THE INFLUENCE OF FOOD WEB STRUCTURE ON THE DIET, MERCURY DYNAMICS AND BIOENERGETICS OF NORTHERN PIKE IN RESERVOIRS OF THE UPPER SOUTH SASKATCHEWAN RIVER BASIN

by

LARS BRINKMANN
Master of Science, University of Lethbridge, 2007

A Thesis
Submitted to the School of Graduate Studies
of the University of Lethbridge
in Partial Fulfilment of the
Requirements for the Degree

DOCTOR OF PHILOSOPHY

Biological Sciences
University of Lethbridge
LETHBRIDGE, ALBERTA, CANADA

TITLE: THE INFLUENCE OF FOOD WEB STRUCTURE ON THE DIET, MERCURY DYNAMICS AND BIOENERGETICS OF NORTHERN PIKE IN RESERVOIRS OF THE UPPER SOUTH SASKATCHEWAN RIVER BASIN

STUDENT NAME: LARS BRINKMANN

Approved by:

Name	Rank	Highest Degree
Joseph B. Rasmussen Supervisor	Professor	PhD
Alice Hontela Thesis Examination Committee Member	Professor	PhD
Cameron P. Goater Thesis Examination Committee Member	Associate Professor	PhD
T. Andrew Hurly Thesis Examination Committee Member	Professor	PhD
Lawrence B. Flanagan Internal Examiner	Professor	PhD
Michael D. Rennie External Examiner	Assistant Professor	PhD
Anthony G. Russell Chair, Thesis Examination Committee	Assistant Professor	PhD

Abstract

This project investigated the influence of community structure in reservoirs on trophodynamics, growth and mercury biomagnification in the apex consumer and fisheries target species northern pike (*Esox lucius*). Where lake whitefish (*Coregonus clupeaformis*) were abundant in the prey community, pike were found to be piscivorous, consuming predominantly lake whitefish and other large-bodied fish species. Benthivorous feeding behavior was observed in reservoirs without abundant lake whitefish stocks, and amphipods formed the predominant prey consumed by pike. Growth rates of pike were found to be significantly lower under the benthivorous trophic regime. Benthivorous pike had lower mercury concentrations, consistent with lower dietary exposure, than piscivorous pike, but higher biomagnification consistent with low food conversion efficiency and slower growth. A bioenergetics analysis showed elevated feeding rates and activity levels in benthivorous pike on a diet of comparatively low energy density, relative to piscivorous pike, confirming a trophic bottleneck in response to trophic simplification and highlighting the importance of trophic coupling between pelagic resources and apex consumers in reservoirs.

Acknowledgements

First of all I would like to thank my supervisor Joe Rasmussen for his countless suggestions on this project, support, and financial generosity.

Thank you to Nexen Inc. and Alberta Conservation Association for the Biodiversity Grant, which also contributed significant funding to this project. Many thanks to NSERC for the operating grant to J.B. Rasmussen.

Thank you to the other committee members Andy Hurly, Cam Goater, Alice Hontela for their time during meetings and suggestions on my work.

Thank you to my assistants during field and lab work, especially Michael Campen, and Rebecca Watson for their help with sample preparations and sampling efforts. I would also like to acknowledge the involvements of David Rolfson and Guy Duke, who were tremendously helpful with a small but significant GIS component in this thesis.

A big Thank you goes out the Tim Oare and Peter Stow at Isomass Scientific, and to Fred Luu at PerkinElmer, all of whom were tremendously helpful with troubleshooting on countless occasions.

Last but not least I would like to thank my parents for their financial support and encouragement.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

Abbreviation	Definition
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BMF	Biomagnification factor
BRST	Brook stickleback
Cs	Cesium
DR	Daily ration
ED	Energy density
ES	Empty stomach
FO	Frequency of occurrence
FWIN	Fall Walleye Index Netting
Hg	Mercury
ICP-MS	Inductively coupled plasma mass spectrometer
IR-MS	Isotope ratio mass spectrometer
LKWH	Lake whitefish
MINN	Minnows (collective cyprinidae)
MMBM	Mercury mass balance model
NRPK	Northern pike
S.D.	Standard deviation
S.E.	Standard error
SC	Stomach content

SI	Stable isotope
SSRB	South Saskatchewan River Basin
TCs	Total Cesium
THg	Total mercury
TP	Trophic position
VBGF	Von Bertalanffy growth function
VIF	Variance inflation factor
YLPR	Yellow perch

Chapter 1

Introduction

1.1 The context of irrigation in Southern Alberta

Settlement of the Canadian western frontier was a key policy in preventing American expansion northward of the 49th parallel. Success of this policy was ascribable in large parts to establishment of infrastructure to support a settling population through an agricultural economy (Topham 1982). This, however, faced severe limitations imposed by climate, especially along the semi-arid western margins of the region known as Palliser's triangle. Captain John Palliser, who led an initiative to explore Canada's western territories essentially for their economic potential, encountered a landscape of shortgrass prairie, salt flats and prickly pear cactus. He reported that the region was "[…] desert or semi-desert in character, which can never be expected to become occupied by settlers". Although overly pessimistic, his assessment anticipated challenges to the envisioned establishment of crop-agriculture, which gambled with a highly variable and often insufficient precipitation regime. The region receives on average less than 250mm of rain during the growing season (Alberta Agriculture 2000). By the end of the 1880s, while enduring a prolonged drought period, farmers began to divert water from streams onto fields in small-scale operations; through cooperatives, ever larger parcels of land fell under simple flood irrigation, exemplifying great potential for securing livelihoods (Alberta Agriculture 2000). From the groundwork laid then, by the early 20th century, almost 250,000 acres fell under irrigation from diversions from the St.Mary, Oldman and Bow Rivers. With greater government support came increasing expansion and the need

for surface storage in addition to further delivery systems arose in the 1930s (Klassen and Gilpin 1999). This initiated the construction of reservoirs throughout the upper South Saskatchewan River Basin (SSRB)(Topham 1982).

1.2 The structure of the irrigation system and its reservoirs

Reservoirs in southern Alberta are primarily intended for the storage of water and can be classified into on-stream and off-stream impoundments. On-stream reservoirs impound rivers directly. As such, they serve to regulate river flows, which peak during snow melt in spring. Stored water is available for release during low-flow periods, thus maintaining adequate supplies to meet allocation needs. Water is diverted from the main stems of rivers into a distribution canal system often far upstream of the agricultural areas where it is used (Figure 1.1). This can be rationalised by the fact that rivers in this area flow through steep-walled coulees, old glacial drainage channels, which can lie up to 60 m below plateau level. Thus, water is diverted into canals on the plateau at suitable locations of low head far upstream. Water flow through the canals is regulated by drop structures and weirs. Off-stream reservoirs are situated along distribution canals and provide additional storage. These reservoirs are constructed in natural depressions in the landscape, such as wetlands enhanced by berms, or by damming dry coulees, which no longer contain major streams. Ultimately the canal system reticulates into a multitude of branches, which deliver water to irrigation equipment located on fields. Excess water is channeled into drainage canals and returned to the river.

Due to the semi-arid climatic regime the southern Alberta landscape features very few natural lakes, which are located mainly along the western prairie-mountain margin. Thus, reservoirs constitute the predominant form of lentic fish habitat. Fish have become

established in reservoirs through stocking or by migration through the canal system. This implies that canals may play a crucial role in the species presence and community structure in off-stream reservoirs, mainly through features and location of their connections to the river, which conditionally represent entry points for fish to the canal system (Rasmussen and Brinkmann 2015).

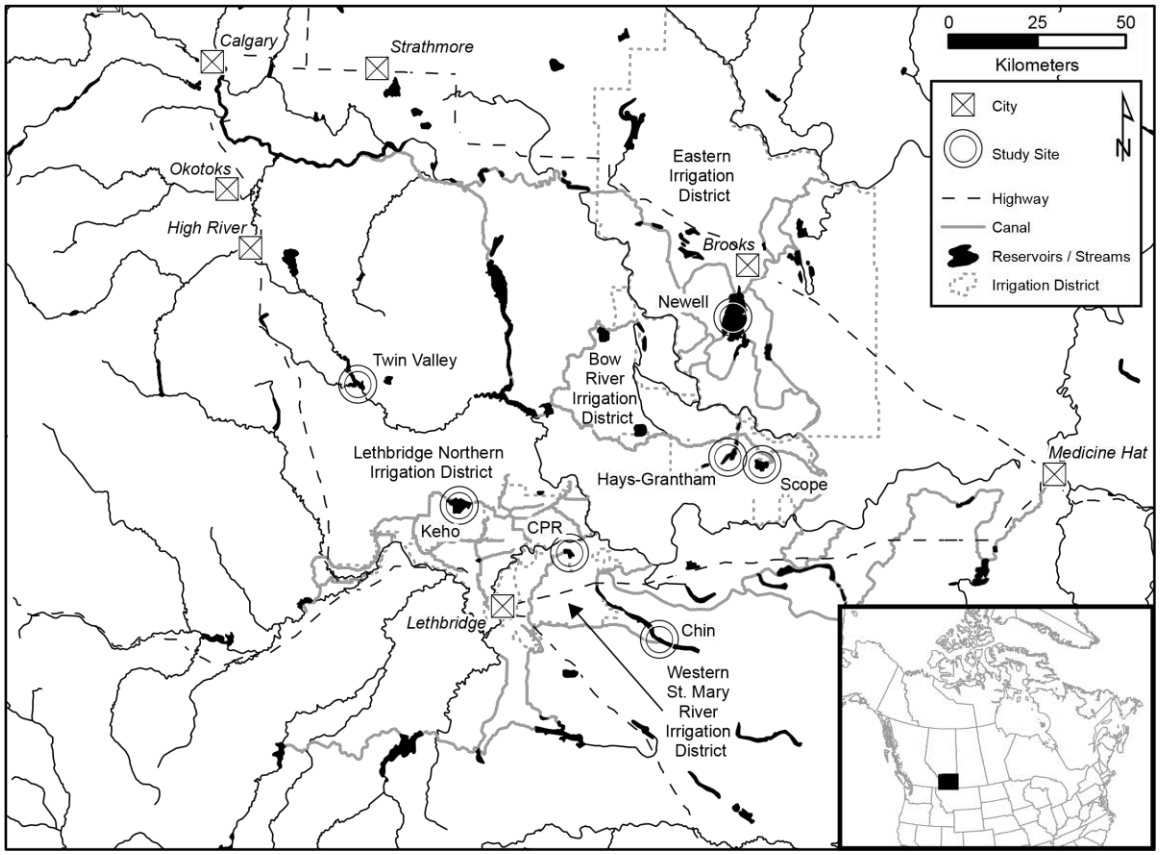


Figure 1.1: Map of southern Alberta irrigation districts, major reservoirs and canal connectivity. Study sites are circled. Reservoirs, which have abundant lake whitefish in the forage fish community are Keho, Newell and Chin. Lake whitefish are completely absent in Twin Valley and Hays-Grantham, and occur only sporadically in Scope and CPR.

1.3 Fish communities in upper SSRB reservoirs

Reservoir construction encompasses conversion of riverine habitat into a state resembling lacustrine habitat. This also entails development of a resident fish community and associated food webs. Unaided by specific management, reservoir fish communities are expected to develop from the fish community in the impounded tributary stream (Fernando and Holcik 1982, Irz et al. 2006). Thus the community structure and food webs in reservoirs depend on the tributary river, which provides the source of fish stocks colonizing reservoirs. The predominant fish species to colonize reservoirs in southern Alberta without management intervention are derived from native lotic fish communities of cool-water species (Mitchell and Prepas 1990, Nelson and Paetz 1992). Northern pike (*Esox lucius*) and burbot (*Lota lota*) may be the omnipresent apex consumers derived from lotic communities. Intermediate consumers include longnose and white sucker (*Catostomus catostomus* and *C. commersonii*), cyprinids, such as lake chub (*Couesius plumbeus*), spottail shiner (*Notropis hudsonius*), and fathead minnow (*Pimephales promelas*). Brook stickleback (*Culaea inconstans*) may also be found in small reservoirs, which have been created from permanent wetlands. Other cool-water fish species, whose distribution has been influenced by a combination of stocking and migration, include walleye (*Stizostedion lucioperca*) and yellow perch (*Perca flavescens*) (Rasmussen and Brinkmann 2015).

Lake whitefish (*Coregonus clupeaformis*), a zooplanktivorous cold-water glacial relic species occuring in subalpine headwater lakes of the Waterton River and St. Mary River tributaries to the Oldman River, have also been introduced into several reservoirs, although some populations may have arisen first by in-migration from upstream stocks

(e.g. the St. Mary reservoir). The barriers to movement of lake whitefish through the irrigation system have not all been fully identified, but may encompass structural barriers along waterways, including dams, drop structures and weirs along canals, which prevent movement upstream (Brinkmann and Rasmussen 2010, Rasmussen and Brinkmann 2015). In addition, physiological barriers, such as high summer temperatures and oxygen levels in reservoirs (Edsall and Rottiers 1976), may further limit establishment of lake whitefish in some reservoirs (Figure 1.1).

In the rivers (stream order 5-6) of southern Alberta, food webs tend to be driven by benthic production and intermediate consumer levels are comprised of benthivorous consumer fish species (Romanuk et al. 2006). Consequently, food webs in reservoirs may naturally develop a similar benthic-driven structure. This energetic pathway, however, may be severely impaired by hydrologic regimes in reservoirs, which through drawdown preclude development of near-shore habitats (Lindstrom 1973, Baxter 1977). Instead, plankton production in the pelagic zone may constitute the most important source of energy, which could support significant fish production long term. Zooplanktivorous fish, such as lake whitefish, are required as intermediate consumers to make this productive pool available to higher trophic levels. Other fish species, which could assume a zooplanktivorous role in reservoirs, are not typically found in tributary rivers in the upper SSRB, as hydromorphological factors preclude a significant zooplankton pathway. Thus, for zooplanktivorous fish to colonize a reservoir haphazardly in this case, an upstream lake with a source population must exist in the watershed (Irz et al. 2006, Miranda et al. 2008), or deliberate stocking must occur.

A recent study proposed that lake whitefish may potentially assume an important trophic coupling role between pelagic production and apex consumers in upper SSRB reservoirs (Brinkmann and Rasmussen 2010). This hypothesis was based on observations made in a newly created reservoir on the Little Bow River (Twin Valley, Figure 1.1), in which access barriers prevented colonization by lake whitefish. The diet of resident northern pike, normally a highly piscivorous species, consisted almost exclusively of benthic invertebrates which led to the interpretation that feeding options for pike were severely limited in the tributary-derived benthic community in this reservoir (Brinkmann and Rasmussen 2012). However, the general importance of lake whitefish as a resource to northern pike or other apex consumers in reservoir communities is largely unknown, and has yet to be properly assessed.

1.4 Fish growth in reservoirs

Growth rates of target fish species is perhaps the most important factor determining the successful establishment of a reservoir fishery. Continuous positive growth occurs when foraging investments produced an energetic profit, over and above basic metabolic and digestive requirements. However, within any given species, growth rates of fish can vary widely across different systems. Field studies have shown that such variation can occur in response to differences in prey base composition (Boisclair and Leggett 1989), which constitutes one of the most important ecological influences on the bioenergetic budget of consumer fish. Prey organisms can vary both in terms of abundance and quality, and the resultant trade-offs constrain the amount of energy allocable for growth in the energy budget of a consumer.

Optimal foraging theory predicts that consumers select prey in order to maximize bioenergetic profit (Stephens and Krebs 1986). Especially in piscivorous fish species, this optimization process may involve a scaling of prey size with consumer body size and gape limitation, which ensures that the time and foraging activity spent to attain bioenergetic reward are minimized (Mittelbach and Persson 1998, Sherwood et al. 2002b). Therefore a series of diet shifts along a spectrum of prey sizes must occur through the course of ontogeny of a consumer, continually 'resetting' foraging cost. Studies have shown that, if options for diet shifting are limited by discontinuity of the prey size spectrum, fish growth rates slow down due to ever increasing foraging cost (Sherwood et al. 2000, Pazzia et al. 2002, Iles and Rasmussen 2005), a phenomenon known as 'trophic bottleneck' (Heath and Roff 1996, Sherwood et al. 2002a). Growth patterns consistent with the trophic bottleneck concept were also observed in northern pike under benthivorous trophic regimes (Venturelli and Tonn 2006), which included Twin Valley reservoir (Brinkmann and Rasmussen 2010). This highlighted the possibility that trophic simplification may impair growth rates of pike, and potentially other piscivorous fish in reservoirs, and may thus adversely affect fisheries potential. This important aspect of reservoir development has received little attention so far. Here, a comparison of growth rates and the underlying bioenergetics of northern pike under benthivorous and piscivorous conditions would greatly enhance our understanding of interrelationships between community structure and fish growth in reservoirs.

1.5 Northern pike feeding ecology

The northern pike may be the most wide-spread top piscivore in southern Alberta and represents an important target species of recreational fisheries. It is well-suited to the

temperature regimes found in reservoirs and tolerant of potentially low oxygen levels during the winter period (Casselman and Lewis 1996), especially in shallow reservoirs. The predatory nature of pike is not a matter of much debate. By its morphological adaptations alone the pike is easily recognized as a specialist piscivore, capable of consuming large prey items relative to its own body size (5-15%). Traits that adapt pike extremely well to ambushing and overcoming large-bodied prey include a large broadened snout, jaws with sharp, regenerable teeth and depressible vomerine teeth, which firmly hold captured prey (Raat 1988). Pike hunt from an ambush and may target prey with the assistance of both visual and lateral-line sensory cues. Feeding strikes typically involve a brief aiming motion followed by a rapid lunge forward towards prey (Webb and Skadsen 1980). Prior to intercepting prey, pike open their mouth widely. This is accompanied by an opening of the opercula to evacuate water from the head past the gills and thus reduce resistance moving forward with an open mouth. Here gill morphology may represent a further important adaptation, in that gill rakers are highly reduced to minute denticles (Raat 1988), which minimizes obstructions to passage of water through the gills. Arguably, while this trait aids in the capture of large prey, it may impose a lower size threshold on the retention of particularly small prey.

Previous studies suggested that strike success rates of pike may be as high as 95% (Webb and Skadsen 1980). Thus, pike is a keystone species, in that its efficient predation on smaller fish has a significant effect on community structure at lower trophic levels (Berg et al. 1997, Skov et al. 2003, Venturelli and Tonn 2005). This has led to the use of pike as a whole-system biomanipulation tool (Berg et al. 1997, Skov et al. 2003). Predation by pike can greatly reduce forage fish densities, and in some cases eliminate

species altogether (Berg et al. 1997). Despite this prey-depletion effect pike can maintain high population densities and may contribute disproportionally to fish biomass in their role as apex predator. In extreme cases pike may even occur as the sole fish species (Venturelli and Tonn 2006). Such configurations are possible only through a high degree of feeding flexibility by pike and suggest that pike can survive on non-fish prey, mainly benthic invertebrates (Venturelli and Tonn 2006). Field studies suggest that the extent of non-piscivorous feeding behaviour in pike is context dependent and may be relatively short-term, seasonal occurences (Chapman and Mackay 1990, Beaudoin et al. 1999, Paradis et al. 2008), or permanent in highly disturbed systems, with species-depauperate fish communities (Venturelli and Tonn 2006, Brinkmann and Rasmussen 2010). Studies are in general agreement that non-piscivorous feeding regimes coincide with significantly reduced growth rates of resident pike, which has led to the interpretation that invertebrate prey bases impose high costs on the energy budgets of pike. The specific bioenergetic consequences of non-piscivory in pike, however, have yet to be addressed in detail.

1.6 Mercury in reservoirs

Mercury is a trace element in the earth's crust and omnipresent in the environment. Its elemental form and most of its compounds exhibit varying degrees of toxicity to most biota. The combination of this characteristic and the fact that mercury is dispersed globally through the atmosphere from both anthropogenic and natural releases makes mercury one of the most important environmental risk factors world-wide.

It is widely recognized that reservoir construction is almost invariably linked with mercury contamination in fish communities (Verta 1990, Hecky et al. 1991, Hall et al.

2005). As such, reservoirs were among the first examples of large-scale mercury contamination not associated with direct industrial releases (Abernathy and Cumbie 1977), such as kraft pulp mills or smelters. It is generally accepted that mercury in reservoirs is released upon inundation, from pools stored in terrestrial soils and vegetation (St Louis et al. 2004). Besides the initial loading, production of methylmercury from inorganic mercury by sulfate-reducing bacteria (Compeau and Bartha 1985), is also likely occurring at elevated rates due to the abundance of decaying organic matter and anoxic conditions. Methylmercury is particularly problematic in the environment, since it can be passed on from prey to consumer through trophic transfer and can accumulate in consumer tissues, and therefore reach high concentrations at the end of food chains (Cabana et al. 1994).

1.7 Mercury biomagnification in fish

Methylmercury (~mercury) in fish can reach concentrations 10^6–fold higher than the abiotic environment, and can therefore expose consumers of fish to toxic levels (Morel et al. 1998). Once mercury enters the food chain at the base it is efficiently passed on from prey organisms to consumers. This trophic transfer process occurs in conjunction with uptake of nutrients, predominantly protein (~energy) (Boudou and Ribeyre 1997). Once assimilated, mercury is eliminated at very slow rates and thus the mercury burden increases in tissues of feeding fish. Energy derived from the diet is allocated towards items on the energy budget of fish and lost efficiently through activity/metabolic processes. The balance can be allocated towards tissue growth, which effectively dilutes the mercury burden stored in tissues. However, due to metabolic losses, combined rates of growth and mercury elimination are always lower than feeding and assimilation, and

thus the mercury concentration increases in consumers relative to their prey, which is termed 'biomagnification' (Thomann 1981). It also follows that the magnitude of biomagnification (biomagnification factor, BMF), defined as $BMF = [Hg_{consumer}]/[Hg_{diet}]$, is inversely proportional to the conversion efficiency of food to consumer tissue (Trudel and Rasmussen 2006, Lepak et al. 2012). Furthermore, biomagnification is cumulative along food chains, and thus consumers at the apex of longer food chains can have high mercury levels, because their diet is also comparatively high in mercury (Cabana et al. 1994, Vander Zanden and Rasmussen 1996). In summary, the accumulation of mercury and bioenergetics in fish are conceptually linked and mercury concentrations in fish are a reflection of both dietary exposure and the efficiency of a trophic link in a food web (Trudel and Rasmussen 2006). Mercury can reach high levels in fish as a result of high exposure and high biomagnification, both factors, which can act independently and in concert.

The link between trophic efficiency and mercury biomagnification was recently illustrated in the context of Twin Valley reservoir. Northern pike exhibited low growth rates while feeding on benthic invertebrates, which suggested a trophic bottleneck. Post-impoundment mercury concentrations in resident northern pike were found to be high compared with reservoirs elsewhere (Brinkmann and Rasmussen 2010), and it was shown that this was in part ascribable to high mercury biomagnification between the invertebrate diet and pike. Elevated biomagnification added further weight to the hypothesis that food web simplification resulted in poor food conversion efficiency in pike. Moreover, this implied that trophic simplification due to incomplete lentic community and habitat development in reservoirs could pose a risk factor to exacerbate mercury concentrations

in predatory fish through biomagnification. This hypothesis remains untested on multiple systems so far, and filling this gap would greatly enhance our understanding of mercury concentrations in reservoir fish populations in southern Alberta, about which very little is known at this point.

1.8 Original research in this thesis

This thesis contains four chapters presenting original research investigating the trophic ecology and mercury dynamics of northern pike in relation to prey community structure in seven reservoirs in southern Alberta. Besides basic ecological and contaminant research objectives, this thesis also presents innovative aspects to generally applicable state-of-the-art isotope tracer and contaminant biokinetics techniques.

Chapter 2 addresses the methodology used to study trophic ecology of fish using nitrogen stable isotope ($\delta^{15}N$) trophic-enrichment techniques. An adaptation of existing procedures to improve methodological accuracy specific to northern pike is presented, based on data drawn from the literature and original research. The chapter discusses the implications of common unverified assumptions for the accuracy of stable isotope techniques and identifies areas in need of further research.

Chapter 3 addresses the trophic ecology of northern pike in response to the prey base found in reservoirs. The chapter presents a dietary breakdown and trophic structure analysis of pike in each reservoir, using a combination of direct observations and stable isotope mixing models, which draw upon the information in chapter 2. The chapter also presents a comparison of growth rates of pike among reservoirs and discusses links between prey type and bioenergetic factors responsible for variable fish growth.

Chapter 4 presents a survey of mercury concentrations in northern pike and their prey. The chapter addresses the effect of trophic structure in reservoirs on dietary mercury exposure and mercury biomagnification in pike. The conceptual links between bioenergetics and fish growth on mercury biomagnification are discussed.

Chapter 5 provides an analysis of bioenergetics of pike in response to trophic regimes in reservoirs. Stable cesium tracer biokinetics in combination with a standard energy budget is used to quantify feeding rates and activity levels of pike. The role of bioenergetics as a factor controlling mercury biomagnification in pike is illustrated by predicting mercury concentrations in pike using mercury biokinetics model. The importance of trophic bottleneck patterns in relation to prey communities as a risk factor contributing to mercury contamination in reservoirs is discussed.

Chapter 2

$\delta^{15}N$ trophic enrichment in northern pike (*Esox lucius*): Is it different from other consumers?

Abstract

Studies of the trophic ecology of fishes have come to rely heavily on analyses of stable isotope ratios of consumers and their diets. Isotopic analyses using $\delta^{15}N$ require key assumptions regarding the enrichment of the isotope between prey and consumer, which is on average 3.4‰ per trophic level. Although trophic position estimates of consumers obtained by this conventional assumption are typically in close agreement with direct dietary estimates, a significant and systematic disagreement in that respect has been observed in northern pike. Here I show that this discrepancy for pike can be explained by a systematic departure in the diet-tissue shift estimate from 3.4‰, and combine original field measurements with a literature review to show that the N-isotopic diet-tissue shift between pike and its average prey is consistently very close to 2.4‰ (±0.48 S.D.). I discuss the significance of anomalous diet-tissue shifts in N-stable isotope studies of food webs and explain how to integrate anomalous diet-tissue shifts into trophic position calculations of apex consumers. Overall, these results highlight the importance of validating estimates of trophic structure based on stable isotope approaches with complementary direct dietary information, especially when the methodology is applied to previously unstudied systems.

2.1 Introduction

In recent decades, the analysis of nitrogen stable isotope ratios has become an invaluable tool in the study of trophic structure. Consumers preferentially eliminate the light isotope of nitrogen (^{14}N) while metabolizing amino acids, resulting in ^{15}N enrichment, relative to prey organisms (Minagawa and Wada 1984). This enrichment process is cumulative along food chains, and therefore the change in the stable isotope ratio can serve as an integrative measure of the trophic positions of organisms in complex communities (Peterson and Fry 1987, Post 2002).

For the purposes of ecological studies, stable isotope ratios are conventionally presented in δ-notation (‰ units), which expresses isotope ratios in unknown samples relative to a known standard isotope ratio using the relationship

$$\delta^{15}N = \left(\frac{\frac{^{15}N}{^{14}N}_{unknown} - \frac{^{15}N}{^{14}N}_{standard}}{\frac{^{15}N}{^{14}N}_{standard}} \right) \quad (1)$$

here using N as an example. Isotopic enrichment between diet and consumer tissues, or the diet-tissue shift ($\Delta^a X$), is defined as the difference between consumer and diet δ-values; for $\delta^{15}N$ specifically, this is written as

$$\Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{diet} \quad (2)$$

(Minagawa and Wada 1984).

Quantitative estimates of trophic positions from $\delta^{15}N$ data are obtained through models assuming an average diet-tissue shift per trophic level. Seminal literature on the

subject reports an average diet-tissue shift of 3.4‰ per trophic level (Post 2002), an estimate based on both laboratory assays as well as field observations. Trophic position estimates using stable isotopes are typically in very close agreement with direct estimates from stomach contents analysis for a spectrum of species and systems (Vander Zanden et al. 1997). Therefore, due to considerably less laborious sampling regimes relative to direct observation, studies in trophic ecology have come to rely solely on stable isotope measurements assuming conventional trophic enrichment.

Accurate estimates of isotopic enrichment are critical for quantitative modeling of trophic relationships with isotope data (Hussey et al. 2010). Although ^{15}N enrichment of 3.4‰ in higher consumers finds widespread application in the literature (Sara and Sara 2007, Logan and Lutcavage 2010), species-specific variability in ^{15}N trophic enrichment cannot be ruled out (Vander Zanden et al. 1997, Vander Zanden and Rasmussen 2001, Vanderklift and Ponsard 2003). Variability in diet-tissue shift estimates has been interpreted from taxonomic, metabolic and other factors (Post 2002, McCutchan et al. 2003, Vanderklift and Ponsard 2003); however, lack of congruency among those lead to considerable ambiguity as to which estimate to use. Jardine et al. (2006) have recently drawn attention to this using the example of northern pike, for which $\Delta^{15}N$ could assume a range of values between 2.0 and 3.4‰. It was also stressed that ambiguity as such may have to be resolved on a species-specific level (Jardine et al. 2006).

Stable isotope techniques and mixing models have greatly enhanced our understanding of the feeding ecology of highly omnivorous consumers with diverse diets across time and space (Jardine et al. 2006). Especially here, however, incomplete knowledge or mere assumptions of isotopic diet-tissue shift estimates may lead to

significant bias in the results of mixing models and affect conclusions about trophic interactions accordingly (Wolf et al. 2009, Varela et al. 2011). Moreover, error in diet-tissue shift estimates is particularly difficult to detect in omnivores, due to the uncertainty in the average $\delta^{15}N$ signature of their diverse prey. In field studies, such uncertainty is particularly difficult to overcome, due to the elaborate sampling regime required for precise diet composition estimates, which are needed to reconstruct isotopic signatures of the average diet of an omnivorous fish population. In addition the feeding ecology of a species can vary greatly among populations, making species-specific deviations in trophic enrichment of N-isotopes from assumed normals difficult to detect.

When comparing stable-isotope derived estimates of trophic position with direct dietary analysis across a number of freshwater fish species, Vander Zanden et al. (1997) found that in northern pike stable isotope based estimates were consistently lower by one half trophic level in comparison with dietary estimates. While such discrepancies can easily result from imprecision in stomach content analysis, this explanation seems unlikely in this case due to the very considerable size of the data set analyzed in this review and its broad seasonal coverage (Vander Zanden et al. 1997).

However, bias in direct dietary methods may result from size-dependent differences in digestion rates of prey (Legler et al. 2010) in highly omnivorous consumers like northern pike. Although little is known in that respect, it may be speculated that digestion of invertebrate prey proceeds more rapidly in pike stomachs, thereby reducing the likelihood of their detection, relative to larger-bodied fish prey. Given that fish prey are assigned a higher trophic level than invertebrates (Vander Zanden et al. 1997), this would consequently bias trophic position estimates of pike using the dietary method. This

potential confounding factor may also result in a systematic underestimation of isotopic enrichment estimates based on field data, since these involve prey isotope signatures weighted to reflect the average diet based on stomach content analysis. Thus, pike populations presumed to rely on fish prey may have to be treated with caution in estimating N-isotopic diet-tissue shift in the field. Northern pike populations known to rely on relatively homogeneous invertebrate diets, and thus occupying a trophic position near the lower species-limit, may naturally control against bias in dietary quantifications. Since these populations will represent unbiased dietary and diet-tissue shift relationships, comparisons of diet-tissue shift estimates between these and piscivorous populations may thus help to identify potential discrimination against small-bodied prey taxa in dietary studies of northern pike in general.

Size-dependent patterns in digestion rates, and thus bias in dietary assessment, would be expected to occur in other omnivorous consumers besides northern pike. However, in walleye for example, which may exhibit similarly diverse feeding habits as pike (Scott and Crossman 1973) trophic position estimates using either method were in close agreement (Vander Zanden et al. 1997). This calls into question that the discrepancy in trophic position estimates is attributable to the dietary method. More consistent with species-specificity, it may be speculated that the observed discrepant pattern in dietary and isotopic trophic position estimates reflects anomalous trophic enrichment of ^{15}N in northern pike. Based on the discrepant pattern I deduce that diet-tissue shift in pike must be considerably lower than the assumed conventional 3.4‰, which causes the stable isotope method to systematically underestimate trophic position of pike. This notion has never been explored in this species, although awareness of

variability in isotopic enrichment in the broader context is increasing (Vanderklift and Ponsard 2003, Auerswald et al. 2010).

In this study original field observations and literature data are assembled into a dataset of N-isotopic diet-tissue shift estimates for northern pike to test the hypothesis that diet-tissue shift in this species differs significantly from the mean commonly assumed in higher consumers (3.4‰). I also propose a system of quality assurance against potential bias in dietary quantifications, by comparing diet-tissue shift among populations at extreme endpoints of the food spectrum of an omnivorous species.

2.2 Methods

2.2.1 *Collections*

Northern pike were collected from five reservoirs by gill-netting and angling between June and September 2009 through 2011. Fish were cooled on ice in the field and later frozen until further processing. In the lab fish length and weight measurements were taken, and a sample of dorsal muscle tissue was collected and dried to constant weight (60°C, 24-48 hrs) for stable isotope analysis. Pike stomachs were dissected and stomach contents sorted and identified. Only well-preserved, undigested stomach contents were used for quantitative bulk analysis. Separated stomach contents were dried to constant weight and percent contributions were calculated on dry weights. Stomach contents in advanced stages of digestion were screened for identifiable parts and recorded only as frequency of occurrence (FO) data. Fish remains, if partially digested, were extrapolated to approximate total length and weights calculated based on length-to-weight regressions for each lake. Skeletized, disjointed fish remains were counted as FO. Frequency of

occurrence data were reported to indicate the entire food spectrum of pike, the proportion of empty stomachs, and individual specialization.

In addition to organisms from stomach contents, samples of benthic and planktonic invertebrates and forage fish samples were collected from each reservoir using Ekman dredge, dip net and gill-nets, respectively. These samples were sorted and dried for stable isotope analysis.

2.2.2 Stable isotope data

Samples of animal tissue were ground to fine homogenous powder and packaged in tin capsules for C-N elemental and stable isotope analysis. Analyses were carried out on a Thermo Finnigan Delta V Advantage isotope ratio mass spectrometer coupled to a Costech ECS 4010 elemental analyzer. Quality was assured through interspersion of working reference materials as well as duplicate samples. Deviations of less than 0.2‰ between duplicates or referenced values were considered acceptable.

A weighted average diet signature was calculated for each system using the mass fraction determined by quantitative bulk analysis of stomach contents. $\delta^{15}N$ diet-tissue shifts ($\Delta^{15}N$) in northern pike for each system were then calculated by inputting sample mean $\delta^{15}N_{pike}$ and net dietary mean signature $\delta^{15}N_{diet}$ into equation. (2).

In addition, stable isotope data were extracted from publications reporting $\delta^{15}N$ signatures for both northern pike and diet organisms. Due to a high degree of omnivory frequently observed in northern pike, with diet taxa ranging several orders of magnitude in weight, dietary information quantified as fraction of bulk weight is required for calculating weighted average dietary isotope signatures. However, not all publications

contained this information in great detail, or omitted altogether. Therefore publications were grouped based on completeness of data: Group 1 omitted diet assessments altogether. Group 2 included papers reporting qualitative stomach contents data (percent of count or frequency of occurrence, %FO) or mentioned stomach observations in the discussion. Group 3 included papers with stable isotope and % bulk weight dietary data. Here, mean diet isotopic signatures were calculated by weighting isotope signatures of prey taxa by their % bulk weight contribution in pike stomachs. In papers providing diet information as % of count or %FO, prey taxa contributing at least 15% to the stomach counts were included and factored into the net dietary signature equally. If the diet was dominated by large prey taxa (typically fish), in combined proportion of approximately 50%, the net dietary signature was based only on these large taxa, weighted equally. If the diet of pike was only mentioned without supporting data, I relied on the statements given by the authors to calculate an approximate average dietary signature. Finally, $\delta^{15}N$ diet-tissue shift was calculated using equation (2).

2.2.3 Statistical analysis

Statistical computations were carried out in R (2012). Initial quality assurance comparisons aimed to identify potential biases in my analyses of diet-tissue shift in three ways: **I)** Literature data varied in completeness, in that dietary data are sometimes not quantitative or omitted. To identify potential bias in my diet-tissue shift estimates due to arbitrariness in diet assessments, I applied confidence rankings to diet-tissue shift estimates from the three groups of publications. Confidence in the accuracy of calculated values was lowest in group 1 papers and highest in group 3 papers. The overall mean diet-tissue shift for pike from all systems was determined using weighted linear

regression with constant independent variable. The model was run three times: 1) with all diet-tissue shift estimates given equal weight; 2) with equal weights assigned only to group 3 estimates and omitting groups 1 and 2; 3) with weights of 1 to 3 assigned to each estimate based on its confidence rank. **II)** Field data and high-confidence literature data were compared statistically using Student's t-test in order to identify potential methodological bias associated with this study. **III)** Focusing on high-confidence observations from field and literature, mean diet-tissue shift estimates were compared among non-piscivorous and piscivorous trophic regimes to test for evidence of general discrimination against invertebrate taxa in direct dietary analysis, resulting in underestimation of isotopic enrichment in piscivorous pike populations.

Finally, in order to test whether diet-tissue shift estimates for northern pike differed from the established mean for other consumers, the entire distribution of observations (n=24) made herein was compared with a simulated normal distribution of n=24 observations with mean 3.4‰ and standard deviation ±1‰ (Post 2002), using Student's *t*-test.

2.3 Results

I assembled dietary and stable isotope data from five reservoir systems in southern Alberta (Table 2.1) and from the published literature (Table 2.2) to estimate N-isotopic diet-tissue shift in northern pike. Diet-tissue shift estimates from original field data averaged 2.3‰ (±0.39) and ranged from 1.7-2.7‰. Literature observations were ranked by level of confidence in diet-tissue shift estimates based on the presence of quantitative dietary data. Regression analysis weighted by confidence ranks produced

similar intercept coefficients in all tested scenarios, with deviations from the non-weighted intercept of less than 0.13‰. The overall average diet-tissue shift, including high and low-confidence observations, was 2.4‰ (S.D. ±0.48‰). Diet-tissue shift estimates based on rank 1 and 2 publications averaged 2.3‰ (S.D. ±0.61‰) and ranged between 1.4 and 3.3‰. Rank 3 observations averaged 2.5‰ (S.D. ±0.23‰).

Original field data and rank 3 publications were not significantly different (*t*-test, $p = 0.164$, $df = 6$) and were treated as one group of high confidence estimates averaging 2.4‰ (S.D. ±0.31) overall.

This analysis included pike populations ranging in feeding habits from invertebrate-dominated to fish only. To test the notion that direct dietary analyses may discriminate against invertebrate components in pike population presumed piscivorous, mean diet-tissue shift estimates were compared among piscivorous and non-piscivorous trophic regimes. Mean diet-tissue shift in piscivorous and non-piscivorous pike populations were 2.3 and 2.5 respectively, and did not differ significantly (*t*-test, $df = 7$, $p = 0.365$).

The average diet-tissue shift estimate of 2.4‰ in northern pike differed significantly from the typical mean diet-tissue shift estimate of 3.4‰ in other consumers (*t*-test, $df = 29$, $p < 0.0005$).

2.4 Discussion

2.4.1 $\delta^{15}N$ trophic enrichment

This represents the first study to investigate $\delta^{15}N$ diet-tissue shift in northern pike. I combined field observations with a literature review to show that $\delta^{15}N$ diet-tissue shift averages 2.4‰ (0.48‰, conservatively) in northern pike. In this and other large-bodied fish species in which the diet history cannot be controlled experimentally, field studies comparing dietary and consumer isotopic signatures may be the only feasible method to obtain isotopic diet-tissue shift estimates. Consequently the precision of quantitative dietary data is the limiting factor to the accuracy of diet-tissue shift estimates, especially in highly omnivorous consumers. As expected, precision in diet-tissue shift estimates herein closely reflected confidence levels in dietary data and was highest among observations relying on system-specific quantitative dietary data. Precision was lowest among observations in which the net dietary signature had to be extrapolated according to the general feeding ecology of pike (Scott and Crossman 1973, Diana 1979, Chapman and Mackay 1990). Notwithstanding increased variance in the data, this generalized dietary break-down did not affect overall mean diet-tissue shift, relative to high confidence field and literature observations. The close agreement among average diet-tissue shift estimates drawn from both high and low confidence analyses supports the overall accuracy of the assessment presented herein and suggests widespread applicability of this diet-tissue shift estimate for northern pike.

It was speculated that in quantitative stomach contents analyses, discrimination against small-bodied taxa due to size-dependent digestion rates may lead to overemphasis

of fish prey in trophic position estimates using direct dietary in highly omnivorous consumers. Due to the potential overemphasis of fish prey in stomachs, it was hypothesized that this phenomenon may reveal itself in significantly higher N-isotopic diet-tissue shift estimates in predominantly non-piscivorous relative to piscivorous pike populations. A comparison between the two trophic regimes assembled from field data and highest confidence level literature data showed no statistical significance in mean diet-tissue shift between the two trophic regimes. Consistency in diet-tissue shift estimates across trophic regimes confirms that effects of prey size dependent digestion rates are not strong enough to introduce significant bias into dietary quantifications in northern pike.

2.4.2 Estimating trophic position with anomalous $\delta^{15}N$ enrichment

As hypothesised, $\delta^{15}N$ trophic enrichment in northern pike deviates significantly from the literature-accepted mean of 3.4‰, frequently used in estimating trophic position of teleost fishes and other consumers (Minagawa and Wada 1984, Post 2002). Estimates similar to that of pike come from different climatic regimes (Vanderklift and Ponsard 2003, Hoeinghaus et al. 2008) or taxonomic groups (Hussey et al. 2010). Although these have been applied in trophic position calculations (Jardine et al. 2006, Drymon et al. 2012), the proper integration of variable enrichment estimates in trophic position calculations has received little attention, especially concerning apex consumers. Standard trophic position models assume constant ^{15}N enrichment along the food chain (Figure 2.1A):

$$TP_{consumer} = \frac{\delta^{15}N_{consumer} - \delta^{15}N_{baseline}}{\Delta^{15}N} + \lambda \qquad (2)$$

where $\Delta^{15}N$ denotes the $\delta^{15}N$ diet-tissue shift and λ is the defined trophic position of the baseline. However, variability in the a priori estimate of $\Delta^{15}N$, often assumed based on seminal literature or an estimate from the respective prey-consumer link, may introduce significant error to trophic position estimates (Vander Zanden and Rasmussen 2001). For example, a reduced diet-tissue shift in the apex prey-consumer link causes an underestimation of the trophic position of the apex consumer (Figure 2.1B). Similarly, assuming the anomalous diet tissue shift as representative of the entire food chain may in error imply additional prey-consumer links at lower trophic levels (Figure 2.1C).

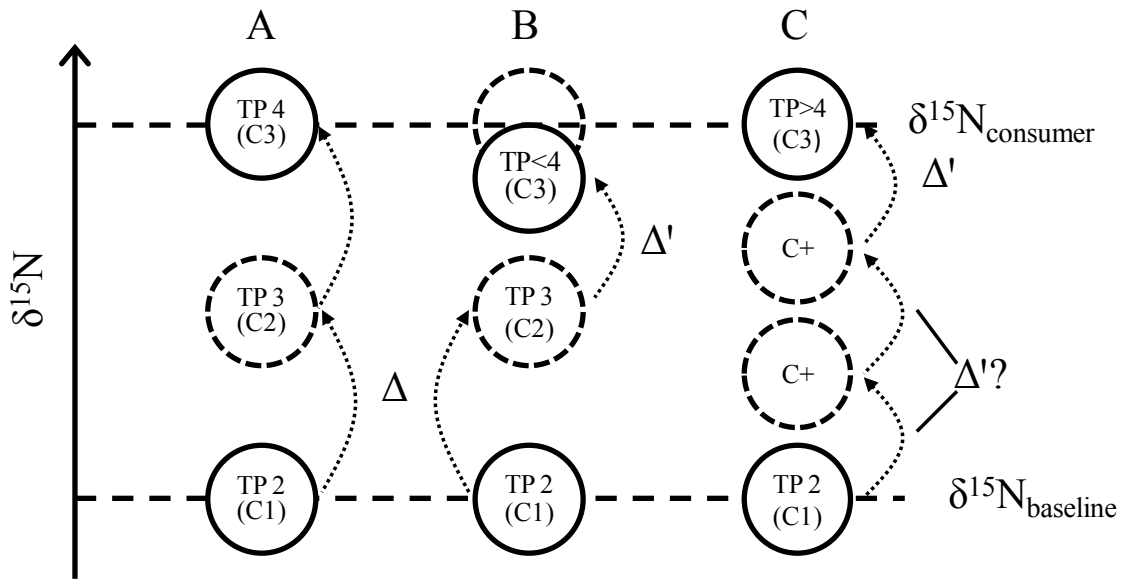


Figure 2.1: Estimating trophic position (TP) from $\delta^{15}N$ data. The difference between consumer and baseline (C1, defined as TP 2) $\delta^{15}N$ is divided by incremental diet-tissue shifts. Dashed circles represent implied consumer levels. A) In the conventional case, known constant diet-tissue shifts (Δ) return three evenly spaced consumer levels. B) The diet-tissue shift Δ' between the consumer C3 and its diet C2 is less than Δ. The conventional trophic position model assigns C3 a TP<4 in error, if Δ' is not known and accounted for (e.g. northern pike in Vander Zanden (1997)). C) A priori, the diet-tissue shift Δ' is based on known relationship between C3 and its diet. If this link exhibits anomalous enrichment Δ', applying this estimate to the entire food web may erroneously imply fewer or additional trophic levels between C1 and C3; in this example, inflating intermediate consumer levels (C+) places C3 at TP>4.

This problem can be overcome by separating anomalous consumers from lower trophic levels in the trophic position calculation. Here it is assumed that 1 trophic level between the anomalous prey-consumer link corresponds to diet-tissue shift $\Delta^{15}N'$ (Vander Zanden and Rasmussen 2001), and thus the trophic position (TP) of the anomalous consumer can be expressed as:

$$TP_{consumer} = \frac{(\delta^{15}N_{consumer} - \Delta^{15}N' - \delta^{15}N_{baseline})}{\Delta^{15}N} + \lambda + 1 \qquad (3)$$

where $\Delta^{15}N$ denotes normal enrichment in trophic links between the anomalous link and baseline and differs from $\Delta^{15}N'$.

Finally, to test the notion that the trophic position of northern pike was previously underestimated by stable isotope methods in broadly applying $\Delta^{15}N = 3.4‰$, I recalculated the trophic position of northern pike. Using the information in Appendix III from Vander Zanden et al. (1997) in equation (3) and letting $\Delta^{15}N' = 2.4‰$ as described herein, hence yields an average TP of 4.2. This estimate is very consistent with the average TP estimated from dietary analysis of 4.24 (Vander Zanden et al. 1997).

Variation in $\Delta^{15}N$ across taxonomic groups is no longer a trivial matter and thus poses a significant complication to the study of ecosystems using quantitative isotope approaches. Several reviews have attempted to distinguish patterns in $\Delta^{15}N$ and increase its predictability for ecological studies based on trophic guild , environment and characteristics of N-metabolism (McCutchan et al. 2003, Vanderklift and Ponsard 2003), however, agreement among these remains poor (Jardine et al. 2006). Variation in $\Delta^{15}N$ has been ascribed to factors such as tissue type (Schmidt et al. 2004), growth stage of the

animal (Tibbets et al. 2008), nutritional quality (Robbins et al. 2005) and nutritional state (Gaye-Siessegger et al. 2007). Unequivocally, variation in pathways of amino acid metabolism is the primary driver here, however, the details remain to be investigated (Martinez Del Rio and Wolf 2005, Wolf et al. 2009).

2.4.3 Potential biochemical factors affecting $\delta^{15}N$ enrichment

All enzyme-mediated pathways involving transfers of amino groups should select for isotopically light precursors going towards product (Figure 2.2). Pathways involving large molecules (dietary assimilation, translation, proteolysis) (Figure 2.2) are generally not expected to affect isotopic discrimination. Deamination of amino acids from the catabolic pool is known to result in isotopically light ammonium (NH_4^-), which is excreted as ammonia in fish. Gaye-Siessegger et al. (2004, 2013) found that enzyme activities within these pathways poorly predict overall $\Delta^{15}N$ in the study organism (Nile tilapia), suggesting that these pathways are not sole drivers of ^{15}N fractionation. Other pathways may also play a role and mask the expected effects.

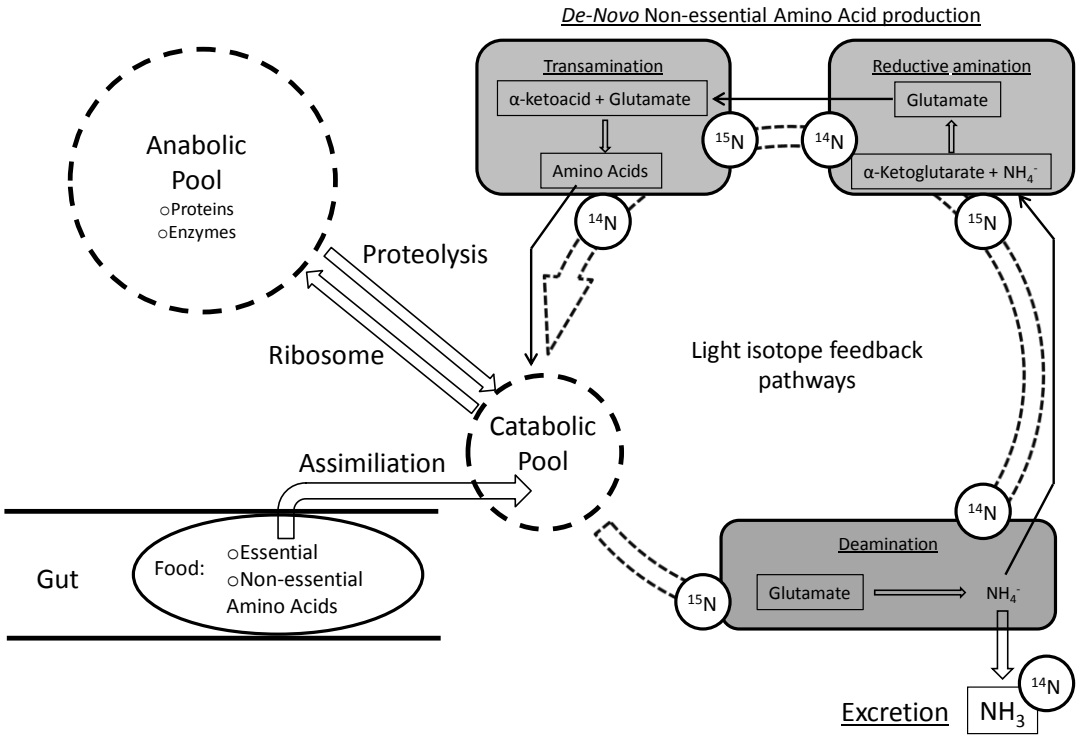


Figure 2.2: Isotope-selective pathways of amino acid metabolism. The broken-line arrow indicates pathways potentially returning light isotope (^{14}N) to the catalytic amino acid pool and circles (^{15}N, ^{14}N) indicate isotopic shifts from substrates to products corresponding to each reaction. Amino-acid turnover (transamination) and ammonia-recycling (reductive amination) are pathways antagonizing ^{15}N enrichment of the anabolic pool, ultimately.

Transamination pathways produce complex non-essential amino acids *de novo* from simpler precursors, such as glutamate. Resultant amino acids are also isotopically lighter than the substrate pool (Figure 2.2) (Macko et al. 1986) and affect the anabolic pool accordingly. Recycling of ammonia is known to occur in fish (Randall and Wright 1987). Isotopically light NH_4^- generated by other pathways can be incorporated into α-ketoacids to form glutamate and other amino acids in reductive amination (Figure 2.2). The pathway involves N-species of low molecular weight and thus products should again

be enriched in ^{14}N relative to the substrate pool. Ammonia-recycling as a pathway hypothetically antagonizing ^{15}N enrichment in animals has, to the best of my knowledge, not been discussed.

2.5 Conclusion

In conclusion, the analysis herein shows that $\Delta^{15}N$ for northern pike is 2.4‰ and thus differs from the conventionally employed estimate of 3.4‰. Close agreement among averages for different trophic regimes confirmed the validity of field-based data. It could be confirmed that stable isotope methods used previously, underestimated the trophic position of northern pike. Using an adapted model incorporating abnormal $\Delta^{15}N$, stable isotope estimates of trophic position were brought into close agreement with direct dietary analyses.

Awareness of variation in $\Delta^{15}N$ in many organisms is increasing. Ecologists should consider such variation as a source of error in interpretations of stable isotope data. Closing the knowledge gap of the role of metabolic factors in determining isotopic fractionation will aid in predicting anomalies a priori and thus reduce the potential for error in ecological studies.

Table 2.1: Stomach contents analysis and average consumer and prey $\delta^{15}N$ signatures in five populations of northern pike (NRPK) in Southern Alberta. Stomach contents were quantified both in bulk, which included all undigested and clearly identifiable stomach samples, and percent frequency of occurence (%FO), which also summarizes digested contents and empty stomachs. $\%FO_{100\%}$ indicates the proportion of pike in the sample which had consumed only one prey taxon.

System	Stomach contents analysis			$\delta^{15}N$		
	Taxon	% bulk weight	%FO ($\%FO_{100\%}$)	NRPK	Weighted mean prey (prey range)	$\Delta^{15}N$
Hays-Grantham (n=21; 11 bulk quantified)	Gammarus	62	42.9 (28.6)	13.41	11.08 (Odonata 9.92 – brook stickleback 13.28)	2.3
	Leeches	17	9.5 (4.8)			
	Odonata	<1	9.5 (4.8)			
	Dytiscidae	2	4.8 (4.8)			
	Brook stickleback	18	19.0 (4.8)			
	Fathead minnow	1.2	4.8 (0)			
	Unidentified fish	–	14.3 (4.8)			
	nil	–	23.8			
Twin Valley reservoir (n=32*; 4 bulk quantified)	Gammarus	71.6	65.6 (28.1)	15.14	12.54 (Gammarus 12.25 – leeches 13.29)	2.6
	Leeches	28.4	6.3 (3.1)			
	Chironomidae	<1	37.5 (3.1)			
	Corixidae	–	6.3 (3.1)			
	Dytiscidae	–	3.1 (0)			
	Limnephilidae	–	3.1 (0)			
	nil	–	21.9			
Keho lake (n=22; 6 bulk quantified)	Lake whitefish	100	27.3 (27.3)	13.33	10.68	2.7
	Unidentified fish*	–	13.6 (13.6)			
	nil	–	59.1			
Lake Newell (n=26; 6 bulk quantified)	Lake whitefish	91	15.4 (15.4)	14.19	12.44 (Yellow perch 11.75 – lake whitefish 12.52)	1.7
	Yellow perch	9	15.4 (15.4)			
	Northern pike*	–	7.7 (7.7)			
	Unidentified fish*	–	3.8 (3.8)			
	nil	–	61.5			
Chin Coulee (n=16; 3 bulk quantified)	Lake whitefish	20	6.3 (6.3)	14.13	11.80 (O.virilis 9.01 – longnose sucker 12.51)	2.3
	Longnose sucker	68	6.3 (6.3)			
	Walleye	2	6.3 (0)			
	O. virilis	10	6.3 (0)			
	Unidentified fish	–	6.3 (6.3)			
	nil	–	75.0			

Table 2.2: Diet-tissue shift estimates in northern pike based literature review. References were ranked based on caveats affecting confidence in the obtained diet-tissue shift result, as indicated (SC: stomach contents).

Reference	Location	System	$\delta^{15}N$ (‰)			Caveat (rank)
			Northern pike	Average diet	$\Delta^{15}N$	
(Vander Zanden et al. 1997)	Quebec	Varied (meta-analysis)	-	-	2.8[1]	none (3)
(Beaudoin et al. 1999, 2001)	Boreal lakes	SPH200	11.4[l]	9.0	2.4	none (3)
			9.2[s]	6.4	2.8	
		LLB20	12.4	9.9	2.5	
		SPH20	17.1	14.7	2.4	
(Venturelli and Tonn 2006)	North-central Alberta	Piche Lake	12.9	10.7	2.2	none (3)
		EXP	10.7	8.0	2.7	
(Jones and Waldron 2003)	England	Stradsett Hall	15.7	13.9	1.8	SC data in "%N" (2)
		Gunthorpe Hall	15.7	13.3	2.4	
		Bayfield Hall	16.7	14.4[2]	2.3	
(Brinkmann and Rasmussen 2010)	Southern Alberta	Twin Valley Res.	13.9	10.7	3.2	SC discussed (2)
(Kidd et al. 1999)[3]	ELA	Lake 110	10.4	7.8	2.6	omit SC data (1)
		Lake 227	9.6	7.0	2.6	
(Kidd et al. 1995, 1998)	Yukon subarctic	Fox	8.2	6.8	1.4	omit SC data (1)
		Laberge	9.9	7.5	2.4	
(Hesslein et al.1989)		Travaillant lake	11.3	8.0	3.3	omit SC data (1)
(Gorski et al. 2003)	Isle Royale	Richie	10.3	8.5	1.8[4]	omit SC data (1)
		Sargent	10.0	8.6	1.6[4]	
(Newbrey et al. 2012)	Central Alberta	Lake Wabamun	14.1	11.4	2.7	omit SC data (1)

[1]Estimate derived from trophic position estimates reported in reference. This reference was the first to report a discrepancy between SC and stable isotope estimates of TP in pike using diet-tissue shift 3.4‰

[l,s] Estimates for small and large pike as reported in the reference

[2]*Asellus* factored into net diet signature

[3]Only 1994 data used from this reference, underlying assumption that food webs found new equilibrium.

[4]Distance between averages for pike and perch. A more conservative estimate between the upper S.D. of pike and the lower S.D. of perch gives a diet-tissue shift estimate of 2.6 for both lakes in this publication.

Chapter 3

Effects of introduced lake whitefish (*Coregonus clupeaformis*) on the diet and growth of northern pike (*Esox lucius*) in prairie irrigation reservoirs.

Abstract

This study investigated the importance of lake whitefish as a resource to northern pike by comparing the trophic ecology and growth of pike in response to the presence or absence of whitefish in the prey community of seven reservoirs in the upper South Saskatchewan River Basin (SSRB). When lake whitefish were present, pike increased significantly in trophic position ($\delta^{15}N$) throughout ontogeny, suggesting gradual diet shifting towards larger, higher trophic position prey organisms. In contrast, population-level diet shifts were generally not observed in non-lake whitefish systems. Stable isotope mixing models ($\delta^{15}N$, $\delta^{13}C$) in combination with stomach contents analysis revealed that fish prey contributed 80.1 to 93.8% to the diets of small and large northern pike, respectively, in the presence of lake whitefish. Although alternative forage fish species were present in non-lake whitefish systems, fish prey played a lesser role in pike diets, which consisted predominantly (78.6%) of amphipods and other invertebrates. Growth rates (length-at-age) of non-piscivorous pike showed evidence of slower growth, whereas pike grew rapidly in systems with lake whitefish dominated prey base. Potential aspects of bioenergetics affecting growth efficiency in piscivorous and non-piscivorous trophic regimes are discussed.

3.1 Introduction

The potential for productive fisheries is one of the key socio-economic benefits of reservoirs. Fisheries productivity depends significantly on properly developed resource pathways between the base of the food web and desired target fish species. This, however, requires the presence of trophic coupler species typical for lentic systems, to form a suitable resource base to higher consumers. Unaided by specific fish introductions, fish assemblages in reservoirs develop from lotic parent communities in impounded stream systems (Fernando and Holcik 1982). Variability in the persistence of fish species is expected, based on their adaptability to newly-created lentic habitat, which may vary considerably in quality due to hydrological engineering requirements specific to individual reservoirs (DiCenzo and Duval 2002) and other factors. Thus the river-reservoir transition may be a stressor resulting in significant restructuring of fish communities and food webs (Wootton et al. 1996). Resource pathways in reservoirs may shift significantly from benthic production and allochtonous inputs in the river towards pelagic production during the river-reservoir transition, and successful exploitation of this pool via zooplankton and zooplanktivorous fish may be an important contributor to fish production at higher trophic levels. The zooplanktivorous functional group, however, may not be present in the riverine parent fish community, especially in lower-order stream systems (Miranda et al. 2008), in which zooplanktivory is not a significant niche, and which feature no lake communities in the watershed upstream. Consequently, zooplanktivores may not establish naturally in some reservoirs and the trophic link between the bulk of reservoir production and higher trophic levels may thus be absent, impairing energy flow to higher consumer levels. In one example to highlight this, it has

been suggested that absence of suitable prey fish species in a newly created reservoir caused unusual reliance on benthic invertebrates in northern pike (Brinkmann and Rasmussen 2010), a species normally expected to exploit fish prey. Reliance on invertebrate prey was accompanied by significant growth impairment in older age classes of pike, consistent with other studies on similarly simplified systems (Venturelli and Tonn 2006). Brinkmann and Rasmussen (2010) suggested that invertivory among pike may be linked to the absence of lake whitefish, which are an important trophic coupler, and occur in other reservoirs in the upper SSRB. However, our understanding of the general feeding ecology of pike in artificial lakes is still fragmentary, and the importance of lake whitefish as a resource to northern pike potentially affecting its growth remains to be shown.

The successful establishment and sustainability of reservoir fisheries may hinge on growth rates of target fish species. Especially in apex consumer species, which are often the most important target species in recreational fisheries, growth rates are highly dependent on efficient energy flow through the food web (Pazzia et al. 2002). Large-bodied apex predators undergo diet shifts towards larger-bodied prey organisms during ontogeny, in order to maximize bioenergetic net benefit during feeding, and thus somatic growth (Mittelbach and Persson 1998, Sherwood et al. 2002a). Diet shifts, however, cannot be realized in the absence of key prey size-classes in disturbed ecosystems, and this has been shown to cause bioenergetic bottlenecks leading to stunting in fish populations (Pazzia et al. 2002, Sherwood et al. 2002a). Therefore, the prey species assemblage may be one of the most critical factors influencing growth of apex predators in reservoirs.

The northern pike is the most common game fish in lakes and reservoirs of the western prairies. It is considered an apex predator, however, in that role, it can assume diverse feeding strategies, consuming a broad prey spectrum within its gape limitation (Hart and Hamrin 1988, Chapman et al. 1989, Craig 2008). Prey size selection is expected to scale with body size during ontogeny, which results in a shift from invertebrate to fish prey early in life (Chapman et al. 1989, Sherwood et al. 2002a). Examples of northern pike maintaining an invertebrate-dominated diet are much less common (Chapman and Mackay 1990, Venturelli and Tonn 2006, Paradis et al. 2008) and are associated with impairment of the forage base (Venturelli and Tonn 2006, Brinkmann and Rasmussen 2010). These pike will maintain a low trophic position throughout their lives, as a consequence of continuous reliance on invertebrate prey.

Shifts in the reliance on fish versus invertebrates should be reflected by a positive correlation between body size of pike and their trophic position (Paradis et al. 2008), since prey fish typically occupy a higher consumer level than invertebrate prey. Positive relationships between fish size and trophic position may thus be an attribute differentiating long-term piscivorous and non-piscivorous feeding strategies of northern pike in reservoirs.

This study investigates prey community effects on the trophodynamics and growth in northern pike in seven reservoirs in the upper South Saskatchewan River Basin. The specific objectives of this study are:

1) to analyze and compare the diet of northern pike among prey communities dominated by lake whitefish relative to non-lake whitefish communities. This

objective will be met using measurements of trophic position ($\delta^{15}N$) and dietary composition analysis using information from stable isotope mixing models and stomach contents analysis.

2) to compare growth rates in northern pike among lake whitefish and non-lake whitefish prey community classes. Here, von Bertalanffy growth models will be fitted to length-at-age measurements back-calculated on bone-structures of northern pike.

3.2 Methods

3.2.1 Sites, community surveys and sampling

The study took place on seven reservoirs that are part of four irrigation districts located in the Upper South Saskatchewan River Basin, Alberta, Canada in 2009-2011. In the semi-arid upper South Saskatchewan River Basin the demand for water by the agricultural industry and municipalities is met by storage of spring runoff in numerous reservoirs, interconnected by canal systems. On-stream reservoirs regulate rivers and water diversion from rivers supplies numerous off-stream reservoirs to create additional storage. Fish assemblages in these lakes include species typical for river systems in this region. Northern pike is the apex predator in most lakes and potential prey species commonly include cyprinids and catostomids, and less commonly, yellow perch and brook stickleback. Lake whitefish were stocked into larger reservoirs in the Eastern, Bow River and Lethbridge Northern irrigation districts to support commercial fisheries for this species, which subsequently invaded other lakes through the interconnecting canal system. However, barriers to movement, such as water control structures and physical

constraints (English 1977), may prevent establishment of lake whitefish in some reservoirs.

With the exception of Twin Valley reservoir lake whitefish were assumed to have access to all reservoirs through the canal system in the study area. Thus reservoirs were individually surveyed and categorized into lake whitefish communities (LKWH+) and non-lake whitefish communities (LKWH-) based on long-term persistence of this species, as established by three criteria: 1) stock densities similar to well established lake whitefish reservoirs (e.g. Keho and Newell, as gathered from FWMIS, Alberta Environment and Sustainable Resource Development), 2) contiguous size distribution from juvenile to adult, suggesting a recruiting population, 3) stable isotope signatures consistent with system-specific baselines suggesting long-term site-fidelity.

Fish communities in study reservoirs were surveyed with the intention to qualitatively establish species-presence, or in connection with periodic population index surveys (Fall Walleye Index Netting, FWIN) on managed reservoirs by the department of Fish and Wildlife. Northern pike and other fish species were collected using multi-mesh gill nets (panel height: 2.4m, stretch mesh size: 25+89mm by 24m each, 25+102mm by 24m each, 76+114+152mm by 30m each), by angling and by beach-seining. Gill nets were set with small mesh sizes starting in macrophyte beds and increasing mesh sizes extended into open water, perpendicular to the edge of the macrophyte bed. Soak times were 20-24hours.

Fish caught were enumerated and relative species abundance was reported as percentage to total fish caught. Fish samples were also collected through netting efforts

coordinated with Alberta Sustainable Resource Development Fish and Wildlife division during annual FWIN surveys in 2009. Northern pike samples consisted of at least 18 individuals. Additional fish samples were collected using a beach seine, mainly when stomach contents of pike suggested reliance on fish prey too small for capture in gill nets.

Benthic invertebrates were collected from 4-6 different sites per lake using an Ekman grab, plankton net, and dip net during the summer months (June- September). Samples were initially cooled in the field and finally frozen for later processing. Benthic invertebrates were identified and separated to species (in amphipods) or family level (insects and hirudinea). Fish were measured for basic biological data (fork/total length and weight) and dissected to collect samples of dorsal muscle tissue, ageing structures (scales or bones), and stomach contents. Fish and invertebrate tissue samples were dried to constant mass at 60°C and ground to fine powder for storage and further analysis. Fish bones were boiled briefly to remove all flesh, and air dried for storage.

Stomach samples of northern pike were separated into invertebrate families, amphipods and fish into species. Quantitative analysis was done on well-preserved and identifiable stomach contents; samples in advanced stage of digestion were recorded only as frequency of occurrence (FO) data. All identified prey items were bulk-quantified, however, individual prey species had to occur more than once in samples to be considered of potential importance at the population level. Whole fish prey items were measured for length and weight, digested fish prey were approximated for length and weights were calculated from length-to-weight regressions determined from regular fish collections. Stomach contents were quantified as % of pooled bulk weight for each lake.

Table 3.1: Reservoir characteristics and community survey results in study systems.

Reservoir	Surface area (ha)	Maximum depth (m)	Mean depth (m)	Fish community	% rel. abundance in survey	Prey community classification	Northern pike sample size (n)
Keho*	2347	7.5	4.1	Lake whitefish	64.5	LKWH+	27
				Northern pike	21.5		22 (+25 angling)
				Spottail shiner	2.1		3
				White sucker	0.5		0
				Other	11.4		0
Newell*	6307	16.8	6.4	Lake whitefish	55.1	LKWH+	14
				Northern pike	6.5		27
				Yellow perch	5.3		3
				Spottail shiner	0.5		0
				Other	32.6		0
Chin*	1569	21	12.8	Lake whitefish	65.4	LKWH+	38
				Northern pike	1.8		18
				Longnose sucker	2.7		9
				White sucker	4.4		0
				Spottail shiner	0.9		2
				Other	24.8		0
CPR	149	n/d	n/d	Northern pike	66.7	LKWH–	28
				White sucker	16.7		7
				Lake whitefish	11.9		5
				Spottail shiner	4.8		2
Hays-Grantham	429	4.5	1.5	Northern pike	94.7	LKWH–	18
				Yellow perch	5.3		1
				Seine hauls:			
				Brook stickleback	75		30
				Fathead minnow	15		6
				Northern pike (yoy)	10		4
Twin Valley	835	21	3.6	Northern pike	58.5	LKWH–	24 (+8 angling)
				White sucker	41.5		17
Scope	809	12.8	2.1	Northern pike	52.2	LKWH–	24
				Yellow perch	28.3		13
				White sucker	10.9		5
				Spottail shiner	4.3		2
				Lake whitefish	4.3		2

* relative abundance data based on FWIN catches. Sample sizes (n) reflect sub-samples collected from FWIN survey catches.

3.2.2 Stable isotope and trophic analysis

Fish and invertebrate tissue samples were analyzed for stable isotope ratios on a Thermo Finnigan Delta V Advantage isotope ratio mass spectrometer coupled to a Costech 4010 Elemental Analyzer. Quality was assured through interspersion of reference materials and duplicate samples. Deviations of less than 0.2 delta units between duplicates or referenced values were considered acceptable. $\delta^{13}C$ values were corrected for lipid content based on C:N elemental ratios, if those exceeded a ratio of 3.5 (Post et al. 2007).

3.2.3 Feeding ecology in northern pike

Dietary habits in northern pike were analyzed through observations of stomach contents and analysis of stable isotope ratios ($\delta^{15}N$, $\delta^{13}C$) in prey and consumer. Trophic position of northern pike was calculated for individuals using the model

$$TP_{pike} = \frac{\left(\delta^{15}N_{pike} - 2.4 - \delta^{15}N_{baseline}\right)}{3.4} + 3$$

which incorporates anomalous $\delta^{15}N$ fractionation known in pike (Chapter 2). Lake-specific baselines were defined as the average $\delta^{15}N$ signatures of Gastropods (*Physa*) and zooplankton in the >1mm (*Daphnia*) size fraction. Diet-shifting in northern pike was identified by regressing trophic position against fork length in each system. The role of potential diet shifts was considered in subsequent analyses of dietary contributions. In systems exhibiting significant trends of trophic position against fork length, isotope data were grouped into size classes <580mm and >580mm, to either side of the mean fork length of the combined pike sample in LKWH+ lakes. Quantitative estimates of dietary

contributions were determined using mixing models in the SIAR package in R (Parnell et al. 2010, R 2012). Models were run on each pike population and included 3 to 5 sources reflecting the known food spectrum evident from stomach analysis, and additional potential sources, not evident in stomach contents. Invertebrate sources were condensed by combining several taxa by trophic guild (Phillips et al. 2005); source Insects included Odonata, Hemiptera and Coleoptera, Amphipods included *Gammarus* and *Hyalella*, and Leeches included Glossiphoniidae, Hirudinidae and Erpobdellidae. Diet-tissue shift parameters were set to 2.4 ± 0.3‰ for $\delta^{15}N$ in northern pike (Chapter 2) and 0.9±1‰ for $\delta^{13}C$ (non-herbivore estimate) (Vander Zanden and Rasmussen 2001). Mixing models were initially run strictly on stable isotope data without prior information. Resulting source contributions were ranked highest to lowest and compared qualitatively with stomach contents data ranked the same way. If stomach data were in agreement with stable isotope mixing models or mixing models suffered from underdetermination, models were re-run including quantitative stomach proportions as prior information (Peterson 1999) to support decisions. For the purpose of inclusion as prior information, sources that were not represented in stomach samples were assigned minimum fractions of 1% and others were rounded down accordingly. If stable isotope mixing models were in disagreement with stomach observations, either in suggesting a significant contribution of sources not detected in stomach contents, or in rejecting the importance of a source indicated by stomachs observations, stable isotope results were given preference.

3.2.4 Growth in northern pike

Northern pike growth rates were determined by back-calculation of length-at-age using the cleithrum as ageing structure. Distances from focus to annulus were measured with a digital caliper on one cleithrum or both, if available, in which case the average of both measurements was used for further analysis. Von Bertalanffy growth models were fitted for each population using a least-squares method (Nelson 2012) in R.

3.2.5 Statistical analysis

Statistical computations were carried out in R version 2.15.2 (R 2012). Linear models were fitted to trophic position against body length data to test the hypothesis that pike shift their diet towards greater proportions of fish prey as they grow. Overall reliance on fish versus invertebrate prey among prey community classes were compared using an ANOVA model with prey community type as factor and percent fish in diet of pike as response. To account for variation in reliance on fish prey in populations exhibiting diet shifts, the response variable was grouped by size class (<580mm and >580mm) of pike and analyzed hierarchically (Nested ANOVA). Fractional data were logit-transformed prior to use in statistical tests.

Growth rates of pike were compared among prey community classes using the likelihood ratio test developed by Kimura (1980) on Von Bertalanffy growth functions. Confidence ellipses (95%) for a graphical representation of error of L_∞ and k parameters were calculated using package “ellipse” (Murdoch and Chow 2013) in R.

3.3 Results

3.3.1 Feeding ecology in northern pike

The feeding ecology of northern pike was assessed by collecting direct dietary information from stomach contents in connection with stable isotope data, which provided information about trophic position and was used in mixing models to obtain quantitative dietary contribution estimates of prey sources in each reservoir. The food spectrum of pike in LKWH+ lakes consisted mostly of fish prey, with lake whitefish the most common prey found (Table 3.2). Stomach contents rarely consisted of more than one species in this class of lakes. The frequency of empty pike stomachs was also high in LKWH+ systems, comprising 59 to 72% of samples. The food spectrum of pike in LKWH─ reservoirs included both invertebrate and system-specific fish prey (Table 3.2). Amphipods and leeches were found in a large proportion of stomachs and composed most of the bulk weight of stomach contents in LKWH─ reservoirs. Fish prey were encountered less frequently, and mostly accompanied by invertebrate prey. The proportion of empty stomachs ranged between 21 and 40% (Table 3.2).

$\delta^{15}N$ signatures of pike were used to quantify trophic position (TP) of pike in each reservoir. Trophic position estimates, regressed against pike body size (fork length) were compared among community class using an analysis of covariance model. Diet shifting of pike was evident in LKWH+ prey communities in which trophic position increased significantly with body size, but this did not occur in LKWH- communities; the interaction term of the ANCOVA was significant (Table 3.4A). The y-intercepts were not different among prey communities, at TP 3.5 (Table 3.4A). Overall the model ascribed

49.4% of the variation in trophic position of northern pike to differences among prey community class. The effect of pike fork length explained 16.3% of the variation. The system-to-length interaction explained 4.5% of the variation.

In order to resolve the effect of diet shifting in subsequent mixing-model analyses of dietary source contributions, the relationships between TP and fork length of pike were modelled individually for each lake to identify populations which exhibited diet shift trends. Such were detected in Keho, Newell, Chin (LKWH+) and Twin Valley (LKWH─) (statistics summarized in Table 3.2), but not in CPR, Scope and Hays-Grantham for the available size ranges of northern pike (Figure 3.1).

Accordingly, samples from reservoirs showing a positive effect for diet shifts were split into two size classes <580mm and >580mm, to accommodate for dietary variability between large and small pike. Results from the stable isotope mixing model combined with stomach contents analysis revealed that dietary sources of northern pike in LKWH+ lakes were dominated by lake whitefish, which on average comprised 56.1 ±20.9% (standard deviation) and 66.8 ±27.8% in size classes <580mm and >580mm by bulk weight, respectively (Figure 3.1). Other prey fish, including yellow perch and sucker, contributed on average 24% and 27% to each size class, respectively. Invertebrate sources (mainly amphipods) played a comparatively minor role in the diet of pike in these systems, contributing on average only 16.9% to the overall diet (Figure 3.1).

In contrast, invertebrates dominated the diets of pike in LKWH─ systems. Amphipods were the most important food source, contributing on average 55.7 ±6.8% to the diet, followed by other invertebrates, including leeches and insects (22.9 ±6.2%). Fish

prey constituted 19.7 ±13.4% of pike diets and varied in species composition among lakes (Table 3.2). To test whether non-piscivory was a significant attribute of pike in LKWH─ communities, the contributions of fish versus invertebrate prey were compared among community classes. The overall reliance on invertebrate food sources relative to prey fish by pike in LKWH─ systems differed significantly from LKWH+ systems (Table 3.4C). Moreover, an effect of diet shifting in LKWH+ communities was accounted for in this comparison by nesting pike size class within the prey community factor. Apart from the greater reliance on fish prey relative to pike in LKWH─ communities, large pike (>580mm in length) also exhibited a significant trend towards increasing reliance on fish prey, by comparison with smaller conspecifics in LKWH+ communities (Table 3.4C).

Several patterns relevant to the feeding ecology of pike in the study systems emerged from the dietary analysis *a posteriori*, and statistical analyses were performed to illustrate these. **1)** The contribution of fish prey in the diet of pike correlated significantly with the ratio of pike:prey fish in gill net sampling surveys (Figure 2A, Table 3.4C). The y-intercept of this relationship was negative and significantly different from 0. The calculated x-intercept was 29.8%. **2)** Similarly, the correlation between the magnitude of diet shift, expressed as the TP to body length regression slope, and the ratio of pike:prey fish was statistically significant (Figure 3.2B, Table 3.4D). **3)** Empty stomachs (ES) were frequently encountered in pike samples from LKWH+ systems. Accordingly, regressing the percentage ES against the degree of piscivory, as quantified by the percent contribution of fish in pike diets, returned a significant result (Figure 3.2C, Table 3.4E).

Table 3.2: Summary of stomach contents analysis and statistics of trophic position models in southern Alberta reservoirs. Stomach contents analysis identified prey organisms consumed by pike (food spectrum), the quantitative contribution of each taxon to the bulk weight of stomach contents and the frequency of occurrence (%FO) in the sample of pike from each reservoir. $\delta^{15}N$ stable isotope measurements were used to calculate the trophic position of pike in each reservoir. Trophic position (TP) information was used to identify diet shifting, as indicated by significant linear regression of TP against pike length (graphical presentation in Figure 1). Mean TP and range of pike are also shown for each reservoir.

Lake whitefish systems:

Reservoir	Community class	Stomach contents analysis			$\delta^{15}N$ Stable isotope analysis	
		Food spectrum	% bulk weight	%FO	Diet shift	Mean TP ±S.D. (Range)
Keho (n=22; 6 bulk quantified, +25 angling)	LKWH+	Lake whitefish Unidentified fish* Nil	100 – –	27.3 13.6 59.1	$TP = 9.27\text{x}10^{-4}*F\text{-}length + 3.19$ ($F_{[1,43]} = 169.1$, $p < 0.0001$, $r^2 = 0.79$)	3.8±0.24 (3.4-4.4)
Newell (n=26; 6 bulk quantified)	LKWH+	Lake whitefish Yellow perch Northern pike* Unidentified fish* Nil	91.0 9.0 – – –	15.4 15.4 7.7 3.8 61.5	$TP = 6.82\text{x}10^{-4}*F\text{-}length + 3.38$ ($F_{[1,25]} = 36.89$, $p < 0.0001$, $r^2 = 0.58$)	3.8±0.19 (3.3-4.1)
Chin (n=18; 4 bulk quantified)	LKWH+	Lake whitefish Longnose sucker Walleye O. virilis Unidentified fish nil	34.0 56.6 1.4 8.0 – –	12.5 6.3 6.3 6.3 6.3 72.2	$TP = 1.29\text{x}10^{-3}*F\text{-}length + 3.04$ ($F_{[1,13]} = 33.87$, $p < 0.0001$, $r^2 = 0.70$)	3.7±0.4 (3.1-4.2)

Table 3.2 continued:

Non-lake whitefish systems:

System	Community class	Stomach contents analysis			$\delta^{15}N$ Stable isotope analysis	
		Food spectrum	% bulk weight	%FO	Diet shift	Mean TP ±S.D. (Range)
CPR (n=28; 9 bulk quantified)	LKWH-	Gammarus	30	28.6	Not significant ($p = 0.78$)	3.7±0.32 (3.2-4.1)
		Leeches	42	53.6		
		Ephemeridae	3	7.1		
		Dytiscidae	1	3.6		
		Phryganeidae	<1	3.6		
		Spottail shiner	24	3.6		
		nil	–	35.7		
Hays-Grantham (n=21; 11 bulk quantified)	LKWH-	Gammarus	62	42.9	Not significant ($p = 0.71$)	3.5±0.13 (3.3-3.9) 3.4 in YoY
		Leeches	17	9.5		
		Odonata	<1	9.5		
		Dytiscidae	2	4.8		
		Brook stickleback	18	19.0		
		Fathead minnow	1.2	4.8		
		Unidentified fish	–	14.3		
		nil	–	23.8		
TVR (n=32*; 4 bulk quantified)	LKWH-	Gammarus	71.6	65.6	TP = $7.23\text{x}10^{-4}$*$F\text{-}length$ + 3.36 ($F_{[1,28]} = 25.75$, $p < 0.0001$, $r^2 = 0.46$)	3.7±0.16 (3.5-4.2)
		Leeches	28.4	6.3		
		Chironomidae	<1	37.5		
		Corixidae	–	6.3		
		Dytiscidae	–	3.1		
		Limnephilidae	–	3.1		
		nil	–	21.9		
Scope (n=23; 7 bulk quantified)	LKWH-	Gammarus	4	34.8	Not significant (p = 0.35)	3.5±0.08 (3.3-3.6)
		Leeches	3	26.1		
		Odonata	2	13.0		
		Lake whitefish	80	4.3		
		Yellow perch	3	8.7		
		White sucker	8	4.3		
		Unidentified fish	–	8.7		
		nil	–	39.1		

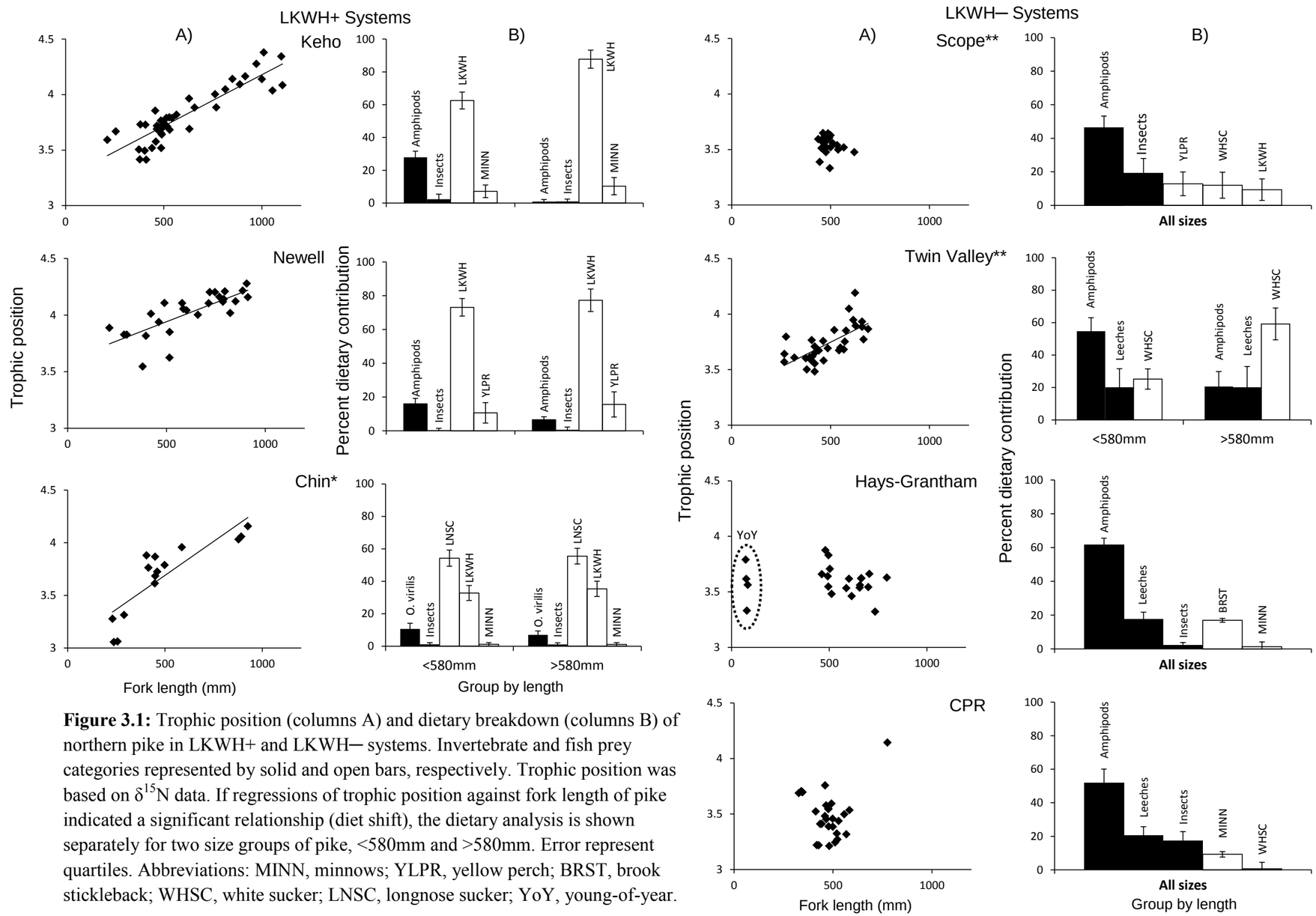


Figure 3.1: Trophic position (columns A) and dietary breakdown (columns B) of northern pike in LKWH+ and LKWH– systems. Invertebrate and fish prey categories represented by solid and open bars, respectively. Trophic position was based on $\delta^{15}N$ data. If regressions of trophic position against fork length of pike indicated a significant relationship (diet shift), the dietary analysis is shown separately for two size groups of pike, <580mm and >580mm. Error represent quartiles. Abbreviations: MINN, minnows; YLPR, yellow perch; BRST, brook stickleback; WHSC, white sucker; LNSC, longnose sucker; YoY, young-of-year.

* Underdetermined mixing model. Decision driven by stomach contents.
** Stomach contents not included as prior information.

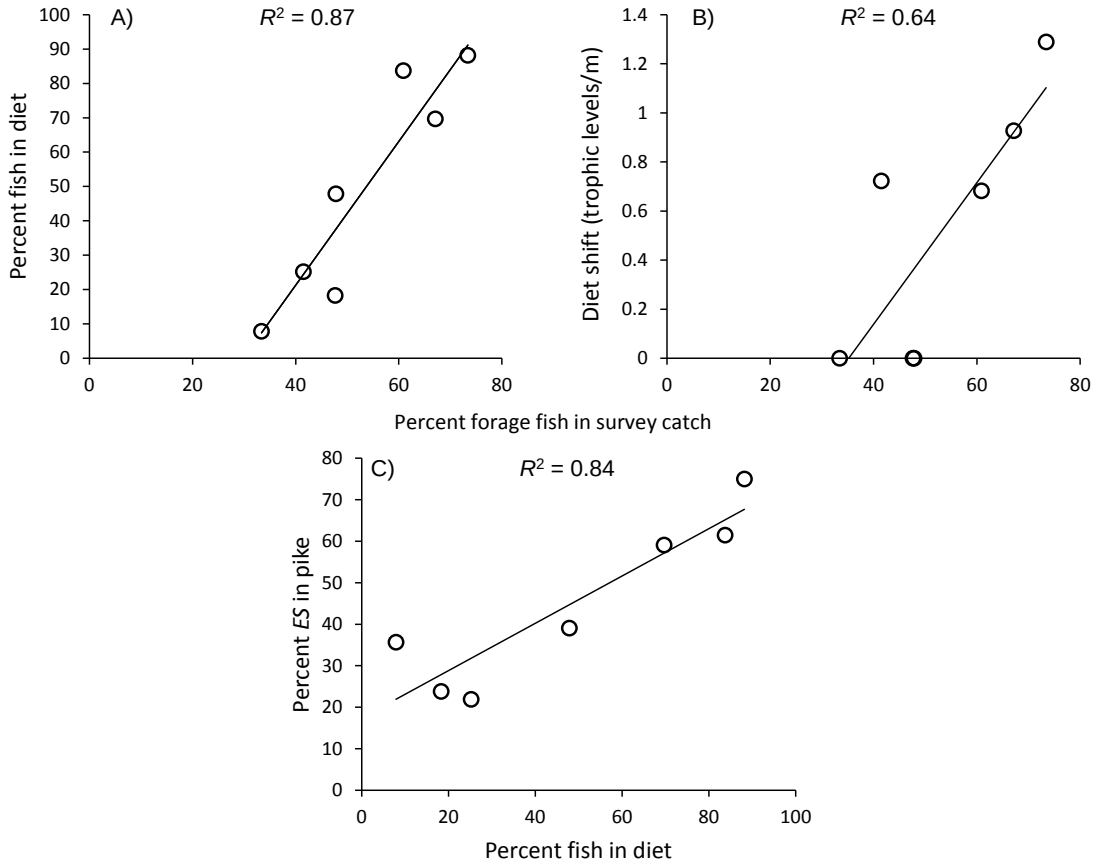


Figure 3.2: Relationships between pike : prey fish ratio in lake surveys and percent fish in the diet of pike (A), and the magnitude of the diet shift observed in pike (trophic levels per meter fork length, Figure 1)(B). C) Correlation between the proportion of fish in the diet and the proportion of empty stomachs (*ES*) in pike samples.

3.3.2 Growth rates of northern pike

Gill net surveys indicated differences in size structures of pike populations among prey community classes. Pike longer than 700mm were infrequent in LKWH─ lakes, whereas numerous pike exceeded 800mm in LKWH+ lakes. Similarly, the age structure was truncated in LKWH─ lakes, with few fish reaching 7+ years. Von Bertalanffy growth parameters (length at age) of northern pike (Figure 3.3, Table 3.3) differed between types of prey community (Table 3.4F). Overall, northern pike in LKWH─ communities reached lower asymptotic length (L_∞) earlier in life (curvature parameters k

Table 3.3: Summary of von Bertalanffy growth functions (VBGF) for northern pike. L_∞: asymptotic length; k: curvature coefficient; t_0: age at 0-length. Models were derived from back-calculated length-at-age series.

	VBGF parameters		
Reservoir	L_∞	k	t_0
Keho	1652	0.094	-0.217
Newell	1175	0.152	-0.270
Chin	1387	0.137	-0.143
Scope	570	0.685	0.238
Twin Valley	668	0.401	-0.152
Hays-Grantham	925	0.189	0.128
CPR	812	0.245	-0.250

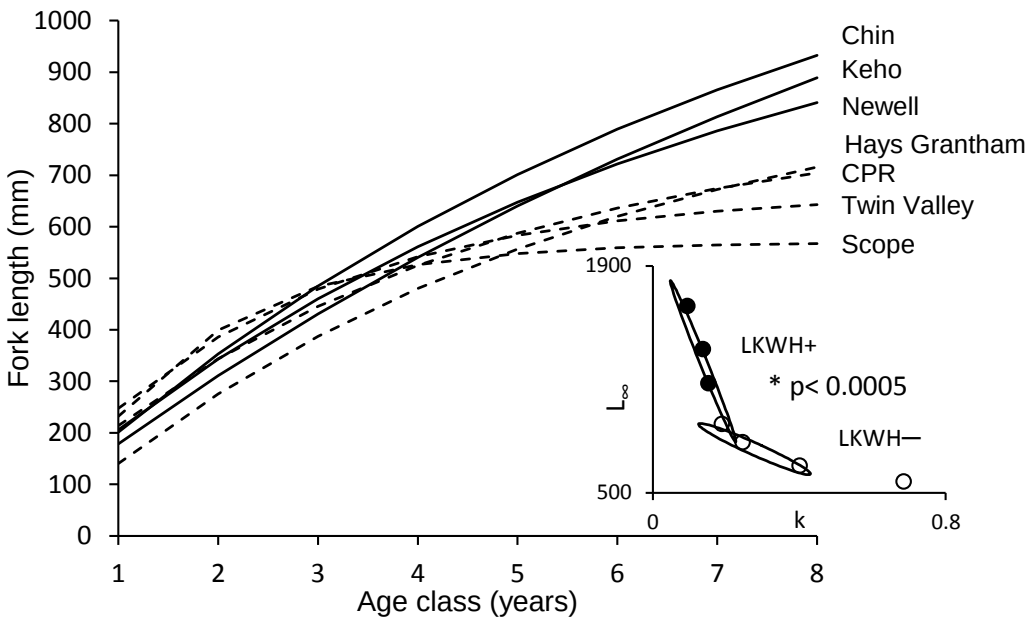


Figure 3.3: Von Bertalanffy growth curves of northern pike in seven Alberta reservoirs. Solid and dashed lines represent LKWH+ and LKWH- prey communities, respectively. Insert: Representation of error-overlap as 95% confidence ellipses for comparison (Likelihood ratio test) of L_∞ and k parameters between prey community classes (Table 3.4F).

were significantly higher) compared with conspecifics in LKWH+ prey communities (Table 3.4F). No significant difference was evident among estimated age at 0 length (t_0 parameters) for the investigated populations (Table 3.4F).

3.4 Discussion

3.4.1 Feeding ecology of northern pike in southern Alberta reservoirs

The objectives of this study were to analyze and compare the dietary composition of northern pike in reservoir food webs with differing prey communities and to establish a link between dietary composition and growth rates of pike. Seven reservoirs were categorized into two classes of prey community, based on the dominance of lake whitefish in the fish assemblage, for comparison.

Northern pike in reservoirs with strong lake whitefish populations exhibited significant correlations between trophic position and body length. The gradual trophic shift in pike indicates that, as they grow, they consume prey of higher trophic position, which is a reflection of scaling prey size and is consistent with a diet shift from invertebrates to lake whitefish and littoral prey fish of increasing size. Lake whitefish, which feed mostly on zooplankton as juveniles and become predominantly benthic feeders during ontogeny, are themselves expected to increase in trophic position as they grow. Thus the relationship between trophic position and body length in pike is evidence for the importance of fish prey to pike in these reservoirs.

Table 3.4: Comparisons of the trophic ecology and growth of northern pike in reservoirs of the upper SSRB. Summary of statistical results.

Comparison	Test	Statistics	Model	Figure
		Trophic ecology of northern pike		
A) Diet shift in response to prey community class	ANCOVA	Effect: Prey community class $F_{(1,187)} = 105.55$; $p < 0.0001$ Effect: Fork length $F_{(1,187)} = 60.38$; $p < 0.0001$ Interaction $F_{(1,187)} = 16.53$; $p < 0.0001$	*LKWH+* TP = 0.00106 • length + 3.49 *LKWH-* TP = 0 • length + 3.49 $R^2 = 0.49$	1) Col. A)
B) Dietary contribution of fish prey relative to invertebrate prey among community classes	Nested ANOVA	Effect: Prey community class $F_{(1,7)} = 57.88$, $p = 0.0001$ Effect: Pike size class nested within community class $F_{(1,7)} = 5.96$, $p = 0.045$	-	1) Col. B)
C) Correlation between pike:prey fish ratio and piscivory	Linear regression	$F_{(1,5)} = 33.04$, $p = 0.0022$	*%fish in diet* = 12.12 • (*% prey fish in survey*) – 6.24 $R^2 = 0.87$	2A)
D) Correlation between pike:prey fish ratio and magnitude of diet shift	Linear regression	$F_{(1,5)} = 8.86$, $p = 0.031$	$R^2 = 0.64$	2B)
E) Correlation between piscivory and FES in pike samples	Linear regression	$F_{(1,5)} = 15.19$, $p = 0.011$	*% ES* = 0.5701*(*% fish in diet*) + 17.401 $R^2 = 0.84$	2C)

Table 3.4 continued:

Comparison	Test	Statistics				Model	Figure
		Growth rates in Northern Pike					
		H_0	*df*	χ^2	*P*		
F) Growth rates of pike among prey community classes	Likelihood ratio test (Kimura 1980)	*LKWH+ = LKWH–:*				LKWH+:	
		$L_\infty^+ = L_\infty^-$	1	10.66	0.001	$L_t = 1311 * \left(1 - e^{-0.137(t+0.169)}\right)$	3)
		$k^+ = k^-$	1	5.06	0.024		
		$t_0^+ = t_0^-$	1	0.12	0.729	LKWH–:	
		$L_\infty^+ = L_\infty^-, k^+ = k^-, t_0^+ = t_0^-$	3	47.46	<0.0005	$L_t = 770 * \left(1 - e^{-0.278(t+0.075)}\right)$	

As expected, stable isotope mixing models pointed to fish-dominated diets in pike, with relatively small invertebrate components mainly in small pike. Although it was expected that lake whitefish played a dominant role in the diet of pike where it was abundant, its contribution to the diet of pike varied considerably and did not differ significantly from the contribution of other fish species. Lake whitefish were confirmed as the predominant fish prey consumed in LKWH+ reservoirs by frequency of occurrence. The bulk weight of stomach contents, however, was not necessarily dominated by lake whitefish as other large-bodied prey fish, such as sucker species and yellow perch also contributed to the diet of pike. The fact that lake whitefish were consumed most frequently may be ascribed to a high relative abundance of this species in the fish community as indicated by surveys.

The presence of a suitable prey fish species of continuous size spectrum, which facilitates fast growth in piscivores, may lead to an overall generalization of the diet towards any large-bodied prey fish species encountered, in order to meet bioenergetic demands. In this case, as faster growth demands larger and larger prey, pike may exploit fish prey opportunistically and may discriminate prey by size rather than identity (Hart and Hamrin 1988, Sammons et al. 1994), and may explain that other prey fish besides the dominant species were consumed by pike in these reservoirs. Overall, the interpretation is supported that pike in LKWH+ reservoirs exhibit a piscivorous feeding ecology typical for this species. In LKWH– communities overall, trophic position of northern pike did not increase with body length. Dietary analysis identified invertebrates as the most important resource to pike. Only the Twin Valley population exhibited trends in trophic position similar to populations in LKWH+ reservoirs, with increasing reliance on fish

prey in larger pike; however still, the mixing-model-predicted fish component in their diet (60%) was lower than that of pike diets in LKWH+ reservoirs (>85%, conservatively), while the large dietary component of benthic invertebrates in small Twin Valley pike compared well with that of other LKWH– populations. The identity of fish prey potentially exploited by pike in Twin Valley was presumed to be white sucker, which appeared to be abundant based on catches in gill nets. However, no fish prey was found in stomach contents to confirm predation by pike. Cannibalism on young conspecifics may be an alternative, however less likely, dietary source to larger pike (Mauck and Coble 1971).

Failure to undergo diet shifts is expected to accompany non-piscivorous feeding habits in pike (Venturelli and Tonn 2005, Paradis et al. 2008). Non-piscivory among northern pike is viewed as an opportunistic response to temporary impairment of the forage base (Chapman and Mackay 1990). Examples of long-term persistence of non-piscivory are rarely confirmed, as significant relationships between body size and trophic position suggest that reliance on invertebrate prey may not be persistent throughout diet ontogeny at the population level (Paradis et al. 2008). Previous reports of persistent non-piscivory in pike stem from disturbed systems, in which prey fish stocks had been lost altogether and pike were the only remaining fish species (Venturelli and Tonn 2006). Noteworthy, and notwithstanding the fact that prey fish were present in all reservoirs investigated here, the fish component in the diet of pike was generally small when lake whitefish were scarce or absent, and benthivory was still the predominant and evidently long-term feeding strategy of pike. This pattern is most likely the result of opportunistic feeding behaviour in response to overall prey fish density and not just a preference for

specific prey fish species by pike. Conceivably, prey fish abundance in reservoir communities supported by plankton-based resource pathways may be greater, relative to reservoirs, in which energy is derived from littoral or benthic food chains. In reservoirs, littoral and benthic habitats may be severely impacted by hydrologic demands on the lake, such as drawdown and rapid water throughput, and may thus contribute only small percentages to overall reservoir productivity and ultimately prey biomass available to higher consumers (Lindstrom 1973).

While gill-net catches may not accurately estimate small fish abundance and this study did not attempt to quantify prey-fish biomass in other ways, the findings herein indicate that the relative abundance of prey fish in the community is a strong predictor of the reliance on fish prey by pike, and accordingly, the tendency to undergo trophic shifts at the population level. However, few studies have addressed the effect of prey fish density on feeding decisions in pike quantitatively, and prey-selective behaviour in pike has not been studied beyond the realm of fish prey (Hart and Hamrin 1988, Hyvarinen and Vehanen 2004). Prey selection to maximize bioenergetic return during feeding is a central tenet of foraging theory (Stephens and Krebs 1986, Sih and Christensen 2001). According to the prey model (Stephens and Krebs 1986), a consumer is expected to switch from small, less profitable prey once encounter rates with large, profitable prey (Werner and Mittelbach 1981) reach a certain threshold. At this threshold preference for large prey increases rapidly. The findings herein indicate that pike incorporate fish into their diet only at a relatively high ratio of prey fish to pike in gill net catches (29%). Encounter rates with prey fish may be governed by factors not quantified for the purpose of this study, such as prey fish density and prey behaviour (Mauck and Coble 1971,

Chapman and Mackay 1990). However, catch ratios of prey:predator in gill nets demonstrate prey presence within the vicinity of the predator and may be a proximal indicator of encounter rates between the two. Since pike are ambush predators, relying on movement of prey into their strike range, effects of prey density and prey behaviour may affect the feeding behaviour of pike in similar ways.

3.4.2 Growth rates of northern pike

Growth rates were lower in non-lake whitefish systems by comparison with lake whitefish dominated prey communities. Several earlier studies have linked stunted growth in northern pike with forage-base constraints, interpreted mainly from the effect of competition in high predator-density scenarios (Margenau et al. 1998, Pierce et al. 2003), or in systems without preferred fish-prey altogether (Venturelli and Tonn 2006, Brinkmann and Rasmussen 2010). Here, the observation of low growth rates in non-lake whitefish lakes is consistent with invertebrate-dominated diets of pike in these systems.

This study observed comparatively high growth rate in pike in lake whitefish prey communities. Others have seen similar patterns in pike in the presence of cisco (*Coregonus artedi*) (Jacobson 1992). The presence of large prey fish is well known to facilitate growth in pike (Diana 1987), and may be the critical factor affecting observed growth differences in southern Alberta reservoirs. The prey base in these reservoirs may influence growth of pike mainly through factors such as dietary quality and bioenergetic cost associated with prey capture (Boisclair and Leggett 1989). For instance, the energy density of amphipods, the main prey organism consumed by benthivorous pike, is only 3.6 kcal/g dry weight (Brinkmann, unpublished data) whereas lake whitefish may have an

energy density of more than 8.9 kcal/g (Madenjian 2006). Fish may compensate for shortcomings of food quality by increasing food consumption rates (Bowen et al.1995). In cases, in which consumed prey organisms are small, increasing consumption may also necessitate greater investment in feeding activity, which distracts from allocation towards somatic growth (Pazzia et al. 2002, Rennie et al. 2005).

It has been noted that piscivorous feeding behaviour is often associated with empty stomachs, indicating a resting period between feeding events (Arrington et al. 2002). This pattern may be caused by a stochastic effect of disproportional abundances of large versus small food items, and thus differences in encounter rates between predator and prey, or in capture success (Breck 1993). Here, frequencies of empty stomachs were significantly higher in piscivorous pike relative to benthivorous conspecifics in non-lake whitefish communities, confirming observations made earlier by Chapman and Mackay (1990). Alternative to a stochastic phenomenon relating to prey size and frequency, Arrington et al. (2002) interpreted this phenomenon as a response to high energetic benefit associated with engulfing large, high-quality food items. Following Arrington's et al. (2002) argument, differences in frequencies of empty stomachs may corroborate the notion that elevated activity levels associated with benthivory impair growth of pike in some reservoirs.

Prey base impairment is a critical factor in preventing ontogenetic diet shift in fish (Sherwood et al. 2002a). Growing fish require prey organisms to scale in size accordingly, in order to maintain bioenergetic profit during feeding. If suitable prey size classes are absent, activity costs associated with exploitation of small prey may deplete the energy budget of growing fish, thus hindering normal growth. As previously

discussed, evidence for trophic shifting in lake whitefish communities was a strong indicator of size-scaling of prey items during ontogeny in pike. Prey communities, which meet such prey size scaling demands by pike and other predatory fish may be the key factor in facilitating fast growth in these target fish species.

3.5 Conclusion

This study presented evidence for considerable variation in feeding strategies among northern pike in southern Alberta reservoirs and showed that benthivory is a common phenomenon. Although an unequivocal preference for lake whitefish relative to other fish species as a resource to pike could not be established, lake whitefish may still play an important role in the diet ontogeny of pike. Furthermore, some findings suggest piscivorous feeding behaviour in pike may follow a threshold response, highlighting the importance of maintaining relatively large prey fish stocks through management practices in reservoirs. Stunted growth patterns in pike were observed in reservoirs without a lake whitefish dominated prey base, which coincided with predominant reliance on invertebrate prey for food. Normal growth rates were associated with fish-dominated prey bases.

Northern pike are known to exert strong top-down effects on prey fish communities and have thus been used as a lake-wide biomanipulation tool (Berg et al. 1997, Byström et al. 2007). Pike may similarly affect prey fish communities in southern Alberta, especially in smaller reservoirs. This may in part explain why samples collected in non-lake whitefish communities were dominated in numbers by pike. The predator-prey interactions resulting in such community structures in southern Alberta reservoirs

would require further investigation to shed light on this. Especially the role of lake whitefish as a trophic coupler in these reservoirs may be addressed in follow-up. I hypothesize that lake whitefish may be a species capable of withstanding predation pressure by littoral predators, such as northern pike, better than other species, due to its preferred pelagic habitat. Thus it may maintain high numbers in many lakes, and in connection with broad movement patterns across littoral-pelagic boundaries, may form a suitable prey base for predatory fish in reservoirs, while controlling its vulnerability to over-exploitation.

Chapter 4

Trophodynamic effects on mercury biomagnification in northern pike (*Esox lucius*) in storage reservoirs of the upper South Saskatchewan River basin

Abstract

This study compared mercury contamination in northern pike under piscivorous and benthivorous trophic regimes in irrigation storage reservoirs and tested for the effects of dietary mercury concentration (C_d) and food chain length on mercury concentrations in pike. Total mercury concentrations (THg) in piscivorous pike, which exploited predominantly lake whitefish (*Coregonus clupeaformis*), were significantly higher than in benthivorous pike, which lived on a diet dominated by amphipods. Further analysis showed that both C_d and trophic position affected pike THg significantly among trophic regimes. Although C_d was 2-3 fold higher in the piscivorous trophic regime than in the benthivorous regime, it affected THg in piscivorous pike at approximately half the rate relative to benthivorous pike. Trophic position, which scaled with body size in pisciviorous but generally not in benthivorous pike, was also a significant predictor of pike THg. However, an interaction effect between trophic position and trophic regime on pike THg was not found, indicating that factors other than diet shifting influenced increasing THg levels through ontogeny in benthivorous pike. These results are consistent with the hypothesis, that benthivorous pike exhibit significantly higher biomagnification factors than piscivorous pike. A discussion of diet energy density, daily ration and foraging costs highlights that bioenergetic effects associated with diet type

may elevate mercury biomagnification in fish, and this may partially offset effects of reduced dietary mercury exposure on mercury concentrations in reservoir fish.

4.1 Introduction

Reservoirs serve critical roles in flow-regulation of rivers, renewable energy generation, and water storage. Yet the socio-economic benefits of reservoirs are often contested on grounds of their detrimental impacts on the ecological integrity of rivers, surrounding watersheds and lifestyles of people who rely on fisheries affected by dam projects (Chevalier et al. 1997, Jackson and Sleigh 2001, Duvail and Hamerlynck 2003). Another major concern in reservoirs is the rise of mercury levels in biota following inundation of soils, which has been identified as a significant risk factor to the viability of fisheries within and below reservoirs worldwide (Lodenius and Malm 1998, Schetagne and Verdon 1999, Larssen 2010).

The primary source of the mercury pulse in reservoirs is release from inundated soils and vegetation, followed by methylation, a process accelerated by decomposition of submerged organic matter (Jackson 1988). In addition to its property to bioaccumulate in micro-biota, methylated mercury is efficiently passed on to higher consumers through trophic transfer, which is the primary transfer route of the contaminant in higher organisms (Bloom 1992, Hrenchuk et al. 2012). Dietary mercury concentration is one of the most important determining factors of mercury concentrations in fish (Trudel and Rasmussen 2006, Stacy and Lepak 2012). Since rates of assimilation and retention of dietary methylmercury in the body burden are greater than consumer body growth, the mercury concentration increases in the consumer relative to its prey. This process, known

as biomagnification, is cumulative along food chains, resulting in an expected positive relationship between the trophic position of consumers and their exposure to dietary mercury (Cabana et al. 1994). Thus comparisons of food chain length among systems can reveal links between the feeding ecology of fish and the mercury levels in their bodies.

It is well understood that the contaminant biomagnification phenomenon is influenced by the energy budget of consumers (Trudel and Rasmussen 2006). Mercury assimilation is coupled with protein assimilation from the diet and is therefore very efficient. However, following assimilation, mercury is mostly added to the existing burden in body tissues and becomes largely decoupled from carbon metabolism, which depends on the bioenergetic budget of the consumer (Trudel and Rasmussen 2006). If the consumer grows at the same rate that mercury is added to the body burden, no change in tissue concentration will occur; as such tissue growth has often been described as a process that dilutes the contaminant. Therefore, consumer growth efficiency plays a critical role in controlling biomagnification, and ultimately constitutes an important predictor of mercury concentrations in fish, in addition to dietary mercury concentration (Simoneau et al. 2005, Ward et al. 2010).

Growth efficiency in fish can vary significantly with system productivity and dietary quality, and numerous studies have shown the effect of growth dilution on fish mercury concentrations in the field (Ward et al. 2010, Lepak et al. 2012). By the same reasoning, reductions in growth efficiency have been implicated as cause of comparatively high mercury levels in fish (Doyon et al. 1998, Trudel and Rasmussen 2001). Recent studies have also shown that ecosystem perturbations causing simplified food webs can have significant negative effects on growth efficiency in fish populations,

mainly through the elimination of key trophic links which facilitate ontogenetic diet shifts in higher consumers (Sherwood et al. 2002b). However, few studies have addressed the effect of trophic simplification on mercury biomagnification in fish, which may be of particular interest in the reservoir context, due to the fact that these systems undergo a mercury pulse in combination with community restructuring. For example, investigating mercury in a newly constructed reservoir, Brinkmann and Rasmussen (2010) reported that mercury biomagnification was elevated in resident northern pike, suggesting that their invertebrate diet and associated stunted growth were contributing factors. This led to the recognition that bioenergetic bottleneck effects in connection with trophic simplification may trade-off against a reduced trophic position, in exacerbating mercury concentrations through enhancement of biomagnification. To summarize, it must be considered that mercury concentrations in fish can reach high levels due to high dietary exposure, which may be associated with food chain length or other factors, but also due to high biomagnification, which is affected by fish bioenergetics. The opposing interplay between food web simplification and mercury biomagnification should receive further attention, in order to evaluate its contribution to the risk of high mercury concentrations in predatory fish in reservoirs.

Natural lakes are rare in the semi-arid upper South Saskatchewan River Basin and storage reservoirs provide most of the stillwater fisheries. Northern pike is the most common apex consumer species in most reservoirs. A recent study investigating the feeding ecology of northern pike in relation to the prey fish community in reservoirs has shown that several pike populations rely predominantly on invertebrate prey rather than prey fish (Chapter 3). In contrast to reservoirs in which pike exploited typical fish prey,

benthivorous pike exhibited no ontogenetic shift in trophic position and showed comparatively low growth rates, consistent with the findings by Brinkmann and Rasmussen (2010) and other studies (Venturelli and Tonn 2006). Assuming that stunted growth is a consequence of higher metabolic cost in benthivorous trophic regimes, it seems plausible to expect higher biomagnification factors in these systems (Brinkmann and Rasmussen 2010). Here, an examination of the effects of trophic regimes on mercury concentrations and biomagnification may provide insights into the potential trade-off between food chain length and bioenergetics constraints on mercury dynamics in reservoirs.

The objective of this study is to investigate mercury concentrations in northern pike in southern Alberta irrigation reservoirs and to examine the role of trophic structure as an explanatory variable from two perspectives: First, the effects of dietary mercury concentration and its potential link with food chain length on mercury concentrations in northern pike will be analyzed. Secondly, biomagnification factors will be compared among piscivorous and benthivorous trophic regimes, in order to help evaluate bioenergetic constraints associated with prey base impoverishment as a risk factor to mercury contamination in reservoir food webs.

4.2 Methods

4.2.1 Sampling and trophic information

This study compares the effect of piscivorous versus benthivorous feeding behavior on mercury dynamics in northern pike in seven irrigation storage reservoirs in the upper South Saskatchewan River Basin. Samples of northern pike and prey fish were

collected using multi-mesh gill nets and a beach seine during the open water seasons from 2009 to 2011. Benthic invertebrate samples were collected by means of Ekman sampler, dip net and plankton trawl net during the same period. Biological collections were cooled on ice for transport and frozen prior to further processing.

In the lab, fish length, weight and sex information was recorded and a sample of skinless and boneless dorsal muscle tissue was collected. Benthic invertebrate samples were sorted to family level. All animal tissue samples were dried (60°C) to constant mass for water contents determination, and finally ground to homogeneous powder for storage and further analyses.

4.2.2 Mercury analysis

Total mercury (THg) concentrations were used as proxy for methylmercury in fish and determined in dried homogenized fish muscle tissue. Samples of ~100mg of tissue were digested at 75°C in 2.0ml concentrated OmniTrace nitric acid in borosilicate glass test tubes sealed gas-tight with PTFE lined caps for 14 hours. Sealed test tubes were left to cool before uncapping. Aliquots of digestate were diluted with a solution of 200ppb gold chloride in nanopure water to achieve ~3% acid concentration for analysis.

Samples were analyzed on a PerkinElmer DRCe inductively-coupled-plasma mass spectrometer (ICP-MS). Mercury concentrations were measured on the ^{201}Hg isotope. Instrument parameters are summarized in Table 4.1. Analytical quality was assured by analysis of interspersed certified reference material DORM-2 and TORT-2, sample duplicates, spike recoveries, and method blanks. The method detection limit was 0.003µg/g Hg in dried muscle tissue.

Table 4.1: ICP-MS instrument parameters for the analysis of Hg in fish tissue digestate.

Instrument parameter	Value
RF-power	1400W
Nebulizer gas flow (Ar)	0.94 L/minute
Acquisition mode	Peak hopping
Dwell time	100 ms
Replicates	3

Invertebrate tissue samples were categorized (amphipods, insects, leeches) and pooled for individual lakes. Subsamples were analyzed for THg and methylmercury at the University of Alberta Biological Analytical Service Laboratory (BASL). Additional invertebrate samples were digested and analyzed for THg using the same protocol applied to fish tissue. These measurements were then adjusted by the proportion of methylmercury in THg determined for each invertebrate prey category and specific to reservoir.

4.2.3 Dietary mercury concentrations and biomagnification factors

Quantitative dietary information on northern pike in each reservoir was obtained from stomach contents and $\delta^{15}N$ and $\delta^{13}C$ stable isotope analysis in combination with a Bayesian mixing model. Furthermore, trophic position (TP) estimates of pike and prey taxa were calculated using $\delta^{15}N$ stable isotope data (Chapter 3).

In order to accurately represent the dietary variability observed among pike in these systems, mean dietary mercury concentrations (C_d) were calculated on a dry-weight basis by weighting mean mercury concentrations [Hg] measured in each dietary taxon i by the estimated proportion p in the diet:

$$C_d = \sum_{i=1}^{n} [Hg]_i p_i \quad (1)$$

Dietary contributions specific to pike size categories were carried over from previous analyses (Chapter 3). Mercury biomagnification factors (BMF) were calculated on an individual basis as

$$\text{BMF} = \frac{C}{C_d} \quad (2)$$

where C is the Hg concentration (dry weight) in individual pike and C_d is the mean dietary Hg concentration specific to the size category to which the individual belonged.

4.2.4 Statistical analysis

Statistical tests were run R version 2.15.2 (2012). Total mercury (THg) in pike response data were log-transformed to normalize residuals and reduce heteroscedasticity. Three outliers were identified by "extreme studentized deviation" (3 S.D.) and eliminated from data sets prior to statistical testing. Values predicted by regression models were back-transformed for the purpose of data display in graphics. Total mercury in pike was initially modeled using analysis of covariance with fork length as covariate to generate regression coefficients for general presentation of mercury levels in each reservoir. Following this, the effect of piscivorous and benthivorous trophic regimes on THg in pike was analyzed using an Analysis of Covariance model, which included TP and C_d as hypothesized covariates of trophic regime. Collinearity between C_d and TP was ruled out by a variance inflation test (VIF = 1.4) and their simultaneous inclusion in the model was permissible. Their respective interaction terms with the main effect were also included, in order to test for differences in regression slopes of pike THg against the covariates among

trophic regime treatments. Trophic position data were y-axis centered by subtracting reservoir-specific means from each data point.

Mercury biomagnification factors were compared among trophic regimes using Analysis of Covariance. The BMF response variable was log-transformed to linearize the distribution and achieve homoscedasticity. Since potential bioenergetic effects of trophic regime are expected to correlate with body size, the model incorporated fork length as covariate, and the trophic regime - fork length interaction term.

4.3 Results

4.3.1 Mercury in northern pike

Mercury concentrations in northern pike varied considerably among study lakes and correlated with body size (Figure 4.1, Table 4.2). Among-reservoir differences explained 90% of variation in mercury concentrations. In relation to the guideline value of 0.5µg/g (wet weight) as a benchmark, mercury levels were highest in Twin Valley, with linear-model-predicted tissue concentrations (parameters back-transformed) exceeding the guideline value at a fork-length of 588mm. Concentrations in excess of 1.0µg/g were measured in Twin Valley. Mercury levels were comparatively low in the remaining three benthivorous populations, Scope, Hays and CPR, with mercury concentrations predicted to exceed the guideline at fork length of 782, 962 and 1013mm, respectively. Size ranges in samples collected from these populations were below the respective length thresholds and mercury concentrations did not exceed 0.33µg/g in wet weight muscle tissue (Figure 4.1).

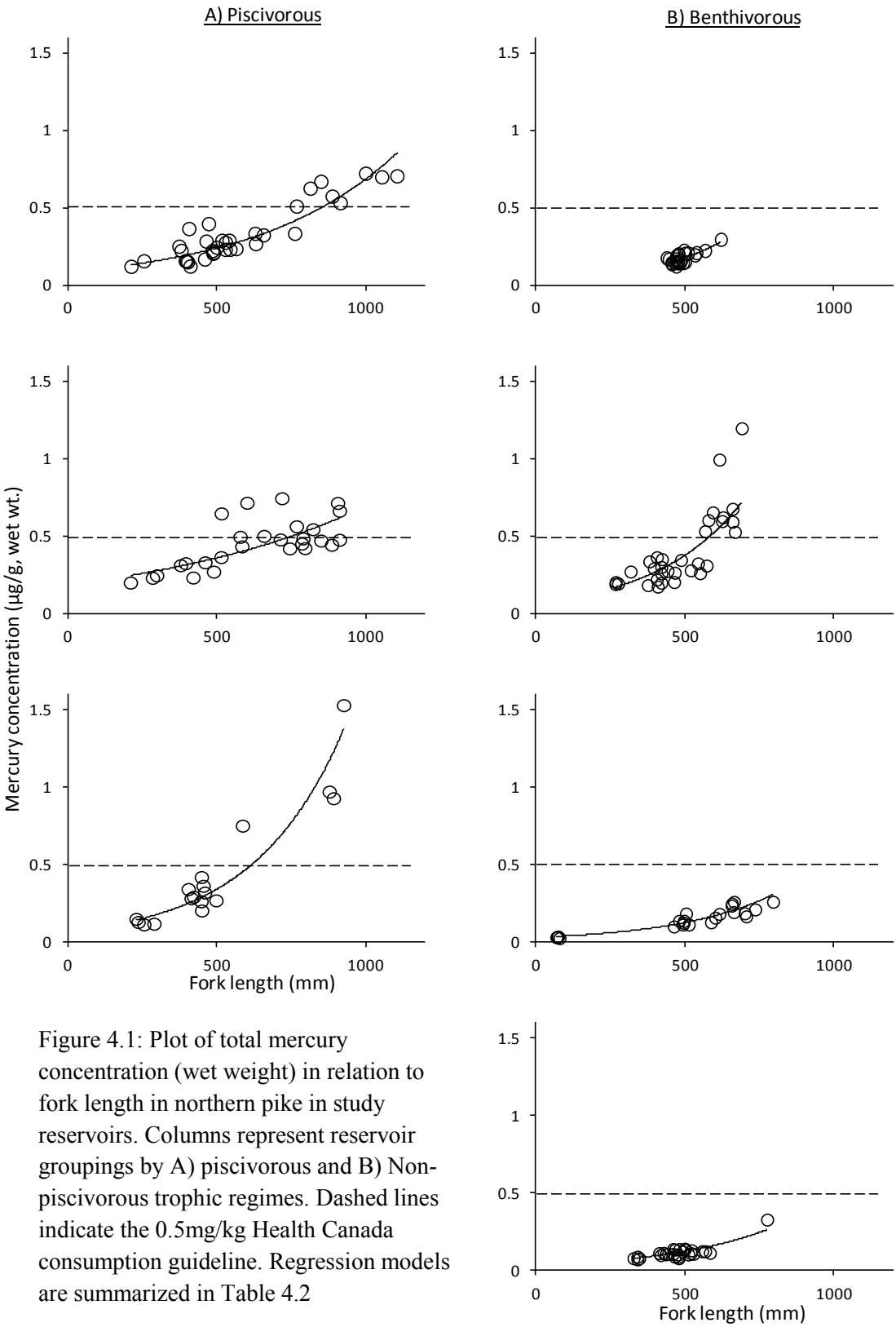


Figure 4.1: Plot of total mercury concentration (wet weight) in relation to fork length in northern pike in study reservoirs. Columns represent reservoir groupings by A) piscivorous and B) Non-piscivorous trophic regimes. Dashed lines indicate the 0.5mg/kg Health Canada consumption guideline. Regression models are summarized in Table 4.2

Among piscivorous populations, pike in Chin had the highest mercury concentrations and exceeded the guideline value at a length of 618mm. Values in the largest pike of the sample exceeded 1.0µg/g. Keho and Newell pike were below 0.5µg/g up to lengths of 851 and 750mm respectively. A separate test comparing piscivorous and benthivorous populations, indicated that among-trophic-regime effects were significant (Table 4.2) and explained 58.7% of variation in pike ln(THg). The ln(THg) regression intercept was higher in the piscivorous trophic regime and differed by a factor of approximately 2.5-fold. The interaction between trophic regime and length was also significant and indicated that ln(THg) increased 1.5-fold more per unit length in piscivorous pike relative to benthivorous pike (Table 4.2).

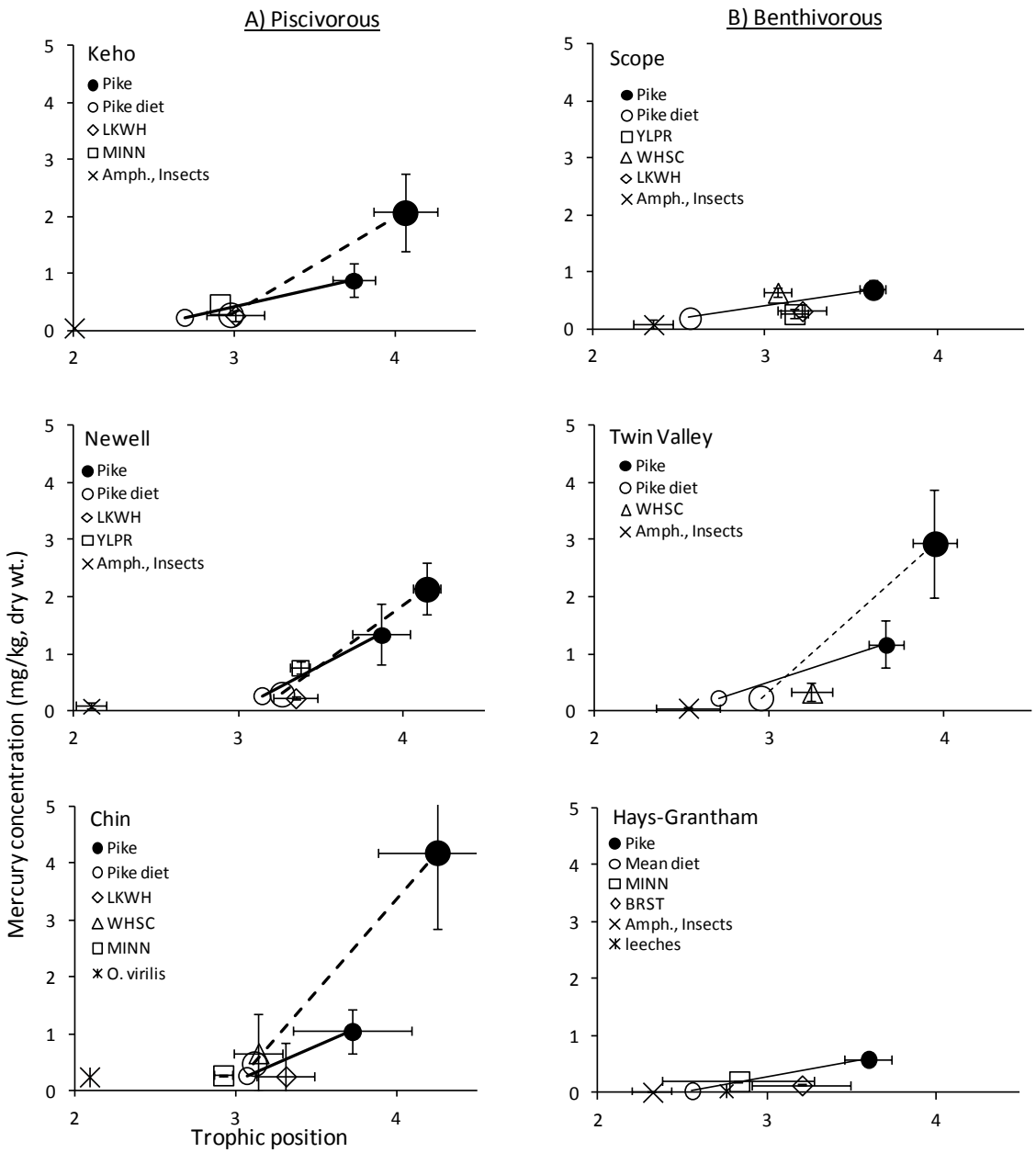
4.3.2 Dietary mercury concentrations and trophic structure

Northern pike diets were dominated either by lake whitefish in piscivorous regimes or by amphipods in benthivorous regimes. Dietary mercury concentrations (C_d) reflected the width of this prey spectrum and ranged between 0.04mg/kg and 0.48mg/kg (dry weight)(Figure 4.2). Average C_d in benthivorous trophic regime reservoirs was 0.09mg/kg. Pike that had exploited primarily fish, had C_d increasing from 0.25mg/kg to 36mg/kg on average, in reflection of a diet shift as pike increase in size, and were thus approximately two to three-fold higher relative to the benthivorous trophic regime. Dietary mercury was a significant predictor of ln(THg) in pike and explained 40.0% of variation when incorporated as a covariate to trophic regime (Figure 4.2, Table 4.2). The trophic regime - C_d interaction was significant and explained a further 1.2% of variation. In total, 62.7% of variation in ln(THg) could be attributed to among-trophic regime effects. Regression coefficients indicated that ln(THg) responded more strongly to C_d in

benthivorous pike populations relative to pisciviorous populations, as indicated by a 30% difference between slope coefficients (Table 4.2).

The plot of mercury in biota against trophic position (Figure 4.2) shows that average diets of pike in the piscivorous trophic regime consisted of fish with an approximate trophic position of 3, whereas the invertebrate-dominated diets consumed by pike in the benthivorous trophic regime were below trophic position 3. Mercury concentrations were expected to correlate with variation in food chain length among trophic regimes. Trophic position was a significant predictor of ln(THg) in pike and could explain 15% of the variation in ln(THg) (Table 4.2). The trophic regime main effect accounted for an additional 21.5% of variation in ln(THg). In combination, trophic position and the trophic position - trophic regime interaction explained 1.7% of the variation in ln(THg) and the majority of variation (61.8%) remained unexplained by this model overall.

A variance inflation factor test confirmed low collinearity between TP and C_d (VIF = 1.4), permitting inclusion of both variables as covariates in a multi-covariates analysis of covariance model. Results from this model, however, yielded little improvement in goodness of fit ($R^2 = 0.67$) relative to the model including only C_d as covariate ($R^2 = 0.63$) (Table 4.2).



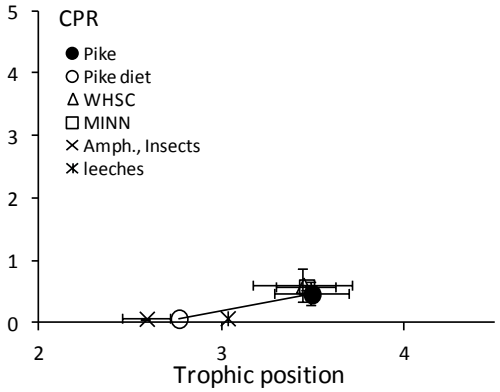


Figure 4.2: Plot of mercury concentration (dry weight) in relation to trophic position in northern pike (solid circles) and prey organisms in study reservoirs. Columns represent reservoir groupings by A) piscivorous and B) Non-piscivorous trophic regimes. Weighted average dietary mercury concentrations of pike are represented by open circles. In systems where diet shifts occurred, two size classes of pike and their diet are indicated as small and large circles, respectively. Errors represent 1 SD. Statistics listed in Table 4.2.

Table 4.2: Statistical comparisons of mercury concentrations in northern pike in relation to trophic regimes in upper SSRB reservoirs.

Comparison	Response	Statistics	Model	Figure
Reservoirs	ln(THg); µg/g wet wt.	Effect: Reservoir $F_{(6,170)} = 143.57$; $p < 0.0001$ Effect: Fork length $F_{(1,170)} = 609.05$; $p < 0.0001$ Interaction $F_{(6,170)} = 9.86$; $p < 0.0001$	*Keho*: ln(THg) = 0.00211 • length -2.49 *Newell*: ln(THg) = 0.00132 • length -1.69 *Chin*: ln(THg) = 0.00329 • length -2.73 *Scope*: ln(THg) = 0.0037 • length -3.59 *Twin Valley*: ln(THg) = 0.00342 • length -2.70 *Hays-Grantham*: ln(THg) = 0.00308 • length -3.65 *CPR*: ln(THg) = 0.00280 • length -3.53 $R^2 = 0.89$	4.1)
Trophic regimes	ln(THg); µg/g wet wt.	Effect: Trophic regime $F_{(1,180)} = 91.31$; $p < 0.0001$ Effect: Fork length $F_{(1,180)} = 155.32$; $p < 0.0001$ Interaction $F_{(1,180)} = 5.88$; $p < 0.016$	*Benthivorous:* ln(THg) = 0.00308 • length - 3.24 *Piscivorous:* ln(THg) = 0.00209 • length - 2.30 ($p < 0.0001$) $R^2 = 0.58$	4.1)
Trophic position	ln(THg); ug/g dry wt.	Effect: Trophic regime $F_{(1,180)} = 61.18$; $p < 0.0001$ Effect: Trophic position $F_{(1,180)} = 43.67$; $p < 0.0001$ Interaction $F_{(1,180)} = 4.94$; $p = 0.028$	*Benthivorous:* ln (THg) = 0.80 • TP – 0.35 *Piscivorous:* ln(THg) = 1.79 • TP + 0.30 ($p < 0.0001$) $R^2 = 0.37$	4.2)
Dietary mercury concentration	ln(THg); ug/g dry wt.	Effect: Trophic regime $F_{(1,180)} = 101.24$; $p < 0.0001$ Effect: C_d $F_{(1,180)} = 192.63$; $p < 0.0001$ Interaction $F_{(1,180)} = 5.70$; $p = 0.018$	*Benthivorous:* ln (THg) = 10.11 • C_d – 1.36 *Piscivorous:* ln(THg) = 7.15 • C_d – 1.67 ($p = 0.243$) $R^2 = 0.63$ Test of collinearity (Variance inflation test) between Cd and TP suggested low collinearity VIF 1.4	4.2)
Biomagnification	ln(BMF)	Effect: Trophic regime $F_{(1,180)} = 94.57$; $p < 0.0001$ Effect: Fork length $F_{(1,180)} = 218.42$; $p < 0.0001$ Interaction: $F_{(1,180)} = 18.11$; $p < 0.0001$	*Benthivorous:* ln (BMF) = 0.0028 • length + 0.70 *Piscivorous:* ln (BMF) = 0.0016 • length + 0.68 ($p = 0.934$) $R^2 = 0.64$	4.3)

4.3.3 Mercury biomagnification

In connection with a significant interacting effect between trophic regime and C_d, mercury biomagnification factors (BMF) in pike, which are defined as $[Hg_{pike}] / [Hg_{diet}]$, were calculated and compared (ANCOVA) among trophic regimes (Figure 4.3, Table 4.2). With fork length as covariate to account for an expected effect of fish size, the analysis showed that 64.8% of variation in ln(BMF) was attributable to among – trophic regime differences, of which fork length accounted for the majority, 42.7%. The effect of trophic regime and its interaction with fork length contributed 18.5% and 3.6%, respectively. The slope coefficient of ln(BMF) against fork length was significantly higher in benthivorous pike (Table 4.2).

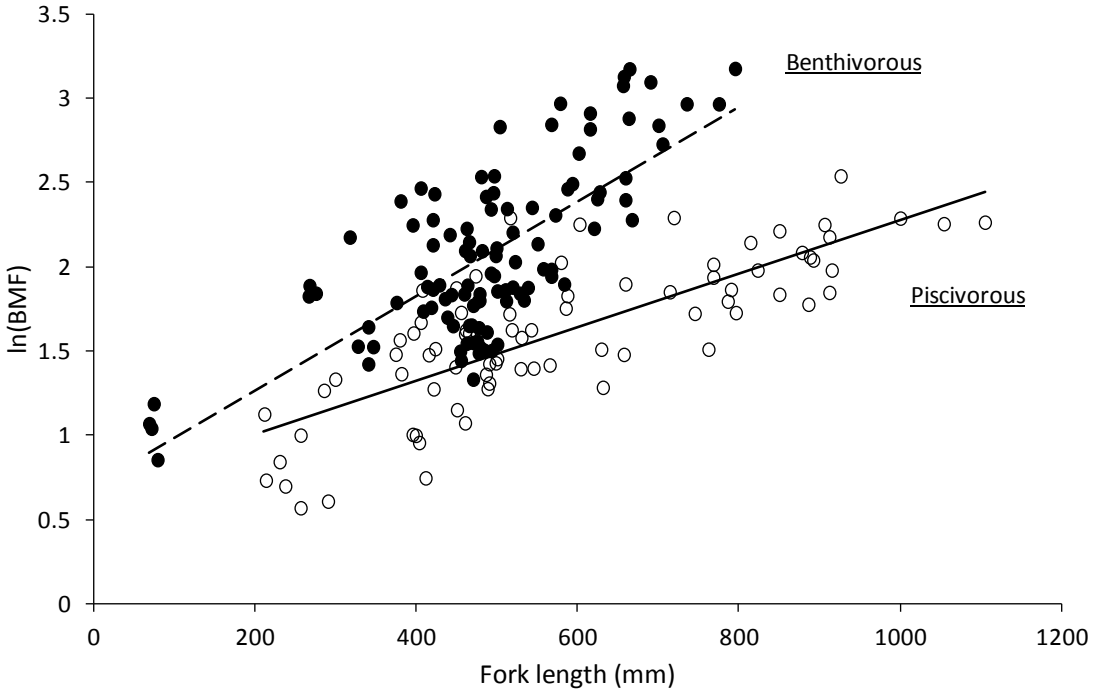


Figure 4.3: Mercury biomagnification factors between northern pike and their weighted average diet in relation to fork length in piscivorous and benthivorous trophic regimes. Calculation based on dry-weight mercury concentrations (for statistics and models refer to Table 4.2).

4.4 Discussion

This study addressed mercury contamination in northern pike in reservoirs in the upper South Saskatchewan River basin in relation to trophodynamic effects. Previous research had found that pike differed significantly in their reliance on fish versus invertebrate prey, correlating strongly with the presence of lake whitefish in the prey fish community of reservoirs. The main objective of this study was to compare mercury concentrations in pike based on the previously identified piscivorous and benthivorous feeding regimes. Specific comparisons aimed to elucidate the role of dietary mercury exposure and potential links with food chain length, which was previously shown to vary among trophic regimes. Furthermore it was hypothesized that biomagnification may differ among piscivorous and benthivorous pike populations, based on differences in growth rates previously observed among trophic regimes.

4.4.1 Mercury concentrations in northern pike

Mercury concentrations in pike varied considerably among reservoirs and increased significantly with fish size at all sites as expected. Values in these reservoirs generally scatter around a reported Canada-wide median concentration of 0.38mg/kg in fresh muscle tissue (specific size of 578mm fork length, 607mm total length for comparison)(Depew et al. 2013). With respect to the Canada-wide median mercury concentration in pike, which excluded estimates from reservoirs and contaminated sites (Depew et al. 2013), Chin and Twin Valley were comparatively high (0.44 and 0.48mg/kg, respectively), Newell was similar (0.40mg/kg), and pike mercury concentrations in all other lakes were comparatively low (<0.28mg/kg). With the exception of Keho (0.28mg/kg) mercury levels in piscivorous pike (reservoirs with lake

whitefish prey community) were also slightly higher than reported values for the Oldman River, in which the predicted mercury concentration in pike of 578mm length was 0.35mg/kg (Brinkmann 2007).

Mercury concentrations ranked highest in Twin Valley among all lakes, although mercury concentrations in pike have declined by more than 50% by comparison with published estimates from 2005, in which regressions showed mercury concentrations near 1.20mg/kg for the specific fish size of 578mm (Brinkmann and Rasmussen 2010). Both the relatively high mercury levels in this reservoir and the observed decline since 2005 may be explained by the relatively young age of this system. Twin Valley was constructed in 2003 and is thus the youngest reservoir in this dataset. Mercury levels increase in reservoirs post-impoundment due to an initial pulse released from inundated terrestrial soils and vegetation (Jackson 1988, St Louis et al. 2004). Fish mercury concentrations in reservoirs typically reach maximum levels after two years of impoundment (Schetagne and Verdon 1999). The period of return to baseline, however, varies by a spectrum of physical factors, including flushing rate and sediment resuspension and may require decades. The influence of these factors on the progression of mercury pulses in reservoirs in the upper South Saskatchewan is largely unstudied. Physico-chemical variables such as relatively high pH levels and seasonally high clay-loads of tributaries, which may help in burial of sedimentary mercury, likely contribute to relatively short pulse duration in the abiotic compartments (e.g. water) in upper SSRB reservoirs (Brinkmann 2007, Brinkmann and Rasmussen 2010).

4.4.2 Dietary mercury concentrations and trophic structure

Increasing mercury concentrations with fish age, or fish size as a proxy thereof, were observed in all reservoirs sampled in this study. Such increases in consumers are attributable to multiple factors, primarily to increasing dietary exposure as a result of diet-shifts towards larger, more contaminated prey organisms (Trudel and Rasmussen 2006). Here, the effect of dietary mercury levels on mercury in pike was significant as expected when compared among trophic regimes. Under the piscivorous trophic regime pike fed predominantly on lake whitefish and other large-bodied prey fish, corresponding to an average (among-lake) dietary mercury concentrations of 0.25mg/g (dry weight) and 0.36mg/g for small and large pike, respectively. This difference in dietary mercury concentrations reflects diet shifting in these pike, towards larger prey fish. By comparison, the invertebrate diet of benthivorous pike had an average mercury concentration of 0.09mg/g and remained overall constant relative to body size, i.e. no significant diet shift occurred in this group. This overall low dietary exposure level among benthivorous pike was consistent with the relatively low mercury concentrations in this group by comparison with piscivorous pike (Lepak et al. 2009).

Mercury concentrations were also found to vary with TP of pike. Previous research had shown that, under the piscivorous trophic regime, pike gradually increased in TP through ontogeny, whereas this pattern did not occur under the benthivorous trophic regime. Since mercury concentrations increase at successive consumer levels, food chain length leading up to a terminal trophic link is an important correlate of dietary mercury exposure and thus concentrations in apex consumers (Cabana et al. 1994, Vander Zanden and Rasmussen 1996), as was seen here with THg in pike. It should be

noted, however, that relatively poor scaling was seen between TP and C_d , which exhibited no significant collinearity. Tests also showed that TP explained only approximately 1/3 of the variation in pike THg relative to C_d. The important implication here is that trophic complexity is not fully responsible for the relatively high mercury levels seen in lake whitefish, as the main prey of piscivorous pike. Other factors, which could affect lake whitefish mercury levels are exposure to an unknown high-mercury diet, or a bioenergetic effect, which increases biomagnification.

Bioenergetic factors generating variation in biomagnification within a diet-consumer link, may influence consumer mercury concentrations to potentially weaken or mask an expected relationship between mercury concentrations and TP in fish, since the effects TP has on C_d and biomagnification are opposing. This becomes apparent, for instance, in those study reservoirs in which pike maintained a constant diet and TP with body size (Chapter 3), but nonetheless showed significant relationships between THg and body size, a pattern which could not be ascribed to diet shifts. This, and the finding discussed above, that invertebrate-derived dietary mercury biomagnifies at a greater rate compared with fish-derived mercury, warrant a discussion of bioenergetic factors which may affect mercury accumulation rates in pike.

4.4.3 Mercury biomagnification factors

Apart from overall differences in dietary mercury exposure among trophic regimes, it is also noteworthy that the effect of C_d on ln(THg) interacted strongly with the trophic regime main effect. The fact that mercury concentrations in benthivorous pike increased more steeply in response to exposure, implies that invertebrate-based diets may affect mercury accumulation in pike differently compared with fish-based diets. Mercury

biomagnification is conceptually coherent with growth efficiency in fish. Mercury is acquired from the diet through trophic transfer in close association with carbon, and added to the existing body burden in somatic tissues. The body burden, in turn, is diluted by growth of somatic tissues. Energy budget allocations that reduce somatic growth reduce the magnitude of dilution that can occur; these are most importantly investments in reproductive products, through which little of the mercury burden is lost (Harris et al. 2003, Trudel and Rasmussen 2006), and activity cost/metabolism (Rennie et al. 2005, Trudel and Rasmussen 2006). Based on this concept, ration size (assuming constant dietary mercury concentration) and somatic growth are factors controlling the uptake and dilution, respectively, of the mercury burden in fish. The interplay of these factors (i.e. growth efficiency) is therefore reflected by contaminant biomagnification rates (Thomann 1981, Doyon et al. 1998, Ward et al. 2010), which can serve as an easy-to-measure proxy of the bioenergetic efficiency of predator-prey interactions.

Here it was shown that mercury biomagnification factors in pike increased with body size and, most noteworthy, this relationship differed among trophic regimes, in that the slope coefficient was approximately 4-fold higher in the benthivorous regime. A general relationship between body size as proxy of fish age and biomagnification factors is expected, and can be explained by declining somatic growth efficiency as a consequence of increasing activity cost and reproductive investment as fish age and mature (Diana 1983, Rowan et al.1996). This interpretation may then explain increasing mercury concentrations with body size in benthivorous pike, which did not increase their mercury exposure through ontogenetic diet shifting, as was the case with piscivorous pike.

However, with respect to the difference in biomagnification pattern among trophic regimes, ontogenetic changes in energy budget allocations also differ among trophic regimes. Particularly the fact that mercury biomagnifies similarly among groups in young pike, but increases more rapidly with body size in benthivorous pike strongly implies that bioenergetic constraints limit growth efficiency of pike in this trophic regime, which is consistent with previously presented evidence of low growth rates in this reservoir context (Figure 4.4) and the published literature (Venturelli and Tonn 2006, Brinkmann and Rasmussen 2010).

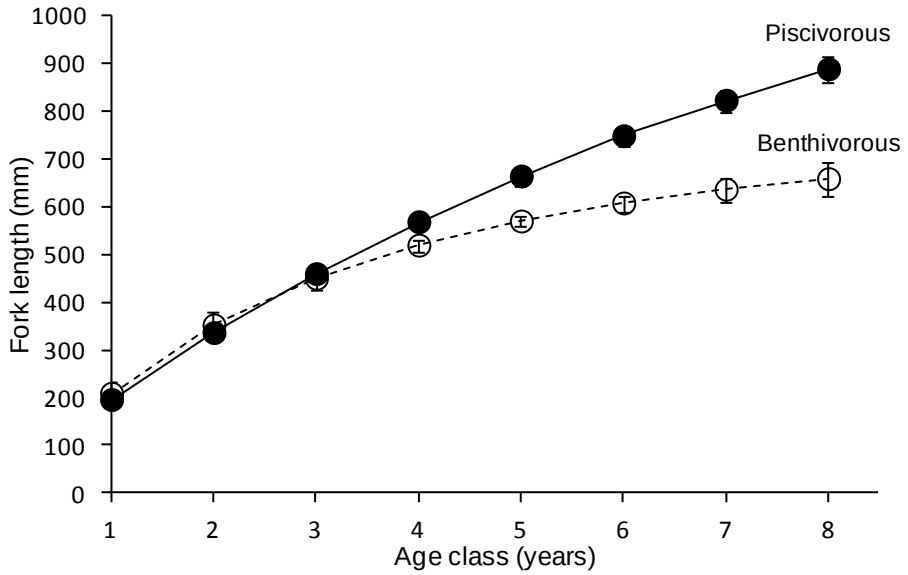


Figure 4.4: Growth rates (fork length at age) of piscivorous and benthivorous northern pike in Southern Alberta reservoirs (also see Chapter 3).

4.4.4 Potential prey-base effects on the energetics of stunting in northern pike

Growing pike, like most apex consumer fish species, require a broad prey size spectrum to undergo a diet shift, which optimizes the predator:prey-size ratio and maximizes the net bioenergetic return per invested unit of feeding activity, thereby

allowing optimal growth (Sherwood et al. 2002b). It is generally accepted, and corroborated by field studies (Sherwood et al. 2002b, Skov et al. 2003), that pike undergo an opportunistic shift towards large (fish) prey early on in life to achieve a relatively low on average, albeit variable, predator:prey-size ratio, which is henceforth gradually maintained throughout diet ontogeny (Diana 1987). The early onset of piscivory is a critical factor for growth in pike and many other piscivorous fish species (Mittelbach and Persson 1998). Herein, this typical life-history was well-reflected by piscivorous pike in that they gradually increased in trophic position in response to living on a fish-dominated diet. Albeit high in mercury, the fish-based diet coincided with comparatively low mercury biomagnification factors, which strongly suggest that high growth rates in piscivorous pike were achieved in considerable part through high growth efficiency (Chapter 3).

Although northern pike are well-known for dietary flexibility (Chapman and Mackay 1984, Sammons et al. 1994, Beaudoin et al. 1999), systems in which invertebrate prey plays a predominant role in the sustenance of pike throughout ontogeny are highly non-typical (Beaudoin et al. 1999, Paradis et al. 2008) and likely a consequence of disturbance and community-restructuring (Venturelli and Tonn 2003, Brinkmann and Rasmussen 2010). In communities missing key species or functional groups, the prey size structure may be discontinuous or truncated, which may impair the diet ontogeny of higher consumers. Accordingly, field studies on yellow perch and lake trout in such simplified food webs have highlighted that failure to optimize the predator:prey-size ratio through diet shifting results in run-away activity cost of foraging, and therefore leads to significant growth impairment and stunting in fish (Kerr 1971, Boisclair and Leggett

1989, Sherwood et al. 2002b). Similarly, studies presenting examples of benthivorous pike populations agree that growth rates of these populations are significantly slowed (Venturelli and Tonn 2006, Brinkmann and Rasmussen 2010), however the bioenergetics of non-piscivory were never fully elucidated. The finding herein, that mercury biomagnification factors were increased rapidly with age of pike supports the interpretation that slowed growth in benthivorous pike is a consequence of run-away activity cost associated with poorly optimized predator:prey-size ratios in this trophic regime.

Previous studies have also discussed the influence of prey quality in terms of energy density on growth dilution of mercury (Trudel and Rasmussen 2006, Stacy and Lepak 2012). The most important dietary component in the diet of benthivorous pike were amphipods, predominantly *Gammarus lacustris*. The energy density of *Gammarus* in southern Alberta was estimated at 3.6 kcal/g (drymass) (bomb calorimetry, Brinkmann 2007, unpublished data), similar to literature-reported estimates of 4.07 kcal/g in amphipods (Hanson et al. 1997). By comparison, energy density estimates in Coregonids are 2 to 3-fold higher, ranging between 8.6 kcal/g (Madenjian et al. 2006) and 10 kcal/g (Pazzia et al. 2002). This suggests that in addition to comparatively greater efforts invested into prey capture, benthivorous pike are faced with lower energetic reward per unit dietary mass, relative to piscivorous pike. In addition to the comparatively low energy density of amphipod prey, the chitinous exoskeleton, which may constitute a significant proportion of ingested prey mass, is likely indigestible to pike and passed in feces. Presumably, benthivorous pike may compensate for low dietary energy density with increased consumption, as was shown to be the case in non-piscivorous lake trout

(Pazzia et al. 2002). Elevated consumption would also increase mercury uptake from the diet and may thus act in unison with elevated activity cost associated with prey capture to exacerbate mercury biomagnification in benthivorous pike.

4.5 Conclusion

This study is the first to compare mercury concentrations in northern pike among reservoirs in the upper South Saskatchewan River Basin. In this semi-arid region, reservoirs constitute virtually all still water fisheries and resident fish communities have developed largely from tributary river communities, which typically exploit benthic resource pathways, with pike being the apex predator. Based on the feeding habits of pike, which were affected significantly by the presence or absence of lake whitefish, reservoirs can be classed into piscivorous and benthivorous trophic regimes. This study revealed that the diet type affected mercury concentrations in pike significantly, in that piscivorous pike were exposed to higher levels of dietary mercury by exploiting lake whitefish, whereas the diet composed of benthic invertebrates in benthivorous pike was comparatively low. Although food chains associated with the benthivorous trophic regime were slightly shorter, this factor had no significant effect on mercury in pike when body size was factored-out. A significant difference in the slope-relationship between body size and mercury biomagnification factors among trophic regimes implicated significant differences in the energy budgets of pike among trophic regimes. This was consistent with previous findings that benthivorous pike exhibited comparatively slow growth rates, showing signs of stunting. Elevated biomagnification factors in this group support the interpretation that pike grow with poor efficiency on a diet of benthic invertebrates, which is likely due to elevated activity cost and consumption rates. This

study demonstrated that, although dietary mercury exposure was lower due to trophic simplification, this may trade-off partially against elevated biomagnification due to bioenergetic bottleneck effects associated with sub-optimal diets. Further research may address the roles of activity cost and consumption rates in affecting mercury concentrations in pike.

Chapter 5

Bionenergetic budgets of piscivorous and benthivorous northern pike in storage reservoirs of the upper South Saskatchewan River Basin

Abstract

In this study the bioenergetic response of northern pike to different prey assemblages in reservoirs was investigated by comparing daily ration (DR) and activity cost, estimated using a stable Cesium (Cs) biokinetics model. Absolute DR (mass basis) was significantly higher in benthivorous pike compared with piscivorous pike and increased more rapidly with body weight in benthivorous pike over the course of ontogeny. Absolute DR expressed in terms of energy contents was significantly higher in piscivorous pike, which exploited predominantly energy-dense lake whitefish (*Coregonus clupeaformis*) and other large-bodied fishes. Consistent with higher mass-based consumption, activity cost in benthivorous pike was also higher and increased more rapidly through ontogeny than in piscivorous pike. In combination these results confirm a significant bioenergetic disadvantage of benthivory for pike, which stems from 1) lower energetic return due to low dietary energy density and 2) higher foraging costs due to consuming more prey mass, and thus explains low growth rates observed in pike under this trophic regime. Furthermore, to test whether bioenergetics parameters and dietary exposure explained differences in mercury biomagnification in pike among trophic regimes, DR estimates were used in a biokinetics model to predict mercury concentrations in pike, followed by comparison with previously measured mercury concentrations. Model-predicted values were similar to measured values, which supports the general validity of the bioenergetics parameters obtained from the Cs tracer kinetics,

and confirms that elevated mercury biomagnification in benthivorous pike can be explained by increased uptake with DR and reduced growth efficiency through elevated activity cost. These results highlight the importance of considering prey assemblages in the management of reservoirs for growth potential and mercury contamination in fisheries target species.

5.1 Introduction

Perturbation of ecosystems can severely alter resource pathways, mainly through factors such as loss of species or functional groups (Sherwood et al. 2002a, Walters and Post 2008), but also species invasions (Vander Zanden et al. 1999, Lepak et al. 2009). The ensuing changes to communities and food webs can greatly attenuate energy flow from the food web base to apex consumer level, especially when important intermediate consumer levels are eliminated (Sherwood et al. 2002a, Rennie et al. 2012).

Fish experience food web restructuring mainly through changes in prey type or prey abundance (Rennie et al. 2012). In such cases survival of fish may hinge on their ability to adapt to a novel prey spectrum and many freshwater fish do exhibit very broad diets, possibly to cope with the stochastic nature of freshwater prey communities (Gerking 1994). Fish species at the apex of food webs are generally piscivores as adults, but exhibit ontogenetic diet shifts as they grow that vary in response to the size structure of the available prey spectrum (Sherwood et al. 2002b). The necessity of diet-shifting for optimal growth requires a broad and continuous prey size spectrum, and makes the energetic budget highly sensitive to community restructuring which can limit the opportunity for ontogenetic diet shifting (Sherwood et al. 2000, Sherwood et al. 2002a). Such re-structuring can result either from extirpations resulting from pollutant impacts

(Iles and Rasmussen 2005, Rasmussen et al. 2008), from species introductions (Vander Zanden et al. 1999, Browne and Rasmussen 2009), or in the present case, from physical alterations such as impoundments.

Field studies have demonstrated effects of food web and community structure on the bioenergetics and growth of several piscivorous fish species. For example, in natural populations of yellow perch, growth rates were shown to scale positively with prey size in the diet of perch (Boisclair and Leggett 1989). Similarly, Sherwood et al. (2002a) showed that a discontinuous prey size spectrum in polluted systems precluded yellow perch from accomplishing a diet shift, and the resulting increase in foraging cost was a primary factor in stunting growth of perch. Pazzia et al. (2002) compared bioenergetics of lake trout under piscivorous and non-piscivorous trophic regimes, and showed that low growth in non-piscivorous lake trout was linked with elevated consumption rates and foraging costs, due to feeding on a small-bodied (invertebrate) diet low in energy density. These examples highlight the sensitivity of energy budgets of piscivores to the composition of their prey base.

Communities in reservoirs can be subject to considerable restructuring while adjusting to newly created lentic habitat following the impoundment of tributary rivers. An important aspect, by which reservoirs differ from their tributary river, is that the bulk of production may occur in the pelagic zone via zooplankton. This pool, however, may remain unexploited and thus unavailable to higher trophic levels in the absence of a zooplanktivorous trophic coupler. In connection with poor littoral habitat development and drawdown, this can limit feeding options for top predators in reservoirs, which may then adopt sub-optimal feeding strategies and exhibit poor growth. This was found to

occur in some reservoirs in the upper SSRB, where northern pike resorted to consuming benthic invertebrates in reservoirs without abundant zooplanktivorous lake whitefish stocks, and consequently exhibited slow growth (Chapter 3). In contrast to this condition, pike were piscivorous and grew normally in reservoirs with an introduced lake whitefish prey base. Here comparatively low growth rates in benthivorous pike may be consistent with the interpretation that higher metabolic cost is involved in the capture of prey of small size and comparatively low energy density. However, the bioenergetics of this system remain to be characterized.

The bioenergetic efficiency of trophic interactions also affects the biomagnification of mercury along food chains. Fish acquire mercury predominantly from their diet through trophic transfer and accumulate it in tissues, because mercury uptake exceeds elimination and tissue growth. Thus mercury biomagnification along food chains depends strongly on losses in biomass conversion between trophic links, due to energy metabolism, and mercury biomagnification can be exacerbated between trophic links in which prey-to-consumer biomass conversion efficiency is particularly low. Ultimately, the items on the energy budget of fish linking conversion efficiency of food webs with mercury biomagnification are 1) the daily ration of fish, which directly factors into mercury uptake, and 2) budget allocations towards metabolic cost, which distract from growth of tissues receiving assimilated mercury. Therefore daily ration and activity cost of fish are important variables to consider in explaining the response of mercury concentrations in fish to differences in the trophic structure of reservoir food webs.

The objective of this study was to test the hypotheses, that

1) Feeding rates are higher in benthivorous pike in order to meet bioenergetic demands from lower quality food,
2) Activity cost incurred by benthivorous pike feeding on a suboptimal prey size spectrum is higher compared with piscivorous pike, which feed on optimal prey size,
3) In combination with growth rate information, the obtained daily ration estimates, used in a biokinetics model, explain observed mercury concentrations in benthivorous and piscivorous northern pike in the upper SSRB reservoir system.

To obtain estimates of daily ration for the testing of the first hypothesis, a published cesium tracer biokinetics model (Rowan and Rasmussen 1996) was used. This method was originally used with the radiotracer ^{137}Cs (Rowan and Rasmussen 1996), however the stable Cesium isotope ^{133}Cs, which is measured with ICP-MS instrumentation, will be used here (Kennedy et al. 2004). Daily ration estimates obtained by this method were used in a bioenergetics model to solve for the activity multiplier (*ACT*) to resting metabolism for the testing of hypothesis 2). Finally, a published mercury kinetics model (Trudel and Rasmussen 2001) is used to predict mercury concentrations in pike from obtained daily ration, and dietary mercury concentrations and growth rates known for each population, in order to test hypothesis 3).

5.2 Methods

5.2.1 Sampling and trophic information

Samples of northern pike, prey fish and benthic invertebrates collected for the analyses presented in previous chapters, were further analysed for total cesium concentration (TCs). Data sets of dietary breakdown and growth rates for pike were taken from Chapter 3.

5.2.2 Cesium analysis

Total Cesium (TCs) concentrations in animal tissues were measured simultaneously with THg using ICP-MS, following the extraction/digestion protocol as outlined in Chapter 4 for THg. Cesium was measured on isotope ^{133}Cs and instrument parameters were set as described in Chapter 4. Analytical quality was assured by analysis of interspersed, sample duplicates, spike recoveries from sample duplicates, and method blanks. The method detection limit was 0.045ng/g Cs in dried muscle tissue.

Table 5.1: ICP-MS instrument parameters for the concomitant analysis of ^{133}Cs and ^{201}Hg in aqueous solution.

Instrument parameter	Value
RF-power	1400W
Nebulizer gas flow (Ar)	0.94 L/minute
Acquisition mode	Peak hopping
Dwell time	100 ms
Replicates	3

5.2.3 Consumption estimates

Feeding rates in pike were estimated using the biokinetics model based on the cesium tracer (Rowan and Rasmussen 1996, Kennedy et al. 2004). The biokinetics model, solved for consumption *(C),* was parameterised closely following Rowan and Rasmussen (1996), however without the need for a decay parameter *D*:

$$C = \frac{\left(Q_t - Q_0 e^{-Et} + Q_g\right)(G + E)}{\alpha \cdot \left[Cs_f\right] \cdot w_0 (e^{Gt} - e^{-Et})} \quad (1)$$

where Q_0, Q_t and Q_g are Cs burdens in somatic tissues at time *0* and time interval *t*, and gonadal tissue, respectively, *E* is Cs elimination, *G* is growth, w_0 is the initial weight of fish, α and $[Cs_f]$ are the assimilation efficiency and concentration Cs in the diet, respectively. Dietary Cs concentrations and diet-taxa specific assimilation factors (Rowan and Rasmussen 1996 and references therein) were incorporated into the model as a composite weighted by proportional contribution of each taxon to the whole diet (Table 5.2). In order to simplify the analysis for populations in which diet-shifting was known to occur, the breakdown for small pike, which were generally more abundant in samples, was assumed representative.

The model was run on the basis of a daily time-step over an interval of 365 days. Daily growth increments were calculated from annual growth in body mass between adjacent age classes, which was determined from known length-at-age growth models (Chapter 3) and weight-to-length regressions specific for each lake. Cesium concentrations in pike muscle were regressed against fork length for each population, in order to interpolate estimates for intermediate age classes not obtained during sampling efforts. Initial and final body burdens of Cs (Q_0 and Q_t, respectively) were determined

using regression-predicted Cs concentrations. Samples of mature gonad tissue were not available for this study and published GSI data was used in this study (Craig 1996 and references therein). Gonad mass was hence expressed as a composite population average weighted by the sex ratio in each sample. The Cs burden in gonads was calculated assuming a constant gonadal/somatic concentration partitioning factor of 0.1.

The elimination of Cs from the body was estimated using the relationship

$$E = -6.583 - 0.111 \cdot (\ln W) + 0.093 \cdot T \tag{2}$$

which requires fish weight W and a temperature T estimate (Rowan and Rasmussen 1995). Temperature was estimated on a whole-lake basis using LandSat imagery available monthly or bimonthly for the study period, to augment sporadic surface water measurements. Surface water temperatures on a given Julian day were taken as representative of the upper 2m of water column, due to overall shallow depth of reservoirs and wind-mixing. LandSat images were selected based on cloud-cover criteria and time of day. Over-night scans were preferred to eliminate artefact due to solar reflection. QGIS 2.0.1 software was used to extract contour polygon areas and surface temperature was calculated as mean weighted by contour area for each reservoir. Seasonal temperature regimes were generated by fitting a cosine-harmonic function to measured temperature data, using an amplitude between +4°C (under ice) and the summer maximum and assuming a 230-day ice-free period between Julian day 90 and 320. Summer maximum temperature as determined from satellite imagery varied by only 1°C among reservoirs. Model input parameters are summarized in Table 5.3.

In order to express absolute daily ration in energy units, the energy density (ED) in diets was estimated by weighting values taken from literature sources (Kelso 1977, Bryan et al. 1996, Hanson et al. 1997, Madenjian et al. 2006, Ward et al. 2008) by the proportion of each prey taxon (Table 5.2). Consumption rates (g/g/day) were then converted from specific mass-basis to absolute daily ration in energy units (kJ/day) using these weighted mean values. Energy density information for northern pike for the calculation of activity cost in energy units was taken from Diana (1983).

5.2.4 Model sensitivity

The model was tested for its sensitivity to bias in sampled sex-ratios, since differences in reproductive investment between males and females are known to affect consumption (Rowan and Rasmussen 1996). This study employed the model to obtain population-level consumption estimates and reproductive investment parameters were thus weighted by sex ratio in samples collected. Skewness in the sex ratio of samples may introduce bias to estimates of consumption obtained through this model. Therefore the effect of sex ratio on the results was assessed by comparing results obtained using original sample sex ratios with results obtained by assuming a 1:1 sex ratio. For piscivorous pike, in which samples were dominated by females on average, this comparison showed that daily ration estimates based on a forced 1:1 sex ratio were on average 12.4% lower across age classes relative to sampled sex ratios. For benthivorous pike, in which samples were skewed towards males, daily ration estimates were on average 2% higher when the sex ratio was set to 1:1. Thus, removal of sex-bias strengthens the effect of trophic regime on daily ration in pike and it is noted that in this

analysis unevenness in sex ratios may have increased the likelihood of Type II error more so than Type I error.

Table 5.2: Prey composition, Cs concentrations, assimilation constants and weighted mean dietary energy density (ED) for the estimation of daily ration in northern pike from reservoirs in the upper SSRB.

Reservoir	Prey taxa	Dietary contribution	[Cs] (ng/g)	Taxon-specific Cs assimilation[1]	Dietary ED (kJ/g)
Keho	Lake whitefish	90%	10.89	0.69	8.90[a,b]
	Cyprinids	10%	4.91	0.69	
Newell	Lake whitefish	75%	5.76	0.69	8.29[a,b]
	Yellow perch	25%	8.79	0.69	
Chin	Lake whitefish	35%	7.35	0.69	5.78[a,b]
	Longnose sucker	55%	8.02	0.69	
	Cyprinids	10%	6.70	0.69	
Scope	Amphipods	47%	14.60	0.18	4.44[a,b,c,d]
	Insects	20%	2.14	0.3	
	Yellow perch	12%	14.14	0.69	
	White sucker	12%	8.63	0.69	
	Lake whitefish	9%	9.77	0.69	
Twin Valley	Amphipods	55%	16.26	0.18	4.13[b,c]
	Insects	20%	2.43	0.3	
	White sucker	25%	3.83	0.69	
Hays-Grantham	Amphipods + Insects	80%	2.43	0.3	3.75[b,c,e]
	Brook stickleback	17%	8.24	0.69	
	Cyprinds	3%	6.91	0.69	
CPR	Amphipods	60%	4.75	0.18	3.80[b,c]
	Insects	30%	3.88	0.3	
	White sucker	5%	6.91	0.69	
	Cyprinids	5%	8.45	0.69	

[1] Values taken from Rowan and Rasmussen (1996)
Energy density data sources: [a]Madenjian et al. (2006), [b]Bryan et al. (1996), [c]Ward et al. (2008), [d]Hanson et al. (1997), [e]Kelso (1977)

Table 5.3: Age groups, Cs concentrations, body weights, growth rates, Cs elimination and resultant feeding rates (C) and activity multipliers (ACT) for northern pike from reservoirs in the upper SSRB.

Reservoir	Age	$[Cs]_t$ (ng/g)	$[Cs]_0$ (ng/g)	w_t (g)	w_0	G (g/g/d)	E (g/g/d)	C (g/g/d)	ACT
				Piscivorous					
Keho	2+	18.70	16.24	588.7	185.45	0.0048	0.0021	0.022	2.035
	3+	19.99	18.70	1176.7	588.7	0.0032	0.0020	0.016	1.651
	4+	20.68	19.99	1920.9	1176.7	0.0019	0.0019	0.014	1.692
	5+	20.99	20.68	3174.0	1920.9	0.0013	0.0018	0.013	1.648
	6+	20.87	20.99	4397.5	3174.0	0.0014	0.0017	0.011	1.547
	7+	20.52	20.87	5854.8	4397.5	0.0009	0.0016	0.010	1.536
Newell	2+	28.30	23.69	777.9	299.3	0.0048	0.0022	0.042	4.708
	3+	29.39	28.30	1451.7	777.9	0.0026	0.0020	0.031	3.840
	4+	28.38	29.39	2238.1	1451.7	0.0017	0.0020	0.024	3.230
	5+	26.43	28.38	3039.7	2238.1	0.0012	0.0019	0.020	2.804
	6+	23.31	26.43	4058.6	3039.7	0.0008	0.0018	0.016	2.383
	7+	19.38	23.31	5186.2	4058.6	0.0008	0.0018	0.012	1.875
Chin	2+	18.84	16.10	828.5	302.9	0.0046	0.0022	0.026	2.632
	3+	19.66	18.84	1880.0	828.5	0.0028	0.0020	0.019	2.095
	4+	18.92	19.66	2899.8	1880.0	0.0022	0.0019	0.013	1.579
	5+	17.35	18.92	4124.8	2899.8	0.0012	0.0019	0.010	1.321
	6+	15.62	17.35	5255.1	4124.8	0.0010	0.0018	0.008	1.112
				Benthivorous					
Scope	2+	24.62	20.55	888.4	549.0	0.0046	0.0022	0.033	3.518
	3+	23.09	24.62	1665.4	888.4	0.0013	0.0021	0.026	2.953
Twin Valley	2+	10.24	8.18	817.4	323.0	0.0049	0.0022	0.042	4.813
	3+	11.05	10.24	1092.8	817.4	0.0025	0.0022	0.031	3.937
	4+	11.93	11.05	1464.7	1092.8	0.0008	0.0021	0.028	3.703
	5+	12.77	11.93	1899.5	1464.7	0.0008	0.0021	0.027	3.779
	6+	13.19	12.77	2145.2	1899.5	0.0007	0.0020	0.024	3.508
Hays-Grantham	2+	6.66	4.78	525.6	184.7	0.0043	0.0026	0.036	3.296
	3+	8.75	6.66	1024.8	525.6	0.0029	0.0024	0.032	3.320
	4+	10.55	8.75	1588.4	1024.8	0.0018	0.0023	0.028	3.147
	5+	10.72	10.55	2106.7	1588.4	0.0012	0.0023	0.024	2.823
	6+	11.05	10.72	2482.3	2106.7	0.0008	0.0022	0.023	2.821
	7+	11.10	11.05	2958.0	2482.3	0.0004	0.0022	0.024	3.172
CPR	2+	10.43	9.44	745.8	468.6	0.0044	0.0023	0.030	3.249
	3+	11.38	10.43	1117.3	745.8	0.0013	0.0021	0.034	3.976
	4+	12.81	11.38	1827.5	1117.3	0.0011	0.0020	0.034	4.397
	5+	13.68	12.81	2404.8	1827.5	0.0014	0.0020	0.029	3.966
	6+	14.24	13.68	2840.5	2404.8	0.0008	0.0020	0.029	4.018
	7+	14.93	14.24	3425.3	2840.5	0.0005	0.0019	0.028	4.079

5.2.5 Activity estimates

Fish activity is expressed as a multiplier to resting metabolism, *M*, in common bioenergetic models. Here, estimates for the activity multiplier for each pike population were obtained with the energy budget equation used by Rowan and Rasmussen (1996 and references therein), which, when solved for ACT assumes the form:

$$ACT = \frac{C - (G + S + F + U)}{M} \tag{3}$$

where *G, S, F, U* represent budget allocation towards growth, specific dynamic action, fecal and urinary losses, respectively. Estimates for *S, F, U* and *M* for pike were obtained from the metabolic information given by Bevelhimer et al. (1985) and Table 1 in Kitchell et al. (1977). Activity cost (*A*) was calculated according to Pazzia (2002) as

$$A = (ACT \cdot R) - R \tag{4}$$

where *R* is absolute resting metabolism expressed in energy units, yielding *A* in units kJ/day.

5.2.6 Mercury mass balance model

The mercury mass balance model (MMBM) to predict the accumulation of mercury in fish tissue over a period of feeding and growth assumed the form discussed by Trudel and Rasmussen (2006):

$$[C_t] = [C_0] \cdot e^{-(E+G)t} + \frac{\alpha \cdot [C_d] \cdot C}{E + G}\left[1 - e^{-(E+G)t}\right] \tag{5}$$

where $[C_0]$ and $[C_t]$ are the mercury concentrations at the beginning and end of time interval *t*, respectively, $[C_d]$ is the mercury concentration in the diet and remaining

variables represent parameters as described for eq. 1. The model was run on a daily time step over a time interval of 365 days. The value for [C_0] was set to the concentration predicted from length-THg regressions and length-at-age models for age 2+ pike. [C_0] for subsequent age classes assumed the predicted final value, [C_t] on day 365, of the preceding age class. Values for [C_d] were obtained from chapter 4. Mercury elimination E was calculated using the body-weight (W) dependent relationship introduced by Trudel and Rasmussen (1997):

$$E = 0.0029 \cdot W^{-0.20} \cdot e^{0.066 \cdot T} \quad (6)$$

where T represents temperature, and assumed an annual cycle as described for eq. 2.

5.2.7 Statistical analysis

Statistical analyses were run R version 2.15.2 (2012). Responses of absolute daily ration (in terms of mass and energy), and activity cost to the effect of trophic regime were tested using ANCOVA models with body weight as covariate. Response variables and the body weight covariate were $\log_{10}$ transformed. The covariate was also x-centered by subtracting respective group means from each value following log-transformation. The performance of the MMBM in predicting [THg] in pike concentrations from dietary and bioenergetics information was tested by analyzing the relationship between MMBM-predicted and observed concentrations (measured in Chapter 4) through a linear model fit. Model coefficients of this relationship were checked for proximity to the diagonal (slope = 1, intercept = 0). In addition, potential differences in model performance in relation to trophic regime were assessed by comparing linear-model slope and intercept coefficients among trophic regimes.

5.3 Results

5.3.1 Feeding rates

Feeding rates in piscivorous pike declined from 0.032 g/g/day at age 2+ to 0.013 g/g/day at age 7+ (Table 5.3). In benthivorous pike feeding rates declined comparatively little with age from 0.034 to 0.026 g/g/day. Feeding rate estimates were similar to piscivorous pike only for age 2+ pike and trended well above estimates for piscivorous pike in age > 3+. Mean absolute daily ration (g/day) ranged from 15.6 to 72.3 g/day in piscivorous pike ages 2+ to 7+, respectively, and from 24.1 to 119.3 g/day in benthivorous pike for the same age range. Differences in $\log_{10}$(DR) on a mass basis between trophic regimes were statistically significant (Figure 5.1A, Table 5.4). The interaction term of the ANCOVA was also significant and suggested that slope coefficients of the relationship between body weight and absolute mass based DR were significantly higher for benthivorous pike relative to piscivorous pike (Figure 5.1A, Table 5.4). When daily ration estimates were expressed in energy units (kJ/day) and analyzed in relation to body weight, piscivorous pike consumed significantly more energy (approximately 1.5-fold on average) than benthivorous pike (Figure 5.1C, Table 5.4). Slope coefficients for the relationship between body weight and absolute energy consumption did not differ significantly among trophic regimes.

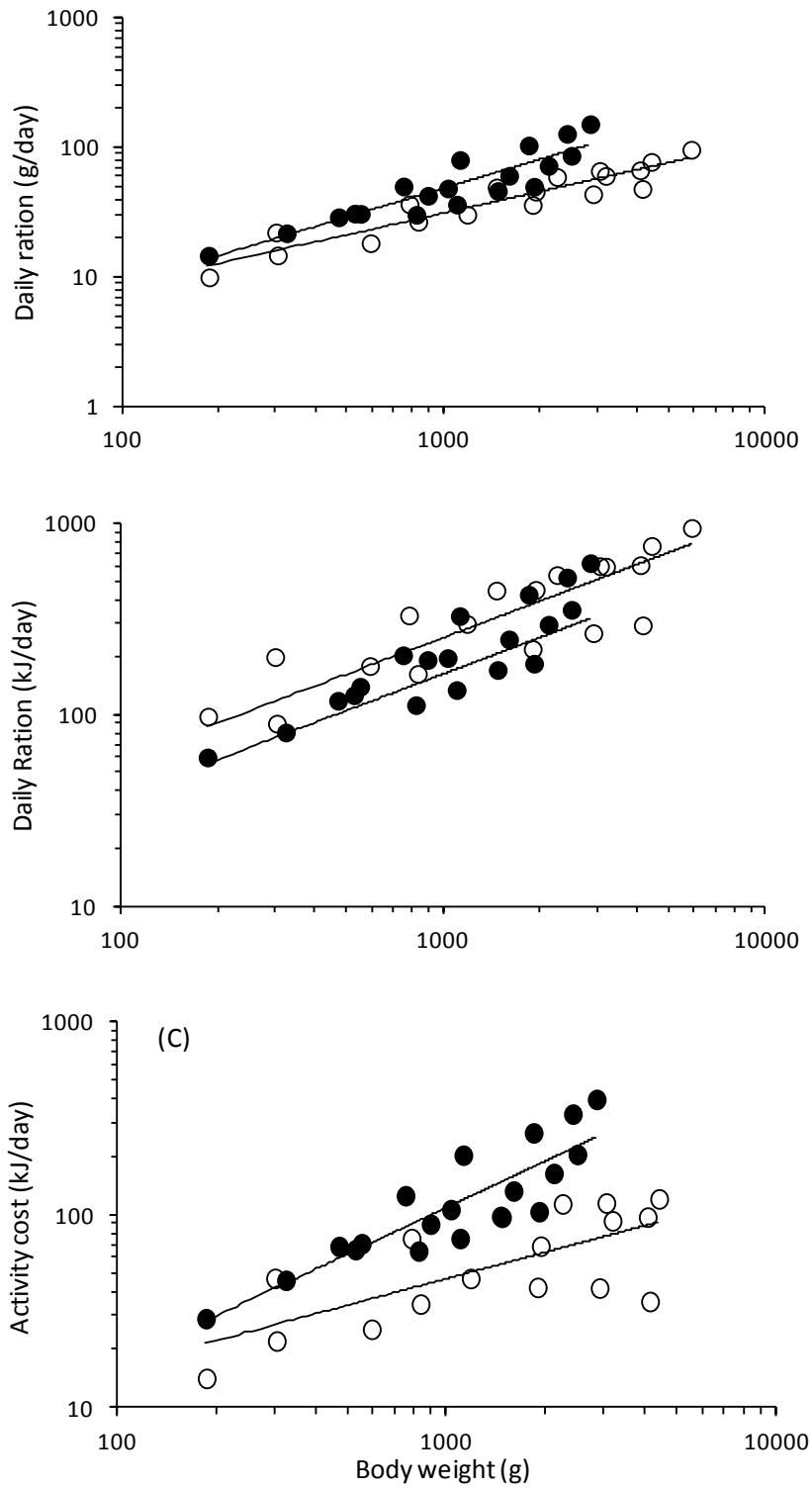


Figure 5.1: Absolute daily ration on mass basis (A), energy basis (B) in relation to body weight in piscivorous (open symbols) and benthivorous (solid symbols) northern pike. (C) Activity cost against body weight in pike among trophic regimes. Each data point in panels (A) through (C) represents one age-class-within-reservoir average. Statistics and model are summarized in Table 5.4.

5.3.2 *Activity cost*

Feeding rates were used to calculate *ACT* in the energy budgets (equation 3) and activity cost estimates generates (equation 4) for individual age classes within each pike population. Activity multipliers in piscivorous pike declined from 3.4 on average in age 2+ pike to 2.0 in age 7+ pike (Table 5.3). In contrast, *ACT* averages in benthivorous pike varied less with age and were maintained around an overall average of 3.5 (Table 5.3). Activity costs (kJ/day) regressed significantly against body weight (age class means for each reservoir) of pike and the slope of the relationship was significantly higher in benthivorous pike. The intercept coefficients at the x-centered point of comparison also differed among trophic regimes (Figure 5.1C, Table 5.4).

5.3.3 Mercury mass balance predictions

To test whether daily ration estimates could help explain differences in mercury biomagnification rates in pike among trophic regimes, mercury concentrations in pike were predicted using a mercury mass balance model. In piscivorous pike, the predicted THg concentration (dry wt.) increased from 0.91 µg/g on average at age 2+ to 2.58 µg/g at age 7+. In benthivorous pike THg increased from 0.33 to 1.20 µg/g over this age-range (Fig. 5.2A). Mass-balance model predictions deviated from measured values by 5.9% and 7.7% on average in piscivorous and benthivorous pike, respectively. The goodness of fit of predicted relative to measured THg values was analysed statistically by fitting linear models. Model coefficients were not significantly different among trophic regimes, passed through the origin and had a regression slope of 0.89. A linear model analysis of observed versus predicted THg relative to the 1:1 diagonal showed that the slope differed

significantly from 1. The coefficient estimate suggests that the biokinetics model may underestimate THg in pike by approximately 7% overall. The overall R^2 of the regression was 0.99 (Fig. 5.2B, Table 5.4).

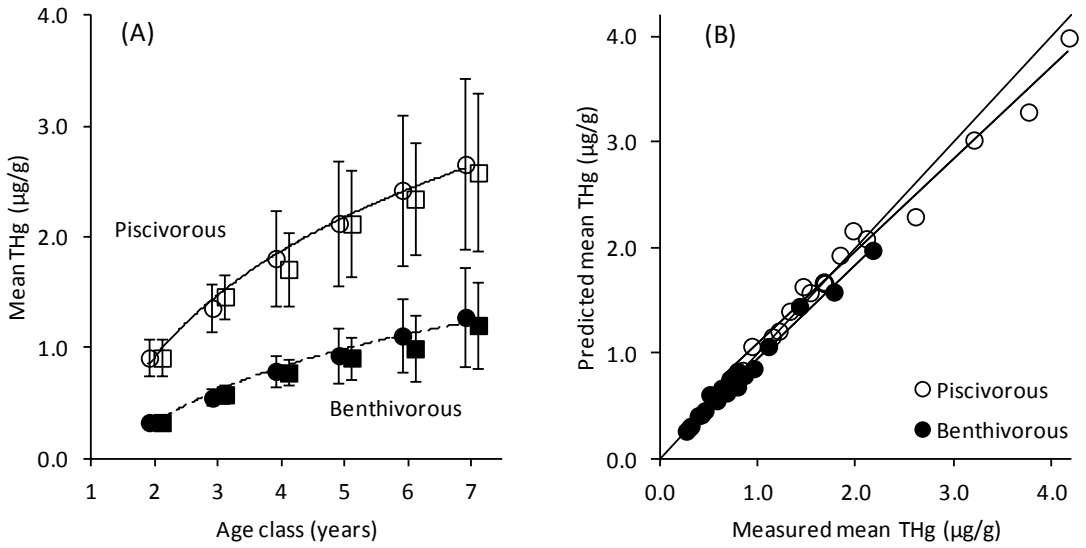


Figure 5.2: A) Measured (circles) and MMBM-predicted (squares) mean total mercury (THg, dry weight basis) in northern pike ages 2-7 in piscivorous (open symbols) and benthivorous (solid symbols) trophic regimes in upper SSRB reservoirs. B) Measured versus predicted mean total mercury in relation to the diagonal (1:1). Data points represent age-classes in individual study reservoirs.

5.4 Discussion

5.4.1 Northern pike feeding rates

This study presents an analysis of bioenergetic variation in northern pike in response to prey base differences creating piscivorous and benthivorous trophic regimes in irrigation reservoirs. For this, feeding rates, estimated using a stable-Cs biokinetics approach, were compared among populations grouped by trophic regime. Furthermore, activity levels were estimated from the energy budgets of pike and compared in relation

to body weight and trophic regime. Specific feeding rates obtained with the stable Cs tracer encompassed ranges between 0.036 and 0.008 g/g/d and are thus similar to estimates obtained using the original radio-tracer method for northern pike (Rowan and Rasmussen 1996). Especially estimates for piscivorous pike herein, which ranged below 0.025g/g/d at age 3 and older, lay within the range of results obtained by others (Rowan and Rasmussen 1996).

Table 5.4: Comparisons of bioenergetics parameters in pike in relation to trophic regimes in the upper SSRB reservoir system. Summary of statistical test results and models.

Comparison	Test	Statistics	Model
		Bioenergetics	
Absolute daily ration (g/day) Figure 5.1(A)	ANCOVA	Effect: Trophic regime $F_{(1,32)} = 13.85$; $p = 0.0008$ Effect: Body weight $F_{(1,32)} = 173.31$; $p < 0.0001$ Interaction $F_{(1,32)} = 4.25$; $p = 0.048$	*Piscivorous:* $log_{10}(DR) = 0.54*log_{10}(BW) + 1.56$ *Benthivorous* $log_{10}(DR) = 0.74*log_{10}(BW) + 1.69$ $R^2 = 0.84$
Absolute daily ration (kJ/day) Figure 5.1(B)	ANCOVA	Effect: Trophic regime $F_{(1,32)} = 14.84$; $p = 0.0005$ Effect: Body weight $F_{(1,32)} = 89.52$; $p < 0.0001$ Interaction $F_{(1,32)} = 2.31$; $p = 0.138$	*Piscivorous* $log_{10}(DR) = 0.74*log_{10}(BW) + 2.49$ *Benthivorous* $log_{10}(DR) = 0.74*log_{10}(BW) + 2.30$ $R^2 = 0.75$
Activity cost (kJ/day) Figure 5.2(C)	ANCOVA	Effect: Trophic regime $F_{(1,32)} = 29.17$; $p < 0.0001$ Effect: Body weight $F_{(1,32)} = 55.65$; $p < 0.0001$ Interaction $F_{(1,32)} = 4.52$; $p = 0.041$	*Piscivorous:* $Log_{10}(AC) = 0.46*log_{10}(BW) + 1.72$ *Benthivorous:* $log_{10}(AC) = 0.80*log_{10}(BW) + 2.04$ $R^2 = 0.71$
		Mercury mass balance model	
Hg mass balance Predicted versus observed [THg] in pike Figure 5.3	Linear regression	Effect: Observed [Hg] $F_{(1,35)} = 2026$; $p < 0.0001$ *Relative to 1:1 line:* Effect: Observed [Hg] $F_{(1,35)} = 18.79$; $p = 0.0001$	$[Hg]_{pred.} = 0.89*[Hg]_{obs.} + 0.059$ (intercept: $p = 0.13$) $R^2 = 0.99$ *Relative to 1:1 line:* $[Hg]_{pred.} = -0.08*[Hg]_{obs} + 0.059$

Specific feeding rates generally declined with age in both trophic regimes beginning at similar levels in the youngest age classes. However, this trend was weaker in

benthivorous pike, and consequently, the oldest benthivorous pike consumed 2-fold higher specific rations than piscivorous pike. Averaged across all ages feeding rates were still 1.5-fold higher on a mass basis in benthivorous pike. This difference in trend with ontogeny was confirmed statistically by the relationship between absolute mass-based daily ration against body weight. Here significantly different slopes (interaction) suggested that benthivorous pike increased their ration at a greater rate with body size compared with piscivorous pike. Significantly different intercept coefficients at the x-centered point of comparison also implied significant divergence in consumption between trophic regimes. However, patterns in daily ration among trophic regimes were reversed when ration estimates were expressed in energy units, which showed that piscivorous pike consumed approximately 1.5-fold more energy relative to benthivorous pike. Thus, comparatively high prey mass consumption by benthivorous pike was not sufficient to yield gross energetic returns similar to those seen for under the pisciviorous regime. Nonetheless, the pattern seen here in pike, albeit more accentuated, is similar to patterns observed in lake trout, as an example of another top-consumer fish species (Pazzia et al. 2002). While non-piscivorous lake trout were shown to consume more prey mass than piscivorous lake trout, their respective energy consumption rates were at least similar, which resulted from differences in the energy density in zooplankton versus fish prey.

As was previously shown for piscivorous and non-piscivorous lake trout (Pazzia et al. 2002), the estimation of feeding rates for pike permits a more meaningful interpretation of their growth rates, which were shown to be significantly reduced in benthivorous pike (Chapter 3). The analysis of bioenergetics herein clearly shows that two factors explain low realized growth in benthivorous pike. For one, the low energy

density of invertebrates resulted in comparatively low energy consumption, even though benthivorous pike consumed more prey mass than piscivorous pike. Furthermore, the analysis demonstrated that increased bioenergetic losses to metabolism may contribute to reducing growth rates in benthivorous pike and several factors may play a role in this.

5.4.2 Northern pike activity

Activity multipliers were on average 1.5-fold higher in benthivorous pike. Fish activity is expected to scale with consumption (Boisclair 1992). Consistent with this, as benthivorous pike maintained comparatively high feeding rates through the course of ontogeny, activity levels remained similarly elevated, whereas declining activity with age was seen for piscivorous pike. Activity cost in absolute energy units increased through ontogeny (with fish size) in both trophic regimes, however more rapidly in benthivorous pike, and patterns were thus in close agreement with those seen in other piscivorous fish species (Sherwood et al. 2000, Pazzia et al. 2002, Henderson et al. 2004). Previous discussions have highlighted that foraging costs increase as the predator:prey-size ratio increases (Boisclair and Leggett 1989, Sherwood et al. 2002b). According to optimal foraging theory (Stephens and Krebs 1986), predators are therefore expected to routinely adjust the predator:prey size ratio through diet shifts towards prey of larger size in order to reduce foraging costs and maintain an energetic surplus to permit growth (Mittelbach and Persson 1998, Giacomini et al. 2013). Failure to realize diet shifts can result in ‘stunted’ growth, known as a trophic bottleneck (Heath and Roff 1996, Sherwood et al. 2002a). Previous dietary and trophic position analyses of pike in the study reservoirs have shown that benthivorous pike did not shift significantly from invertebrates to fish prey with increasing body size. In contrast, under the piscivorous regime pike increased in

trophic position by ~0.5-0.7 levels, which indicated a continuous shift towards larger prey fish, predominantly highly energy-dense lake whitefish, throughout ontogeny. Thus, diet shifting in the presence of suitable forage fish communities, may result in an energy surplus for piscivorous pike, which permits a reduction in overall consumption and thus minimizes foraging cost following juvenile stages.

Another factor that may exacerbate foraging cost for pike feeding on invertebrate prey may be their morphology, which is clearly best adapted for the capture of large prey from an ambush, making pike poorly suited to forage actively on small prey items. Invertebrates present a small visual target, and may be difficult for pike to track, when in the 'blind spot' immediately in front of the mouth. Thus target acquisition prior to capture may require significant rotation and repositioning movements for comparatively small bioenergetic reward. Furthermore, the gill rakers of pike are highly reduced to permit the easy passage of water during speed strikes with an open mouth (Webb and Skadsen 1980, Craig 1996). This could result in a significant portion of small prey escaping from the oral cavity, and therefore increased costs of foraging. Thus, albeit more abundant than fish prey in the vicinity of foraging pike, the process of capture of invertebrate prey may be less efficient for pike than the capture of fish prey.

In consideration of Jobling's (1981) work, the possibility that specific dynamic action (SDA) may change with both ration size and dietary quality should also be discussed. Here SDA was modeled as a function of ration size (Bevelhimer et al. 1985) and food quality was not considered for simplicity. In any case, however, according to Jobling (1981), SDA scales positively with digestible energy in the diet, and SDA-associated metabolic cost should therefore be lower in benthivorous pike compared with

pike feeding on a more energy-dense fish diet. Thus metabolic activity associated with digestion of invertebrate prey cannot immediately explain the observed reduced growth in benthivorous pike.

5.4.3 Mercury mass balance predictions

One major goal for the estimation and comparison of feeding rates in piscivorous and benthivorous pike stemmed from the mercury dynamics observed in this study system. Piscivorous pike were observed to have higher mercury concentration, consistent with longer food chains and overall higher dietary exposure, than benthivorous pike. However, benthivorous pike were shown to exhibit higher biomagnification relative to piscivorous pike, which, in combination with differences in growth rates, formed the basis for the idea that pike bioenergetics associated with each feeding regime affected the mercury dynamics in these reservoirs. In order to test this hypothesis, ration estimates obtained were used with previously collected information on dietary mercury concentrations and growth to predict mercury concentrations in pike through their ontogeny (Trudel and Rasmussen 2001), followed by a comparison with measured values. Although the mercury biokinetics model may slightly underestimate expected values, which may be associated with uncertainty in the elimination kinetics of mercury in pike (Van Walleghem 2013), predicted mercury concentrations were nonetheless in very close agreement with measured values. The most important factors determining mercury accumulation in fish are mercury uptake and fish growth (Trudel and Rasmussen 2006). Mercury uptake is a function of dietary concentrations and feeding rates. Mercury concentrations in the invertebrate prey consumed by benthivorous pike were 3 to 4-fold lower than in the prey fish consumed by piscivorous pike (compare 0.09µg/g versus 0.25-

0.36µg/g, respectively). However, feeding rates were up to 2-fold higher in older age-class benthivorous pike and it becomes clear that lower mercury concentrations in their diet are partially offset by their elevated consumption. Assimilated mercury is stored in body tissues and is effectively diluted by body growth (Trudel and Rasmussen 2006, Lepak et al. 2012). Here, the analysis showed that benthivorous pike consumed lower amounts of energy for a comparatively higher cost associated with foraging on invertebrates. Thus less energy is available for conversion to body tissue and therefore to dilute assimilated mercury in benthivorous pike. Consequently the concentration of mercury in pike tissue relative to prey tissue increases at a faster rate in benthivorous pike relative to piscivorous pike. Thus, the importance of available prey forage in influencing mercury dynamics in the upper SSRB system of reservoirs is supported by the mercury kinetics model.

Secondarily, it is noteworthy that through accurately predicting mercury dynamics in these reservoirs the mercury mass balance model also served to independently validate bioenergetics estimates made with the stable-Cs tracer. Although there is little reason to expect that stable Cs would behave differently than the radio-isotope, on which this method was originally developed, and has been previously used as a bioenergetics tracer (Kennedy et al. 2004, Guenard et al. 2008), the method has not been validated and remains under-applied. The results obtained here corroborate that stable Cs may be a suitable substitute tracer for radioactive Cs in bioenergetics studies, while bearing the advantages of higher environmental abundance and relatively economical measurement and co-measurement with other elements on ICP-MS (also see Kennedy et al. 2004).

Chapter 6

Conclusion

6.1 Thesis summary

One of the main objectives of this study was to examine the trophodynamics of northern pike in response to the prey community structure in irrigation reservoirs in the upper South Saskatchewan River basin. In order to accomplish this, data collection included observations from both direct stomach contents analysis and $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratio measurements. An initial comparison of $\delta^{15}N$ values for pike with values for stomach contents of pike showed that diet-tissue isotope ratio shifts in pike were 2.4‰ and differed from the widely assumed value of 3.4‰. Accurate diet tissue shifts are required when $\delta^{15}N$ values are used in trophic position analyses and in mixing models. This analysis resolved a long-standing discrepancy between trophic position estimates derived from stomach contents data and stable isotope analysis in northern pike (Vander Zanden et al. 1997). Furthermore, the model commonly used to estimate trophic position from $\delta^{15}N$ values was amended to incorporate anomalous diet-tissue shifts in terminal prey-consumer links, thus bringing trophic position analyses from stable isotopes into agreement with stomach contents analysis.

The feeding ecology of pike was compared among reservoirs with and without lake whitefish present in the prey fish community. Where present, lake whitefish were found to represent a major component in the diet of pike. Lake whitefish also had an effect on the diet ontogeny of pike, which increased in trophic position with age, indicating that pike exploited progressively larger prey as they grew. In the comparison group of reservoirs without lake whitefish, it was important to find that pike exploited

predominantly benthic invertebrates rather than other fish, suggesting that the prey fish community may have been too unproductive, or in other ways unsuitable to be exploited by pike. Under this trophic regime pike maintained a low trophic position through ontogeny, feeding continuously on invertebrate prey. Thus, benthivorous trophic regimes for pike are not uncommon among reservoirs in the upper SSRB, and appear to coincide with poor coupling between pelagic resources and consumers at the apex of food webs. Growth rates of pike were significantly higher in piscivorous regimes, relative to benthivorous regimes, in which flat growth curves indicated a trophic bottleneck in response to simplified food webs (Sherwood et al. 2000, Sherwood et al. 2002a).

This research project also conducted an analysis of pike mercury concentrations in relation to food web structure in reservoirs. Mercury concentrations were significantly higher in pike under piscivorous trophic regimes, which was attributable largely to relatively high dietary exposure associated with fish prey. The invertebrate prey consumed by benthivorous pike was 3 to 4-fold lower in mercury, which explained comparatively low mercury concentrations in pike tissue. However, mercury biomagnification was significantly higher in benthivorous pike relative to piscivorous pike, suggesting poor food conversion efficiency in pike feeding on invertebrates and supporting that trophic regimes had significant bioenergetic consequences.

The consequences of trophic simplification on pike bioenergetics were confirmed with an energy budget analysis using a cesium-biokinetics method. Benthivorous pike consumed more prey mass than piscivorous pike, however, the pattern was reversed when consumption rates were corrected for dietary energy densities, which were lower in invertebrates than in fish prey. Due to increased prey consumption by mass, activity cost

was significantly higher in benthivorous pike, which explained the low food conversion efficiency seen in benthivorous pike. In combination with dietary exposure, the role of bioenergetics in determining mercury concentrations in pike was shown by a mercury-biokinetics model, which yielded predictions of mercury accumulation in pike in very close agreement with observed values.

Trophic transfer and biomagnification of mercury and cesium permits their use as research tools to quantify important bioenergetics parameters, such as feeding rates and activity in biokinetics models (Trudel and Rasmussen 2006). Using the stable isotope of cesium as trophic tracer, which is relatively abundant in the environment and with confidence meets the steady-state assumption, is an improvement over the use of radiocesium, which is limited in its application by high-clay environments (e.g. upper SSRB), and by requiring decay-rate parameters to accounts for violations of steady-state assumptions due to pulse-releases.

6.2 Management directions for fish growth

Reservoir projects are often contested by critics citing the adverse effects of impoundments on river health. It is often not fully realized that the reservoir formed by river impoundment has potential as lentic habitat, and may thus provide social benefits in the form of fisheries, over and above basic hydrological engineering objectives. The successful establishment of fisheries in reservoirs depends on growth rates and yields of target species, which are often apex consumers in inland recreational fisheries, and some commercial fisheries (Kitchell et al. 1997). Some have extended the application of models predicting fish yields in lakes, which are often based on simple correlations with primary productivity (Adams et al. 1983, Downing et al. 1990), to reservoirs (Jenkins and

Oglesby 1982, Jones and Hoyer 1982). While this may apply in the case of some very large projects (Baxter 1977), others have highlighted that perturbations associated with reservoir operations can impair the resource base at multiple levels in food webs, which suggests that trophic linkage can also become an important factor in limiting fish production in reservoirs (Ploskey and Jenkins 1982). As an example, water level fluctuations (=drawdown) may severely impair inshore habitat development and therefore limit feeding opportunities for large predatory fish species, which alternatively, may have to rely on prey sources linking into pelagic production in reservoirs (Lindstrom 1973). Research presented here suggested that introduced zooplanktivorous lake whitefish perform an important coupling role between pelagic production and apex consumers in southern Alberta reservoirs. Where present in abundance, lake whitefish formed an important component in the diet of northern pike, the predominant apex consumer and fisheries target species. Of particular significance, however, was the finding that, when lake whitefish were not present in significant numbers, pike resorted to exploiting mainly benthic invertebrates. This suggests that littoral and benthic prey fish communities, which were derived from lotic tributary communities without specific management intervention, could not form a suitable prey base for pike. An analysis of growth rates and bioenergetics of pike in relation to trophic regime showed that pike exploited lake whitefish highly efficiently. The relatively high energy density of lake whitefish permitted an overall reduction of prey consumption by mass, which in turn reduced foraging costs for pike and enhanced their growth. Thus, the bioenergetic advantage of a lake whitefish prey base is clearly established. In summary, reservoir fisheries may ultimately depend on the establishment of a prey base tolerant of operational practices

and capable of coupling production in the pelagic zone with apex consumers in reservoirs.

6.3 Management directions for mercury mitigation

Mercury can reach high levels in reservoirs due to releases from soil and vegetation as a direct result of inundation. This, in conjunction with factors conducive to the production of highly bioavailable and biomagnifiable methylmercury, can increase mercury concentrations in reservoir fish populations to levels of concern to human and wildlife health for several decades post-impoundment (Verta 1990, Bodaly et al. 2007). Mercury contamination is therefore one of the principle risk factors distracting from the socio-economic benefits realized from creating fisheries on reservoirs. Several strategies have been developed to mitigate mercury contamination, and the efficacy and cost-effectiveness of each may be context-dependent (Mailman et al. 2006). For example, removal of standing vegetation prior to flooding partially removes mercury from the terrain subject to inundation. Furthermore, less biomass is ultimately available for decomposition under water, which reduces the potential for methylmercury production. This process can also be influenced by adjustment of water chemistry, such as neutralising pH or the addition of selenium, which greatly reduce bioavailability of mercury and increase depuration. In the context of the upper SSRB, and its grasslands dominated landscapes, burning of vegetation may not reduce mercury loadings to reservoirs significantly, however, the practice may alleviate loadings of organic matter during flooding and thus reduce mercury methylation potential post-impoundment. pH levels in the upper SSRB are also naturally high (7.5-9.0), thus acidity is not a major

factor contributing to the mercury problem in these reservoirs. Selenium addition may be a strategy worth evaluating in these systems.

Higher organisms acquire mercury mainly through trophic transfer from their diet. Mercury then becomes concentrated, or 'biomagnifies', in the consumer relative to its diet, because mercury assimilation rates are higher than elimination and growth. Thus, alternative approaches to mitigating mercury problems in reservoirs may involve reducing bioaccumulation or biomagnification of mercury through food webs (Mailman et al. 2006). For example, nutrient enrichment increases algal biomass and effectively distributes a given amount of bioavailable methylmercury among a larger number of cells, a process known as 'algal bloom dilution' (Chen and Folt 2005), which reduces mercury concentrations at the food web base, and may secondarily contribute to sedimentation and burial of mercury in sediments. In organisms at higher trophic levels biomagnification can be controlled by ensuring an abundant and optimally exploited prey base, thereby maximizing growth efficiency of consumers (Lepak et al. 2012). The potential for this strategy to reduce mercury in southern Alberta reservoir developments was explored in Chapter 3, which compared mercury biomagnification in response to differences among the prey base. Mercury biomagnification factors were shown to be lower in pike feeding on lake whitefish, consistent with fast, efficient growth in pike under this trophic regime. However, mercury concentrations in pike in lake whitefish reservoirs were still higher than in the comparison group, which was attributable to up to 4-fold higher mercury concentrations in the lake whitefish-dominated diet. Therefore lake-whitefish may not constitute a prey base particularly suited to lower mercury concentrations in piscivorous fish consuming them.

6.4 Future research on reservoir fish communities

Prospective research may address the trophic ecology and mercury exposure dynamics in lake whitefish. For instance, lake whitefish are known to exploit predominantly zooplankton at smaller sizes and shift towards benthos as they grow. This diet shift may coincide with an increase in their dietary mercury exposure, which may explain relatively high mercury concentrations in their tissues (Eagles-Smith et al. 2008). The timing of this shift away from zooplankton during the ontogeny of lake whitefish may also be important, in that an early shift may coincide with higher mercury concentrations in smaller sizes of lake whitefish (all else equal), which form the predominant resource base to piscivorous consumers in these reservoirs.

One factor potentially influencing the timing of the diet shift in lake whitefish, may be the reservoir operational regime, which can affect the zooplankton resource base in a given reservoir. Zooplankton biomass is positively correlated with water residence time, which varies considerably among reservoirs in southern Alberta, in some cases impairing zooplankton production (Campen, unpublished). Given impoverishment of the zooplankton resource base, lake whitefish in affected reservoirs may adopt benthic resources earlier during their ontogeny, compared with reservoirs rich in zooplankton, and therefore result in higher mercury exposure earlier in their life. Clearly, our understanding of the trophic ecology of lake whitefish in these reservoirs and of the potential repercussions of operational regimes is highly rudimentary at this point. Future studies should investigate lake whitefish growth in relation to resource base, the nature and timing of potential diet shifts, and the influence this may have on mercury exposure pathways in reservoir food webs.

Another aspect worth considering is that lake whitefish are cold-water, glacial relic species, occurring naturally in cold, subalpine lakes in southern Alberta. Reservoir temperatures, however, can exceed 23°C during the summer, which is close to the reported upper temperature tolerance of ~25°C (Edsall and Rottiers 1976). Based on this it is conceivable that lake whitefish may come under considerable temperature stress during the summer months, which may elevate their metabolic rate and thereby reduce their growth efficiency, which would result in elevated mercury biomagnification relative to their diet (Doyon et al. 1998). Furthermore, dissections of lake whitefish in the lab also revealed that nearly all were infected by the parasite *Ichthyocotylurus* sp., which was found encysted in the pericardium. Parasite loads were found to vary among reservoirs (Polo et al. 2011, personal comm.). Considering that parasites are normally a stress factor in animals (Marcogliese and Pietrock 2011) and therefore elicit a stress response elevating metabolic costs constraining growth in lake whitefish, one would expect to find a correlation between parasite loads and growth in lake whitefish. This may constitute another factor influencing mercury biomagnification patterns in lake whitefish.

6.5 Mercury pulse dynamics in reservoirs

Post-impoundment, mercury is released rapidly from inundated soils and vegetation, which provide the initial source pool of the mercury pulse in reservoirs (Montgomery et al. 2000, St Louis et al. 2004). This initial pulse is then transferred through successive compartments of the ecosystem, beginning with water and ending with fish and sediments, and presumably occurring with slight time-delays depending on transfer rates between compartments. The persistence of the pulse in each compartment depends on clearance rates, which are also variable. For example, the clearance rate of

mercury in the water column will depend largely on water residence time in a given reservoir (Rowan 2013). Since metabolic clearance in biota is relatively slow, clearance rates in biotic compartments will depend most importantly on the longevity of organisms comprising the compartment. For example, turnover rates of zooplankton and invertebrates are much faster compared with fish. Thus, in combination, duration of the mercury pulse is expected to be much shorter in water and invertebrates than in fish. It follows that a large amount of mercury in a reservoir may become associated with fish, whose concentrations continue to increase, after concentrations in water, zooplankton and benthic invertebrates are already in a state of decline. This also implies that, during later stages of the post-impoundment mercury pulse fish and their by-products (carcasses, excrement) may become a significant source of mercury in a reservoir. Based on this idea, one may propose that a stocking regime of a low trophic position fish species to 'trap' mercury in its tissues, followed by its targeted removal, could significantly shorten the duration of the mercury pulse in large recreational target fish species, such as pike and walleye. Large-scale fish removal from reservoirs has been proposed as a strategy to lower fish-mercury concentrations, mainly by reducing intraspecific competition for a limited resource base and thereby increasing growth efficiency and growth dilution of mercury (Verta 1990, Mailman et al. 2006). However, the effects of timed fish removal on mercury pulse dynamics in a reservoir ecosystem have generally received little research attention.

In reservoirs of the upper SSRB, a post-impoundment stocking regime may first encompass stocking of small lake whitefish, which may be sourced from annual fish-rescue efforts in draining irrigation canals. The successful establishment of lake whitefish

may require concomitant control of piscivores, mainly pike, through near-shore netting efforts with mesh sizes to select against stocked lake whitefish size classes. Stocking should continue for at least a few years. Mercury will accumulate in lake whitefish and potentially reach a plateau within 2-3 years. The population dynamics, trophic ecology and mercury concentrations in lake whitefish should be monitored and the decision to start fish removal should be based on this information. Removal efforts may focus on large size classes of lake whitefish, which exploit benthic invertebrates and incorporate this high-mercury pool into their tissues. The exact timing and procedures of lake whitefish removal, and ultimately piscivore introduction, are hypothetical at this point and will require experimentation and modelling research to draft definitive recommendations and protocols.

References

Abernathy, A.R., and Cumbie, P.M. 1977. Mercury accumulation by largemouth bass (*Micropterus salmoides*) in recently impounded reservoirs. Bull. Environ. Contam. Toxicol. **17**(5): 595-602.

Adams, S.M., Kimmel, B.L., and Ploskey, G.R. 1983. Sources of organic-matter for reservoir fish production - a trophic-dynamics analysis. Can. J. Fish. Aquat. Sci. **40**(9): 1480-1495.

Alberta Agriculture, P.F.R.D. 2000. Irrigation in Alberta. Irrigation Branch, Lethbridge.

Arrington, D.A., Winemiller, K.O., Loftus, W.F., and Akin, S. 2002. How often do fishes "Run on empty"? Ecology **83**(8): 2145-2151.

Auerswald, K., Wittmer, M., Zazzo, A., Schaufele, R., and Schnyder, H. 2010. Biases in the analysis of stable isotope discrimination in food webs. J. Appl. Ecol. **47**(4): 936-941.

Baxter, R. 1977. Environmental effects of dams and impoundments. Annu. Rev. Ecol. Syst. 255-283.

Beaudoin, C.P., Prepas, E.E., Tonn, W.M., Wassenaar, L.I., and Kotak, B.G. 2001. A stable carbon and nitrogen isotope study of lake food webs in Canada's boreal plain. **46**(4): 465-477.

Beaudoin, C.P., Tonn, W.M., Prepas, E.E., and Wassenaar, L.I. 1999. Individual specialization and trophic adaptability of northern pike (*Esox lucius*): An isotope and dietary analysis. Oecologia **120**(3): 386-396.

Berg, S., Jeppesen, E., and Søndergaard, M. 1997. Pike (*Esox lucius* l.) stocking as a biomanipulation tool 1. Effects on the fish population in Lake Lyng, Denmark. Hydrobiologia **342**: 311-318.

Berkeley, S.A., Hixon, M.A., Larson, R.J., and Love, M.S. 2004. Fisheries sustainability via protection of age structure and spatial distribution of fish populations. Fisheries **29**(8): 23-32.

Bevelhimer, M.S., Stein, R.A., and Carline, R.F. 1985. Assessing significance of physiological differences among three esocids with a bioenergefics model. Can. J. Fish. Aquat. Sci. **42**(1): 57-69.

Bloom, N.S. 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. Can. J. Fish. Aquat. Sci. **49**(5): 1010-1017.

Bodaly, R.A., Jansen, W.A., Majewski, A.R., Fudge, R.J.P., Strange, N.E., Derksen, A.J., and Green, D.J. 2007. Postimpoundment time course of increased mercury concentrations in fish in hydroelectric reservoirs of northern Manitoba, Canada. Arch. Environ. Contam. Toxicol. **53**(3): 379-389.

Boisclair, D. 1992. Relationship between feeding and activity rates for actively foraging juvenile brook trout (*Salvelinus fontinalis*). Can. J. Fish. Aquat. Sci. **49**(12): 2566-2573.

Boisclair, D., and Leggett, W.C. 1989. Among-population variability of fish growth. 2. Influence of prey type. Can. J. Fish. Aquat. Sci. **46**(3): 468-482.

Boudou, A., and Ribeyre, F. 1997. Mercury in the food web: Accumulation and transfer mechanisms. *In:* Metal ions in biological systems. Marcel Dekker AG. pp. 289-320.

Bowen, S.H., Lutz, E.V., and Ahlgren, M.O. 1995. Dietary-protein and energy as determinants of food quality - trophic strategies compared. Ecology **76**(3): 899-907.

Breck, J.E. 1993. Foraging theory and piscivorous fish: Are forage fish just big zooplankton? Trans. Am. Fish. Soc. **122**(5): 902-911.

Brinkmann, L. 2007. Mercury biomagnification in the upper South Saskatchewan River Basin., MSc thesis. Biological Sciences, The University of Lethbridge, Lethbridge, AB.

Brinkmann, L., and Rasmussen, J.B. 2010. High levels of mercury in biota of a new prairie irrigation reservoir with a simplified food web in southern Alberta, Canada. Hydrobiolgia **641**(1): 11-21.

Brinkmann, L., and Rasmussen, J.B. 2012. Elevated mercury levels in biota along an agricultural land use gradient in the Oldman River basin, Alberta. Can. J. Fish. Aquat. Sci. **69**(7): 1202-1213.

Browne, D.R., and Rasmussen, J.B. 2009. Shifts in the trophic ecology of brook trout resulting from interactions with yellow perch: An intraguild predator-prey interaction. Trans. Am. Fish. Soc. **138**(5): 1109-1122.

Bryan, S.D., Soupir, C.A., Duffy, W.G., and Freiburger, C.E. 1996. Caloric densities of three predatory fishes and their prey in Lake Oahe, South Dakota. J. Freshwater Ecol. **11**(2): 153-161.

Byström, P., Karlsson, J.A.N., Nilsson, P.E.R., Van Kooten, T., Ask, J., and Olofsson, F. 2007. Substitution of top predators: Effects of pike invasion in a subarctic lake. Freshwater Biol.**52**(7): 1271-1280.

Cabana, G., Tremblay, A., Kalff, J., and Rasmussen, J.B. 1994. Pelagic food-chain structure in ontario lakes - a determinant of mercury levels in lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. **51**(2): 381-389.

Casselman, J.M., and Lewis, C.A. 1996. Habitat requirements of northern pike (*Esox lucius*). Can. J. Fish. Aquat. Sci. **53**(S1): 161-174.

Chapman, C., and Mackay, W. 1984. Versatility in habitat use by a top aquatic predator, *Esox lucius* l. J. Fish Biol. **25**(1): 109-115.

Chapman, L.J., and Mackay, W.C. 1990. Ecological correlates of feeding flexibility in northern pike (*Esox lucius*). J. Fish Biol. **5**(3): 313-322.

Chapman, L.J., Mackay, W.C., and Wilkinson, C.W. 1989. Feeding flexibility in northern pike (*Esox lucius*) - fish versus invertebrate prey. Can. J. Fish. Aquat. Sci. **46**(4): 666-669.

Chen, C.Y., and Folt, C.L. 2005. High plankton densities reduce mercury biomagnification. Environ. Sci. Technol. **39**(1): 115-121.

Chevalier, G., Dumont, C., Langlois, C., and Penn, A. 1997. Mercury in northern québec: Role of the mercury agreement and status of research and monitoring. Water Air Soil Poll. **97**(1-2): 75-84.

Compeau, G.C., and Bartha, R. 1985. Sulfate-reducing bacteria - principal methylators of mercury in anoxic estuarine sediment. Appl. Environ. Microb. **50**(2): 498-502.

Craig, J. 1996. Pike: Biology and exploitation. Springer.

Craig, J.F. 2008. A short review of pike ecology. Hydrobiologia **601**: 5-16.

Depew, D.C., Burgess, N.M., Anderson, M.R., Baker, R., Bhavsar, S.P., Bodaly, R.A., Eckley, C.S., Evans, M.S., Gantner, N., Graydon, J.A., Jacobs, K., LeBlanc, J.E.,

St Louis, V.L., and Campbell, L.M. 2013. An overview of mercury concentrations in freshwater fish species: A national fish mercury dataset for canada. Can. J. Fish. Aquat. Sci. **70**(3): 436-451.

Diana, J.S. 1979. Feeding pattern and daily ration of a top carnivore, the northern pike (*Esox lucius*). Can. J. Zool. **57**(11): 2121-2127.

Diana, J.S. 1982. An experimental-analysis of the metabolic-rate and food utilization of northern pike. Comp. Biochem. Physiol. A-Physiol. **71**(3): 395-399.

Diana, J.S. 1983. Growth, maturation, and production of northern pike in 3 michigan lakes. Trans. Am. Fish. Soc. **112**(1): 38-46.

Diana, J.S. 1987. Simulation of mechanisms causing stunting in northern pike populations. Trans. Am. Fish. Soc. **116**(4): 612-617.

DiCenzo, V.J., and Duval, M.C. 2002. Importance of reservoir inflow in determining white bass year-class strength in three virginia reservoirs. North Am. J. Fish Manage. **22**(2): 620-626.

Downing, J.A., Plante, C., and Lalonde, S. 1990. Fish production correlated with primary productivity, not the morphoedaphic index. Can. J. Fish. Aquat. Sci. **47**(10): 1929-1936.

Doyon, J.F., Schetagne, R., and Verdon, R. 1998. Different mercury bioaccumulation rates between sympatric populations of dwarf and normal lake whitefish (*Coregonus clupeaformis*) in the La Grande complex watershed, James Bay, Quebec. Biogeochemistry **40**(2-3): 203-216.

Drymon, J.M., Powers, S.P., and Carmichael, R.H. 2012. Trophic plasticity in the atlantic sharpnose shark (*Rhizoprionodon terraenovae*) from the north central Gulf of Mexico. Environ. Biol. Fish. **95**(1): 21-35.

Duvail, S., and Hamerlynck, O. 2003. Mitigation of negative ecological and socio-economic impacts of the Diama dam on the Senegal River delta wetland (Mauritania), using a model based decision support system. Hydrol. Earth Syst. Sc. **7**(1): 133-146.

Eagles-Smith, C.A., Suchanek, T.H., Colwell, A.E., and Anderson, N.L. 2008. Mercury trophic transfer in a eutrophic lake: The importance of habitat-specific foraging. Ecol. Appl. **18**(8): A196-A212.

Edsall, T.A., and Rottiers, D.V. 1976. Temperature tolerance of young-of-year lake whitefish, *Coregonus clupeaformis*. J. Fish. Res. Board Can. **33**(1): 177-180.

English, W.G. 1977. The possible impact of high summer temperatures on the lake whitefish (*Coregonus clupeaformis*) populationj in scope reservoir. Government of Alberta, Lethbridge, Alberta, Canada.

Fernando, C.H., and Holcik, J. 1982. The nature of fish communities - a factor influencing the fishery potential and yields of tropical lakes and reservoirs. Hydrobiologia **97**(2): 127-140.

Gaye-Siessegger, J., Focken, U., Abel, H., and Becker, K. 2004. Individual protein balance strongly influences $\delta^{15}N$ and $\delta^{13}C$ values in nile tilapia, *Oreochromis niloticus*. Naturwissenschaften **91**(2): 90-93.

Gaye-Siessegger, J., Focken, U., Abel, H., and Becker, K. 2007. Starvation and low feeding levels result in an enrichment of ^{13}C in lipids and ^{15}N in protein of nile tilapia *Oreochromis niloticus* l. J. Fish Biol. **71**(1): 90-100.

Gaye-Siessegger, J., Mamun, S.M., Brinker, A., and Focken, U. 2013. Improving estimates of trophic shift (delta delta(trophic)) for diet reconstruction studies using enzyme activities. Comp. Biochem. Phys. A **164**(4): 579-583.

Gerking, S.D. 1994. Feeding ecology of fish. Academic Press San Diego.

Giacomini, H.C., Shuter, B.J., and Lester, N.P. 2013. Predator bioenergetics and the prey size spectrum: Do foraging costs determine fish production? J. Theor. Biol. **332**: 249-260.

Gorski, P.R., Cleckner, L.B., Hurley, J.P., Sierszen, M.E., and Armstrong, D.E. 2003. Factors afffecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. Sci. Total Environ. **304**(1-3): 327-348.

Guenard, G., Boisclair, D., Ugedal, O., Forseth, T., and Jonsson, B. 2008. Comparison between activity estimates obtained using bioenergetic and behavioural analyses. Can. J. Fish. Aquat. Sci. **65**(8), 1705-1720.

Hall, B.D., St Louis, V.L., Rolfhus, K.R., Bodaly, R.A., Beaty, K.G., Paterson, M.J., and Cherewyk, K.A. 2005. Impacts of reservoir creation on the biogeochemical cycling of methyl mercury and total mercury in boreal upland forests. Ecosystems **8**(3): 248-266.

Hanson, P., Johnson, T., Schindler, D., and Kitchell, J. 1997. Fish bioenergetics 3.0 software and manual. Sea Grant Institute.

Harris, H.H., Pickering, I.J., and George, G.N. 2003. The chemical form of mercury in fish. Science **301**(5637): 1203-1203.

Hart, P., and Hamrin, S.F. 1988. Pike as a selective predator. Effects of prey size, availability, cover and pike jaw dimensions. Oikos **51**(2): 220-226.

Heath, D.D., and Roff, D.A. 1996. The role of trophic bottlenecks in stunting: A field test of an allocation model of growth and reproduction in yellow perch, *Perca flavescens*. Environ. Biol. Fishes **45**(1): 53-63.

Hecky, R., Ramsey, D., Bodaly, R., and Strange, N. 1991. Increased methylmercury contamination in fish in newly formed freshwater reservoirs. *In:* Advances in Mercury Toxicology. Springer. pp. 33-52.

Henderson, B.A., Morgan, G.E., and Vaillancourt, A. 2004. Growth, ingestion rates and metabolic activity of walleye in lakes with and without lake herring. J. Fish Biol. **65**(5): 1270-1282.

Hesslein, R.H., Fox, D.E., and Capel, M.J. 1989. Sulfur, carbon, and nitrogen isotopic composition of fish from the Mackenzie River Delta region and other arctic drainages. Can. Data Rep. Fish. Aquat. Sci. **728**(iv): + 11 p.

Hladik, M., Kubecka, J., Mrkvicka, T., Cech, M., Drastik, V., Frouzova, J., Hohausova, E., Matena, J., Matenova, V., Kratochvil, M., Peterka, J., Prchalova, M., and Vasek, M. 2008. Effects of the construction of a reservoir on the fish assemblage in an inflow river. Czech J. Anim. Sci. **53**(12): 537-547.

Hoeinghaus, D.J., Winemiller, K.O., and Agostinho, A.A. 2008. Hydrogeomorphology and river impoundment affect food-chain length of diverse neotropical food webs. Oikos **117**(7): 984-995.

Hrenchuk, L.E., Blanchfield, P.J., Paterson, M.J., and Hintelmann, H.H. 2012. Dietary and waterborne mercury accumulation by yellow perch: A field experiment. Environ. Sci. Technol. **46**(1): 509-516.

Hussey, N.E., MacNeil, M.A., and Fisk, A.T. 2010. The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. Hydrobiologia **654**(1): 1-5.

Hyvarinen, P., and Vehanen, T. 2004. Effect of brown trout body size on post-stocking survival and pike predation. Ecol. Freshw. Fish **13**(2): 77-84.

Iles, A.C., and Rasmussen, J.B. 2005. Indirect effects of metal contamination on energetics of yellow perch (*Perca flavescens*) resulting from food web simplification. Freshw. Biol. **50**(6): 976-992.

Irz, P., Odion, M., Argillier, C., and Pont, D. 2006. Comparison between the fish communities of lakes, reservoirs and rivers: Can natural systems help define the ecological potential of reservoirs? Aquat. Sci. **68**(1): 109-116.

Jackson, S., and Sleigh, A.C. 2001. The political economy and socio-economic impact of China's Three Gorges dam. Asian Studies Review **25**(1): 57-72.

Jackson, T.A. 1988. The mercury problem in recently formed reservoirs of northern Manitoba (Canada): Effects of impoundment and other factors on the production of methyl mercury by microorganisms in sediments. Can. J. Fish. Aquat. Sci. **45**(1): 97-121.

Jacobson, D.C. 1992. Analysis of factors affecting growth of northern pike in Minnesota. Investigational Report 424, Minnesota DNR, St. Paul, MN.

Jardine, T.D., Kidd, K.A., and Fisk, A.T. 2006. Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environ. Sci. Technol. **40**(24): 7501-7511.

Jenkins, R.M., and Oglesby, R.T. 1982. The morphoedaphic index—concepts and practices: The morphoedaphic index and reservoir fish production. Trans. Am. Fish. Soc. **111**(2): 133-140.

Jobling, M. 1981. The influences of feeding on the metabolic-rate of fishes - a short review. J. Fish Biol. **18**(4): 385-400.

Jones, J.I., and Waldron, S. 2003. Combined stable isotope and gut contents analysis of food webs in plant-dominated, shallow lakes. Freshw. Biol. **48**(8): 1396-1407.

Jones, J.R., and Hoyer, M.V. 1982. Sportfish harvest predicted by summer chlorophyll-a concentration in midwestern lakes and reservoirs. Trans. Am. Fish. Soc. **111**(2): 176-179.

Kelso, J.R.M. 1977. Unexploited percid populations of West Blue Lake, Manitoba, and their interactions. J. Fish. Res. Board Can. **34**: 1655-1669.

Kennedy, B.P., Klaue, B., Blum, J.D., and Folt, C.L. 2004. Integrative measures of consumption rates in salmon: Expansion and application of a trace element approach. J. Appl. Ecol. **41**(5): 1009-1020.

Kerr, S.R. 1971. Prediction of fish growth efficiency in nature. J. Fish. Res. Board Can. **28**(6): 809-&.

Kidd, K.A., Paterson, M.J., Hesslein, R.H., Muir, D.C.G., and Hecky, R.E. 1999. Effects of northern pike (*Esox lucius*) additions on pollutant accumulation and food web structure, as determined by delta c-13 and delta n-15, in a eutrophic and an oligotrophic lake. Can. J. Fish. Aquat. Sci. **56**(11): 2193-2202.

Kidd, K.A., Schindler, D.W., Hesslein, R.H., and Muir, D.C.G. 1998. Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in yukon territory. Can. J. Fish. Aquat. Sci. **55**(4): 869-881.

Kidd, K.A., Schindler, D.W., Muir, D.C.G., Lockhart, W.L., and Hesslein, R.H. 1995. High-concentrations of toxaphene in fishes from a sub-arctic lake. Science **269**(5221): 240-242.

Kimura, D.K. 1980. Likelihood methods for the von Bertalanffy growth curve. Fish. Bull. **77**(4): 765-776.

Kitchell, J.F., Schindler, D.E., Ogutu-Ohwayo, R., and Reinthal, P.N. 1997. The nile perch in Lake Victoria: Interactions between predation and fisheries. Ecol. Appl. **7**(2): 653-664.

Kitchell, J.F., Stewart, D.J., and Weininger, D. 1977. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. **34**(10): 1922-1935.

Klassen, S., and Gilpin, J. 1999. Alberta irrigation in the old and new millennium. Can. Water Resour. J. **24**(1): 61-69.

Larssen, T. 2010. Mercury in chinese reservoirs. Environ. Poll. **158**(1): 24-25.

Legler, N.D., Johnson, T.B., Heath, D.D., and Ludsin, S.A. 2010. Water temperature and prey size effects on the rate of digestion of larval and early juvenile fish. Trans. Am. Fish. Soc. **139**(3): 868-875.

Lepak, J.M., Kinzli, K.D., Fetherman, E.R., Pate, W.M., Hansen, A.G., Gardunio, E.I., Cathcart, C.N., Stacy, W.L., Underwood, Z.E., Brandt, M.M., Myrick, C.A., and Johnson, B.M. 2012. Manipulation of growth to reduce mercury concentrations in sport fish on a whole-system scale. Can. J. Fish. Aquat. Sci. **69**(1): 122-135.

Lepak, J.M., Robinson, J.M., Kraft, C.E., and Josephson, D.C. 2009. Changes in mercury bioaccumulation in an apex predator in response to removal of an introduced competitor. Ecotoxicology **18**(5): 488-498.

Lienesch, P.W., Lutterschmidt, W.I., and Schaefer, J.F. 2000. Seasonal and long-term changes in the fish assemblage of a small stream isolated by a reservoir. Southw. Natural. **45**(3): 274-288.

Lindqvist, O., Johansson, K., Aastrup, M., Andersson, A., Bringmark, L., Hovsenius, G., Hakanson, L., Iverfeldt, A., Meili, M., and Timm, B. 1991. Mercury in the swedish environment - recent research on causes, consequences and corrective methods. Water Air Soil Pollut. **55**(1-2): R11-&.

Lindstrom, T. 1973. Life in a lake reservoir fewer options decreased production. Ambio **2**(5): 145-153.

Lodenius, M., and Malm, O. 1998. Mercury in the amazon. *In* Reviews of environmental contamination and toxicology. Springer. pp. 25-52.

Logan, J.M., and Lutcavage, M.E. 2010. Stable isotope dynamics in elasmobranch fishes. Hydrobiologia **644**(1): 231-244.

Macko, S.A., Estep, M.L.F., Engel, M.H., and Hare, P.E. 1986. Kinetic fractionation of stable nitrogen isotopes during amino-acid transamination. Geochim. Cosmochim. Acta **50**(10): 2143-2146.

Madenjian, C.P., O'Connor, D.V., Pothoven, S.A., Schneeberger, P.J., Rediske, R.R., O'Keefe, J.P., Bergstedt, R.A., Argyle, R.L., and Brandt, S.B. 2006. Evaluation of a lake whitefish bioenergetics model. Trans. Am. Fish. Soc. **135**(1): 61-75.

Mailman, M., Stepnuk, L., Cicek, N., and Bodaly, R.A. 2006. Strategies to lower methyl mercury concentrations in hydroelectric reservoirs and lakes: A review. Sci. Total Environ. **368**(1): 224-235.

Marcogliese, D.J., and Pietrock, M. 2011. Combined effects of parasites and contaminants on animal health: Parasites do matter. Trends Parasitol. **27**(3): 123-130.

Margenau, T.L., Rasmussen, P.W., and Kampa, J.M. 1998. Factors affecting growth of northern pike in small northern Wisconsin lakes. N. Am. J. Fish. Manage. **18**(3): 625-639.

Martinez Del Rio, C., and Wolf, B.O. 2005. Mass-balance models for animal isotopic ecology. *In:* Physiological and ecological adaptations to feeding in vertebrates. *Edited by* M.A. Starck and T. Wang. Science Pub Inc, Enfield, New Hampshire. pp. 141-174.

Mauck, W.L., and Coble, D.W. 1971. Vulnerability of some fishes to northern pike (*Esox lucius*) predation. J. Fish. Res. Board Can. **28**(7): 957-969.

McCutchan, J.H., Lewis, W.M., Kendall, C., and McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**(2): 378-390.

Minagawa, M., and Wada, E. 1984. Stepwise enrichment of n-15 along food-chains - further evidence and the relation between delta-n-15 and animal age. Geochim. Cosmochim. Acta **48**(5): 1135-1140.

Miranda, L.E. 2008. Extending the scale of reservoir management. *In:* Balancing fisheries management and water uses for impounded river systems. *Edited by* M.S. Allen, S. Sammons and M.J. Maceina. Amer Fisheries Soc, Bethesda. pp. 75-102.

Miranda, L.E., Habrat, M.D., and Miyazono, S. 2008. Longitudinal gradients along a reservoir cascade. Trans. Am. Fish. Soc. **137**(6): 1851-1865.

Mitchell, P., and Prepas, E.E. 1990. Atlas of Alberta lakes. University of Alberta.

Mittelbach, G.G., and Persson, L. 1998. The ontogeny of piscivory and its ecological consequences. Can. J. Fish. Aquat. Sci. **55**(6): 1454-1465.

Montgomery, S., Lucotte, M., and Rheault, I. 2000. Temporal and spatial influences of flooding on dissolved mercury in boreal reservoirs. Sci. Total Environ. **260**(1-3): 147-157.

Morel, F.M.M., Kraepiel, A.M.L., and Amyot, M. 1998. The chemical cycle and bioaccumulation of mercury. Annu Rev Ecol Syst: 543-566.

Murdoch, D., and Chow, E.D. 2013. Ellipse: Functions for drawing ellipses and ellipse-like confidence regions [online]. Available from http://CRAN.R-project.org/package=ellipse

Nelson, G.A. 2013. Fishmethods: Fisheries methods and models in r [online]. Available from http://CRAN.R-project.org/package=fishmethods

Nelson, S.J., and Paetz, M.J. 1992. The fishes of alberta. University of Alberta Press, Edmonton, Alberta.

Newbrey, J.L., Paszkowski, C.A., and Gingras, B.A. 2012. Trophic relationships of two species of grebe on a prairie lake based on stable isotope analysis. Hydrobiologia **697**(1): 73-84.

Paradis, Y., Bertolo, A., and Magnan, P. 2008. What do the empty stomachs of northern pike (*Esox lucius*) reveal? Insights from carbon (delta ^{13}C) and nitrogen (delta ^{15}N) stable isotopes. Environ. Biol. Fishes **83**(4): 441-448.

Parnell, A.C., Inger, R., Bearhop, S., and Jackson, A.L. 2010. Source partitioning using stable isotopes: Coping with too much variation. Plos One **5**(3).

Pazzia, I., Trudel, M., Ridgway, M., and Rasmussen, J.B. 2002. Influence of food web structure on the growth and bioenergetics of lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. **59**(10): 1593-1605.

Peterson, B.J. 1999. Stable isotopes as tracers of organic matter input and transfer in benthic food webs: A review. Acta Oecol.-Int. J. Ecol. **20**(4): 479-487.

Peterson, B.J., and Fry, B. 1987. Stable isotopes in ecosystem studies. Annu. Rev. Ecol. Syst. **18**: 293-320.

Phillips, B.W., and Johnston, C.E. 2004. Fish assemblage recovery and persistence. Ecol. Freshw. Fish **13**(2): 145-153.

Phillips, D.L., Newsome, S.D., and Gregg, J.W. 2005. Combining sources in stable isotope mixing models: Alternative methods. Oecologia **144**(4): 520-527.

Pierce, R.B., Tomcko, C.M., and Margenau, T.L. 2003. Density dependence in growth and size structure of northern pike populations. N. Am. J. Fish. Manage. **23**(1): 331-339.

Ploskey, G.R., and Jenkins, R.M. 1982. Biomass model of reservoir fish and fish-food interactions, with implications for management. N. Am. J. Fish. Manage. **2**(2): 105-121.

Post, D.M. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. Ecology **83**(3): 703-718.

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

R Development Core Team 2012. R: A language and environment for statistical computing [online]. R Foundation for Statistical Computing, Vienna, Austria. Available from http://www.R-project.org/

Raat, A.J. 1988. Synopsis of biological data on the northern pike: *Esox lucius* linnaeus, 1758. Food & Agriculture Org.

Randall, D., and Wright, P. 1987. Ammonia distribution and excretion in fish. Fish Physiol. Biochem. **3**(3): 107-120.

Rasmussen, J.B., and Brinkmann, L. 2015. Distribution of yellow perch in lakes, reservoirs and rivers of alberta and British Columbia, in relation to their climatic tolerance, dispersal and invasive ability. *In* In press.

Rasmussen, J.B., Gunn, J.M., Sherwood, G.D., Iles, A., Gagnon, A., Campbell, P.G.C., and Hontela, A. 2008. Direct and indirect (foodweb mediated) effects of metal exposure on the growth of yellow perch (*Perca flavescens*): Implications for ecological risk assessment. Hum. Ecol. Risk Assess. **14**(2): 317-350.

Rennie, M.D., Collins, N.C., Shuter, B.J., Rajotte, J.W., and Couture, P. 2005. A comparison of methods for estimating activity costs of wild fish populations: More active fish observed to grow slower. Can. J. Fish. Aquat. Sci. **62**(4): 767-780.

Rennie, M.D., Johnson, T.B., and Sprules, W.G. 2012. Energy acquisition and allocation patterns of lake whitefish (*Coregonus clupeaformis*) are modified when dreissenids are present. Can. J. Fish. Aquat. Sci. **69**(1): 41-59.

Robbins, C.T., Felicetti, L.A., and Sponheimer, M. 2005. The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. Oecologia **144**(4): 534-540.

Romanuk, T.N., Jackson, L.J., Post, J.R., McCauley, E., and Martinez, N.D. 2006. The structure of food webs along river networks. Ecography **29**(1): 3-10.

Rowan, D. J. 2013. Bioaccumulation factors and the steady state assumption for cesium isotopes in aquatic foodwebs near nuclear facilities. J. Environ. Radioactiv., **121**, 2-11.

Rowan, D.J., and Rasmussen, J.B. 1995. The elimination of radiocaesium from fish. J. Appl. Ecol. **32**(4): 739-744.

Rowan, D.J., and Rasmussen, J.B. 1996. Measuring the bioenergetic cost of fish activity in situ using a globally dispersed radiotracer (Cs-137). Can. J. Fish. Aquat. Sci. **53**(4): 734-745.

Rowan, D.J., Chant, L.A., and Rasmussen, J.B. 1998. The fate of radiocesium in freshwater communities - why is biomagnification variable both within and between species? J. Environ. Radioact. **40**(1): 15-36.

Sammons, S.M., Scalet, C.G., and Neumann, R.M. 1994. Seasonal and size-related changes in the diet of northern pike from a shallow prairie lake. J. Freshw. Ecol. **9**(4): 321-329.

Sara, G., and Sara, R. 2007. Feeding habits and trophic levels of bluefin tuna *Thunnus thynnus* of different size classes in the mediterranean sea. J. Appl. Ichthyol. **23**(2): 122-127.

Schetagne, R., and Verdon, R. 1999. Post-impoundment evolution of fish mercury levels at the La Grande complex, Québec, Canada (from 1978 to 1996). *In:* Mercury in the biogeochemical cycle. Springer. pp. 235-258.

Schetagne, R., and Verdon, R. 1999. Post-impoundment evolution of fish mercury levels at the La Grande complex, Québec, Canada (from 1978 to 1996). *In:* Mercury in the biogeochemical cycle. Springer. pp. 235-258.

Schmidt, K., McClelland, J.W., Mente, E., Montoya, J.P., Atkinson, A., and Voss, M. 2004. Trophic-level interpretation based on delta ^{15}N values: Implications of tissue-specific fractionation and amino acid composition. Mar. Ecol.-Prog. Ser. **266**: 43-58.

Scott, W.B., and Crossman, E.J. 1973. Freshwater fishes of canada. Bulletin of the Fisheries Research Board Canada, Ottawa, Ontario.

Sherwood, G.D., Kovecses, J., Hontela, A., and Rasmussen, J.B. 2002a. Simplified food webs lead to energetic bottlenecks in polluted lakes. Can. J. Fish. Aquat. Sci. **59**(1): 1-5.

Sherwood, G.D., Pazzia, I., Moeser, A., Hontela, A., and Rasmussen, J.B. 2002b. Shifting gears: Enzymatic evidence for the energetic advantage of switching diet in wild-living fish. Can. J. Fish. Aquat. Sci. **59**(2): 229-241.

Sherwood, G.D., Rasmussen, J.B., Rowan, D.J., Brodeur, J., and Hontela, A. 2000. Bioenergetic costs of heavy metal exposure in yellow perch (*Perca flavescens*): In situ estimates with a radiotracer (Cs-137) technique. Can. J. Fish. Aquat. Sci. **57**(2): 441-450.

Sih, A., and Christensen, B. 2001. Optimal diet theory: When does it work, and when and why does it fail? Anim. Behav. **61**: 379-390.

Simoneau, M., Lucotte, M., Garceau, S., and Laliberte, D. 2005. Fish growth rates modulate mercury concentrations in walleye (*Sander vitreus*) from eastern canadian lakes. Environ. Res. **98**(1): 73-82.

Skov, C., Lousdal, O., Johansen, P.H., and Berg, S. 2003. Piscivory of 0+pike (*Esox lucius* l.) in a small eutrophic lake and its implication for biomanipulation. Hydrobiologia **506**(1-3): 481-487.

St Louis, V.L., Rudd, J.W.M., Kelly, C.A., Bodaly, R.A., Paterson, M.J., Beaty, K.G., Hesslein, R.H., Heyes, A., and Majewski, A.R. 2004. The rise and fall of mercury methylation in an experimental reservoir. Environ. Sci. Technol. **38**(5): 1348-1358.

Stacy, W.L., and Lepak, J.M. 2012. Relative influence of prey mercury concentration, prey energy density and predator sex on sport fish mercury concentrations. Sci. Total Environ. **437**: 104-109.

Stephens, D.W., and Krebs, J.R. 1986. Foraging theory: Monographs in behavior and ecology.

Thomann, R.V. 1981. Equilibrium-model of fate of microcontaminants in diverse aquatic food-chains. Can. J. Fish. Aquat. Sci. **38**(3): 280-296.

Tibbets, T.M., Wheeless, L.A., and del Rio, C.M. 2008. Isotopic enrichment without change in diet: An ontogenetic shift in delta ^{15}N during insect metamorphosis. Funct. Ecol. **22**(1): 109-113.

Topham, H.L. 1982. History of irrigation in western Canada. P.F.R.A Regina.

Trudel, M., and Rasmussen, J.B. 1997. Modeling the elimination of mercury by fish. Environ. Sci. Technol. **31**(6): 1716-1722.

Trudel, M., and Rasmussen, J.B. 2001. Predicting mercury concentration in fish using mass balance models. Ecol. Appl. **11**(2): 517-529.

Trudel, M., and Rasmussen, J.B. 2006. Bioenergetics and mercury dynamics in fish: A modelling perspective. Can. J. Fish. Aquat. Sci. **63**(8): 1890-1902.

Vander Zanden, M.J., and Rasmussen, J.B. 1996. A trophic position model of pelagic food webs: Impact on contaminant bioaccumulation in lake trout. Ecol. Monogr. **66**(4): 451-477.

Vander Zanden, M.J., and Rasmussen, J.B. 2001. Variation in delta ^{15}N and delta ^{13}C trophic fractionation: Implications for aquatic food web studies. Limnol. Oceanogr. **46**(8): 2061-2066.

Vander Zanden, M.J., Cabana, G., and Rasmussen, J.B. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (delta ^{15}N) and literature dietary data. Can. J. Fish. Aquat. Sci. **54**(5): 1142-1158.

Vander Zanden, M.J., Casselman, J.M., and Rasmussen, J.B. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature **401**(6752): 464-467.

Vanderklift, M.A., and Ponsard, S. 2003. Sources of variation in consumer-diet delta(15)n enrichment: A meta-analysis. Oecologia **136**(2): 169-182.

Van Walleghem, J. L., Blanchfield, P. J., Hrenchuk, L. E., & Hintelmann, H. 2013. Mercury elimination by a top predator, *Esox lucius*. Environ. Sci. Technol. **47**(9), 4147-4154.

Varela, J.L., Larranaga, A., and Medina, A. 2011. Prey-muscle carbon and nitrogen stable-isotope discrimination factors in atlantic bluefin tuna (*Thunnus thynnus*). J. Exp. Mar. Biol. Ecol. **406**(1-2): 21-28.

Venturelli, P.A., and Tonn, W.M. 2003. Introduction of northern pike into a fishless lake: Does a diet of macroinvertebrates affect predators or prey? **133**: 294-294.

Venturelli, P.A., and Tonn, W.M. 2005. Invertivory by northern pike (*Esox lucius*) structures communities of littoral macroinvertebrates in small boreal lakes. J. N. Am. Benthol. Soc. **24**(4): 904-918.

Venturelli, P.A., and Tonn, W.M. 2006. Diet and growth of northern pike in the absence of prey fishes: Initial consequences for persisting in disturbance-prone lakes. **135**(6): 1512-1522.

Verta, M. 1990. Changes in fish mercury concentrations in an intensely fished lake. Can. J. Fish. Aquat. Sci. **47**(10): 1888-1897.

Walters, A.W., and Post, D.M. 2008. An experimental disturbance alters fish size structure but not food chain length in streams. Ecology **89**(12): 3261-3267.

Ward, D.M., Nislow, K.H., Chen, C.Y., and Folt, C.L. 2010. Rapid, efficient growth reduces mercury concentrations in stream-dwelling atlantic salmon. Trans. Am. Fish. Soc. **139**(1): 1-10.

Ward, M., Willis, D., Herwig, B., Chipps, S., Parsons, B., Reed, J., and Hanson, M. 2008. Consumption estimates of walleye stocked as fry to suppress fathead minnow populations in west-central minnesota wetlands. Ecol. Freshw. Fish. **17**(1): 59-70.

Webb, P.W., and Skadsen, J.M. 1980. Strike tactics of *Esox*. Can. J. Zool.-Rev. Can. Zool. **58**(8): 1462-1469.

Werner, E.E., and Mittelbach, G.G. 1981. Optimal foraging - field-tests of diet choice and habitat switching. Am. Zool. **21**(4): 813-829.

Wolf, N., Carleton, S.A., and Martínez del Rio, C. 2009. Ten years of experimental animal isotopic ecology. Funct. Ecol. **23**(1): 17-26.

Wootton, J.T., Parker, M.S., and Power, M.E. 1996. Effects of disturbance on river food webs. Science **273**(5281): 1558-1561.

Wright, P.J. 2013. Methodological challenges to examining the causes of variation in stock reproductive potential. Fish Res. **138**: 14-22.