EVALUATING THE TRANSFER AND ACCUMULATION OF POLYUNSATURATED FATTY ACIDS IN FRESHWATER FOOD WEBS: A MODELING APPROACH

by

Jennifer M. Sawyer

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Department of Geography
University of Toronto
© Copyright by Jennifer M. Sawyer 2014
EVALUATING THE TRANSFER AND ACCUMULATION OF POLYUNSATURATED FATTY ACIDS IN FRESHWATER FOOD WEBS: A MODELING APPROACH

Jennifer M. Sawyer
Doctor of Philosophy
Department of Geography
University of Toronto
2014

ABSTRACT

Polyunsaturated fatty acids (PUFAs) are crucial nutrients for fish, affecting metabolic activity, growth rates and reproduction. A generic model was developed for a freshwater fish that estimates uptake, elimination and content of n-3 (α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid) and n-6 (linoleic acid, arachidonic acid) PUFAs. The model includes dietary uptake, absorption efficiency, egestion, transformation and β-oxidation. Multivariate analysis calculated regression equations for rate constants based on variables such as mass, diet and predator/prey interactions. All regression equations had adjusted-$R^2 \geq 0.47$ and $p$-values $< 0.001$. The model was applied to Yellow Perch (Perca flavescens) from the upper Bay of Quinte, Canada. Modeled PUFA contents were within a standard deviation of measured values. β-oxidation was the dominant loss process of ALA, DHA and LIN. The model was expanded and applied to an 11-group food web in the upper Bay of Quinte for which the PUFA content was measured and reported. Food web model PUFA content was within 20% of measured values for the lower food web and within an order of magnitude in fish 83% of the time. The model showed that deficiencies in PUFAs at the base of the food web can be somewhat overcome by transformation but trophic transfer is critical. The PUFA food web model was used to explore the impacts of eutrophication, invasive species and climate change on n-3 PUFAs in the upper Bay of Quinte. Reduced eutrophication resulted in ~2-fold increase in EPA and DHA.
content. Zebra Mussels introduction caused a further increase of 30 and 50% in EPA and DHA content, respectively, in large piscivores due to changes in the dietary matrix and PUFA food quality. Increased abundance of the invasive Round Goby could provide the efficient transfer of n-3 PUFAs between Zebra Mussels and higher trophic levels. Increasing temperatures associated with climate change could cause more frequent cyanobacteria blooms, which in turn resulted in an EPA and DHA decrease >80% in fish compared to the model with ‘mixed’ phytoplankton input. The model showed that upper trophic levels must aggressively transform ALA in an attempt to meet their EPA and DHA requirements and compensate for poor food quality.
ACKNOWLEDGMENTS

This thesis would not have been possible without the help and support of many people. First I would like to thank my friends and family for their continuous support and encouragement. In particular, I would like to thank my husband, Wojtek and my son, Ben, both of whom are now PUFA experts in their very own right.

A very big thank you to my supervisor, Professor Miriam Diamond, for taking me on as a graduate student. Your guidance, support, encouragement and enthusiasm throughout the past years have been unwavering.

Thank you to Professor George Arhonditsis for you input and expertise in food web modeling and statistical analysis. Thank you to Professor Michael Arts for sharing your vast PUFA knowledge, both in the laboratory and in natural systems. I am thankful that Agnes Richards-Blukacz shared her knowledge surrounding the upper Bay of Quinte food web. Thanks you Gregg Tomy, Donald Jackson and Paul Helm for providing input on my thesis during my external defense. Thank you to Myrna Simpson and Harvey Shear who sat on my committee at various points throughout my time at the University of Toronto.

Thank you to the Diamond laboratory, past and present members. I thank Nilima Gandhi and Susie Csiszar for their guidance and support in mathematical modeling. I thank Matthew Robson and Lisa Melymuk for their patience and help in the laboratory. Thank you to Amanda Giang, Elli Papangelakis and Christiana Dean for your help with benthic invertebrate sampling and sorting. It could not have been accomplished without you.

Thank you to Robert Bonnell and Ashley Bedford from the Department of Fisheries and Oceans for taking me sampling. Going on the ‘Leslie J’ was an experience I will never forget! Thank you Tim Johnson and Jaclyn Brush for sharing PUFA data with me. Thank you to Jerry Chao for processing my PUFA samples when I was unable to do so.

Finally, thank you to the Department of Geography at the University of Toronto for their support and guidance throughout my thesis.
# TABLE OF CONTENTS

ACKNOWLEDGMENTS .................................................................................................................. iv  
TABLE OF CONTENTS .............................................................................................................. v 
LIST OF TABLES ........................................................................................................................ viii  
LIST OF FIGURES ...................................................................................................................... ix 
LIST OF APPENDICES ............................................................................................................... xi 

## CHAPTER 1: GENERAL INTRODUCTION .................................................................................. 1

1.1 Review of uptake and elimination processes in freshwater organisms: ingestion, egestion, transformation and \( \beta \)-oxidation .......................................................................................................................... 3  
1.1.1 Ingestion .......................................................................................................................... 3  
1.1.2 Egestion ........................................................................................................................ 4  
1.1.3 Transformation .............................................................................................................. 4  
1.1.4 \( \beta \)-oxidation ............................................................................................................... 6  
1.2 Review of existing PUFA models ......................................................................................... 6  
1.3 Freshwater ecosystems and food web transfer ................................................................... 8  
1.4 Food web stressors: changes to phytoplankton PUFA content ......................................... 9  
1.5 Thesis Aim and Structure ................................................................................................... 10 

## CHAPTER 2: A GENERAL MODEL OF POLYUNSATURATED FATTY ACID (PUFA) UPTAKE AND ELIMINATION IN FRESHWATER FISH ........................................................................ 12

2.1 Introduction ......................................................................................................................... 12  
2.2 Model Development ........................................................................................................... 14  
2.2.1 PUFA Fate and Transport: Compartmental Analysis .................................................... 16  
2.3 Model Application .............................................................................................................. 20  
2.4 Results and Discussion ...................................................................................................... 21  
2.4.1 Rate Constants and Model Outcome ............................................................................. 21
4.3.4 Climate Change .............................................................................................................. 79
4.3.5 Model Uncertainties and Limitations ........................................................................ 80
4.3.6 Implications .................................................................................................................. 82

CHAPTER 5: GENERAL CONCLUSIONS ........................................................................... 89
  5.1 Unique contributions ....................................................................................................... 89
  5.2 Specific conclusions ....................................................................................................... 92
  5.3 Recommendations for future work ............................................................................... 93

CHAPTER 6: REFERENCES .................................................................................................. 94

APPENDICES ....................................................................................................................... 109
LIST OF TABLES

Table 2.1. Summary of 1 fish PUFA model inputs................................................................. 27

Table 2.2. Empirical statistical relationships for PUFA egestion, transformation and \( \beta \)-oxidation.
.................................................................................................................................................. 29

Table 3.1. Summary of model inputs......................................................................................... 57

Table 3.2. Average measured PUFA content for aquatic biota and fauna in the upper Bay of
Quinte (mg g\(^{-1}\) D.W.).................................................................................................................. 59

Table 4.1. Modeled scenarios. ..................................................................................................... 83

Table 4.2. Modeled large piscivore DHA ingestion, egestion, transformation and \( \beta \)-oxidation as
absolute (\( \mu \)mol fish\(^{-1}\) d\(^{-1}\)) and relative (%) values as well as residence times (h) for scenarios A1,
A2, B1, B2, C1, C2 and ‘base case’ model................................................................................... 84
LIST OF FIGURES

Figure 2.1. Major PUFA metabolic pathways in a freshwater fish. ......................................................... 31

Figure 2.2. PUFA model Schematic for a single freshwater fish. ............................................................... 32

Figure 2.3. Yellow Perch 2009 measured values (n=3) (Johnson, unpubl. data, 2011) in the upper Bay of Quinte, Canada compared to the 1 fish modeled data ................................................................. 33

Figure 2.4. Modeled PUFA content (C; mg g$^{-1}$ D.W.) and fluxes (arrows; µmol fish$^{-1}$ d$^{-1}$ and relative contribution; %) in a Yellow Perch in the upper Bay of Quinte ................................................. 35

Figure 3.1. Upper Bay of Quinte measured (2009) and modeled invertebrate PUFA content. Measured values were unavailable for zooplankton and Round Goby and large piscivore ALA. Error bars represent standard deviation. ........................................................................................................ 60

Figure 3.2. Model scenarios illustrate the impact of transfer and accumulation of n-3 PUFAs through the upper Bay of Quinte’s food web when dietary ALA and EPA contributions are minimized. Base case results are presented as a comparison. ........................................................................................................ 65

Figure 4.1. Modeled n-3 PUFA accumulation (mg g$^{-1}$ D.W.) in the upper Bay of Quinte’s upper food web for the pre-phosphorus controls (A1), post-phosphorus controls (A2) and post Zebra Mussel invasion (B1) periods. The food web structure has been simplified to show primary (phytoplankton, macrophytes and detritus), secondary (zooplankton, benthic invertebrates and bivalves/Zebra Mussels (post 1994)) and tertiary (planktivores, benthivores, Round Goby (post 1994), small piscivores and large piscivores) trophic levels. ........................................................................................................ 85

Figure 4.2. Modeled DHA accumulation in the upper Bay of Quinte for Zebra Mussel invasion (B1) and hypothetical scenarios of increases of Round Goby biomass by 100-fold (B2). Phytoplankton, macrophytes and detritus are measured values as adapted from Ahlgren et al. (1992) and Chapter 3. ........................................................................................................ 86

Figure 4.3. Modeled climate warming scenario C1 when phytoplankton ALA was reduced by 40% and C2 where *Microcystis* is the dominant cyanobacteria in the upper Bay of Quinte. ‘Base
case’ modeled values as reported in Chapter 3. Measured data were unavailable for ALA in Round Goby and large piscivores.
LIST OF APPENDICES

Appendix A. Major PUFA transformation elongation/desaturation pathways (as modified from Bézard et al., 1994). .......................................................... 109

Appendix B. Polyunsaturated fatty acid (PUFA) datasets utilized for multivariate regression analysis for the 1 fish PUFA model uptake and elimination rates................................. 110

Appendix C. Measured versus predicted PUFA egestion rate constants….. ...................... 125

Appendix D. Measured versus predicted PUFA transformation rate constants. .................... 128

Appendix E. Measured versus predicted PUFA β-oxidation rate constants....................... 129

Appendix F. 1 fish PUFA model sensitivity analysis. Egestion, transformation, β-oxidation and ingestion rate constants were varied in increments of ±10%, up to a variation of ±30%......... 132

Appendix G. Major metabolic pathways of PUFA (Chapter 2)........................................ 137

Appendix H. Upper Bay of Quinte (circled), Ontario Canada.............................................. 138

Appendix I. Vertebrate rate constant equations for egestion, trasformation and β-oxidation. .. 139

Appendix J. Dietary matrix (% volume) for the upper Bay of Quinte food web model. .......... 141

Appendix K. Dietary matrix (% volume) for the upper Bay of Quinte food web model as modified from Koops et al. (2006) and Blukacz-Richards and Koops (2012)......................... 143


Appendix M. Modeled ALA and EPA accumulation in the upper Bay of Quinte for Zebra Mussel invasion (B1) and hypothetical scenarios of increases of Round Goby biomass by 100-fold (B2). Phytoplankton, macrophytes and detritus are measured values as adapted from Ahlgren et al. (1992) and Chapter 3........................................................................ 151
Fish consumption has many beneficial effects, namely the high concentration of polyunsaturated fatty acids (PUFAs). Consuming PUFAs has been found to present numerous health benefits such as those related to neurodevelopmental effects and lower risk of heart disease (i.e. Connor, 2000; Lemaitre et al., 2003). In aquatic ecosystems, PUFAs are nutritionally critical molecules, determining trophic transfer efficiency, secondary production, and functionality of aquatic food webs (Perhar and Arhonditsis, 2009). In animals, the physiologically important fatty acids include n-3 PUFAs α-linolenic acid (ALA; 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and n-6 linoleic acid (LIN; 18:2n-6) and arachidonic acid (ARA, 20:4n-6).

EPA and ARA are precursors of eicosanoids and have several functions in reproduction, ion and water transport, and neurotransmission (Harrison, 1990; Stanley-Samuelson and Pedibhotla, 1996). DHA is strongly retained within cell membranes where it significantly alters many membrane properties including maintaining fluidity (Stillwell and Wassall, 2003).

Most of the nutritional literature on PUFAs comes from marine fish and seafood which are the main dietary source for most people (Arts et al., 2001). Less information is available on PUFA content in freshwater fish, which typically have lower, but still appreciable long chain (LC) PUFA (LC PUFA = PUFA with 20 or more carbon atoms) concentrations (Arts et al., 2001, Pantazopoulos et al., 2013).

ALA and EPA are synthesized de novo only by plants including phytoplankton, periphyton, and macrophytes in aquatic systems. PUFAs accumulate in fish by trophic transfer through the food web (Arts et al., 2001). As such their levels at the top of food webs are strongly influenced by synthesis at the base of the food web. Aquatic ecosystems play the unique role in the biosphere as the principal source of n-3 EPA and DHA for animals, including those in terrestrial ecosystems (Gladyshev et al., 2009).

Although fish obtain most of their PUFAs from their diet, little is known surrounding the essential link between PUFA content in the lower food web and their role for fish (Kainz et al., 2004). As well, the extent to which transformation in the upper food web can make up for poor
PUFA quality in the lower food web is unknown. Thus, studies of PUFAs and their transfer within aquatic food webs should include knowledge of PUFA contents in natural animal populations with careful consideration of taxonomic position, age and diet of each species (Sushchik et al., 2003) as these can ultimately influence PUFA accumulation in a predator.

Mathematical models of energy and chemical dynamics within a single organism and chemical transfer within food webs have provided considerable insight into ecosystem dynamics. I used the information presented by previous rat, human and fish models to aid in the construction of the 1-fish and food web PUFA models, which will be reviewed below in section 1.2 Review of existing PUFA models.

The overarching goal of this thesis is to evaluate the transfer and accumulation of PUFAs in freshwater environments under various environmental conditions. By developing a mathematical model for 1-fish and then an entire food web, I explore the impact that the uptake (i.e. diet) and elimination (i.e. egestion, transformation and β-oxidation) rates have on PUFA accumulation.

The thesis is comprised of five chapters. In this introductory chapter, I first discuss the key uptake and elimination processes responsible for PUFA accumulation in freshwater systems. Second, I review the existing PUFA uptake and accumulation models both in humans and fish. Third, I provide an overview of PUFA transfer in aquatic food webs. Finally, I discuss food web stressors that may impact the base of the food web thereby impacting PUFA transfer and accumulation. I conclude this chapter with the aim and outline of this thesis. Chapter 2 presents a model of n-3 and n-6 PUFA uptake, loss and transformation within a single fish. Chapter 3 expands the 1 fish model to consider a food web. The upper Bay of Quinte, located in northeastern Lake Ontario, is used as a case study for the application and evaluation of the model. Chapter 4 uses the food web model presented in Chapter 3 to examine how stressors that have affected the upper Bay of Quinte affect PUFA accumulation. These stressors include excessive nutrients, reduction of nutrient levels, and the colonization of the bay by invasive Zebra Mussels (Dreissena polymorpha) and then Round Goby (Neogobius melanostomus). This chapter ends by using the model to explore potential consequences to PUFA accumulation in the bay’s ecosystem as a result of climate change. The thesis concludes with a summary of the major findings and recommendations for future work.
1.1 Review of uptake and elimination processes in freshwater organisms: ingestion, egestion, transformation and β-oxidation

The assimilation and retention of key nutrients in consumers is fundamental to the optimal physiological performance of animals in aquatic food webs (Kainz et al., 2004) and dietary fatty acids composition is reflected in fish tissues (Teoh et al., 2011). Following ingestion, a PUFA is either assimilated and metabolized or egested. With assimilation, PUFA are found primarily as, but not limited to, constituents of phospholipids, triacylglycerol and/or free fatty acids. After dietary uptake, a PUFA may be transformed (i.e. elongated and/or desaturated) to LC PUFA or β-oxidized for energy production. Below I discuss the uptake and elimination processes of ingestion, egestion, transformation and β-oxidation, considered to be the most physiologically important process in controlling PUFA accumulation in freshwater organisms.

1.1.1 Ingestion

Uptake into enterocytes was originally thought to occur by passive diffusion for all fatty acids, including PUFAs in fish. However, it is now widely believed that whereas medium-chain fatty acids are primarily absorbed by simple diffusion, the uptake of long-chain fatty acids involves various transport proteins (Andre et al., 2000; Concha et al., 2002; Denstadli et al., 2004). Sire and Vernier (1981) suggested that the transport proteins in fish have similar characteristics to the fatty acid binding proteins found in mammals.

Fatty acid uptake depends on many factors. Fatty acids are absorbed mainly as sn-2-monoacylglycerols and free fatty acids which are produced by lipase-aided hydrolysis of triacylglycerols (Simonetti et al., 2008). A substrate esterified in triacylglycerols or phospholipids needs to be hydrolyzed prior to absorption, whereas free fatty acids can be directly absorbed in the enterocyte (Denstadli et al., 2004). Free fatty acid absorption into enterocytes is followed by re-esterification and incorporation of triacylglycerols in chylomicrons, such that the newly formed triacylglycerols retain, in the sn-2 position, fatty acid from the original dietary triacylglycerols. In comparison, the fatty acids in the sn-1 and sn-3 positions of chylomicron triacylglycerols are randomly esterified from the available pool of free fatty acids and thus are partially substituted by endogenous fatty acids (Simonetti et al., 2008). The constituent molecular composition of triacylglycerol and phospholipids is very important for the absorption
of dietary fat since it has a significant effect on the quantity of lipid absorbed from the intestine and on the subsequent rate of lipid metabolism (Simonetti et al., 2008). For example, a diet rich in neutral lipid (i.e. triacylglycerol) has been associated with the accumulation of large lipid droplets in the enterocytes of common carp (Cyprinus carpio), arctic char (Salvelinus alpinus) and rainbow trout (Oncorhynchus mykiss) (Fontagné et al., 1998; Olsen et al., 1999; Caballero et al., 2002), potentially reducing lipid transport rates across the intestinal epithelium.

The absorption of fatty acids depends on both chain length and degree of saturation as this affects the physical properties of the molecules, namely their water solubility and melting point, which ultimately affect the rate of diffusion (Morais et al., 2005). In addition to the constituent molecular composition, the transport of PUFA depends on chain length and degree of saturation. Fatty acid digestibility tends to decrease with increasing chain length while increasing with the degree of unsaturation (Denstadli et al., 2004; Teoh et al., 2011).

1.1.2 Egestion

The rate constant for PUFA egestion can be influenced by several factors, including temperature, diet, nutritional history, body size and stress (Persson, 1979; Storebakken et al., 1999). It has been reported previously that saturated fatty acids are present at higher levels in the feces compared to PUFAs and short chain (≥18-carbon) monounsaturated fatty acids (Lie et al., 1987; Johnsen et al., 1993). PUFA content in fecal matter is low, typically ranging from 5-12% D.W. of total fatty acid (Bjerkeng et al., 1999; Ng et al., 2003; Olsen et al., 2004; Oxley et al., 2009).

1.1.3 Transformation

Fish can have high dietary PUFA demands that differ substantially from what their diet provides. As such, fish (particularly freshwater species) have evolved to compensate for inadequate dietary PUFA content by metabolically transforming PUFA (Weers et al., 1997; Nanton and Castell, 1998; Bec et al., 2003). PUFA transformation can follow 2 pathways. The pathways differ in that one uses n-3 PUFA precursors while the other uses n-6 PUFAs. The n-3 pathway elongates and desaturates ALA to EPA and EPA is further elongated and desaturated to DHA. The n-6 pathway uses LIN for conversion to ARA. Details outlining the elongation and
desaturation process can be found elsewhere (e.g. Bézard et al., 1994; Nakamura et al., 2004; Sargent et al., 2002).

A major difference between marine and freshwater fish is that the freshwater species studied to date appear to have a greater ability to desaturate and elongate dietary n-3 and n-6 PUFAs to LC PUFA (Karahadian and Lindsay, 1989; Sargent et al., 1999). It is believed that almost all freshwater fish have the ability to convert ALA to EPA and DHA and LIN to ARA (Turchini et al., 2006), with higher rates observed for those freshwater fish that have high proportions of ALA or LIN and limited DHA or ARA, respectively, in their natural diet (Sargent et al., 2002; Hessen and Leu, 2006).

Inter- as well as intra-species variation (attributable to differences in life history stages) can also contribute to differences in PUFA transformation efficiency (Alhazzaa et al., 2011). Teoh et al. (2011) reported that tilapia (Oreochromis sp.) fed a low-PUFA diet exhibited efficient Δ-6 and Δ-5 desaturation and elongation of both n-3 and n-6 PUFA. A decreased supply of dietary long chain PUFA has been reported to stimulate desaturation and elongation rates involved in the FA transformation pathway for freshwater fish (Francis et al., 2007). Sargent et al. (1999) have shown that transformation rate constants can depend on diet, as competitive interactions exist with the conversion of LIN to ARA competing with the conversion of ALA to EPA and DHA. Consequently, a diet rich in LIN but poor in ALA generates a relative excess of ARA and a relative deficiency of EPA and DHA (Sargent, 1997).

Little is known about transformation among invertebrates. Taipale et al. (2011) hypothesize that the ability to transform PUFAs varies greatly from one species to another. Sushchik et al. (2003) report that mayflies and caddisflies transform ALA to EPA and LIN to ARA, although they are unable to further transform EPA to DHA. However, the enzymes used for desaturating and elongating EPA to DHA are present in copepods, suggesting their capacity to perform this transformation (Persson and Vrede, 2006). The EPA:DHA ratio differs because cladocerans often mature and reproduce more rapidly and frequently than copepods (Mauchline, 1998), which may necessitate a greater supply of EPA (Smyntek et al., 2008). In contrast to the short, seasonal life cycle of many cladocerans, copepods generally have longer generation times and often remain active over the winter (Farkas, 1979). This ability is believed to be facilitated by accumulation of DHA which is associated with increased membrane fluidity (Stillwell and Wassall, 2003).
1.1.4 β-oxidation

β-oxidation is the process by which fatty acids are broken down to generate acyl-CoA, the entry molecule for the Krebs cycle (Marýın-Garcýía et al., 2002). Short and medium chain fatty acids are β-oxidized in cell mitochondria, whereas long-chain fatty acids are β-oxidized in peroxisomes (Froyland et al., 1998). Acyl-CoA is a temporary compound formed when coenzyme A (CoA) attaches to the end of a long chain fatty acid. EPA and DHA, in their fatty acyl-CoA forms, are esterified to cellular lipid and undergo β-oxidation and other metabolic transformations including oxygenation reactions to eicosanoids (Arts et al., 2001). ALA, EPA, DHA and LIN are good substrates for β-oxidation, especially when provided at high levels (Richard et al., 2006). In contrast, PUFAs are limited in the diet they are retained or deposited in the tissues (Karalazos et al., 2010). Teoh et al. (2011) observed that competition between β-oxidation and transformation of ALA was affected by dietary lipid source, as transformation was higher in fish fed a diet deprived of LC PUFA compared to a LC PUFA-rich diet. Morais et al. (2005) found that DHA was only minimally catabolized for energy purposes, consistent with its essential role for membrane structure. Thus, β-oxidation capabilities are affected by various factors, including diet and dietary fatty acid composition.

1.2 Review of existing PUFA models

Prior to Cunnane and Anderson (1997), the extent to which the conversion of shorter-chain to LC-PUFA occurs (i.e. LIN to ARA) in the whole animal was unclear. They examined partitioning in rats consuming low but not deficient ALA and LIN diets considering dietary uptake, egestion, transformation and β-oxidation. Whole-body fatty acid balance analysis indicated that the majority of the LIN consumed was β-oxidized (76%), and the rest accumulated (20%), transformed (3%), and egested (1%). For ALA, 85% was β-oxidized, 12%, accumulated, 2% egested and 1% transformed.

Pawlosky et al. (2001) were the first to propose a physiological compartmental model of n-3 PUFA metabolism in humans. Kinetic parameters (i.e. uptake, transformation), half-lives and flow rates of the n-3 PUFAs in the plasma were determined for each of the 8 human subjects. The model predicted plasma values for the n-3 PUFAs that were in good agreement with measured steady-state concentrations. They found that the inefficiency of the conversion of
ALA to EPA indicates that the transformation of EPA and DHA from ALA acid is minimal. In contrast, they found a much greater rate of transformation from EPA to DHA suggesting that dietary EPA may be used to synthesize DHA in humans. Pawlosky et al. (2003) further utilized their PUFA metabolism model to test the hypothesis that the fat content of a fish-based diet would inhibit the kinetics of the metabolism of n-3 PUFAs compared with a beef-based diet. They concluded that the main effect of a fish-based diet n-3 PUFA metabolism involved processes that inhibited the transformation of EPA to DHA.

In 2006, Turchini and coworkers used a whole-body fatty acid balance method to explore PUFA metabolism in Murray Cod (Maccullochella peelii peelii) using 2 different diets in an aquaculture environment. They found that Murray Cod were able to transform ALA and LIN. Additionally, their findings indicated that Murray Cod showed a preferential order of PUFA utilization for β-oxidation where ALA>LIN. They also found that increased dietary ALA was responsible for elevated transformation rates. In 2007, Turchini and coworkers introduced a further development of the whole-body fatty acid balance method for the estimation of the transformation of fatty acids, noting that previous methods were time consuming and expensive. Their method requires knowledge of the fish’s initial and final body weight, initial and final quantitative fatty acid composition of the whole body, the total feed intake, the quantitative fatty acid content of the diet, and the fatty acid digestibility of the quantitative fatty acid content of the total feces produced during the experiment. Using their mass balance method it is possible to measure the fate of individual fatty acids towards transformation and β-oxidation. This method has been well received due to its simplicity and reliability. However, this method requires data made available through feeding trials of sufficient duration and thus cannot be applied to fish in natural environments.

Currently, there are no mass balance models that examine the uptake and elimination of PUFAs in fish in natural environments. As a result, there is a lack of understanding regarding the dynamics surrounding the processes concerning PUFA uptake, egestion, transformation and β-oxidation.
1.3 Freshwater ecosystems and food web transfer

Higher trophic organisms rely on lower trophic organisms for dietary PUFAs. Animals cannot synthesize ALA and LIN de novo at rates sufficient for survival although phytoplankton can (Bézard et al., 1994; Rai et al., 1997; Kainz et al., 2004). For example, Daphnids have de novo synthesis rates which are generally <2% of the accumulated total fatty acids (Goulden and Place 1990) and most animals grow best when provided with direct sources of LC PUFAs (Brett and Müller-Navarra, 1997). Once synthesized at the phytoplankton level, PUFAs are transferred and can accumulate at progressively higher trophic levels in the biomass of aquatic organisms. Therefore, phytoplankton nutritional content is critical for establishing overall ecosystem health and survival.

The PUFA composition of phytoplankton can vary considerably amongst taxa (Brett et al., 2009). Diatoms, cryptophytes and dinoglagellates, whose growth is favored under oligotrophic conditions, are quite nutritious, having high proportions of EPA and DHA although low ALA and LIN acid content (Harwood, 1996; Tocher et al., 1998). Although chlorophytes may only have traces of EPA, DHA and ARA, they have high ALA and LIN content (Hessen and Leu, 2006). Cyanophytes, with the lowest PUFA quality, thrive in phosphorus-rich conditions and have virtually no EPA and DHA, and often very little ALA and LIN.

When comparing benthic and pelagic species, differences in PUFA content have been observed. Ahlgren et al. (1997) found that net plankton consistently had higher values of PUFA, especially the n-3 PUFAs, than sediment trap samples concluding that suspended particulate matter has better quality n-3 PUFAs than sedimenting matter. Similarly, Goedkoop et al. (2000) found that surficial sediments were 96% lower in PUFA content compared to sedimenting matter due to rapid PUFA decomposition in the sediment.

In freshwater food webs, herbivorous zooplankton is functionally important, providing the major link between primary producers and secondary consumers in these food webs (Arts et al., 2001; Persson et al., 2007; Smyntek et al., 2008). As phytoplankton and zooplanktivorous fish can have vastly different PUFA compositions, zooplankton bridge a gap between the trophic levels by transforming and concentrating physiologically important PUFAs (Ravet et al., 2010).

Fatty acid composition of fish lipids generally reflects the fatty acid profile of the diets (Richard et al., 2006; Teoh et al., 2011). Retaining more long-chain unsaturated fatty acids in tissues benefits consumer growth and reproduction (Persson et al., 2007; Smyntek et al., 2008;
Brett et al., 2009) and allows for more efficient dietary trophic transfer (Müller-Navarra et al., 2000). The preferential uptake, elimination or modification of PUFAs by predators may ultimately influence fisheries ecology, as well as trophic cascades in lakes (Ravet et al., 2010). PUFAs accumulate through aquatic food webs from their source in algae at the base of the food web. DHA is generally the most highly retained PUFA in many freshwater fishes (Kainz et al., 2008).

Currently, there are no mass balance models that examine the uptake and elimination of PUFAs in freshwater food webs. This results in a dearth of knowledge concerning food web interactions between primary, secondary and tertiary producers and the drivers that influence PUFA accumulation.

1.4 Food web stressors: changes to phytoplankton PUFA content

Changes in fish PUFAs are important given the role of LC PUFA, particularly EPA and DHA to human health, as well as the increasing demand of consumers for nutritious and health promoting products (Karalazos et al., 2010). PUFAs accumulate through food webs; therefore, ecosystem structure changes are hypothesized to impact their transfer and through the aquatic systems to higher trophic level organisms (Bélanger et al., 2006; Kainz et al., 2006). Lakes and reservoirs have global perimeters of about 2 orders of magnitude greater than the length of ocean shoreline. Therefore, freshwater ecosystems are a main source of essential LC PUFA for terrestrial ecosystems (Gladyshev et al., 2009); thus the likely decrease of LC PUFA levels in freshwater systems is believed to have global consequences.

Algae account for more than half the total primary production at the base of the food web (Arts et al., 2009). Due to the importance of phytoplankton as PUFA synthesizers, information on temperature effects on PUFA content is necessary when considering climate change (Fuschino et al., 2011). A decrease in PUFAs with an increase in temperature is commonly seen as an adaptive mechanism and is valid across a wide range of temperatures encompassing cold, temperate and thermal algae (Jiang and Gao 2004; Gushina and Harwood, 2009).

Eutrophication and rising water temperature favors the dominance of cyanobacteria (Müller-Navarra et al., 2004; Paerl and Huisman, 2008), many of which are known to be depleted in LC PUFA (Ahlgren et al., 1992). The nutritional status of organisms at higher
trophic levels may be compromised because of changes in the PUFA content of their phytoplankton food base, if water temperatures in small lakes increase in response to changing climate (Fuschino et al., 2011). As they are highly retained in freshwater aquatic food webs, any changes in the quantity and quality of PUFAs in phytoplankton as a consequence of eutrophication or climate change could impact growth rates, reproductive capacities and general health (e.g. disease resistance) of freshwater organisms.

It is extremely difficult to measure the impact of PUFA transfer and accumulation in freshwater systems under changing phytoplankton conditions. A PUFA food web model would enable the examination of how stressors such as eutrophication and climate change impact PUFA accumulation through a food web. Currently there are no mass balance models that examine potential uptake and elimination of PUFAs in freshwater food webs.

1.5 Thesis Aim and Structure

The overall aim of this thesis is to understand and quantitatively estimate PUFA dynamics within freshwater fish and through a freshwater food web. I focus on the use of statistical tools and develop a new mechanistic mass balance model to highlight ingestion, egestion, transformation and β-oxidation pathways in n-3 ALA, EPA and DHA and n-6 LIN and ARA in freshwater systems. Three specific objectives are addressed in this thesis, each corresponding to a chapter.

The first objective is to create global equations to predict the elimination rates for egestion, transformation and β-oxidation in freshwater fish. I then use these equations in a generic, 1 fish PUFA uptake and elimination model. The second objective is to expand the 1 fish model to elucidate PUFA transfer and accumulation from primary producers through to upper trophic level species. Finally, the third objective is to explore how stressors such as nutrient management controls and climate change impact phytoplankton PUFA content and its accumulation through the food web. This work advances science as there is currently no method available to evaluate PUFA accumulation and transfer in freshwater food webs in natural systems, although the model is also applicable to laboratory environments. The 1 fish and food web models can examine how changing environments (i.e. eutrophication, invasive species, climate change) impact PUFA accumulation in higher trophic species. This is of interest to those
working with aquatic food webs as PUFA content impacts the health and reproductive success of freshwater environments. This is also of interest to terrestrial systems, as freshwater fish are a vector for LC PUFA content to terrestrial systems.
CHAPTER 2: 
A GENERAL MODEL OF POLYUNSATURATED FATTY ACID (PUFA) UPTAKE AND ELIMINATION IN FRESHWATER FISH

2.1 Introduction

Understanding and quantifying fish tissue fatty acid (FA) profiles is of great importance to human health as polyunsaturated fatty acids (PUFAs) play a role in lowering cardiovascular disease (Lemaitre et al., 2003; Yokoyama et al., 2007), moderating tissue inflammation (Connor, 2000; Ruxton et al., 2004) and contributing to the development of nervous (Burdge, 1998), reproductive (Sidhu, 2003), and visual (Kim and Mendis, 2006) systems. The essentiality of some PUFAs stems from their many health benefits and disease prevention (Parrish, 2009), plus the inability of most animals, including humans, to synthesize them de novo and/or, in the case of long chain PUFA (LC PUFA = PUFA with 20 or more carbon atoms) at rates sufficient to maintain optimal health (Bell and Tocher, 2009).

In addition to a vital role in human health, lipids are critical components in fish nutrition as sources of energy, essential fatty acids (EFAs) and sterols (Hansen et al., 2011). PUFAs are crucial for fish because they affect metabolic activity, growth rates and reproduction. When available in adequate supply, EFAs reduce the probability that fish will exhibit various pathologies (Watanabe 1982). Additionally, PUFAs are required for regulating hormonal processes (Arts and Kohler, 2009). In fish, the physiologically-essential PUFAs include the n-3 FA α-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and the n-6 FAs linoleic acid (LIN, 18:2n-6) and arachidonic acid (ARA, 20:4n-6). Parrish (2009) has proposed that docosapentaenoic acid (DPA, 22:5n-3) is also an EFA, but I do not consider it further because of a lack of information.

The low melting points of PUFAs (around -50°C) is an important factor in the maintenance of cell membrane fluidity and consequently PUFAs play an important role in biochemical adaptation to cold, aquatic environments (Arts and Kohler, 2009). As noted above, fish cannot synthesize ALA, EPA and DHA or LIN and ARA at rates sufficient to meet their basic biochemical requirements. As such, these PUFAs must be obtained largely through the
diet (Bézard et al., 1994; Kainz et al., 2004; Hessen and Leu, 2006). These requirements for specific PUFAs contributes to the general finding that dietary PUFA composition is, at least in part, reflected in fish tissues (Richard et al., 2006; Karalazos et al., 2011; Teoh et al., 2011). Thus, PUFAs have been used in conjunction with other FAs as biomarkers to elucidate trophic relationships (Béringe and Barnathan 2005; Gomes et al., 2010; McMeans et al., 2013).

Freshwater fishes can have high metabolic PUFA demands that differ substantially from what their diet provides. One major difference between marine and freshwater fishes is that the freshwater species studied to date appear to have a greater ability to transform dietary ALA and LIN to LC PUFAs (Krahadian and Lindsay, 1989; Sargent et al., 1999; Simonetti et al., 2008). This is considered an evolutionary adaptation of freshwater fishes to compensate for inadequate dietary PUFA content. PUFA transformation can follow two pathways (Appendix A). The n-3 pathway elongates and desaturates ALA to EPA and EPA is further transformed to DHA. Alternatively, the n-6 pathway uses LIN for transformation to ARA. Details outlining the transformation process can be found elsewhere (Bézard et al., 1994; Sargent et al., 2002; Nakamura and Nara, 2004). Although the process of PUFA transformation by freshwater fishes has been documented, the extent to which it occurs in wild fish has not been reported.

β-oxidation is the process by which FAs are broken down to generate acetyl-CoA, the entry molecule for the Krebs cycle (Marýin-Garcýia and Goldenthal, 2002). Acyl-CoA is a temporary compound formed when coenzyme A (CoA) attaches to the end of a long chain FA. EPA and DHA, in their fatty acyl-CoA forms, are esterified to cellular lipid and undergo β-oxidation and other metabolic transformations including oxygenation reactions to eicosanoids (Arts et al., 2001). The use of PUFAs as a substrate for β-oxidation would be expected only when the need for PUFAs has been satisfied and supply is in excess. Information on rates largely comes from the aquaculture literature where fish diet is controlled, however the importance of β-oxidation in wild fish is not known.

Several FA transport and accumulation models have been proposed for rats, humans and fish. Cunnane and Anderson (1997) first proposed a whole-body FA mass balance model for rats, emphasizing the importance of accumulation, transformation and β-oxidation pathways. Pawlosky and coworkers (2001, 2003) examined the metabolism of ALA in humans using stable isotope tracers and developed a multi-compartmental model to elucidate FA contributions to the maintenance of n-3 PUFAs in human plasma. Finally, Turchini and coworkers (2006, 2007)
were the first to develop a compartmental FA mass balance method for fish, focusing on the freshwater Murray Cod (*Maccullochella peelii*). Their method used experimental FA data to calculate the fraction of total FA that is transformed versus β-oxidized. They found that Murray Cod were able to transform both ALA and LIN. For ALA and LIN they found that β-oxidation was the dominant process (74% and 77% for ALA and LIN, respectively). A limitation of their model is that it cannot be used to calculate the transformation and β-oxidation rates of FA in organisms without a feeding trial in which the initial and final body weight, initial and final quantitative fatty acid composition of the whole body, the total feed intake, the quantitative fatty acid composition of the diet and the fatty acid digestibility or the quantitative fatty acid composition of the total feces produced during the experiment are known.

I present a whole-body mass balance model for PUFA-specific uptake and elimination in a “generic” predatory freshwater fish to be used as a heuristic tool that builds on the approach previously proposed by Cunnane and Anderson (1997), Pawlosky et al. (2001, 2003), and Turchini et al. (2006, 2007). This PUFA model, which aims to be generally applicable to freshwater fishes, simultaneously examines the uptake and elimination of inter-related n-3s ALA, EPA and DHA and n-6s LIN and ARA in an individual fish consuming multiple prey species. The model uses compartmental analysis to simplify the complexity of PUFA metabolism, and explicitly considers the processes of dietary uptake, egestion, transformation (interconversion among n-3 or n-6 PUFAs by elongation and/or desaturation), and β-oxidation. Unlike the model of Turchini et al. (2006, 2007), empirical relationships were developed using literature data and multivariate regression analysis to parameterize the rate constants for these processes. I provide justification for the governing model equation and discuss the associated empirical regression relationships. I then apply the model to a single fish species, Yellow Perch (*Perca flavescens*), to illustrate the model’s utility and insights gained.

### 2.2 Model Development

Both marine and freshwater fish PUFA profiles are affected by and reflective of their dietary PUFAs (Richard et al., 2006; Turchini et al., 2006; Bendito-Palos et al., 2011). Following ingestion, a PUFA is either digested or egested (Fig. 2.1). Upon digestion, a PUFA
may be transformed by elongation and/or desaturation to longer chain PUFA or β-oxidized for
energy production (Fig. 2.1).

Chemical similarities of PUFAs can lead to competitive interactions in the biochemical
and physiological reactions undergone by the parent compounds, precursors and products
(Sargent et al., 1999). The model presented here considers uptake, loss and transformation of 5
PUFAs (ALA, EPA, and DHA, LIN and ARA). All PUFA content is represented as dry weight
(D.W.) values.

I express the mass balance for PUFA metabolism in a predator using the generic equation
as follows:

\[
\frac{dm_{lx}}{dt} = \sum_{i,j=1}^{n} \gamma_{Gl,x} m_{jx} k_{Aji,x} - m_{lx} (k_{El,x} + k_{Ol,x}) \pm m_{Tlx,y} (k_{Tlx,y})
\]  

(2.1)

Where ‘m’ is the content (mg D.W.) of precursor PUFA ‘x’ and end product PUFA ‘y’. Five
dependent mass balance equations are generated for ALA, EPA or DHA and LIN or ARA. The
consumer (i.e. predator) and diet (i.e. prey) are ‘i’ and ‘j’, respectively. \( \gamma_G \) is a gut absorption
coefficient (unitless). The ‘k’ values are uptake and elimination rate constants (h\(^{-1}\)) and
subscripts A, E and O are diet (i.e. food), egestion and β-oxidation, respectively. Subscript ‘T’ is
the net transformation for PUFA ‘x’ through elongating and/or desaturating to PUFA ‘y’. If
PUFA ‘x’ transforms to PUFA ‘y’, then the sign is negative as PUFA ‘x’ is ‘lost’. The sign is
positive when PUFA ‘x’ has an additional input from PUFA ‘y’ that has transformed to PUFA
‘x’. I assume that a transformed PUFA is subsequently available for additional elimination
processes (i.e. egestion and β-oxidation).

Under steady-state conditions, Eq. 2.1 can be rewritten as (Eq. 2.2, Fig. 2.2):

\[
m_{lx} = \frac{\sum_{i,j=1}^{n} \gamma_{Gl,x} m_{jx} k_{Aji,x} \pm m_{Tlx,y} k_{Tlx,y}}{k_{El,x} + k_{Ol,x}}
\]  

(2.2)

As this is a first generation model, a steady-state solution is presented rather than an unsteady-
state model because of the former’s mathematical simplicity and a paucity of data for
parameterization and evaluation. The mass of the 5 PUFAs, as expressed in Eq. 2.2, can be
solved for simultaneously using a matrix form modified from Gandhi et al. (2006):
\[ Am = 0 \] (2.3)

Where matrix A includes the dietary matrix as well as all possible uptake and elimination pathways. For 5 PUFAs the matrix is:

\[
\begin{bmatrix}
  A^a & -T^{ba} & -T^{ca} & 0 & 0 \\
  -T^{ab} & A^b & -T^{cb} & 0 & 0 \\
  -T^{ac} & -T^{bc} & A^c & 0 & 0 \\
  0 & 0 & -T^{de} & A^d & -T^{ed} \\
  0 & 0 & 0 & -T^{de} & A^e
\end{bmatrix}
\begin{bmatrix}
m^a \\
m^b \\
m^c \\
m^d \\
m^e
\end{bmatrix}
= \begin{bmatrix}
0 \\
0 \\
0 \\
0 \\
0
\end{bmatrix}
\] (2.4)

Where a, b, c, d and e are ALA, EPA, DHA, LIN and ARA, respectively.

\subsection*{2.2.1 PUFA Fate and Transport: Compartmental Analysis}

Empirical regression equations for the general model (Eq. 2.2) were developed from a data training set to estimate the rate constants for uptake, egestion, transformation and \( \beta \)-oxidation for each of the 5 PUFAs (Fig. 2.2). These regression equations were developed to estimate rate constants for these processes for freshwater fish species in general, rather than using rate constants from the literature (which were developed under specific conditions and therefore cannot be used in a general manner, i.e. Alhazzaa et al. 2011; Teoh et al., 2011). The outcomes of these regressions (expressed in units of mg PUFA g\(^{-1}\) h\(^{-1}\)) were mathematically manipulated (as presented below in Eqs. 2.5-2.17) to provide a rate constant (h\(^{-1}\)) as best-fits to a data training set compiled from 6 studies (Appendix B). This training set, derived from studies by Turchini et al. (2006), Francis et al. (2007 and 2009), Turchini and Francis (2009), Alhazzaa et al. (2011) and Teoh et al. (2011) includes a total of 22 data points for the freshwater species Rainbow Trout (\textit{Oncorhynchus mykiss}) (2 data points), Barramundi (\textit{Lates calcarifer}) (freshwater life-cycle phase; 3 data points), Nile Tilapia (\textit{Oreochromis niloticus}) (4 data points) and Murray Cod (\textit{Maccullochella peelii}) (13 data points). Diet, temperature, fish weight and study duration varied within and among studies. I note that these studies were done within the context of aquaculture in which the PUFA content of fish could be significantly different from that under natural conditions. Grubbs’ Test (1969) was used to determine data exclusion and outliers \((p<0.05)\) were not included in the multivariate analysis.
Empirical regression equations to predict egestion, transformation and β-oxidation rate constants were tested using a multivariate analysis algorithm (Statistica 7.0). Various permutations were evaluated for their overall fit (adjusted-$R^2$), residuals and variance using multivariate linear regression. The final regression equations for each rate constant (except for uptake) had the overall best fit and that were consistent with current understanding of physiological processes. A summary of all model inputs for the regressions and definitions can be found in Table 2.1. Generally, the egestion, transformation and β-oxidation rate constants (Eqs. 2.5-2.17) were highly correlated with body weight and the amounts of PUFA taken up from the diet.

Below I present detailed discussions of the egestion, transformation and β-oxidation processes, followed by the PUFA-specific regression equation formulations.

2.2.1.1 Egestion

In the regression analysis, species-specific egestion rates from the literature for individual species and dietary content of ALA and LIN were ln(x)-transformed while content of EPA, DHA and ARA were ln(x+1)-transformed prior to conducting the multivariate regression analysis (Appendix A). The regression equations for the egestion rate constants of each PUFA considered (Eqs. 2.5-2.9) had adjusted $R^2$-values ≥0.69 and $p<0.001$ (Table 2.2). No major problems of heteroscedasticity or influential outliers were observed (Appendix C). Egestion rate constants (h$^{-1}$) were therefore calculated as:

$$k_{ELALA} = (3.13 \times 10^{-6} \times [ALA]^{1.19} \times ([EPA] + 1)^{0.47}) / [ALA]$$  (2.5)

$$k_{EL EPA} = (1.45 \times 10^{-5} \times ([EPA] + 1)^{0.79}) / [EPA]$$  (2.6)

$$k_{EL DHA} = (1.49 \times 10^{-5} \times ([EPA] + 1)^{-1.68} \times ([DHA] + 1)^{2.66}) / [DHA]$$  (2.7)

$$k_{EL LIN} = (1.24 \times 10^{-5} \times [LIN]^{0.43} \times [ALA]^{0.43} \times ([EPA] + 1)^{0.36}) / [LIN]$$  (2.8)

$$k_{EL ARA} = (1.64 \times 10^{-5} \times ([ARA] + 1)^{2.25}) / [ARA]$$  (2.9)
Where \([x]_i\) is the dietary content of PUFA ‘x’ in predator ‘i’(mg FA\(_j\) g\(_i\)\(^{-1}\) D.W.).

2.2.1.2 Transformation

Species-specific transformation rates and content of dietary and predator ALA and LIN from the literature were ln(x)-transformed while dietary content of EPA, DHA and ARA were ln(x+1)-transformed prior to performing multivariate regression analysis (Appendix A). For the regression models, I considered precursor PUFA ‘x’ transforming to end product PUFA ‘y’ individually because n-3 and n-6 PUFA follow distinct transformation pathways (Eqs. 2.10-2.12).

The final, regression equations for ALA and LIN (Eqs. 2.10 and 2.12) had adjusted \(R^2\)-values above 0.47 and \(p<0.001\) (Table 2.2) and no major problems of heteroscedasticity or influential outliers were observed (Appendix D). A statistically significant relationship was not found for EPA transforming to DHA. Therefore, until further data become available, I recommend modeling this transformation using the same regression equation for ALA transforming to EPA but replacing the ALA content input with EPA (Eq. 2.11). \(k_{Ti,xy}\) is the transformation rate constant of precursor PUFA ‘x’ to end product PUFA ‘y’ in h\(^{-1}\) (Eqs. 2.10-2.12).

\[
k_{Ti,ALA\rightarrow EPA} = (8.19 \times 10^{-5} \times [ALA]^{0.73})/[ALA] \quad (2.10)
\]

\[
k_{Ti,EPA\rightarrow DHA} = (8.19 \times 10^{-5} \times ([EPA] + 1)^{0.73})/[EPA] \quad (2.11)
\]

\[
k_{Ti,LIN\rightarrow ARA} = (6.08 \times 10^{-5} \times ([EPA] + 1)^{-0.69} \times [LIN]^{0.98})/[LIN] \quad (2.12)
\]

2.2.1.3 \(\beta\)-oxidization

To obtain equations for calculating \(\beta\)-oxidation rate constants, dietary content of ALA and LIN in the diet and prey were ln(x)-transformed. The content of EPA, DHA and ARA in the species-specific diet were ln(x+1)-transformed prior to multivariate regression analysis (Appendix A). The species-specific ARA \(\beta\)-oxidation rates were ln(x+1)-transformed in order to obtain a normal distribution of the data. All resultant regression equations had adjusted \(R^2\)-
values >0.80 and \( p<0.001 \) (Table 2.2). The resultant dataset was homoscedastic and had no influential outliers (Appendix E). The following equations for calculating the \( \beta \)-oxidation rate constants (Eqs. 2.13-2.17) were arrived at after analysis of various options:

\[
\begin{align*}
    k_{O_{L,ALA}} &= (2.84\times10^{-4} \times ALA^{0.94} \times ([EPA] + 1)^{-0.18})/[ALA] \\
    k_{O_{L,EPA}} &= (3.25\times10^{-5} \times ([DHA] + 1)^{2.39} \times ([ARA] + 1)^{-5.00} \times [LIN]^{2.39})/[EPA] \\
    k_{O_{L,DHA}} &= (8.57\times10^{-5} \times ([DHA] + 1)^{2.92} \times ([EPA] + 1)^{-2.39})/[DHA] \\
    k_{O_{L,LIN}} &= (1.46\times10^{-4} \times [LIN]^{1.07} \times ([ARA] + 1)^{-0.62})/[LIN] \\
    k_{O_{L,ARA}} &= (([ARA] + 1)^{1.68\times10^{-4}} - 1/[ARA])
\end{align*}
\]

Where \( k_{O_{L,xy}} \) is the \( \beta \)-oxidation rate of PUFA ‘x’ in h\(^{-1}\).

2.2.1.4 Other Elimination Processes

Other elimination processes in addition to egestion, transformation and \( \beta \)-oxidation occur; however, their contribution to PUFA elimination is limited. For example, some conversion of EPA and ARA to eicosanoid products occurs, but at low rates (Turchini et al., 2007). In rats, the rate of conversion was found to be less than 1 \( \mu \)g rat\(^{-1}\) d\(^{-1}\) (Hansen and Jensen, 1983). Therefore, I did not explicitly model this elimination process. Additionally, Turchini and co-workers (2006, 2007, 2009) considered PUFA transformation to dead-end products (i.e. ALA transformation to eicosatrienoic acid (ETE), LIN elongation to eicosadienoic acid (EDA)) in their mass-balance equations. However, Turchini et al. (2007) reported less than 2% transformation of ALA to its dead-end products relative to its total net intake. Thus, I did not include these processes in this model.

2.2.1.5 Dietary Uptake and Gut Absorption Coefficient

The regression model for the dietary uptake rate \( R_{Ai} \) (kg d\(^{-1}\)) was previously defined by Arnot and Gobas (2004) for freshwater fish as:
\[ R_{Al,x} = 0.022 BW_i^{0.85} e^{0.06 \times Temp} \] (2.18)

Where Temp is water temperature (°C) and BW\(_i\) is the mass (kg w.w.) of the predator. This equation is specific for species and temperature, but not PUFA. The equation was modified for expression as a dietary uptake rate constant \(k_{Al,x}(\text{h}^{-1})\). The dietary fraction (\(\beta_A\)) of species ‘i’ ingesting species ‘j’ can be multiplied by the dietary uptake rate constant to quantify the contribution of specific dietary sources.

Uptake into enterocytes was originally thought to occur by passive diffusion for all FAs, including PUFAs. However, it is now widely believed that whereas medium-chain FAs are primarily taken up by passive diffusion, the uptake of long-chain FAs involves various transport proteins and thus, uptake in the gut is highly efficient (Concha et al., 2002; Andre et al., 2004; Denstadli et al., 2004). Sire and Vernier (1981) suggested that the transport proteins in fish have similar characteristics to the FA binding proteins found in mammals. The gut absorption coefficient (\(\gamma_{G,i}\)), ranging from 0-1 (unitless), is the fraction of PUFA that the predator absorbs from its diet. In a situation where PUFA content is limited, the predator would absorb all available PUFAs (i.e. \(\gamma_{G,i} = 1\)). Where PUFA content is abundant, the predator may not physiologically require the entirety of the PUFA available in its diet and \(\gamma_{G,i} < 1\).

### 2.3 Model Application

I applied the model (Eq. 2.2 and rate constants Eqs. 2.5-2.18) to a small, piscivorous Yellow Perch in the upper Bay of Quinte, Ontario, Canada. The system’s average fall water temperature is 20°C and the Yellow Perch maintains a diet of bivalves (15%), benthic invertebrates (70%), planktivores (5%) and benthivores (10%) (dietary fractions as modified from Koops et al. (2006)). The Yellow Perch’s weighted diet contains 0.28, 1.4, and 2.3 mg of ALA, EPA and DHA and 0.38 and 0.90 mg of LIN and ARA, respectively (T. Johnson, Ministry of Natural Resources, Picton Ontario, Canada, personal communication). A sensitivity analysis was performed by changing each parameter by increments of ±10% in order to identify sensitive parameters. I used a Monte Carlo analysis to propagate the error associated with the regression equations through the PUFA model equations. The standard error of the estimate (SEE; Table
2.2) for each PUFA’s regression equations was used for each parameter’s normalized distribution.

2.4 Results and Discussion

2.4.1 Rate Constants and Model Outcome

In all cases, excluding EPA’s β-oxidation rate constant equation (Eq. 14), rate constant equations were positively correlated with the corresponding PUFA’s dietary content. In 8 of 13 rate constant equations, competition existed between PUFAs. For example, in Eq. 14 DHA, LIN and ARA content in the diet divided by the predator body weight all contribute to the β-oxidation rate constant equation for EPA. The interpretation of this equation was that in the dataset used to develop the equations, the rate of β-oxidation of EPA increased as a function of the content of DHA, LIN and ARA in the fish’s diet which makes sense intuitively. In contrast, in Eq. 10 only ALA content in the diet divided by the predator body weight is considered when calculating the ALA to EPA transformation rate constant, i.e. the more ALA available the greater its rate of conversion to EPA. Competition did not exist for rate constant equations predicting ARA values. Whereas some dependencies were mechanistically explicable, as noted above, others were not. In 6 of the 8 rate constant equations that exhibited competition, EPA was most often (75% of the time) the competitive PUFA that appeared in rate constant equation, suggesting that dietary EPA content plays an important role in rate determination. The multivariate regression analysis picked up some competitive interactions of transformation (i.e. EPA content in the diet impacted transformation of LIN to ARA, Eq. 2.12) where competing elongase and desaturase processes exist between n-3 and n-6 PUFAs.

Using multivariate analysis, dietary PUFA content divided by predator body weight predicted egestion, transformation and β-oxidation rate constants in fish. Interestingly, temperature was not a significant predictor variable, despite previous studies (i.e. Jiang and Gao 2004) indicating that increased temperature over a range of 15°C decreases organism PUFA content. Temperature may not have been a significant predictor variable due to a lack of temperature variation in the data used in the multivariate analysis, as 22 of the 24 data points (90%) had study temperatures >24°C.

The modeled values for Yellow Perch of EPA and DHA were within 20% and 10% of the 2009 measured values from upper Bay of Quinte values while the ALA content was within one
standard deviation of measured values ($r^2 = 0.94$; Fig. 2.3a). I note that the measured values were quite variable, i.e. measured DHA content was 10.1 mg g$^{-1}$ ± 7.4 (Fig. 2.3b). The model was able to capture the general trend of the n-3 PUFAs in a Yellow Perch, where ALA<EPA<DHA. Although in line with the general n-3 trend, the model underestimated the ALA content by 3 fold. For both n-6 PUFAs the modeled values were within 25% of the measured values. However, the model had difficulty capturing the LIN<ARA trend, producing similar values (~2.7 mg g$^{-1}$) for LIN and ARA. Compared to the measured values, the model overestimated LIN by 10% while underestimating ARA by 25%.

In terms of model structure, the model included all dominant uptake and elimination pathways for PUFAs in a fish. Uptake from diet, egestion, transformation and β-oxidation are major pathways that others (i.e. Turchini et al., 2006) have considered and highlighted as important. Although there are other pathways that could have been considered, I do not believe that they would have significantly altered the model’s outcome. For example, I only consider PUFA transformation but not retro-conversion (i.e. DHA transformation to EPA through chain shortening and increased saturation) based on reports that the latter is of minimal quantitative importance (Buzzi et al., 1997). A possible exception could be for DPA (22:5n-3) which retro-converts to EPA (20:5n-3). However, this primarily occurs when DPA is abundant and EPA is scarce (G. Turchini, Deakin University, Australia, personal communication, 2011), which is an unlikely scenario in freshwater environments.

### 2.4.2 Rates of Intake and Loss

The rates of intake and loss varied according to PUFA and pathway. Intake of all PUFAs was primarily from diet with the exception of DHA (as discussed below). Dietary intake was 100% for ALA and LIN as this is the only mechanism for uptake. EPA and ARA dietary intake were 94% and 86%, respectively, with remaining uptake from transformation.

For ALA, the dominant loss mechanism was β-oxidation (66%), followed by transformation (33%) with minimal egestion (1%) (Fig. 2.4a). LIN had a similar distribution of loss pathways in that β-oxidation was dominant, followed by transformation and egestion (60%, 28% and 12%, respectively; Fig. 2.4b). This agrees with evidence that these PUFAs are good substrates for β-oxidation, especially when available through the diet at high levels (Richard et al., 2006; Karalazos et al., 2011; Teoh et al., 2011).
For EPA, the dominant loss mechanism was transformation to DHA (84%), followed by egestion (15%) with minimal β-oxidation (1%). EPA’s predominant uptake pathway was through ingestion (94%) while only 6% of the uptake was via transformation from ALA (Fig. 2.4c). This suggests that in this case study, dietary EPA was sufficient to meet the Yellow Perch’s dietary requirement placing minimal demand for transformation to meet the fish’s physiological EPA needs. Conversely, 87% of DHA was supplied by transformation from EPA suggesting that the DHA dietary content was insufficient and hence transformation of EPA to DHA was necessary to meet the Yellow Perch’s physiological demand for DHA (Fig. 2.4d). Thus, the model showed that transformation was critical for supplying the fish with adequate DHA whereas sufficient ALA and EPA could be acquired primarily through diet.

Interestingly, β-oxidation was the dominant loss pathway for DHA (81%) as typically DHA is not considered to be a good substrate for β-oxidation due to its essential role in membrane structure (Morais et al., 2005). It is possible that similar to ALA and LIN, excess accumulated DHA may be used in processes (i.e. β-oxidation) where it would not be used under conditions where the PUFA is limiting. Another explanation is that this high rate was related to the diet of fish in the training set (from aquaculture studies) that differed significantly from conditions considered in the upper Bay of Quinte, as mentioned above. It is also possible and perhaps most likely that high rate of β-oxidation of DHA is an artifact of the steady-state assumption and in reality, fish accumulate DHA in summer when DHA is abundant rather than utilizing it for β-oxidation.

The model did not replicate the n-6 LIN<ARA trend, rather LIN≈ARA. While the transformation of ALA to EPA and DHA was able to supplement any n-3 deficiency in the diet, the LIN to ARA transformation rate was unable to compensate do to so and therefore unable to capture the n-6 LIN<ARA trend, in spite of appreciable accumulation compared to measured values (Fig. 2.3b). The underestimated ARA accumulation could be attributed to a transformation rate (0.42 µmol fish\(^{-1}\) d\(^{-1}\)) derived from the training set that was not applicable to the upper Bay of Quinte situation. Some fish display an affinity for the delta-6 desaturase used in the transformation process towards ALA over LIN (Sargent et al., 2002; Stubhaug et al., 2007; Francis et al., 2009). Although the model calculated similar ALA and LIN transformation rates (0.38 µmol fish\(^{-1}\) d\(^{-1}\)) compared to 0.42 µmol fish\(^{-1}\) d\(^{-1}\) for ALA and LIN transformation,
respectively), the relative contribution of transformation of ALA to EPA was 5% greater than that of LIN to ARA. LIN had a greater proportion lost to egestion (11%) relative to ALA (1%).

2.4.3 Model Sensitivity and Limitations

The dominant uptake and elimination pathways have been accounted for in the model suggesting that discrepancies between modeled and measured PUFA content was largely due to uncertainty in the rate constants, that in turn, would be due to the nature of the data in training set. Results of the sensitivity analysis indicate that each PUFA displays a unique sensitivity to the model parameters (Appendix F). ALA and LIN are most sensitive to the dietary uptake and β-oxidation rate constants, e.g., a 30% β-oxidation decrease produces a 22% increase in ALA and LIN. The ingestion rate constant equation (Eq. 2.18) is well established, and as such, I have high confidence in this parameter’s estimation. Conversely, the β-oxidation rate constants are poorly studied and hence I am less confident of these regressions (Eqs. 2.13-2.17). Moreover, it is possible that these rate constants reflect a bias in the training set, as mentioned above.

Not surprisingly, EPA was highly sensitive to the EPA to DHA transformation rate; a 30% increase EPA to DHA transformation resulted in a 20% decrease EPA content. This rate constant (Eq. 2.11) was the only rate that did not produce a statistically significant relationship to any of the predictor variables. Consequently, I used the ALA to EPA transformation rate constant equation (Eq. 2.10) rather than a unique equation, substituting EPA for ALA (Eq. 2.11).

As mentioned above, an important weakness in the model application is that the studies in the training set used to formulate the regression equations are from the aquaculture literature that examined how changing contents of ALA and LIN influence EPA, DHA and ARA accumulation. In these studies, high ALA and/or LIN content was incorporated into the fish diet, while dietary EPA, DHA and/or ARA were kept low or completely excluded. Under such conditions, egestion, transformation and/or β-oxidation may only reflect a limited set of natural freshwater conditions, i.e. a cyanobacteria bloom where phytoplankton ALA content is high while EPA and DHA content is very low (Müller-Navarra et al., 2004).

Excess dietary ALA can result in stimulated β-oxidation rates. Although ALA is a good substrate for β-oxidation, an overly aggressive rate constant reflective of the training set conditions could account for the model under-predicting ALA content as its rate constant (0.77 µmol fish⁻¹ d⁻¹) was 50% and 100% faster than those for transformation and egestion,
respectively. Although the model overestimated LIN content by 10%, it too had a fast β-oxidation rate relative to the other elimination rates. The LIN β-oxidation rate (0.88 µmol fish\(^{-1}\) d\(^{-1}\)) was 50% and 80% faster than the transformation and egestion rates, respectively. Both ALA and LIN were most sensitive to changing β-oxidation rates.

Due to logistical impracticalities, egestion, transformation and β-oxidation rates have not been measured in natural freshwater fish. The current data training is adequate for a first generation model as it has elucidated the major uptake and elimination pathways. However, as a model’s outcome is only as strong as its inputs, measured rates in freshwater fish under natural conditions and/or learning more about these rates from another application of the model would be a useful next step towards improving the model.

### 2.4.4 Implications

PUFAs are crucial for fish because they affect metabolic activity, growth rates and reproduction. The 1 fish PUFA mass balance model simultaneously examines the uptake and elimination of ALA, EPA and DHA, LIN and ARA in freshwater fish. The model simplifies the physiological dynamics of PUFA metabolism, by focusing on the most significant pathways of PUFA accumulation: ingestion, egestion, transformation and β-oxidation. The model advances the knowledge of PUFA dynamics in freshwater fish as it is based on the mechanistic understanding of PUFA dynamics and empirical data. The derived regression equations for rate constants rely on measurable variables such as body mass and reflect the interconnection of PUFAs, e.g., the rate constant for LIN transformation considers EPA and LIN prey content.

The PUFA-specific rate constants used for egestion, transformation and β-oxidation in the PUFA mass balance model were developed from empirical regression coefficients, based on experimental data from the literature (i.e. Turchini et al., 2006; Francis et al., 2007; Francis et al., 2009; Turchini and Francis, 2009; Alhazzaa et al., 2011; Teoh et al., 2011). Rather than using static numerical inputs, these rate constants are connected with meaningful predictor variables, namely diet and body weight. Using this method, I am able to better define their uncertainty and the generic model parametric error is now replaced by the SEE of the regression models (used to perform Monte Carlo analysis). This method offers a more reliable solution to using a numerical PUFA model when guiding environmental management under a wide variety of conditions (i.e.
changing diet due to invasive species and/or climate change) as the model’s rate constants are not bound by one set of parameterization and are uniquely calculated for each scenario. The application of the model to estimate PUFA dynamics in a Yellow Perch produced ALA, EPA and DHA, LIN and ARA content that was consistent with measured values. The dominant elimination pathways varied according to PUFA. ALA and LIN elimination pathways were governed by β-oxidation while the predominant elimination pathway for EPA was transformation. The model results showed that transformation plays an integral role in PUFA accumulation in freshwater fish, contributing significantly to PUFA accumulation (i.e. DHA).

As a heuristic, rather than a predictive tool, the model can be applied to natural freshwater environments, facilitating a greater understanding of PUFA transfer and accumulation in fish. As a result of using regression analysis to formulate rate constant equations as opposed to using ‘fixed’ input values, the model is designed to be applicable for a wide range of scenarios and is not limited by the parameterization used in the aforementioned Yellow Perch model scenario. This model could be further expanded to quantify the transfer of PUFAs through a food web and used to examine overall food web health including various potential food web disturbances (i.e. invasive species, global warming). The model can be used to examine different management, invasive species and climate scenarios where the system may experience changing food web structure and/or PUFA food quality. Additionally, this model can be applied to aquaculture scenarios, as the rate constant equations were developed using aquaculture data which may bias results for species under natural conditions.
2.5 Tables

Table 2.1. Summary of 1 fish PUFA model inputs.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Units</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>i</td>
<td>--</td>
<td>The consumer (i.e. predator) (subscript notation)</td>
</tr>
<tr>
<td>j</td>
<td>--</td>
<td>The diet (i.e. prey) (subscript notation)</td>
</tr>
<tr>
<td>x</td>
<td>--</td>
<td>‘Generic’ PUFA notation (subscript notation)</td>
</tr>
<tr>
<td>y</td>
<td>--</td>
<td>‘Generic’ PUFA notation (subscript notation)</td>
</tr>
<tr>
<td>γ</td>
<td>--</td>
<td>Gut absorption coefficient</td>
</tr>
<tr>
<td>k</td>
<td>h⁻¹</td>
<td>Uptake/elimination rate constant</td>
</tr>
<tr>
<td>R</td>
<td>kg d⁻¹</td>
<td>Uptake/elimination rate</td>
</tr>
<tr>
<td>A</td>
<td>--</td>
<td>PUFA-based diet (i.e. food) of the consumer</td>
</tr>
<tr>
<td>E</td>
<td>--</td>
<td>PUFA egestion</td>
</tr>
<tr>
<td>O</td>
<td>--</td>
<td>PUFA β-oxidation</td>
</tr>
<tr>
<td>T</td>
<td>--</td>
<td>PUFA transformation (i.e. elongation/desaturation)</td>
</tr>
<tr>
<td>[LIN]</td>
<td>mg LINj g⁻¹</td>
<td>Mass fraction of linoleic acid (mg) in the diet per gram of predatory fish</td>
</tr>
<tr>
<td>[ARA]</td>
<td>mg ARAj g⁻¹</td>
<td>Mass fraction of arachidonic acid (mg) in the diet per gram of predatory fish</td>
</tr>
<tr>
<td>[ALA]</td>
<td>mg ALAj g⁻¹</td>
<td>Mass fraction of α-linolenic acid (mg) in the diet per gram of predatory fish</td>
</tr>
<tr>
<td>[EPA]</td>
<td>mg EPAj g⁻¹</td>
<td>Mass fraction of eicosapentaenoic acid (mg) in the diet per</td>
</tr>
<tr>
<td>Symbol</td>
<td>Unit</td>
<td>Description</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>-------------</td>
</tr>
<tr>
<td>[DHA]</td>
<td>mg DHA(_j) g(^{-1})</td>
<td>Mass fraction of docosapentaenoic acid (mg) in the diet per gram of predatory fish</td>
</tr>
<tr>
<td>m</td>
<td>g</td>
<td>Mass of PUFA ‘x’</td>
</tr>
<tr>
<td>BW</td>
<td>kg</td>
<td>Body weight</td>
</tr>
<tr>
<td>(\beta_A)</td>
<td>Dietary fraction</td>
<td>The fraction of species ‘j’ consumed by species ‘i’</td>
</tr>
<tr>
<td>Temp</td>
<td>°C</td>
<td>Water temperature</td>
</tr>
</tbody>
</table>