sijm D.T.H.M., Seinen W. and A. Opperhuizen. 1992. Life-cycle biomagnification in fish. Environmental Science and Technology 26: 2162-2174.

Thomann R.V. and J.P. Connolly. 1984. Model of PCB in the Lake Michigan lake trout food chain. Environmental Science and Technology 18: 65-71.

Wania F. and D. Mackay. 1996. Tracking the distribution of persistent organic pollutants. Environmental Science and Technology 30: 390A-396A.

Winberg G.G. 1954. Rate of metabolism and food requirements of fishes. Fisheries Research Board of Canada Translation Series No. 194. Ottawa, Ontario.

Wszolek P.C., Wachs T., Youngs W.D. and D.J. Lisk. 1979. Persistence of polychlorinated biphenyls and 1, 1 – Dichloro - 2, 2 – bis (*p* – chlorophenyl) ethylene (*p, p'* – DDE) with age in lake trout after 8 years. Environmental Science and Technology 13: 1269-1271.
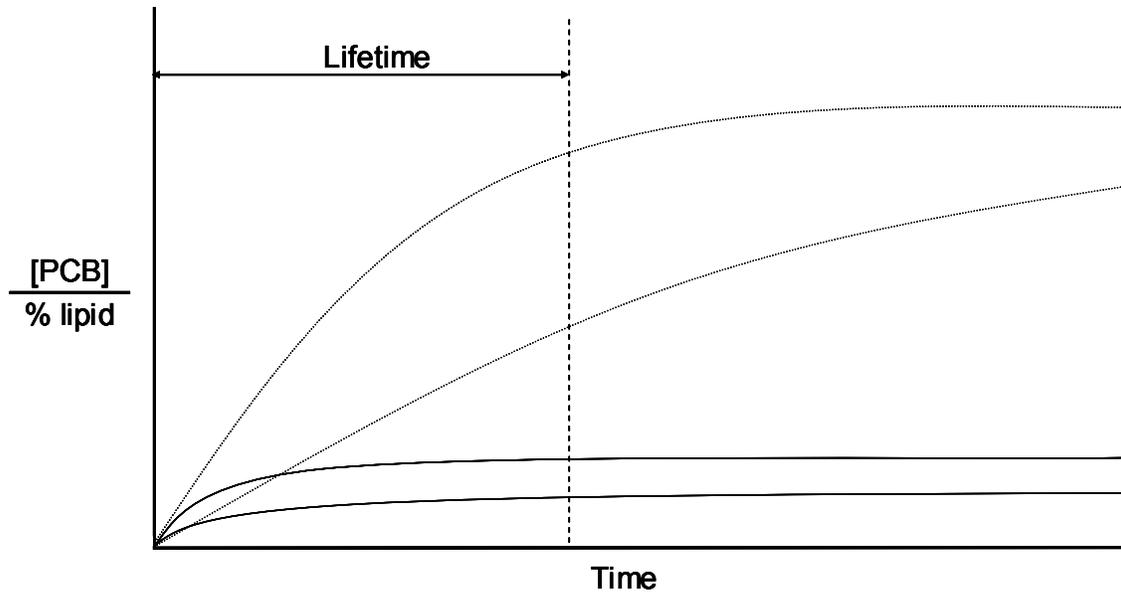
Figure 1.1: Theoretical bioaccumulation of PCB congeners across a range of low to high hydrophobicity (K_{ow}) where congeners represented by solid lines achieve steady state within the lifetime of the individual and dotted lines represent PCB congeners that also achieve steady state, but do so over 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) $$- d[PCB]/dt = k_2[PCB]$$

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

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

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) $$\ln [PCB]_t = -k_2 t + \ln [PCB]_0$$

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

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

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

k_2 values for a limited number of species have been measured at variable or non-optimal temperatures (Spigarelli et al. 1984; Paterson et al. 2007*b*). Paterson *et al.* (2007*b*) 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. (2007*b*) 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 logK_{ow} from 5.02 to 8.18 in yellow perch (*Perca flavescens*) raised under two constant temperature regimes of 23 and 13ºC, the influence of temperature on k_2 was determined. An optimal temperature was chosen, 23ºC (Kitchell et al. 1977), where feeding rate is high and growth potentials are maximized. A lower temperature of 13ºC 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 13ºC. 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 23ºC 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 23ºC 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 –30ºC 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 Na_2SO_4 (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

13

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 110ºC

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

Na_2SO_4 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 250ºC, injection volume

was 2μL (spitless), carrier gas was helium (flow rate of 1 mL/min) and detector temperature was

280ºC. GC column temperature was programmed from 90 to 280ºC. 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. (2007*a*) 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:

$$(2.5) \qquad\qquad\qquad t_{90} = \ln(10) / k_2$$

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:

$$(2.6) \qquad [PCB]_{Growth} = [PCB]_{Sacrifice} \cdot (Mass_{Sacrifice} / Mass_{t=0})$$

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

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 23ºC tank was 23.09 ± 0.04ºC and the 13ºC tank was 12.99 ± 0.01ºC. 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 23ºC (28.6 ± 1.14 g) and 13ºC (32.1 ± 8.27 g) tanks at the beginning of the experiment (Paired t-test, $P > 0.05$). The sampled 13ºC 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 23ºC, 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 13ºC (8.97 ± 0.34 %) and 23ºC (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 13ºC and 23ºC 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 23ºC and 13ºC, 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 23ºC and 13ºC 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 23ºC was a mean 2.30 fold higher than k_2 at 13ºC (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 23ºC and 13ºC. 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 23ºC than the fish at 13ºC, 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

18

strong evidence that metabolic rate has a substantial impact only on the k_2 of chemicals with low

K_{ow} (i.e. logK_{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 23ºC 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. logK_{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

Barron M.G., Stehly G.R. and W.L. Hayton. 1990. Pharmacokinetic modeling in aquatic animals I. Models and Concepts. Aquatic Toxicology 18: 61-86.

Brett J.R. and T.D.D. Groves. 1979. Physiological Energetics. *In* Fish Physiology. Edited by W.S. Hoar, D.J. Randall, and J.R. Brett. Academic Press, New York. Pp 279 – 352.

Buckman A.H., Brown S.B., Hoekstra P.F., Solomon K.R. and A.T. Fisk. 2004. Toxicokinetics of three polychlorinated biphenyl technical mixtures in rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry 23: 1725-1736.

Buckman A.H., Brown S.B., Small J., Muir D.C.G., Parrott J., Solomon K.R. and A.T. Fisk. 2007. Role of temperature and enzyme induction in the biotransformation of polychlorinated biphenyls and formation of hydroxlyated polychlorinated biphenyls by rainbow trout (*Oncorhynchus mykiss*). Environmental Science and Technology 41: 3856-3863.

Clark K.E., Gobas F.A.P.C. and D. Mackay. 1990. Model of organic chemical uptake and clearance by fish from food and water. Environmental Science and Technology 24: 1203-1213.

Coristine S., Haffner G.D., Ciborowski J.J.H., Lazar R., Nanni M.E. and C.D. Metcalfe. 1996. Elimination rates of selected di-*ortho*, mono-*ortho* and non-*ortho* substituted polychlorinated biphenyls in rainbow trout (*Oncorhynchus mykiss*). Environmental Toxicology and Chemistry 15: 1382-1387.

Daley J.M., Leadley T.A. and K.G. Drouillard. (*In Press*). Evidence for bioamplification of nine polychlorinated biphenyl (PCB) congeners in yellow perch (*Perca flavescens*) eggs during incubation. Chemosphere (2009), doi:10.1016/j.chemosphere.2009.02.013

Drouillard K.G. and R.J. Norstrom. 2003. The influence of diet properties and feeding rates on PCB toxicokinetics in the ring dove. Archives of Environmental Contamination and Toxicology 44: 97-106.

Drouillard K.G., Hagen H. and G.D. Haffner. 2004. Evaluation of chloroform/methanol and dichloromethane/hexane extractable lipids as surrogate measures of sample partition capacity for organochlorines in fish tissues. Chemosphere 55: 395-400.

Enders E.C., Boisclair D., Boily P. and P. Magnan. 2006. Effect of body mass and water temperature on the standard metabolic rate of juvenile yellow perch, *Perca flavescens* (Mitchill). Environmental Biology of Fishes 76: 399-407.

Fisk A.T., Norstrom R.J., Cymbalisty C.D. and D.C.G. Muir. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their

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

Gewurtz S.B., Laposa R., Gandhi N., Christensen G.N., Evenset A., Gregor D. and M.L. Diamond. 2006. A comparison of contaminant dynamics in arctic and temperate fish: a modeling approach. Chemosphere 1328-1341.

Gobas F.A.P.C., Zhang X. and R. Wells. 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environmental Science and Technology 27: 2855-2863.

Jenson A.L., Spigarelli S.A. and M.M. Thommes. 1982. PCB uptake by five species of fish in Lake Michigan, Green Bay of Lake Michigan and Cayuga Lake, New York. Canadian Journal of Fisheries and Aquatic Sciences 39: 700-709.

Jimenez B.D., Cirmo C.P. and J.F. McCarthy. 1987. Effects of feeding and temperature on uptake, elimination and metabolism of benzo(a)pyrene in the bluegill sunfish (*Lepomis macrochirus*). Aquatic Toxicology 10: 41-57.

Kidd K.A., Schindler D.W., Hesslein R.H. and D.C.G. Muir. 1998. Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in Yukon Territory. Canadian Journal of Fisheries and Aquatic Sciences 55: 869-881.

Kiriluk R.M., Servos M.R., Whittle D.M., Cabana G. and J.B. Rasmussen. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Canadian Journal of Fisheries and Aquatic Sciences 52: 2660-2674.

Kitchell J.F., Stewart D.J. and D. Weininger. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). Journal of the Fisheries Research Board of Canada 34: 1922-1935.

Lazar R., Edwards R.C., Metcalfe C.D., Gobas F.A.P.C., and G.D. Haffner. 1992. A simple, novel method for the quantitative analysis of coplanar (non-*ortho*-substituted) polychlorinated biphenyls in environmental samples. Chemosphere 25: 493-504.

Leney J.L., Drouillard K.G. and G.D. Haffner. 2006. Does metamorphosis increase the susceptibility of frogs to highly hydrophobic contaminants? Environmental Science and Technology 40: 1491-1496.

Mackay D. 1982. Correlation of bioconcentration factors. Environmental Science and Technology 16: 274-278.

Madenjian C.P., Carpenter S.R. and P.S. Rand. 1994. Why are the PCB concentrations of Salmonine individuals from the same lake so highly variable? Canadian Journal of Fisheries and Aquatic Sciences 51: 800-807.

Morrison H.A., Gobas F.A.P.C., Lazar R., Whittle D.M. and G.D. Haffner. 1997. Development and verification of a benthic/pelagic food web bioaccumulation model for PCB congeners in western Lake Erie. Environmental Science and Technology 31: 3267-3273.

Niimi A.J., and B.G. Oliver. 1983. Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole fish and muscle of rainbow trout (*Salmo gairdneri*). Canadian Journal of Fisheries and Aquatic Sciences 40: 1388-1394.

Paterson G., Huestis S.Y., D.M. Whittle, Drouillard K.G. and G.D. Haffner. 2005. In situ measurement of tissue turnover and energy conversion efficiencies in lake trout (*Salvelinus namaycush*) using a novel toxicokinetic approach. Canadian Journal of Fisheries and Aquatic Sciences 62: 464-471.

Paterson G., Drouillard K.G. and G.D. Haffner. 2007*a*. Long-term polychlorinated biphenyl elimination by three size classes of yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences 64: 1222-1233.

Paterson G., Drouillard K.G. and Haffner, G.D. 2007*b*. PCB elimination by yellow perch (*Perca flavescens*) during an annual temperature cycle. Environmental Science and Technology 41: 824-829.

Scott W.B. and E.J. Crossman. 1973. Freshwater fishes of Canada. Bulletin of the Fisheries Research Board of Canada No. 184.

Spigarelli S.A., Thommes M.M. and W. Prepejchal. 1983. Thermal and metabolic factors affecting PCB uptake by adult brown trout. Environmental Science and Technology 17: 88-94.

Veith G.D., DeFoe D.L. and B.V. Bergstedt. 1979. Measuring and estimating the bioconcentration factor and chemicals in fish. Journal of the Fisheries Research Board of Canada 36: 1040-1048.

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

a)

23ºC	logK_{ow}	Experiment Day							
		0	8	48	84	114	178	235	254
PCB 19	5.02	ND	ND	-	ND	-	-	-	ND
PCB 18	5.24	111.6222	77.19582	-	83.94081	-	-	-	96.37534
PCB 31/28	5.67	99.7057	101.1238	-	102.3977	-	-	-	149.5375
PCB 52	5.84	135.9484	139.3761	-	132.6957	-	-	-	282.2422
PCB 95	6.13	123.2817	150.9327	-	134.6885	-	-	-	273.4042
PCB 101	6.38	184.2003	235.1969	-	189.4505	-	-	-	384.1899
PCB 110	6.48	107.0464	123.6049	-	95.10662	-	-	-	228.3722
PCB 149	6.67	161.1927	197.5686	-	151.5215	-	-	-	308.1495
PCB 153/132	6.92	522.7651	559.0735	-	470.6967	-	-	-	816.4856
PCB 177	7.08	ND	32.50683	-	23.71369	-	-	-	62.74663
PCB 180	7.36	120.3103	109.8583	-	80.15566	-	-	-	182.9874
PCB 201	7.62	ND	9.579017	-	ND	-	-	-	58.98725
PCB 194	7.8	ND	ND	-	ND	-	-	-	21.34445
PCB 206	8.09	ND	ND	-	ND	-	-	-	7.326714

b)

13ºC	logK_{ow}	Experiment Day						
		0	5	10	20	40	80	180
PCB 19	5.02	1.399315	1.102351	1.852938	1.178126	1.587394	2.080377	2.080377
PCB 18	5.24	10.46145	7.673383	13.29399	8.86063	12.3372	15.83444	15.83444
PCB 31/28	5.67	24.78817	21.96786	29.72377	19.7529	27.77316	34.05232	34.05232
PCB 52	5.84	34.81733	32.83872	42.68673	30.87634	40.13826	48.8388	48.8388
PCB 95	6.13	40.06624	35.82585	47.57873	34.94655	46.36765	53.11409	53.11409
PCB 101	6.38	72.90365	69.18221	78.36643	61.39346	79.56008	93.32886	93.32886
PCB 110	6.48	41.69315	39.29433	46.45298	35.16542	45.58198	53.07912	53.07912
PCB 149	6.67	56.97377	54.06196	65.83972	49.10101	61.33213	76.27358	76.27358
PCB 153/132	6.92	159.4891	157.3155	170.7537	133.9219	163.8765	202.0912	202.0912
PCB 177	7.08	10.52559	10.07172	12.35639	9.055336	10.73452	14.14557	14.14557
PCB 180	7.36	40.25946	37.4506	38.61799	29.90558	37.89975	45.97755	45.97755
PCB 201	7.62	7.834052	7.691003	7.431665	6.273289	8.283326	8.766429	8.766429
PCB 194	7.8	4.595363	4.41241	4.933183	3.208144	5.777713	6.241405	6.241405
PCB 206	8.09	2.535823	2.879907	1.52584	2.567069	5.197449	5.443196	5.443196

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

23°C	$\log K_{ow}$	P	k_2	t_{90}
PCB 19	5.02	0.00	0.0141 ± 0.0035	163.4
PCB 18	5.24	0.00	0.0107 ± 0.0013	215.3
PCB 31/28	5.67	0.00	0.0059 ± 0.0013	388.7
PCB 52	5.84	0.05	0.0030 ± 0.0015	754.9
PCB 95	6.13	0.22	0.0018 ± 0.0014	1267.4
PCB 101	6.38	0.59	0.0008 ± 0.0014	2945.3
PCB 110	6.48	0.47	0.0010 ± 0.0013	2305.2
PCB 149	6.67	0.78	0.0004 ± 0.0014	5805.8
PCB 153/132	6.92	0.85	0.0003 ± 0.0015	8153.3
PCB 177	7.08	0.66	0.0007 ± 0.0015	3385.7
PCB 180	7.36	0.80	0.0000 ± 0.0014	∞
PCB 201	7.62	0.88	0.0002 ± 0.0015	10354.4
PCB 194	7.8	0.64	0.0000 ± 0.0014	∞
PCB 206	8.09	0.17	0.0000 ± 0.0013	∞

13°C	$\log K_{ow}$	P	k_2	t_{90}
PCB 19	5.02	0.00	0.0077 ± 0.0011	298.8
PCB 18	5.24	0.00	0.0041 ± 0.0009	557.6
PCB 31/28	5.67	0.02	0.0024 ± 0.0009	962.0
PCB 52	5.84	0.17	0.0014 ± 0.0010	1623.7
PCB 95	6.13	0.29	0.0010 ± 0.0010	2197.1
PCB 101	6.38	0.48	0.0007 ± 0.0010	3237.6
PCB 110	6.48	0.43	0.0008 ± 0.0010	2940.4
PCB 149	6.67	0.53	0.0006 ± 0.0010	3565.8
PCB 153/132	6.92	0.54	0.0007 ± 0.0011	3358.6
PCB 177	7.08	0.42	0.0009 ± 0.0011	2475.2
PCB 180	7.36	0.64	0.0005 ± 0.0011	4389.5
PCB 201	7.62	0.38	0.0011 ± 0.0012	2108.6
PCB 194	7.8	0.49	0.0008 ± 0.0012	2776.9
PCB 206	8.09	0.59	0.0007 ± 0.0013	3150.1
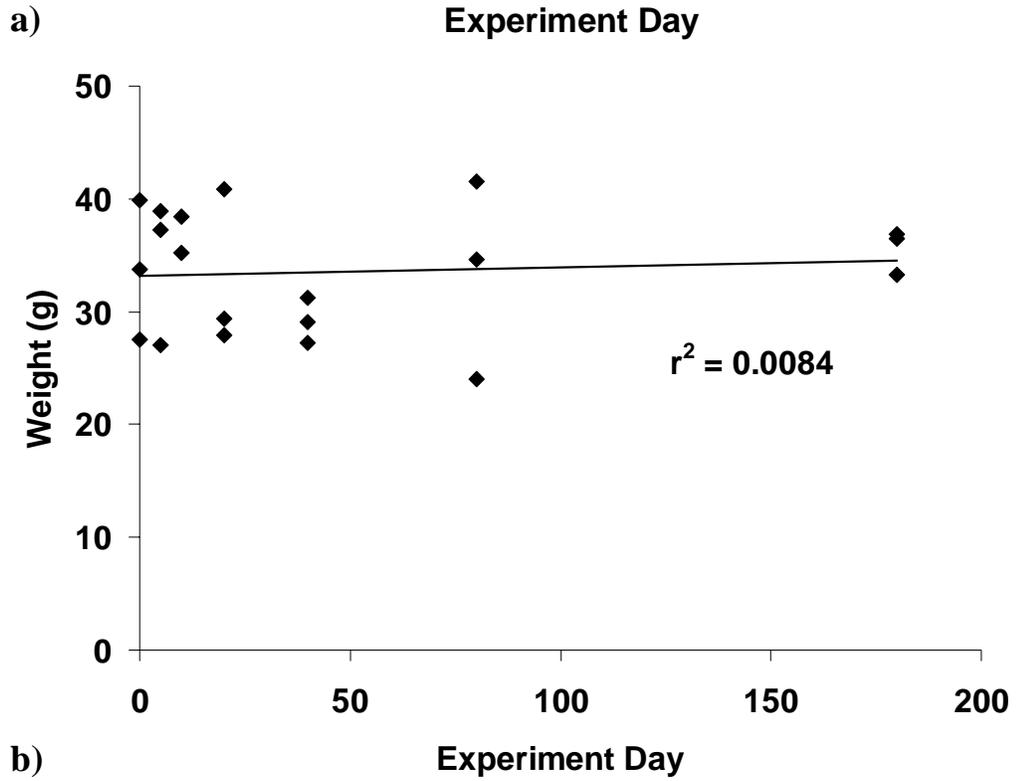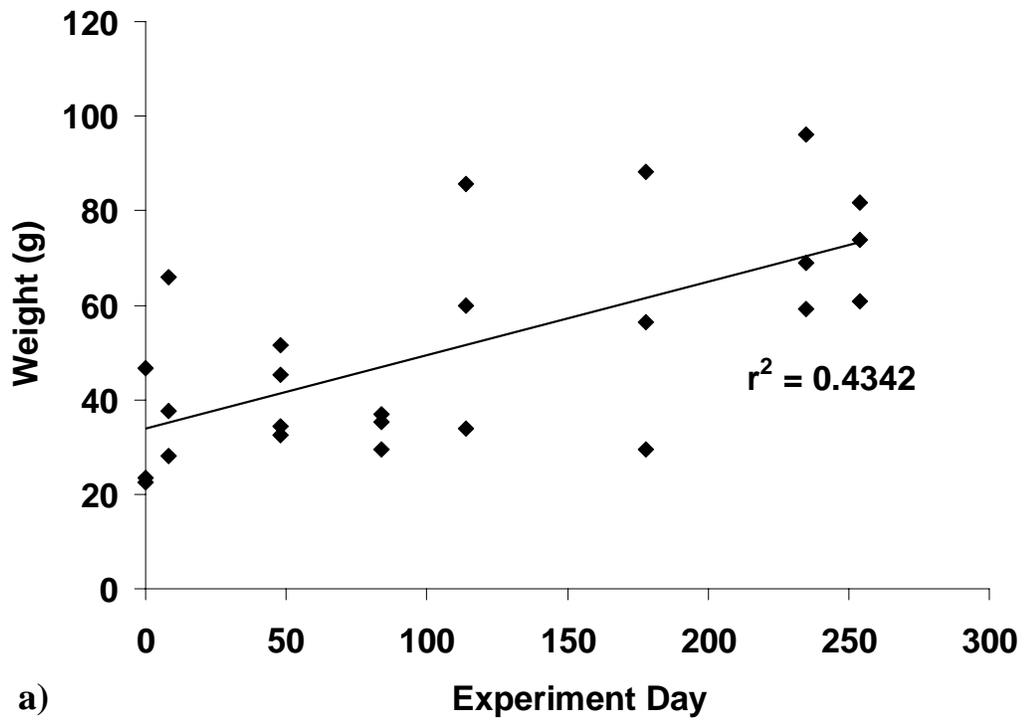
Figure 2.1: Body mass of individual fish at (a) 23ºC and (b) 13ºC. Solid lines represent the linear least squares regression lines.
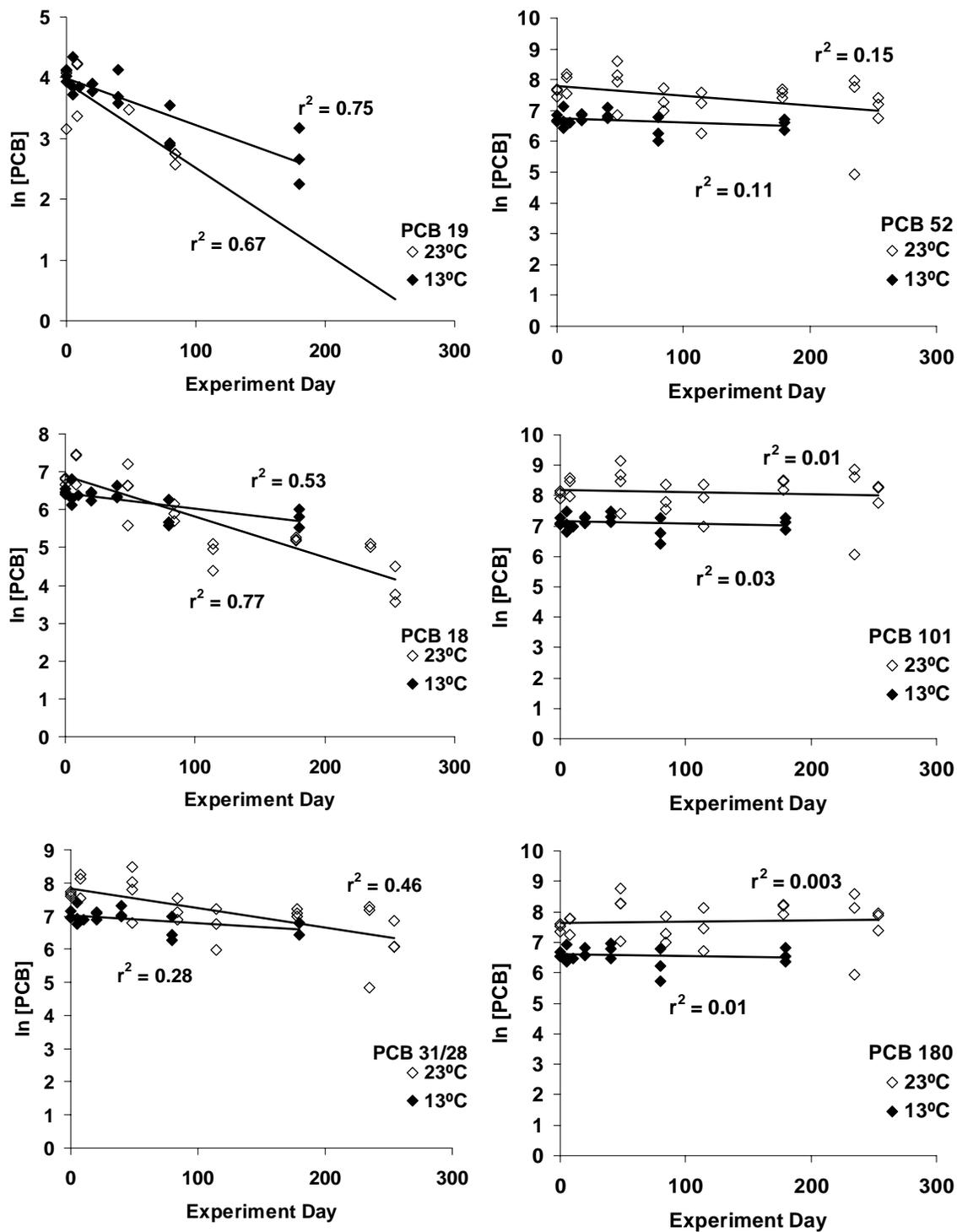
Figure 2.2: Elimination of PCBs (ng/g) across the entire K_{ow} range of tested congeners over a 254 day period at 23ºC (◊) and a 180 day period (♦). Each point represents an individual fish and solid lines represent the least squares regression lines.
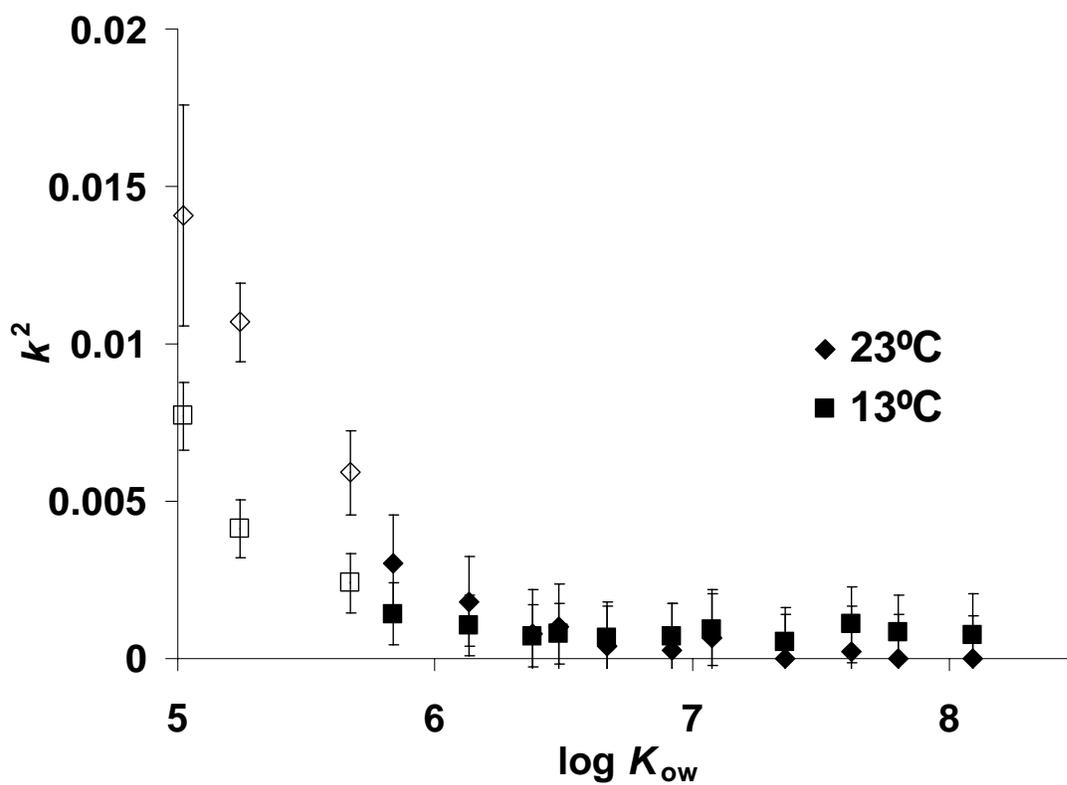
Figure 2.3: PCB congener k_2 values ($\pm$ 1 standard error) at 23ºC ($\blacklozenge$) and 13ºC ($\blacksquare$). k_2 values were calculated from Eq. (2.3). Open and solid symbols represent congeners that were significantly and not significantly eliminated, respectively.

CHAPTER 3: STEADY AND NON-STEADY STATE KINETICS IN NATURAL POPULATIONS OF BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS*) AND CISCO (*COREGONUS ARTEDII*).

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

$$(3.1) \qquad\qquad f = C/Z$$

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

$$(3.2) \qquad\qquad Z = X_L K_{ow} / H$$

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

29

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. 2007*a*, *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. 2007*b*).

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 30ºC during the summer in temperate lakes (Cherry et al. 1977). Cisco are cold water, pelagic species that are confined mainly to the colder (8-15ºC) 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

32

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 (44.46ºN, 76.41ºW) from May to September 2006. Sharbot Lake is a cold, relatively deep ($Z_{max} \sim 34m$) 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 ºC 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_2SO_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 110ºC 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

Na_2SO_4 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 250ºC, injection volume

was 2µL (spitless), carrier gas was helium (flow rate of 1 mL/min) and detector temperature was

280ºC. GC column temperature was programmed from 90 to 280ºC. 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 [13]C – labelled PCB

52 and [13]C – 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:

$$(3.3) \quad \delta X = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where δX (‰) 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:

$$(3.4) \qquad L_t = L_\infty \cdot (1 - e^{-k(t-t_0)})$$

where L_t is the total length (cm) of the fish at time t, L_∞ 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_∞ ($t_{0.50}$ and $t_{0.90}$) were also calculated as outlined in eqs. (3.5) and (3.6) below respectively.

$$(3.5) \qquad t_{50} = \ln(2) / k_2$$

$$(3.6) \qquad t_{90} = \ln(10) / k_2$$

In investigating chemical bioaccumulation across age, a cohort analysis approach was taken. This approach was taken to investigate PCB bioaccumulation, δ^{15}N and δ^{13}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;

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

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_∞) 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 $2.7 \pm 0.1\%$ in 2 year old fish to $1.0 \pm 0.4\%$ 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 relicta*) 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^{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^{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^{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^{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^{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 (δ^{13}C) and nitrogen (δ^{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 δ^{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 δ^{15}N as this species aged. Cisco were observed to significantly enrich in δ^{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 δ^{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 1995*a*, *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. (2007*a*) 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_∞) 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} (logK_{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 the 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

Aku P.M.K., Rudstam L.G. and Tonn W.M. 1997. Impact of hypolimnetic oxygenation on the vertical distribution of cisco (*Coregonus artedii*) in Amisk Lake, Alberta. Canadian Journal of Fisheries and Aquatic Sciences 54: 2182-2195.

Balcer M.D., Korda N. L., and S.I. Dodson. 1984. Zooplankton of the Great Lakes: a guide to the identification and ecology of the common crustacean species. University of Wisconsin Press.

Bentzen E., Lean D.R.S., Taylor W.D., and D. Mackay. 1996. Role of food web structure on lipid and bioaccumulation of organic contaminants by lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences 53: 2397-2407.

Borgmann U., and D.M. Whittle. 1992. Bioenergetics and PCB, DDE, and mercury dynamics in Lake Ontario lake trout (*Salvelinus namaycush*): a model based on surveillance data. Canadian Journal of Fisheries and Aquatic Sciences 49: 1086-1096.

Cherry D.S., Dickson K.L., Cairns J. Jr. and J.R. Stauffer. 1977. Preferred, avoided and lethal temperatures of fish during rising temperature conditions. Journal of the Fisheries Research Board of Canada 34: 239-246.

Clark K.E., Gobas F.A.P.C. and D. Mackay. 1990. Model of organic chemical uptake and clearance by fish from food and water. Environmental Science and Technology 24: 1203-1213.

Connolly J.P. and C.J. Pederson. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. Environmental Science and Technology 22: 99-103.

Debruyn A.M.H. and F.A.P.C. Gobas. 2006. A bioenergetic biomagnification model for the animal kingdom. Environmental Science and Technology 40: 1581-1587.

Drouillard K.G., Hagen H. and G.D. Haffner. 2004. Evaluation of chloroform/methanol and dichloromethane/hexane extractable lipids as surrogate measures of sample partition capacity for organochlorines in fish tissues. Chemosphere 55: 395-400.

Evans M.S., Bathelt R.W. and C.P Rice. 1982. PCBs and other toxicants in *Mysis relicta*. Hydrobiologia 93: 205-215.

Fisk A.T., Hoekstra P.F., Borgå K. and D.C.G. Muir. 2003. Correspondence: biomagnification. Marine Pollution Bulletin 46: 522-524.

France R.L. 1995*a*. Carbon-13 enrichment in benthic compared to planktonic algae: foodweb implications. Marine Ecology Progress Series 124: 307-312.

France R.L. 1995*b*. Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. Limnology and Oceanography 40: 1310-1313.

Gobas F.A.P.C., Muir D.C.G. and D. Mackay. 1988. Dynamics of dietary bioaccumulation and faecal elimination of hydrophobic organic chemicals in fish. Chemosphere 17: 943-962.

Gobas F.A.P.C., Zhang X. and R. Wells. 1993. Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals. Environmental Science and Technology 27: 2855-2863.

Gobas F.A.P.C., Wilcockson J.B., Russell R.W. and G.D. Haffner. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. Environmental Science and Technology 33: 133-141.

Gray J.S. 2002. Biomagnification in marine systems: the perspective of an ecologist. Marine Pollution Bulletin 45: 46-52.

Hagen H.J. 2005. Distribution of bioavailable organic contaminants in the lower food web of morphologically distinct freshwater lakes. MSc. Thesis. University of Windsor, Windsor, ON, Canada.

Hattula M.L. and O. Karlog. 1972. Toxicity of polychlorinated biphenyls (PCB) to Goldfish. Acta Pharmacologica et Toxicologica 31: 238-240

Hawker D.W. and D.W. Connell. 1988. Octanol-water partition coefficients of polychlorinated biphenyl congeners. Environmental Science and Technology 22: 382-387.

Hrabik T.R., Jensen O.P., Martell S.J.D., Walters C.J. and J.F. Kitchell. 2006. Diel vertical migration in the Lake Superior pelagic community. I. Changes in vertical migration of coregonids in response to varying predation risk. Canadian Journal of Fisheries and Aquatic Sciences 63: 2286-2295.

Jenson A.L., Spigarelli S.A. and M.M. Thommes. 1982. PCB uptake by five species of fish in Lake Michigan, Green Bay of Lake Michigan and Cayuga Lake, New York. Canadian Journal of Fisheries and Aquatic Sciences 39: 700-709.

Jensen O.P., Hrabik T.R., Martell S.J.D., Walters C.J. and J.F. Kitchell. 2006. Diel vertical migration in the Lake Superior pelagic community. II. Modeling trade-offs at an intermediate trophic level. Canadian Journal of Fisheries and Aquatic Sciences 63: 2296-2307.

Johannsson O.E., Leggett M.F., Rudstam L.G., Servos M.R., Mohammadian M.A., Gal G., MDermott R.M. and R.H. Hesslein. 2001. Diet of *Mysis relicta* in Lake Onatrio as revealed by stable isotope and gut content analysis. Canadian Journal of Fisheries and Aquatic Sciences 58: 1975-1986.

Keast A. 1977. Mechanisms expanding niche width and minimizing intraspecific competition in two centrarchid fishes. Evolutionary Biology 10: 333-397.

Kiriluk R.M., Servos M.R., Whittle D.M., Cabana G. and J.B. Rasmussen. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Canadian Journal of Fisheries and Aquatic Sciences 52: 2660-2674.

Lazar R., Edwards R.C., Metcalfe C.D., Gobas F.A.P.C., and G.D. Haffner. 1992. A simple, novel method for the quantitative analysis of coplanar (non-*ortho*-substituted) polychlorinated biphenyls in environmental samples. Chemosphere 25: 493-504.

Leblanc G.A. 1995. Trophic-level differences in the bioconcentration of chemicals: implications in assessing environmental biomagnification. Environmental Science and Techology 29: 154-160.

Leney J.L., Drouillard K.G. and G.D. Haffner. 2006. Metamorphosis increases biotransformation of polychlorinated biphenyls: a comparative study of polychlorinated biphenyl metabolism in green frogs (*Rana clamitans*) and leopard frogs (*Rana pipiens*) at various life stages. Environmental Toxicology and Chemistry 25: 2971-2980.

Lumb C.E., Johnson T.B., Cook H.A., and J.A. Hoyle. 2007. Comparison of lake whitefish (*Coregonus clupeaformis*) growth, condition, and energy density between Lakes Erie and Ontario. Journal of Great Lakes Research 33: 315-325.

Mackay D. 1981. Calculating fugacity. Environmental Science and Technology 15: 1006-1014.

Mackay D. 1982. Correlation of bioconcentration factors. Environmental Science and Technology 16: 274-278.

Mackay D. and A.I. Hughes. 1984. Three-parameter equation describing the uptake of organic compounds by fish. Environmental Science and Technology 18: 439-444.

Minagawa M. and E. Wada. 1984. Stepwise enrichment of 15N along food chains: further evidence and the relation between δ15N and animal age. Geochimica et Cosmochimica Acta 48: 1135-1140.

Oliver B.G. and A.J. Niimi. 1988. Trophodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in the Lake Ontario ecosystem. Environmental Science and Technology 22: 388-397.

Paterson G., Huestis S.Y., D.M. Whittle, Drouillard K.G. and G.D. Haffner. 2005. In situ measurement of tissue turnover and energy conversion efficiencies in lake trout (*Salvelinus namaycush*) using a novel toxicokinetic approach. Canadian Journal of Fisheries and Aquatic Sciences 62: 464-471.

Paterson G., Drouillard K.G. and G.D. Haffner. 2006. An evaluation of stable nitrogen isotopes and polychlorinated biphenyls as bioenergetic tracers in aquatic systems. Canadian Journal of Fisheries and Aquatic Sciences 63: 628-641.

Paterson G., Drouillard K.G. and G.D. Haffner. 2007a. Long-term polychlorinated biphenyl elimination by three size classes of yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences 64: 1222-1233.

Paterson G., Drouillard K.G. and G.D. Haffner. 2007b. PCB elimination by yellow perch (*Perca flavescens*) during an annual temperature cycle. Environmental Science and Technology 41: 824-829.

Peckarsky B.L., Fraissinet P.R., Penton M.A., and D. R. Conklin Jr. 1990. Freshwater macroinvertebrates of northeastern North America. Cornell University Press.

Post D.M., Layman C.A., Arrington D.A., Takimoto G., Quattrochi J. and C.G. Montana. 2007. Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152: 179-189.

Rasmussen J.B, Rowan J.D., Lean D.R.S. and J.H. Carey. 1990. Food chain structure in Ontario lakes determines PCB levels in lake trout (*Salvelinus namaycush*). Canadian Journal of Fisheries and Aquatic Sciences 47: 2030-2038.

Rau G. 1978. Carbon-13 depletion in a subalpine lake: carbon flow implications. Science 201: 901-902.

Reiser D.W., Greenberg E.S., Helser T.E., Branton M. and K.D. Jenkins. 2004. *In situ* reproduction, abundance, and growth of young-of-the-year and adult largemouth bass in a population exposed to polychlorinated biphenyls. Environmental Toxicology and Chemistry 23: 1762-1773.

Rudstam L.G. and J.J. Magnusson. 1985. Predicting the vertical distribution of fish populations – analysis of cisco, *Coregonus artedii* and yellow perch, *Perca flavescens*. Can J. Fish. Aquat. Sci. 42: 1178-1188.

Russell R.W., Gobas F.A.P.C. and Haffner, G.D. 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in food webs. Environ. Toxicol. Chem. 18: 1250-1257.

Scott W.B. and E.J. Crossman. 1973. Freshwater fishes of Canada. Bulletin of the Fisheries Research Board of Canada No. 184.

Shuter B.J., Lester N.P., LaRose J., Purchase C.F., Vascotto K., Morgan G., Collins N.C. and P.A. Abrams. 2005. Optimal life histories and food web position: linkages among somatic growth, reproductive investment, and mortality. Canadian Journal of Fisheries and Aquatic Sciences 62: 738-746.

Sijm D.T.H.M., Seinen W. and A. Opperhuizen. 1992. Life-cycle biomagnification study in fish. Environmental Science and Technology 26: 2162-2174.

SYSTAT 2004. SYSTAT for Windows version 11.0. SYSTAT Software Inc. Richmond, CA.

Thomann R.V. and J.P. Connolly. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. Environmental Science and Technology 22: 99-103.

Wszolek P.C., Wachs T., Youngs W.D. and D.J. Lisk. 1979. Persistence of polychlorinated biphenyls and 1, 1 – dichloro- 2, 2 – bis (p – chlorophenyl) ethylene (p, p' – DDE) with age in lake trout after 8 years. Environmental Science and Technology 13: 1269-1271.

Table 3.1. Summarized biological, stable carbon (δ^{13}C) and nitrogen (δ^{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.

Species	Age (yrs)	*N*	Average mass (g)	Average length (cm)	Average lipid (%)	Average δ^{13}C (‰)	Average δ^{15}N (‰)
Bluegill	2	3	13.0 (0.4)	9.9 (1.2)	2.7 (0.1)	-28.3 (0.3)	8.6 (0.1)
	3	7	26.1 (4.7)	12.0 (1.1)	1.5 (0.3)	-28.2 (0.4)	8.9 (0.3)
	4	9	45.4 (5.3)	14.4 (1.3)	1.3 (0.2)	-27.4 (0.5)	9.2 (0.2)
	5	3	69.8 (13.6)	16.3 (2.9)	1.0 (0.4)	-28.7 (0.9)	8.9 (0.3)
Cisco	2	6	33.1 (6.6)	16.0 (0.8)	2.4 (0.3)	-32.4 (0.4)	10.3 (0.5)
	3	8	53.4 (12.0)	18.0 (1.1)	3.9 (0.7)	-33.1 (0.5)	10.7 (0.3)
	4	10	63.8 (6.9)	19.8 (1.3)	2.2 (0.4)	-33.3 (0.4)	11.4 (0.2)
	5	6	75.6 (10.4)	20.8 (2.0)	3.6 (0.4)	-33.0 (0.4)	11.3 (0.3)

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.

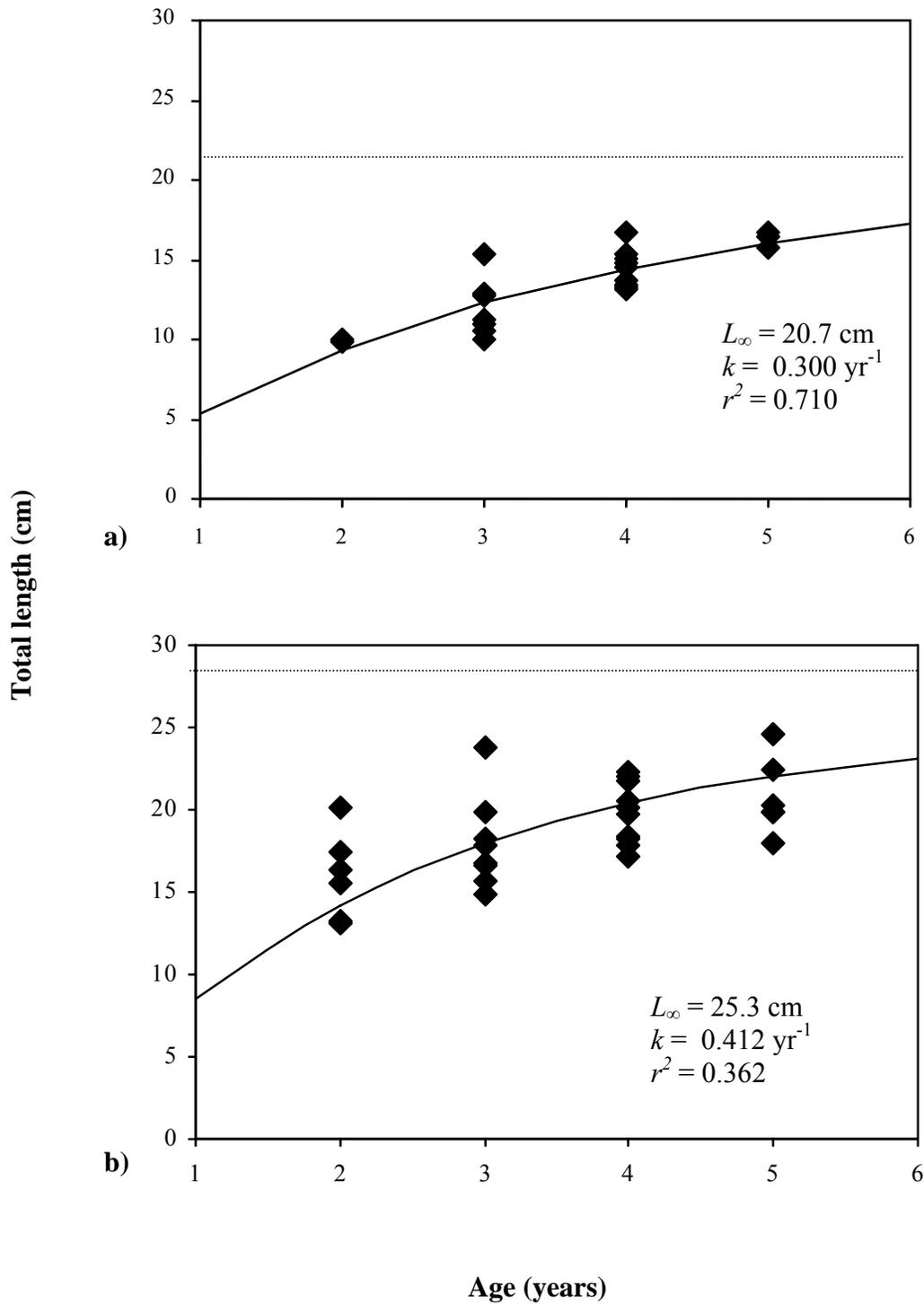|	Age classes			
Prey category	2	3	4	5
Bluegill				
Amphipoda	-	16.7	11.1	-
Coleoptera	-	8.3	0.7	-
Diptera (Chironomidae)	1.7	16.7	11.6	-
Emphemeroptera	15.0	41.7	14.8	-
Hymenoptera	29.2	-	12.1	-
Gastropoda	14.6	-	-	-
Odonata	1.4	-	23.1	-
Trichoptera	-	-	1.5	22.0
Zooplankton (Cladocera + Copepoda)	16.7	-	22.2	58.0
Detritus/other	21.5	16.7	2.8	20.0
Cisco				
Amphipoda	-	-	-	8.3
Diptera (Chironomidae)	40.0	40.0	20.0	33.3
Bivalvia	-	-	1.0	-
Mysidacea	-	20.0	39.0	10.0
Odonata	-	-	-	25.0
Zooplankton (Cladocera + Copepoda)	60.0	40.0	40.0	15.0
Detritus/other	-	-	-	8.3

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_∞; cm; dotted lines) and von Bertalanffy growth coefficient (k; year^{-1}) estimates are also provided.

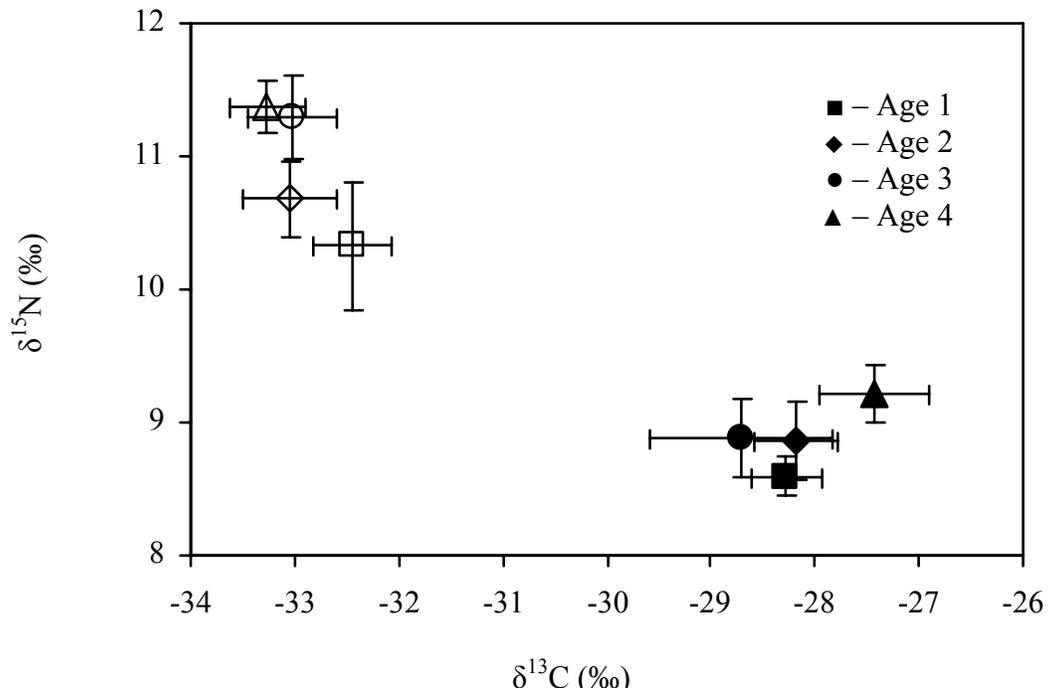**Figure 3.2**: Average carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope signatures in bluegill sunfish (*Lepomis macrochirus*) and cisco (*Coregonus artedii*) age classes. Bluegill and cisco are indicated by the solid and open symbols, respectively. Error bars for both $\delta^{13}C$ and $\delta^{15}N$ indicate $\pm$ 1 standard error.
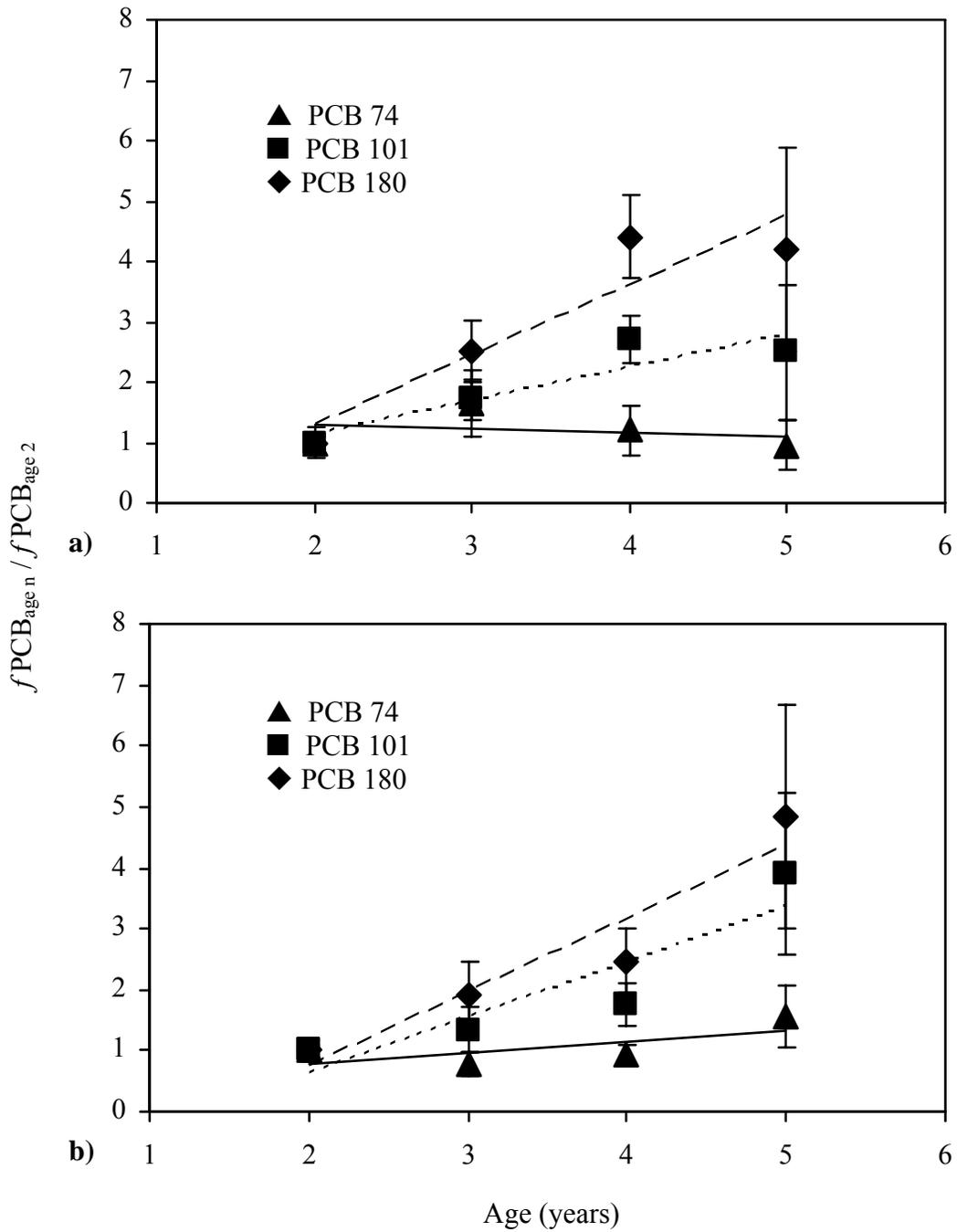
Figure 3.3: Relationships between age 2 normalized PCB congener fugacities (f) and age for (a) bluegill sunfish (*Lepomis macrochirus*) and (b) cisco (*Coregonus artedii*). Solid, dotted and dashed lines represent the best fit least squares regression lines for PCB congeners 74, 101, and 180, respectively. Error bars in both panels represent ± 1 standard error.
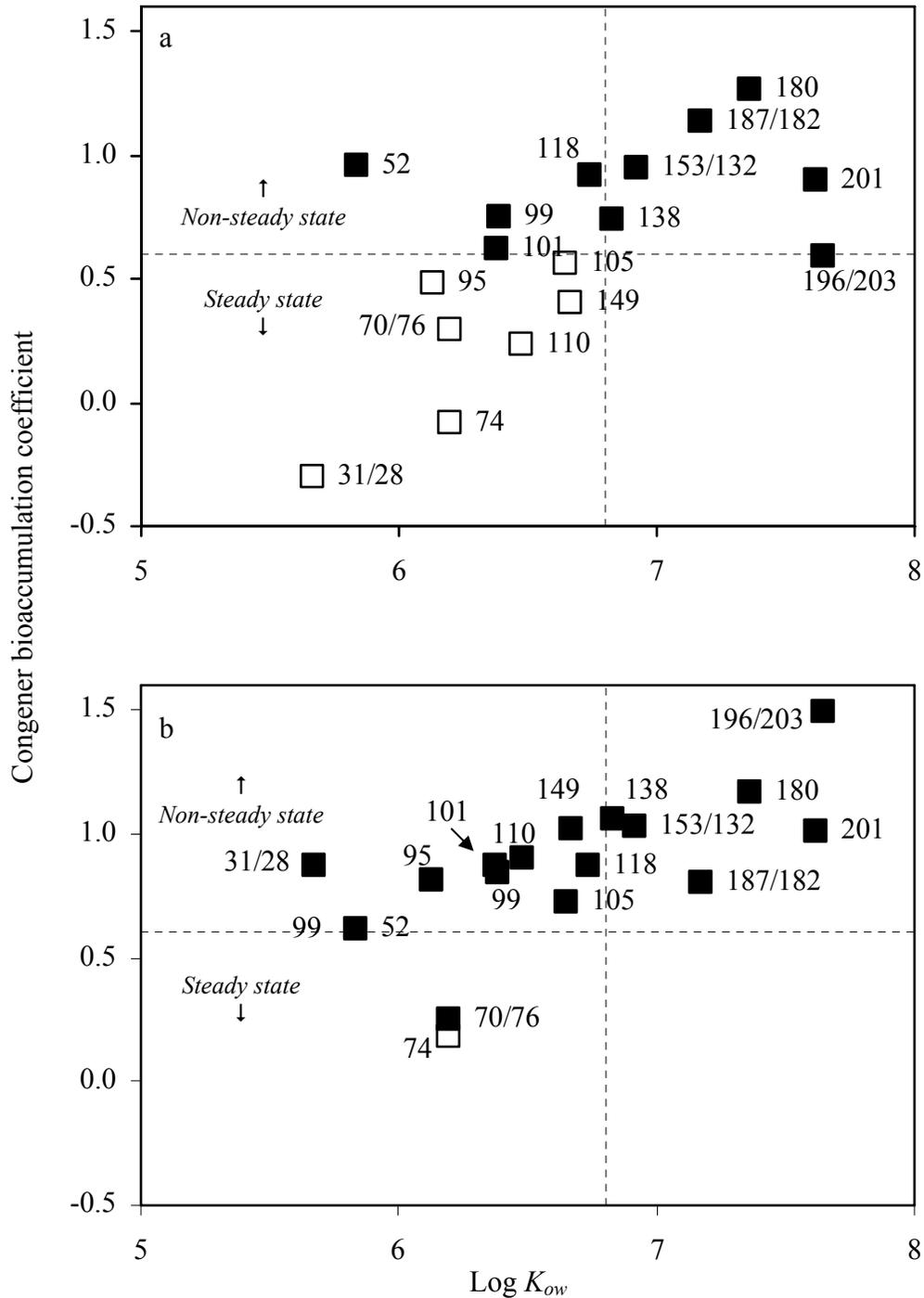
Figure 3.4: Relationships between congener bioaccumulation coefficients and log K_{ow} for 17 PCB congeners measured in (a) bluegill sunfish (*Lepomis macrochirus*) and (b) cisco (*Coregonus artedii*) age classes. Open symbols represent those PCB congeners that did not demonstrate significant ($P > 0.10$) 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. 2007*a,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; Debruyn 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.

61

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. logK_{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

environment. 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

Arnot J. A. and F. A. P. C. Gobas. 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environmental Toxicology and Chemistry 23: 2343-2355.

Dabrowska H., Fisher S.W., Dabrowski K. and A.E. Staubus. 1999. Dietary uptake efficiency of 2,2',4,4',5,5' - hexachlorobiphenyl in yellow perch and rainbow trout: role of dietary and body lipids. Environmental Toxicology and Chemistry 18: 938-945.

Debruyn A.M.H. and F.A.P.C. Gobas. 2006. A bioenergetic biomagnification model for the animal kingdom. Environmental Science and Technology 40: 1581-1587.

Fisk A.T., Norstrom R.J., Cymbalisty C.D. and D.C.G. Muir. 1998. Dietary accumulation and depuration of hydrophobic organochlorines: bioaccumulation parameters and their relationship with the octanol/water partition coefficient. Environmental Toxicology and Chemistry 17: 951-961.

Gewurtz S.B., Laposa R., Gandhi N., Christensen G.N., Evenset A., Gregor D. and M.L. Diamond. 2006. A comparison of contaminant dynamics in arctic and temperate fish: a modeling approach. Chemosphere 1328-1341.

Hrabik T.R., Jensen O.P., Martell S.J.D., Walters C.J. and J.F. Kitchell. 2006. Diel vertical migration in the Lake Superior pelagic community. I. Changes in vertical migration of coregonids in response to varying predation risk. Canadian Journal of Fisheries and Aquatic Sciences 63: 2286-2295.

Jenson A.L., Spigarelli S.A. and M.M. Thommes. 1982. PCB uptake by five species of fish in Lake Michigan, Green Bay of Lake Michigan and Cayuga Lake, New York. Canadian Journal of Fisheries and Aquatic Sciences 39: 700-709.

Jensen O.P., Hrabik T.R., Martell S.J.D., Walters C.J. and J.F. Kitchell. 2006. Diel vertical migration in the Lake Superior pelagic community. II. Modeling trade-offs at an intermediate trophic level. Canadian Journal of Fisheries and Aquatic Sciences 63: 2296-2307.

Kiriluk R.M., Servos M.R., Whittle D.M., Cabana G. and J.B. Rasmussen. 1995. Using ratios of stable nitrogen and carbon isotopes to characterize the biomagnification of DDE, mirex, and PCB in a Lake Ontario pelagic food web. Canadian Journal of Fisheries and Aquatic Sciences 52: 2660-2674.

Paterson G., Drouillard K.G. and G.D. Haffner. 2007*a*. Long-term polychlorinated biphenyl elimination by three size classes of yellow perch (*Perca flavescens*). Canadian Journal of Fisheries and Aquatic Sciences 64: 1222-1233.

Paterson G., Drouillard K.G. and Haffner, G.D. 2007*b*. PCB elimination by yellow perch (*Perca flavescens*) during an annual temperature cycle. Environmental Science and Technology 41: 824-829.

Russell R.W., Gobas F.A.P.C. and G.D. Haffner. 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. Environmental Toxicology and Chemistry 18 : 1250-1257.

Schrap S.M. amd A. Opperhuizen. 1988. Elimination kinetics of two unmetabolized polychlorinated biphenyls in *Poecilla reticulate* after dietary exposure. Bulletin of Environmental Contamination and Toxicology 40: 381-388.

Sijm D.T.H.M., Seinen W. and A. Opperhuizen. 1992. Life-cycle biomagnification in fish. Environmental Science and Technology 26: 2162-2174.

Sijm D.T.H.M. and A. Van der Linde. 1995. Size-dependant bioconcentration kinetics of hydrophobic organic chemicals in fish based on diffusive mass transfer and allometric relationships. Environmental Science and Technology 29: 2769-2777.

Wszolek P.C., Wachs T., Youngs W.D. and D.J. Lisk. 1979. Persistence of polychlorinated biphenyls and 1, 1 – Dichloro - 2, 2 – bis (*p* – chlorophenyl) ethylene (*p, p'* – DDE) with age in lake trout after 8 years. Environmental Science and Technology 13: 1269-1271.
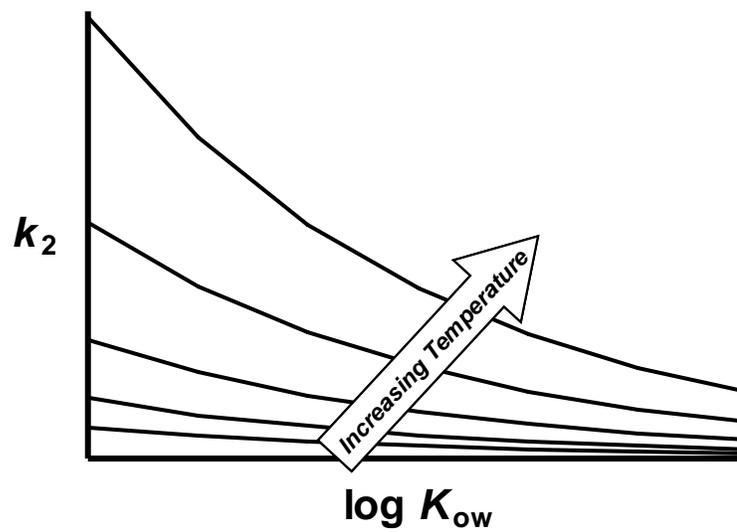
Figure 4.1: Hypothetical relationships of k_2 versus K_{ow} at different temperatures. The affect of temperature on k_2 may be either (a) equal across K_{ow} or (b) greater as K_{ow} increases.
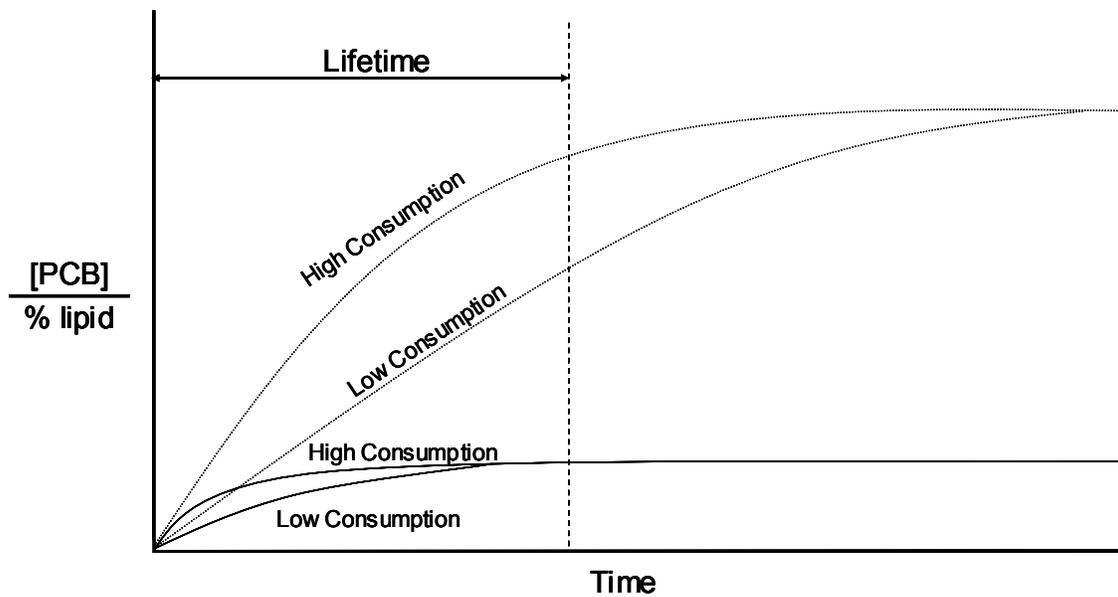
Figure 4.2: The accumulation of a high (dotted lines) and low (solid lines) K_{ow} PCB at high and low consumption rates is presented. Regardless of food consumption rates, all PCBs eventually achieve steady state. The lower K_{ow} PCB is able to do so within the lifetime of the organism while the higher K_{ow} does not.

VITA AUCTORIS

NAME: Michael Daniel Burtnyk

PLACE OF BIRTH: Etobicoke, Ontario, Canada

YEAR OF BIRTH: 1981

EDUCATION: Sacred Heart Catholic High School, Newmarket, Ontario,
1995-1999

Queen's University, Kingston, Ontario
1999-2004

Great Lakes Institute for Environmental Research,
University of Windsor, Windsor, Ontario
2005-2009