

# **Studies of the Upper Rainy River Food Web and Variations in Spawning Critical Habitats in Relation to Flow**

by

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## **Author's Declaration**

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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## Abstract

While the impacts of large hydroelectric dams are now well recognized, there is growing awareness of the pivotal role of the flow regime as a key driver of the ecology of rivers. Specifically, studies have identified low flow requirements for recruitment and spawning of fish species. Flow variation has also been shown to be a key structural influence on the food webs of regulated rivers. Overall, the alteration of flow regimes is considered one of the most serious and continuing threat to ecological integrity of rivers. In 2001, the International Joint Commission (IJC) issued an order prescribing a method for regulating the levels of the boundary waters of Rainy and Namakan lakes. The resulting hydrograph of the Rainy River is thought to have lost the natural seasonal flow pattern as a result of the upstream management practices. To date only limited biological monitoring has been conducted on Rainy River. Given the paucity of work on the Rainy River and importance of the flow regime for the ecology of lotic systems, the general purpose of the MSc research described here was to fill gaps in the available scientific knowledge, relating to population condition, food web and critical spawning habitat of the target species.

Study findings included weight-length relationships (WLRs) for walleye (*Sander vitreus*), lake sturgeon (*Acipenser fulvescens*) and logperch (*Percina caprodes*), captured from April-June, 2012 and 2013. Weight models and condition indices for Rainy River target species were near the upper limits of values reported previously in the literature for the species. Growth models indicated no significant changes in growth patterns for walleye in comparison to the Rainy River/Lake of the Woods population in the late 1990s. In contrast, lake sturgeon population exhibited faster growth and condition than estimated for 2002 Rainy Lake populations.

Application of stable isotope analysis provided us with the opportunity to explore the feasibility of using pectoral fin clips as an alternative to dorsal muscle tissue in feeding studies for adult lake sturgeon. The strong relationships between fin-clip and muscle tissue signatures demonstrated that fin-clips should be considered as good surrogates for muscle tissue. The result will allow trophic studies to accurately adjust for the effect of differential lipid accumulation between individuals and, for adult lake sturgeon, will effectively limit the need for lethal sampling to complete additional trophic studies. We applied the use of fin-clip tissue in stable isotope analysis to examine the dietary patterns of lake sturgeon inhabiting the upper reach of the

Rainy River. In general the Rainy River supports a healthy food web with typical vertical trophic structure consisting of four trophic levels, with adult walleye sitting at the apex position. The study findings provide further evidence for lake sturgeon reliance on consumption of fish eggs or other substrate deposited fish material. The reliance on fish derived protein has been similarly reported in Lake Winnebago, Wisconsin (Stelzer et al., 2008), while historical studies report consumption of fish eggs (e.g. Cuerrier, 1966).

Walleye and lake sturgeon spawning sites on the Rainy River were generally separated, with only a single location overlap. Both species showed preference for coarse substrates dominated by bedrock, bedrock with cobble/boulder, or cobble/boulder. The depths and velocities experienced by adults, eggs or larvae present at spawning grounds, however, varied significantly and encompassed reported values from previous studies. Although the proportions of preferred spawning habitats did not change significantly as water surface elevation (WSE) rose, the total availability of preferred spawning substrate types increased, more than doubling for both species. In addition to maintaining a constant WSE to prevent dewatering of spawning sites, or a reduction of water depths over selected lake sturgeon spawning sites as noted above, a minimum WSE of 328m must be maintained to optimize lake sturgeon spawning if the lower spawning habitat requirements and population numbers prevail. Walleye and lake sturgeon spawning does not appear to be limited within the Rainy River system (Mosindy and Rusak, 1991), although the quality and quantity of these areas is directly affected by fluctuations in river flow.

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# Chapter 1 General Introduction

## 1.1 Hydroelectric impacts

The impacts of large hydroelectric dams are now well recognized (Sabater, 2008). While the more obvious impacts of large impoundments and hydro peaking practices such as impeding fish passage, stranding and egg exposure are well studied (Coutant, 2000; Halleraker et al., 2003; Berland et al., 2004; Tuhtan et al., 2012; Fisk et al., 2012), less is known about more subtle impacts on resident fish. For example, the rapid fluctuation of water levels, as a result of hydroelectric peaking, has potentially large implications for life-stages, such as eggs, that cannot move with the changing water level and those species that depend on shallow, slow velocity habitats along the shore (O'Shea, 2005).

There is growing awareness of the pivotal role of the flow regime as a key driver of the ecology of rivers (Poff et al., 2010). Changes to the hydrological regime are a major threat to river ecosystems (Sabater, 2008). Natural, uncontrolled hydrologic conditions allow for the creation and maintenance of suitable habitats for resident species (Poff et al., 1997). Altering flow regimes will have a direct impact on a diverse array of ecological processes and patterns in streams and rivers (Hart and Finelli, 1999).

Previous studies have focused on the effects of altered flow regimes on aquatic biodiversity, (Bunn and Arthington, 2002), and noted that flow is a major driver of physical habitat, and through its effect on habitat is a major determinant of biotic composition (Poff et al., 1997). Studies have also focused on life-history responses to altered flows. Specifically, studies have identified low flow requirements for recruitment and spawning of fish species (Milton and Arthington, 1983 & 1984; Humphries and Lake, 2000). Some fish species have been shown to use natural flow variation as cues for migration and spawning (King et al., 1998; Humphries et al., 1999). Modified temperature regimes below dams have also been shown to delay timing of spawning events (Zhong and Power, 1996). Apart from effects on fish, flow variation has also been shown to be a key structural influence on the food webs of regulated rivers (Marty et al., 2009). In productive rivers, current velocity has been shown to affect baseline carbon sources (Findlay, 1999). Marty et al. (2009) found that the ramping rate regime influences organisms at

the base of food web, with a reduction in food web length. Overall, the alteration of flow regimes is often claimed to be the most serious and continuing threat to ecological integrity and sustainability of rivers (Bunn and Arthington, 2002).

## **1.2 Scope of Work**

To avoid the more obvious implications of altered flow regimes, the International Joint Commission (IJC) issued an order in 2001 prescribing a method for regulating the levels of the boundary waters of Rainy and Namakan lakes (Figure 1.1). The 2000 rule curves will be subject to review in 2015, where the IJC will consider supplementary monitoring information that may indicate the ecosystem effects of flow changes (IJC, 2001). Since 2001, a number of government agencies have been conducting studies to evaluate the effects of the 2000 rule curve change, e.g. Minnesota Department of Natural Resources, Ontario Ministry of Natural Resources and U.S. Geological Survey. The primary focus of the studies has been on monitoring the aquatic and riparian ecosystems of the upstream Rainy Lake and Namakan Reservoir, both of which influence Rainy River water levels.

The operators of the International Falls Dam (IFD) use the 2000 rule curves to control the outflow of Rainy Lake and, correspondingly, the discharge rate into the Rainy River. Under the current operation plan, Rainy Lake elevation must be maintained between a prescribed minimum and maximum elevation. As long as the water level of Rainy Lake is maintained within the limits, the discharge rate into, and the water level of, Rainy River is unrestricted. The resulting hydrograph of Rainy River is thought to have lost the natural seasonal flow pattern and has increased short-term variability in discharge due to hydro peaking (O'Shea, 2005). O'Shea (2005) modelled hydraulic conditions on the Rainy River to assess the biological impacts of flow fluctuations and to recommend flow conditions to protect the ecologically significant portions of the hydrograph.

Two study locations (Manitou Rapids and Long Sault Rapids) were examined in the O'Shea (2005) study. The two areas were identified as two of the three most important sites for lake sturgeon spawning, and the third site was at the base of the IFD (Mosindy and Rusak, 1991). Habitat preference curves from the Minnesota Department of Natural Resources (MNDNR) were

used to assess the quality and quantity of habitat using data on species' life-stage preference for depth, velocity, and substrate. Twelve fish species and/or life-stages, chosen to address a variety of the habitat guilds, were combined with depth and velocity discharge curves for the river to produce weighted useable area habitat curves. The relationships between habitat and discharge were then used to identify instream flow prescriptions (IFP), which served to define the point where “no additional water should be artificially removed from the system or held back at the dam” (O’Shea, 2005). O’Shea (2005) also made recommendations to move towards a more “natural” flow regime. The rapid fluctuation of water levels, as a result of hydroelectric peaking, has a large effect on life-stages, such as eggs, that cannot move with the changing water level and those species that depend on shallow, slow velocity habitats along the shore (O’Shea, 2005).

To date only limited biological monitoring has been conducted on the Rainy River (Kallemeyn et al., 2009). Although the O’Shea (2005) report addressed many of the concerns about the impacts of varying water levels on key fish species in the Rainy River, it was spatially limited and did not address concerns for areas immediately below IFD. Given the paucity of information relating to the ecological state of the river in relation to variations in river water levels, the general purpose of the MSc research described here will be to fill gaps in the available scientific knowledge, providing useful data for a scientific evaluation of the existing rule curves and discharge practices on the Rainy River.

### **1.3 Target Species**

The IJC identified three key species as target species: lake sturgeon (*Acipenser fulvescens*), walleye (*Sander vitreus*) and logperch (*Percina caprodes*). The target species were chosen based on ecological significance, spawning times and trophic guilds. While the chosen species cover a range of functional ecological characteristics, they are all spring or summer spawning species. Fall spawners, however, are represented by only a few fish species in the river, including lake whitefish (*Coregonus clupeaformis*) and cisco (*Coregonus artedi*) (Eibler and Anderson, 2004).

Walleye make up the largest component of the Rainy River fishery, Payer (1987) tagged over 10000 walleye in the Lake of the Woods and found that a large portion of Lake of the Woods walleye spawn in Rainy River. Many walleye populations are known to migrate to rivers



or tributary streams characterized by fast flowing water and gravel-coarse rock substrates to spawn (Ivan et al., 2010; Geiling et al., 1996; DePhilip et al., 2005). Spawning occurs in early spring (late April to late May) when temperatures range from 4 - 10 °C (Kerr et al., 1997). Walleye are known to move upstream along the shoreline in an attempt to avoid the highest water velocities as they are not strong swimmers (Kerr et al., 1997). Therefore, resting areas such as boulders, backwaters, and instream debris are particularly important during spawning in streams and rivers with faster currents (Cholmondeley, 1985).

Logperch spawning occurs numerous times in the warmer months of the year (late spring to early summer) when temperatures reach the 17 – 21.5 °C range (Holm et al., 2009). Holm et al. (2009) indicated that logperch eggs are often buried in sandy sediments where aeration must occur via running water or waves

Lake sturgeon abundance in the Rainy River is currently only a fraction of what it once was and is considered a species of special concern in Ontario (COSEWIC, 2006). The population once supported a native trade and subsistence fishery. Mosindy and Rusak (1991) identified three main areas for lake sturgeon spawning on the Rainy River; two at Long Sault and Manitou rapids and a third immediately below the IFD. Lake sturgeon spawning generally occurs in late May through to late June when water temperatures range from 8.5 – 18 °C, although optimum temperatures range between 14 – 16 °C (Houston, 1986). Current velocity, substrate particle size and depth are considered to be important factors for spawning habitat selection (Johnson et al., 2006). Specific spawning site selection can vary between rivers and lakes. General spawning habitat characteristics include: fast-flowing waters, usually below waterfalls, rapids, dams or headwaters, and clean hard substrate types (COSEWIC, 2006; Auer, 1996; Adams et al., 2006).

The research presented here provided us with the opportunity to explore the use of stable isotope analysis in the assessment of lake sturgeon diet and development of a non-lethal, non-invasive sampling alternative to past diet studies. While walleye are an important sport fish in the Lake of the Woods/Rainy River system and logperch represent the forage fish available in the system, the work presented in chapters 3 and 4 will primarily focus on lake sturgeon due to their conservation issues, native significance and novelty of methods being applied.

## 1.4 Objectives

Given the importance of the flow regime for the ecology of lotic systems and the gaps in knowledge available to assess the effects of the flow regime on the upper Rainy River the following objectives were addressed:

The second chapter addressed the characterization and cataloging of the existing condition and age-class structure of the identified species. Specific objectives included: estimation of weight-length relationships, von Bertalanffy growth models, relative condition factors and the correlations of condition with age and sex for Rainy River populations. Where possible, comparisons were made with published literature on Lake of the Woods/Rainy River populations prior to the 2000 rule curve change.

In the third chapter, I evaluated the feasibility of using pectoral fin clips as an alternative to dorsal muscle tissue in stable isotope studies for adult lake sturgeon. A specific objective was to test the hypothesis that there is a significant relationship between the stable isotope measures of pectoral fin-clip and dorsal muscle tissue samples. Furthermore, I sought to evaluate the influence of lipid extraction and mathematical normalization models on the obtained pectoral fin-clip and muscle tissue stable isotope comparisons as a means of determining whether lipid extraction and/or correction is routinely required for lake sturgeon stable isotope studies. With the development and validation of non-lethal sampling methods, the chapter sought to extend the applicability of stable isotope methods to the study of lake sturgeon.

In the fourth chapter, I sought to characterize the Rainy River food web using stable isotope analysis and provide a baseline for the assessment of the Rainy River food web and trophic structure. Specifically, I used stable isotope data gathered on the target species to: [1] examine intraspecific diet variability and ontogenetic shifts in diet; [2] determine the proportions of prey items contributing to diets; and, [3] characterize trophic position and isotopic niche space both within the river as a whole, and as a function of identified geomorphic units. A central focus of the chapter was on lake sturgeon, about which relatively few isotope studies exist. Data collected for walleye and logperch are included in Appendix A.

The objective of the fifth chapter was to identify and characterize the critical spawning habitat of the target species. Specifically I sought to [1] identify and characterize the spawning locations of lake sturgeon and walleye in the upper Rainy River based on temperature, velocity, depth and substrate; and, [2] determine if substrate, water level and depth characteristics of the spawning sites were affected by changes in water surface elevation.

## 1.5 Figures

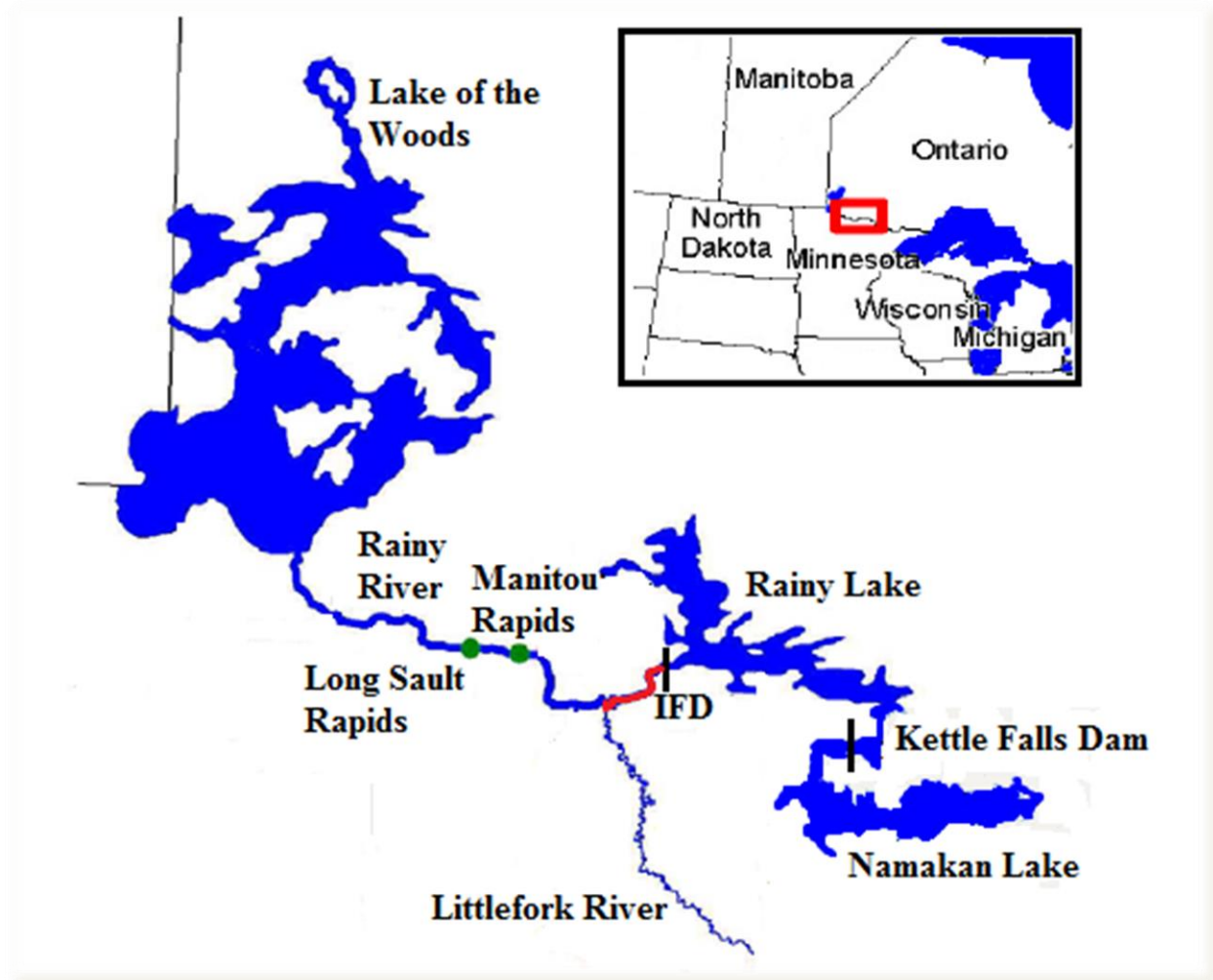


Figure 1.1 Location of Rainy River, International Falls Dam (IFD), Long Sault and Manitou rapids. Study reach indicated begins at the IFD and ends at the confluence with Littlefork River.

## **Chapter 2 Weight-Length relationships, condition and growth of walleye (*Sander vitreus*), lake sturgeon (*Acipenser fulvescens*) and logperch (*Percina caprodes*) from Rainy River, ON.**

### **2.1 Introduction**

Since the beginning of the 20<sup>th</sup> century, weight-length relationships (WLRs) have been widely used to estimate relative weights and to compute condition factors for comparing the well-being of fish populations for ecological studies (Hayes et al., 1995). Individual condition can be determined through a variety of methods including: weight-length based methods such as relative condition (LeCren, 1951) and relative weight (Murphy et al., 1990), somatic indices such as hepatosomatic and gonadosomatic indices (Htun-han, 1978; King, 1995; Kaufman et al., 2007) and calorific analysis (Hails, 1983; Booth and Keast, 1986). Each approach has its own merits and relies on different assumptions. Of all the methods, weight-length based measures of condition are most easily obtained and provide a non-invasive method for managers to assess the general state of well-being of fish populations which makes them attractive for routine assessment purposes (Craig et al., 2005; Froese, 2006; Kaufman et al., 2007; De Robertis and Williams, 2008).

The exact relationship between weight and length differs among species (i.e., is dependent on body shape) and within species (i.e., is dependent on the condition of individuals) (Le Cren, 1951). Thus while determination of the relative condition of individuals is useful, it is often not suitable for comparisons among populations as it assumes that the WLR remains constant over the period of study (Schneider et al., 2000). For example, relative condition can be reflective of seasonal changes brought on by reproduction (Hansen and Nate, 2005), stage of development or sex (Bruch et al., 2011), latitude and longitude of the population (Power and McKinley, 1997) and gear selection biases (Treasurer, 1976).

In addition to information on the relative condition of fish populations, data on the size and age of fish are very important for fisheries management, as they form the basis for the calculation of growth and mortality rates (Csirke et al., 1987). To model the growth of a species, a method for accurate ageing is required (Casselman, 1987). Fisheries scientists have developed

several methods for ageing of fish including: using various calcified structures such as vertebrae, spines, scales and otoliths and using length-frequency distributions (Casselman, 1976). Of all the calcified structures, otoliths are considered the most accurate for age determination. The collection of otoliths requires lethal sampling, which is not ideal if large sample sizes are needed or the species is considered at risk (Casselman, 1987). Nevertheless, accurate growth information helps managers understand reproductive potential of a population given that growth is related to fecundity and can be used to infer the overall health of the population (Le Cren, 1951; de Veen, 1970; Schmitt and Skud, 1978).

Here data for three target species identified by the International Joint Commission (IJC) as important are reported on. The species include: lake sturgeon (*Acipenser fulvescens*), walleye (*Sander vitreus*) and logperch (*Percina caprodes*). The objective was to characterize and catalogue the existing condition and age-class structure of the identified species. Specific objectives included: estimation of WLRs, von Bertalanffy growth models, relative condition factors and the correlations of condition with age and sex for Rainy River populations. Where possible, comparisons are made with previously published literature on Lake of the Woods/Rainy River populations.

## **2.2 Methods**

All fish species used in the study were captured on the Rainy River, Ontario (48°36' N, 93°24' W), between the International Falls Dam and the confluence of Little Fork River, as part of a larger study assessing the impact of flow regulation on the three target species. The Rainy River is located on the Minnesota-Ontario border and flows westward from Rainy Lake to the south end of Lake of the Woods. It is the largest tributary of Lake of the Woods, contributing 70% of the annual input flows and has a drainage area of approximately 70 000km<sup>2</sup> (Eibler and Anderson, 2004).

The fish were collected by Fisheries and Oceans Canada in the spring and summer of 2012 and 2013. Collection of each target species coincided with the typical spawning period for the species, i.e. walleye in early spring, lake sturgeon in late spring, and logperch in early summer. Multiple methods were used to capture the highest numbers of each species and reduce

capture size bias. Walleye were captured by electrofishing at night. Lake sturgeon were also captured by electrofishing at night and with 25.4 and 30.5 cm mesh gill nets set overnight following methods described in Dubreuil and Cuerrier (1950). In 2012, logperch were caught by boat electrofishing, and in 2013 supplemental sampling was completed using a 38mm inner mesh mini-Missouri trawl net.

Biologically relevant information, including: length (cm), weight (kg or g), girth (cm) and sex (if possible) were obtained from all captured individuals. Sex determination for walleye and lake sturgeon was limited to release of gametes at the time of capture as neither species demonstrate reliable sexual dimorphism (Craig et al., 2005). Logperch do not demonstrate reliable sexual dimorphism and sex was determined via dissection (Winn, 1958). For ageing purposes the first dorsal fin ray (Overman and Parrish, 2001) and a 1cm section of the pectoral fin ray (Rossiter et al., 1995) were removed, respectively, from walleye and lake sturgeon. Otoliths were removed from logperch for aging (Casselman, 1987). Aging of the all species was completed by Fisheries and Oceans Canada, Great Lakes Laboratory for Fisheries and Aquatic Sciences following standardized protocols outlined in DeVries and Frie (1996). Briefly, spines, pectoral fin rays and otoliths were mounted using Cold Cure epoxy and thin-sectioned transversely with an Isomet low-speed saw. Ages were determined under magnification using transmitted light by two separate agers, with blind readings compared for consistency afterward and discrepancies resolved via a final consensus reading.

Weight-length relationships were determined for all species using the standard allometric equation (Schneider et al., 2000) expressing weight (W) in g as a function of length (L) in cm:

$$W = aL^b \quad [1]$$

The parameters a and b were estimated via linear regression using the following transformation of the weight-length relationship (Wootton, 2003):

$$\text{Log}_{10} W = \text{log}_{10}(a) + b \text{log}_{10}(L) \quad [2]$$

The estimated parameters from the WLR relationship were then used to estimate relative condition as follows:

$$K_n = W / aL^b \quad [3]$$

where  $K_n$  is the relative condition of an individual, and  $W$  and  $L$  are the measured weight and length, respectively (LeCren, 1951).

Age and length data were combined to estimate a von Bertalanffy growth model for each species where possible (von Bertalanffy, 1938) as follows:

$$L_t = L_{MAX} (1 - e^{-K(t-t_0)}) \quad [4]$$

where  $L_t$  is length at age  $t$  and estimated parameters  $L_{MAX}$  is the maximum length,  $K$  is the growth coefficient defining how fast the maximum length is reached (growth rate) and  $t_0$  is theoretical age at zero length. Model parameters were estimated using non-linear methods (Bates and Watts, 1988) using the Levenberg-Marquardt algorithm in Statistica, version 8 (Statsoft Inc., Tulsa, OK). Where data on ages <5 years for walleye and lake sturgeon were lacking, the models were forced through the origin, estimated using the default convergence criterion and varying starting values as a check for convergence stability (Bates and Watts, 1988).

The normality and variance homogeneity of the obtained data were assessed prior to statistical analyses using the Shapiro-Wilk  $W$  test (Shapiro and Wilk, 1965) and Levene's  $F$  test (Levene, 1960). Owing to unequal variances, Welch ANOVAs followed by Tukey's post-hoc HSD were used to compare length, weight, girth, age and condition between males and females from each species when possible. Linear regression was used to test for significant linear relationships between relative condition and age and girth (Zar, 2010). JMP 7.0 (SAS Institute) was used to perform all reported statistical analyses, with level of significance judged at  $\alpha=0.05$ . Significance differences between growth models of different years was established using the analysis of the residual sum of squares as outlined in Ratkowsky (1983) and Chen et al. (1982).

## **2.3 Results**

### **2.3.1 Lake sturgeon**

Sampling in 2012 and 2013 resulted in the capture of 385 lake sturgeon. Due to a lack of reliable sexual dimorphism in this species (Craig et al. 2005) the majority of individuals could not be sexed. Meaningful comparisons between sexes were not possible for lake sturgeon due to

small sample sizes. Mean and standard error of biologically relevant variables are presented in Table 2.1.

Logarithmic WLRs estimated for lake sturgeon are plotted in Figure 2.1. WLR coefficient estimates are reported in Table 2.1. WLRs explained 90% of the variation in the overall data. When compared to an assumed slope of 3, lake sturgeon demonstrated allometric growth ( $df=1$ ,  $t=6.83$ ,  $P<0.050$ ). Corresponding relative condition was determined for all individuals using the appropriate regression equations if sex was known and the overall equation when sex was unknown. Variations in relative condition by age are plotted in Figure 2.2. Lake sturgeon evidenced a significant, but weak positive relationship between relative condition and age ( $F_{1, 261}= 6.0$ ,  $r^2=0.02$ ,  $P=0.020$ ) and girth ( $F_{1, 344}=13.04$ ,  $r^2=0.15$ ,  $P=0.004$ ).

WLR regressions were compared when individuals were separated by year of capture, with no significant differences found between relationships estimated for 2012 and 2013 for lake sturgeon (ANCOVA  $F_{3, 342}=1018.8$ ,  $r^2=0.83$ ,  $P=0.770$ ) with the result that pooled data was used in the plots presented in Figure 2.1. A significant difference was observed between the slope of the overall equation and that for males ( $F_{3, 342}=1038.9$ ,  $r^2=0.90$ ,  $P=0.005$ ).

Table 2.2 presents the estimated parameters for the von Bertalanffy growth models developed for lake sturgeon samples from this study (2012/13) as well as lake sturgeon collected from Rainy Lake from 2002-2004 by Adams et al.(2006). Also presented are the growth parameters estimated by Mosindy and Rusak (1991) for lake sturgeon in the Rainy River from 1987-1989. Overall models were developed using mean length at age data and had high explanatory power (all models:  $P\leq 0.001$ ,  $r^2\geq 0.83$ ). Mean length at age models and literature derived models are presented in Figure 2.3. For lake sturgeon, the 2012 model differed significantly from the 2002 model ( $F_{6,62}=5.18$ ,  $P=0.003$ ). Comparisons with the model from Mosindy and Rusak (1991) were not possible as mean length at age data was not available.

### **2.3.2 Walleye**

Sampling in 2012 and 2013 resulted in the capture of 426 walleye. Due to a lack of reliable sexual dimorphism in this species (Craig et al. 2005) some individuals could not be sexed. Mean



and standard error of biologically relevant variables are presented in Table 2.1. Sex-specific comparisons were made for walleye with significant differences (all tests:  $P < 0.001$ ) seen for length ( $F_{1, 242} = 173.2$ ,  $r^2 = 0.42$ ), weight ( $F_{1, 242} = 268.1$ ,  $r^2 = 0.53$ ), girth ( $F_{1, 242} = 314.8$ ,  $r^2 = 0.57$ ) and age ( $F_{1, 235} = 146.7$ ,  $r^2 = 0.18$ ), while female walleye were found to be generally larger, they were also significantly older.

Logarithmic WLRs estimated for walleye are plotted in Figure 2.1. WLR coefficient estimates are reported in Table 2.1. The overall WLR explained 97% of the variation in the data. When compared to an assumed slope of 3, walleye demonstrate allometric growth ( $df = 1$ ,  $t = 6.38$ ,  $P < 0.001$ ). Corresponding relative condition was determined for all individuals using the appropriate regression equations if sex was known and the overall equation when sex was unknown. No significant pattern was observed for condition with age or girth.

WLR regressions were compared when individuals were separated by year of capture, with no significant differences found between relationships estimated for 2012 and 2013 for walleye (ANCOVA  $F_{3, 417} = 5314.1$ ,  $r^2 = 0.97$ ,  $P = 0.220$ ) with the result that pooled data was used in the plots presented in Figure 2.1. Using ANCOVA analysis, the regression for males and females were shown to be significantly different in slope ( $F_{3, 240} = 610.2$ ,  $r^2 = 0.88$ ,  $P = 0.280$ ).

Table 2.2 presents the estimated parameters for the von Bertalanffy growth models developed for walleye samples from this study (2012/13) as well as walleye collected in Lake of the Woods from 2002 and 1997 by Mosindy and Mucha (2005). Overall models were developed using mean length at age data and had high explanatory power (all models:  $P \leq 0.001$ ,  $r^2 \geq 0.88$ ). Sex specific models for females were not statistically significant ( $P > 0.800$ ) and therefore not presented here while the model for males demonstrated high explanatory power (all models:  $P \leq 0.001$ ,  $r^2 \geq 0.96$ ). Mean length at age models and literature derived models are presented in Figure 2.4 for walleye. For overall walleye models there is no statistical difference between the models among the years ( $F_{6, 23} = 2.09$ ,  $P = 0.090$ ). For the male walleye models no significant difference was found between the models among the years ( $F_{6, 20} = 1.27$ ,  $P = 0.330$ ).

### **2.3.3 Logperch**

Sampling in 2012 and 2013 obtained 28 logperch. Meaningful sex based comparisons were not possible for due to small sample sizes. Mean and standard error of biologically relevant variables are presented in Table 2.1.

Logarithmic WLRs estimated for logperch are plotted in Figure 2C. WLR coefficient estimates are reported in Table 2.1. All WLRs explained >90% of the variation in the data. When compared to an assumed slope of 3, logperch demonstrated isometric growth ( $df=1$ ,  $t=1.64$ ,  $P=0.150$ ). Corresponding relative condition was determined for all individuals using the appropriate regression equations if sex was known and the overall equation when sex was unknown. No significant pattern was observed between condition age and girth. Year of capture, sex-specific comparisons and growth model for logperch were not considered statistically due to small sample sizes

## **2.4 Discussion**

### **2.4.1 Lake Sturgeon**

It has previously been recognized that for species at risk analysis of weight-length, condition and growth can be a practical non-lethal tool for biologists and managers to gauge the overall health of the population (Craig et al., 2005). Today lake sturgeon abundance in the Rainy River/Lake of the Woods is only a fraction of what it once was and lake sturgeon is currently listed as a species of special concern in Ontario (COSEWIC, 2006). The establishment of a hydroelectric dam in 1909 and two paper mills at International Falls and Fort Frances in 1907 and 1914, respectively, a commercial lake sturgeon fishery, increasing urbanization and agriculture and declining water quality have all contributed to the decline in lake sturgeon abundance (Mosindy and Rusak, 1991). However, improvements to water quality in the 1970s and the end of the commercial fishery in 1995 have led to some population recovery since (Eibler and Anderson, 2004).

WLRs and condition of lake sturgeon have been previously studied for populations throughout their geographic range (e.g. Beamish et al., 1996, Fortin et al., 1996; Power and McKinley, 1997). The WLR (slope= 3.41; 95% CI (3.50, 3.31)) and condition ( $1.01\pm 0.008$ ) for

the Rainy River population is fairly consistent with populations throughout the species range, in which slope is frequently observed at approximately 3.3 (3.0-3.4) and condition of approximately 1 (0.98-1.05) (Beamish et al., 1996; Craig et al., 2005; Trested and Isely, 2011). A previous study of lake sturgeon population in Rainy Lake (Adams et al., 2006) reported a WLR of  $\log_{10}(W) = -2.29 + 3.033(\log_{10}TL)$ . While the relationship on the Rainy River indicates a greater slope one must consider the comparative limitations when only a portion of the population is sampled (i.e. spawning individuals as with this study) as sex and maturity can influence the overall relationship (Craig et al., 2005). Insufficient females were captured to analyze the WLR and condition separately, however the relationship for males was found to have a significantly decreased slope from the overall equation (2.68; 95% CI (3.03, 2.34)). A study by Craig et al. (2005) of St Clair River lake sturgeon found no difference in WLRs or condition between males and females but noted that studies should use caution when assessing sex differences in growth and condition until a non-lethal method for sex determination is developed, which would provide larger sample sizes for each sex and refinement of WLRs.

The inability to determine sex of lake sturgeon impacts possible comparisons as relative condition calculations typically do not account for the changes due to reproductive stage. Fully developed individuals are thought exhibit the highest relative condition, however lake sturgeon exhibit intermittent spawning, generally only up to 45% of the spawning population may be reproductively developed during a season (Bruch, 1999; Bruch and Binkowski, 2002). The protracted spawning cycle of lake sturgeon may also mean mature fish months away from final gonad development would likely also exhibit high relative condition factors. A weak positive correlation was seen between age and relative condition for lake sturgeon on the Rainy River. Condition seems to be generally greater for individuals of age greater than 14yrs which corresponds to the age at sexual maturity of lake sturgeon (10-12 for males and 15-20 for females) (COSEWIC, 2006).

Several studies and reviews have fitted a von Bertalanffy growth equation to length and age for lake sturgeon throughout their geographic range (Fortin et al., 1996; Power and McKinley, 1997; Adams et al., 2006; Trested and Isely, 2011). The study by Adams et al. (2006) on the Rainy Lake population of lake sturgeon allowed for direct comparison of growth models.

The comparison demonstrated that sturgeon in the Rainy River in 2012 are growing more quickly than sturgeon from Rainy Lake in 2002. While direct comparisons with the Rainy River/Lake of the Woods population from Mosindy and Rusak (1991) were not possible, the estimated parameters suggest that the results from our current study are biologically reasonable in comparison and that growth of Rainy River lake sturgeon has not changed markedly since the late 1980s.

#### **2.4.2 Walleye**

The walleye population in Rainy River is not considered at risk as they make up the largest component of the Lake of the Woods recreational fishery. Nevertheless they are targeted for sampling as part of annual assessments of the lake (Eibler and Anderson, 2004). In 1987, Payer et al. successfully tagged over 10000 walleye in Lake of the Woods and found that a large portion of this population migrate to spawn in the Rainy River.

WLRs and condition of walleye have been previously studied for a variety of populations (e.g. Murphy et al., 1990; VanDeValk et al., 2008). The WLR (slope=3.15; 95% CI (3.20, 3.11)) and condition ( $1.0 \pm 0.008$ ) for the Rainy River population is consistent with a mean slope of 3.18 from the equation derived from 114 populations throughout the species range reported in Murphy et al. (1990). Our results are also consistent with the range of slopes (2.96-3.24) reported by Mosindy and Mucha (2005) for Lake of the Woods walleye caught in 1997 and 2002.

Significant differences were seen between identified males and females in overall length, weight, girth, and age with females being significantly larger in general. Results were, therefore, consistent with evidence that walleye growth is sexually dimorphic (Sass and Kitchell, 2005). No difference was found between the WLRs of males and females; however the slope of the equation for males was significantly lower than the combined equation. The same caution should be exercised with the walleye data as was noted for lake sturgeon when assessing sex differences in growth and condition until a reliable non-lethal method for sex determination is developed.

Several studies and reviews have fitted a von Bertalanffy growth equation to length and age for walleye (Craig et al., 1995; Lester et al., 2000; Quist et al., 2003; He et al., 2005;

Mosindy and Mucha, 2005). A previous study by Mosindy and Mucha (2005) allowed for direct comparisons between the current estimated parameters and model from 2002 and 1997 Lake of the Woods for the overall population and male specific models. No differences were found between years for the overall population or male only models between 2012/13, 2002 and 1997 indicating that walleye growth has not changed over the last 16 years. Therefore our current study indicates that walleye growth has not changed markedly since the late 1990s.

### **2.4.3 Logperch**

Very little to no work has been completed on growth and condition of logperch populations. Generally, any literature reports on logperch have occurred as part of stream assemblage studies (e.g. Aadland et al., 1991, Hall and Rudstam, 1999) or species interaction studies (e.g. Balshine et al., 2005; Bergstrom and Mensinger, 2009). To our knowledge, this is the first study to present WLRs for logperch. A weight-length review by Schneider et al. (2000) suggested the use of the WLR for blackside darter (*Percina maculata*) could be applied to logperch. The relationship for blackside darter ( $\log_{10}(W) = -5.49 + 3.24 * \log_{10}(L)$ ) was developed for fish in Illinois waters, while both species demonstrate similar slopes, the intercepts of the regressions were significantly different. There were no relationships seen between logperch condition, sex, age or girth. However, the sample size remains small and did not allow for robust comparisons or growth curve calculation.

## 2.5 Figures and Tables

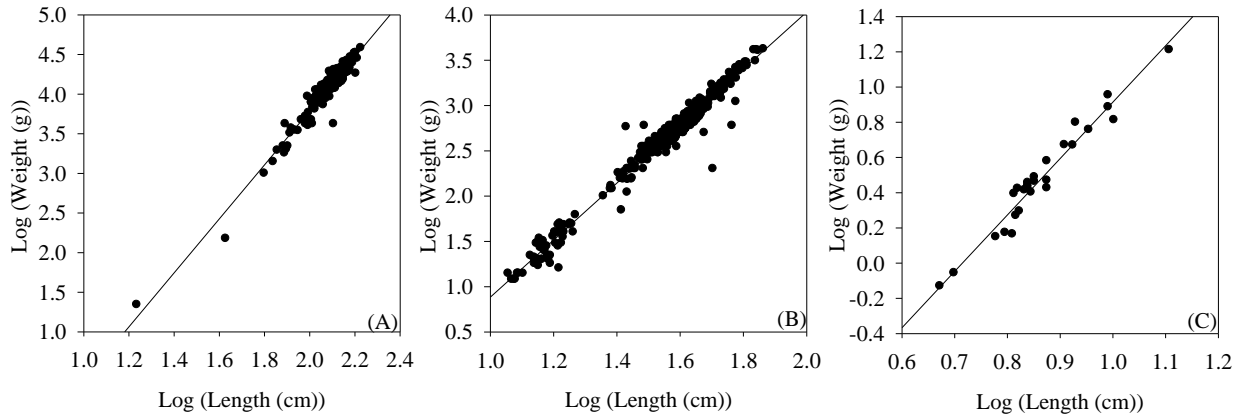


Figure 2.1 Weight-length relationships for lake sturgeon (A), walleye (B) and logperch (C) captured on the Rainy River during the spawning season, Data combines all years (2012 and 2013) and all sexes.

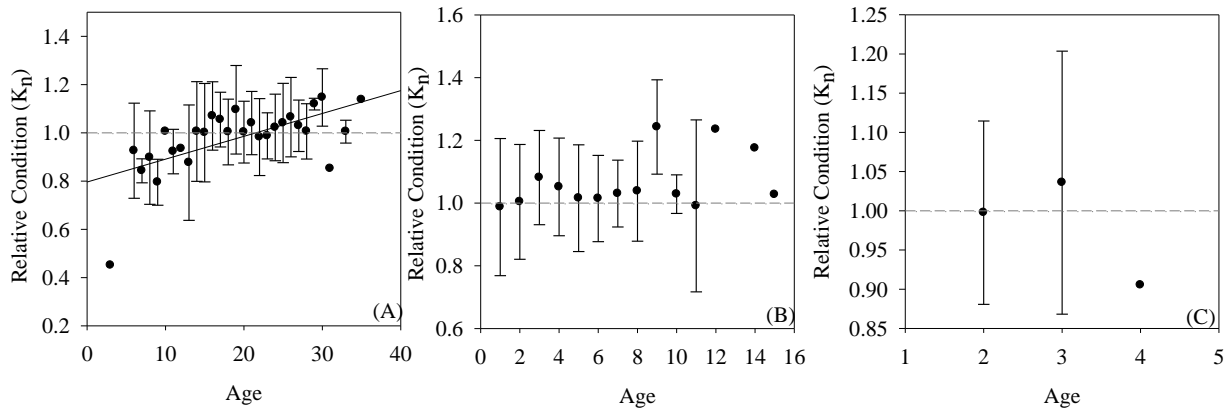


Figure 2.2 Relationships between age and relative condition for lake sturgeon (A), walleye (B) and logperch (C) captured on the Rainy River during spawning seasons of 2012 and 2013. Points represent mean condition at age with STDEV bars. Significant regression lines indicated by black line, and relative condition of 1.0 indicated by grey dashed line.

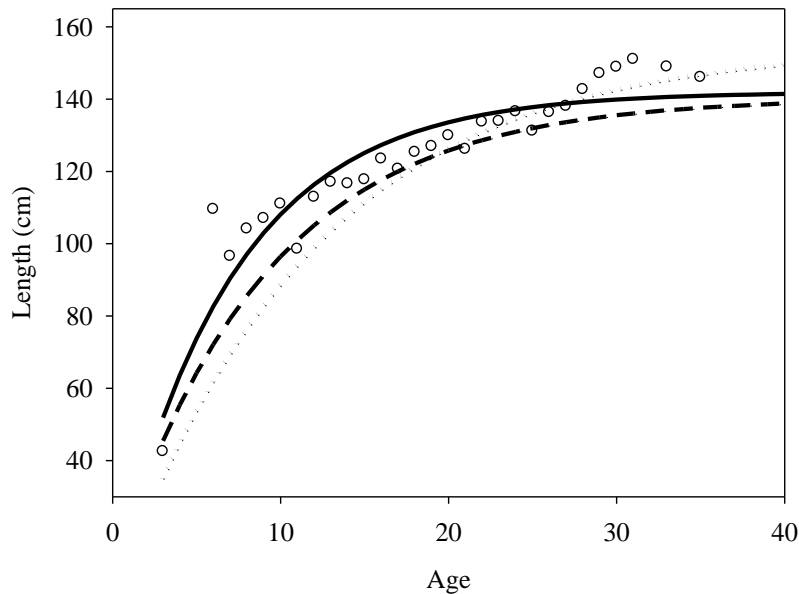


Figure 2.3 Mean length at age and von Bertalanffy growth model (solid line) developed for lake sturgeon caught on the Rainy River from 2012-2013. The dotted line indicates model previously reported by Mosindy and Rusak (1991) for lake sturgeon on the Rainy River caught from 1987-1989. The dashed line indicates a non-linear model developed from mean length at age data from Adams et al. (2006) for lake sturgeon caught on Rainy Lake from 2002-2004.

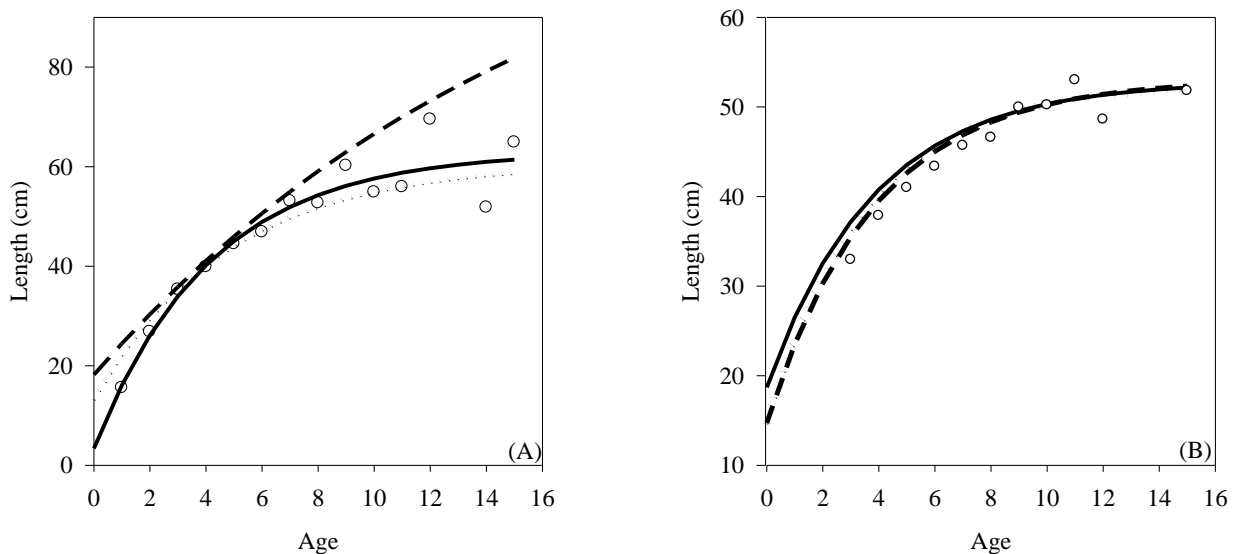


Figure 2.4 Mean length at age (white circles) and von Bertalanffy growth model (solid line) developed for all walleye (A) and male walleye (B) caught on the Rainy River from 2012-2013. The dotted and dashed lines indicate models previously reported by Mosindy and Mucha (2006) from 2002 and 1997, respectively, for all walleye (A) and male walleye (B) caught in Lake of the Woods.

Table 2.1 Summary biological data for walleye, lake sturgeon and logperch caught on the Rainy River. Mean  $\pm$ SE or 95% confidence intervals (when indicated) of the mean shown in brackets. Significant differences between males and females ( $\alpha=0.05$  level of significance) indicated with an asterisk. Data for confirmed males and females is reported.

Species		n	Mean Length (cm)	Mean Weight (g)	Girth (cm)	Mean Age	Weight-Length Regression			Relative Condition
							Slope (95% CI)	Intercept (95% CI)	R <sup>2</sup>	
Walleye	Males	215	41.8* ( $\pm 0.4$ )	713.5* ( $\pm 21.7$ )	21.0* ( $\pm 0.2$ )	4.6* ( $\pm 0.1$ )	2.73 (2.93, 2.54)	-1.61 (-1.29, -1.93)	0.78	1.00 ( $\pm 0.02$ )
	Females	29	57.0* ( $\pm 1.4$ )	2120.7* ( $\pm 171.8$ )	31.2* ( $\pm 0.8$ )	7.0* ( $\pm 0.4$ )	3.03 (3.30, 2.76)	-2.02 (-1.55, -2.50)	0.95	1.01 ( $\pm 0.01$ )
	Overall	426	37.4 ( $\pm 0.6$ )	680.8 ( $\pm 31.0$ )	20.6 ( $\pm 0.3$ )	4.2 ( $\pm 0.1$ )	3.15 (3.20, 3.11)	-2.26 (-2.20, -2.35)	0.97	1.00 ( $\pm 0.008$ )
Lake sturgeon	Males	85	133.3 ( $\pm 1.1$ )	17246 ( $\pm 435.6$ )	49.7 ( $\pm 0.5$ )	21 ( $\pm 0.5$ )	2.68 (3.03, 2.34)	-1.48 (-0.74, -2.21)	0.77	1.04 ( $\pm 0.01$ )
	Overall	385	127.7 ( $\pm 0.9$ )	15012 ( $\pm 289.3$ )	48.3 ( $\pm 0.4$ )	20 ( $\pm 0.3$ )	3.38 (3.50, 3.26)	-3.23 (-2.71, -3.23)	0.90	1.01 ( $\pm 0.008$ )
Logperch	Males	5	6.0 ( $\pm 0.4$ )	1.5 ( $\pm 0.2$ )		2.4 ( $\pm 0.2$ )	2.75 (3.30, 2.19)	-1.98 (-1.55, -2.42)	0.98	1.01 ( $\pm 0.06$ )
	Females	8	8.8 ( $\pm 0.8$ )	6.2 ( $\pm 1.7$ )		3 ( $\pm 0.19$ )	3.21 (3.83, 2.60)	-2.32 (-1.75, -2.90)	0.96	0.99 ( $\pm 0.02$ )
	Overall	28	7.5 ( $\pm 0.3$ )	4 ( $\pm 0.6$ )		2.7 ( $\pm 0.1$ )	3.23 (3.53, 2.94)	-2.31 (-2.05, -2.57)	0.95	1.03 ( $\pm 0.03$ )

\*p-value <0.001



Table 2.2 Estimated parameters for growth model of walleye and lake sturgeon on the Rainy River. Overall models were developed using individual length and age data (individuals) as well as mean length-at-age data (means). NS indicates the model was not statistically significant at  $\alpha=0.05$ .

			Overall			Males		
			Estimated	95% CI		Estimated	95% CI	
Walleye	Rainy River 2012/13	L <sub>max</sub>	63.04	71.25	54.83	52.86	56.29	49.42
		K	0.24	0.38	0.1	0.26	0.39	0.13
		T <sub>0</sub>	-0.23	0.96	-1.42	-1.68	0.16	-3.51
	Lake of the Woods 2002	L <sub>max</sub>	60.66	66.21	55.11	52.62	55.01	50.24
		K	0.21	0.27	0.16	0.28	0.34	0.22
		T <sub>0</sub>	-1.17	-0.85	-1.49	-1.17	-0.75	-1.59
	Lake of the Woods 1997	L <sub>max</sub>	125.48	259.48	-8.53	53.11	61.4	44.82
		K	0.06	0.16	-0.03	0.26	0.39	0.13
		T <sub>0</sub>	-2.61	-1.04	-4.18	-1.25	-0.53	-1.98
Lake Sturgeon	Rainy River 2012/13	L <sub>max</sub>	141.93	148.17	135.68			
		K	0.14	0.17	0.11			
		T <sub>0</sub>	-0.25	0.6	-1.1			
	Rainy Lake 2002	L <sub>max</sub>	140.38	144.45	136.31			
		K	0.11	0.15	0.07			
		T <sub>0</sub>	-0.56	3.1	-4.22			
	Rainy River 1991*	L <sub>max</sub>	154.5					
		K	0.085					
		T <sub>0</sub>	-0.03					

\* Mosindy (1991) model determined for FL, final values converted to TL using the eqn  $TL = 1.06 FL + 38.93$

## **Chapter 3 Non-lethal sampling of lake sturgeon for stable isotope analysis: comparing pectoral fin-clip and dorsal muscle for use in trophic studies.**

### **3.1 Introduction**

Owing to the predictable changes that occur in stable isotope ratios as energy flows through food webs, stable isotope analysis (SIA) has become a commonly used method for obtaining time-integrated information about feeding relationships in food web studies (Peterson and Fry, 1987; Finlay and Kendall, 2007). When used to study the feeding ecology of fish, dorsal white muscle is commonly sampled because it has an intermediate turnover rate, low isotopic variability, and frequently has low lipid content (Pinnegar and Polunin, 1999).

Obtaining dorsal muscle tissue for SIA can pose problems for many species as laws or management plans may constrain, or prohibit, the lethal sampling needed to obtain the tissue (e.g., Haley, 1998, Brosse et al., 2002). On larger species, the utilization of muscle biopsy plugs has been perfected (Hanisch et al., 2010; Nelson et al., 2011; Carlisle et al., 2012) as one means of reducing the need for lethal sampling, but biopsies are often viewed as undesirable for juveniles or species of special concern (COSEWIC, 2007).

In place of muscle tissue, a number of studies have considered alternative non-lethal sampling including: mucus (Church et al., 2009), scales (Sinnatamby et al., 2008) and fin clips (e.g., Willis et al., 2013). Fin clip tissue has been considered a suitable surrogate to dorsal muscle tissue in several reviews including: Australian tropical and temperate fishes (Jardine et al., 2011), European freshwater fishes (Tronquart et al., 2012) and North American freshwater species (Willis et al., 2013). Within North American studies salmonids, in particular, dominate in study comparisons (42%) as a result of their threatened status and the associated demands for non-lethal sampling alternatives (Sanderson et al., 2009; Hanisch et al., 2010).

While there is some evidence that regional multi-species models for fin-muscle relationships may be applied in fish food web related studies (Jardine et al., 2011; Tronquart et al., 2012), the fin-muscle stable isotope relationship has frequently been shown to vary by species (Kelly et al., 2006; Hanisch et al., 2010; Willis et al., 2013). Furthermore, fin-muscle

relationships often fail (28% of test cases) to exhibit a 1:1 slope indicative of an unbiased conversion of one tissue SIA value to another (Willis et al., 2013). Therefore, while strong relationships between the isotope signatures of fin clip and muscle tissues available in the literature demonstrate that fin clips can be used in place of muscle tissue, evidence of conversion biases and the dominance of salmonid studies highlight the need for further studies on non-salmonid species.

One species for which tissue comparisons have not been made to date is lake sturgeon (*Acipenser fulvescens*). Lake sturgeon population sizes have decreased in recent decades due to poor water quality, overfishing and damming (COSEWIC, 2006; Fisheries and Oceans Canada, 2008), with recent studies having identified the need for further non-lethal sampling information on sturgeon diet and foraging in areas where populations are affected by invasive species (McCabe et al., 2006) or other anthropogenic stressors (e.g., Haxton and Findlay, 2008). Furthermore, lake sturgeon represent a unique fin-muscle comparison given the high muscle tissue C:N ratios (typically > 5) as compared to previously examined species (e.g., Tronquart et al., 2012 C:N $\approx$ 3.5).

A strong relationship has been found between the C:N ratio and lipid content in animals (Post et al. 2007). Lipids are isotopically depleted in carbon relative to carbohydrates and proteins due to differing fractionation during lipid synthesis (DeNiro and Epstein, 1978; Griffiths, 1991). As such, some researchers have employed direct chemical extraction of lipids or mathematical normalization techniques to minimize the bias caused by heterogeneous lipid content among tissue samples (McConnaughey and McRoy, 1979; Sweeting et al., 2006; Hoffman and Sutton, 2010), with the technique being recommended when the C:N ratio is relatively high (above 3.5) or highly variable (Post et al., 2007).

In light of the above, the aim of the present study was to explore the use of non-lethal sampling for SIA of adult lake sturgeon. A specific objective was to test the hypothesis that there is a significant relationship between the stable isotope measures of pectoral fin-clip and dorsal muscle tissue samples. Furthermore, we sought to address a critical analytical issue (e.g., Arrington et al., 2006; Kiljunen et al., 2006; Logan et al., 2008; Fagan et al., 2011) by evaluating

the influence of lipid extraction and mathematical normalization models on the obtained pectoral fin-clip and muscle tissue stable isotope comparisons as a means of determining whether lipid extraction and/or correction is routinely required for lake sturgeon stable isotope related studies.

### **3.2 Methods**

The lake sturgeon used in the study were captured on the Rainy River, Ontario (48<sup>0</sup>36' N, 93<sup>0</sup>24' W) immediately below the International Falls Dam. The Rainy River is located on the Minnesota-Ontario border and flows westward from Rainy Lake to the south end of Lake of the Woods. The fish were collected by the staff of Fisheries and Oceans Canada by boat electro-fishing at night or with 25.4 and 30.5 cm mesh gill nets set overnight in early to late May 2012 and 2013. Nets were set following protocols described in Dubreuil and Cuerrier (1950), i.e., parallel or at an angle to river flow in currents and back eddies and strategically placed to optimize lake sturgeon capture. All capture was completed coincident with lake sturgeon spawning congregations on spawning beds or immediately downstream of the spawning areas.

Fin clips were obtained with a circular paper punch applied to the pectoral fin membrane so as to avoid sampling of the fin ray (Tyus et al., 1999). One or two muscle plugs were taken from each individual using a 3mm dermal biopsy punch from behind the third dorsal scute to obtain at least 1mg of muscle tissue (Tyus et al., 1999). The resulting wounds were sealed using 3M Vetbond Tissue Adhesive™. A total of 91 lake sturgeon were biopsied in two sampling seasons (66 in 2012 and 25 in 2013). Muscle and fin clip samples were kept frozen until returned to the laboratory where they were dried at 50°C for 24-48h and ground into a fine powder using a mortar and pestle.

Lipids were extracted from half the available muscle tissue from 30 individuals using methanol and chloroform following Folch et al. (1957) as revised by Kauffman et al. (2007). Approximately 0.5 mg of a tissue sample was added to 8ml of 2:1 (by volume) Chloroform: Methanol solution; left to soak and centrifuged for (10 min at 1000 x g) after 12-24hrs. The lipid extracted muscle tissue was then rinsed with distilled water and allowed to air dry before SIA.

Fin clip, muscle, and lipid extracted muscle tissue samples were processed for SIA, with all analyses performed at the Environmental Isotope Laboratory, University of Waterloo, on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Carlo Erba elemental analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy). Machine analytical precision was  $\pm 0.1\%$  and  $\pm 0.2\%$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , respectively, and was determined by repeat analysis of duplicates (one in ten). All resulting measurements are expressed using standard delta notation as parts per thousand differences (‰) with respect to the international reference standards, carbonate rock from the Peedee Belemnite formation for  $\delta^{13}\text{C}$  (Craig, 1957) and nitrogen gas in the atmosphere for  $\delta^{15}\text{N}$  (Mariotti, 1983). The carbon-to-nitrogen (C:N) ratio by mass was determined for each sample before and after lipid extraction.

Several lipid correction mathematical models exist in the literature (McConnaughey and McRoy 1979; Kiljunen et al., 2006; Post et al., 2007; Logan et al., 2008). Models differ in both approach, i.e., lipid normalization, arithmetic mass balance and regression based (Hoffman and Sutton, 2010) and the data sets used for estimation i.e., strictly aquatic, mixed aquatic and terrestrial (Logan et al., 2008) and functional form i.e., linear, non-linear (Fagan et al., 2011). Differences in model approach, however, may matter less than the accuracy with which model parameters are estimated or the specificity of the data used to estimate the model (Logan et al., 2008).

Accordingly here we have chosen two differing approaches. The first entails the use of the commonly applied general non-linear model developed by McConnaughey and McRoy (1979) and modified by Kiljunen et al. (2006) specific application to freshwater fish. The model was selected because the C:N ratio values used in its estimation were comparable to those observed in this study (3-12). Furthermore, in a comparative analysis of predictive performance, the McConnaughey and McRoy (1979) adjusted model proved statistically superior in terms of having the lowest mean absolute percent predictive error when tested against an extensive lake whitefish (*Coregonus clupeaformis*) data set (Fagan et al., 2011). The use of the model requires the estimation and application of two equations, as defined below.

Equation 1:

$$L = \frac{93}{1+(0.246 \times C:N-0.775)^{-1}}$$

Equation 2:

$$\delta^{13}C_N = D \left( I + \frac{3.90}{1 + \frac{287}{L}} \right)$$

Where  $L$  is the proportional lipid content of the sample,  $\delta^{13}C_N$  is the lipid-normalized value of the sample,  $C:N$  is the ratio of elemental carbon and nitrogen in the sample,  $\delta^{13}C$  is the measured value of the sample,  $D$  is the isotopic difference between protein and lipid and  $I$  is a constant. The parameter values used here were  $D = 7.018$  and  $I = 0.048$  as re-estimated by Kiljunen et al. (2006).

For the second approach an arithmetic mass balance model using tissue  $C:N$  ratios as a proxy for tissue lipid content (Alexander et al., 1996; Fry et al., 2003) was developed and applied. The model can be applied with the estimation of two parameters derived from stable isotope data available for bulk and lipid extracted samples: the  $C:N_{\text{protein}}$  of extracted tissue and the isotopic depletion factor for lipid,  $\Delta \delta^{13}C_{\text{lipid}}$  (Hoffman and Sutton, 2010; Logan et al., 2008) as follows:

Equation 3:

$$\delta^{13}C_{\text{protein}} = \delta^{13}C_{\text{bulk}} + (\Delta \delta^{13}C_{\text{lipid}} \times (C:N_{\text{protein}} - C:N_{\text{bulk}}))/C:N_{\text{bulk}}$$

where  $\delta^{13}C_{\text{protein}}$ ,  $\delta^{13}C_{\text{bulk}}$ ,  $\Delta \delta^{13}C_{\text{lipid}}$ ,  $C:N_{\text{protein}}$  and  $C:N_{\text{bulk}}$ , respectively are the standardized  $\delta^{13}C$  values for lipid free muscle protein, the  $\delta^{13}C$  of bulk muscle tissue (i.e. non-lipid extracted), the change in the  $\delta^{13}C$  value resulting from lipid extraction, the  $C:N$  ratio of lipid extracted tissue and the  $C:N$  ratio of bulk muscle tissue.

The normality and variance homogeneity of the obtained data were assessed prior to statistical analyses using the Shapiro-Wilk W test (Shapiro and Wilk, 1965) and Levene's F test (Levene, 1960). Owing to unequal variances, Welch ANOVAs followed by Tukey's post-hoc HSD were used to compare muscle, fin-clips, lipid extracted muscle, and the mathematically normalized stable isotope values. Linear regression was used to test for significant linear relationships between the SIA signatures of all tissues and treatment types. Standard t-test significance tests were used to determine whether regression intercepts and slopes, respectively, deviated significantly from zero and unity (Zar, 2010), with deviations from both zero and one indicating errors or biases in the use of one SIA value to predict another (Sinnatamby et al., 2008). Where necessary, differences between estimated regression models were subsequently tested using ANCOVA (Zar, 2010) to test the applicability of common slope and intercept models. JMP 7.0 (SAS Institute) was used to perform all reported statistical analyses, with level of significance judged at  $\alpha=0.05$ .

### 3.3 Results

Lake sturgeon sampled in this study were primarily adult fish with a mean age ( $\pm$  standard deviation) of 18.4 ( $\pm 6.5$ ) but covered a significant length range (42.5-168.5cm) (Table 3.1). Of the 68 lake sturgeon sampled for both muscle and fin tissue, 30 (12 in 2012 and 18 in 2013) yielded sufficient muscle tissue (approximately 1mg) for the SIA of both dorsal muscle (DM) and lipid extracted dorsal muscle ( $DM_E$ ) tissue from which the  $C:N_{\text{protein}}$  and  $\Delta \delta^{13}C_{\text{lipid}}$  parameters required for the mass balance correction model were estimated, respectively, as 3.51 and -5.8‰.

Significant differences in the SIA values were observed between tested and treated tissue types. Welch ANOVA comparisons (Table 3.2) yielded significant differences among the  $\delta^{13}C$  values by tissue type and treatment ( $F_{4,302} = 133.0, P < 0.001$ ). Tukey's post-hoc HSD indicated that DM had a significantly lower mean  $\delta^{13}C$  relative to fin clip (FC) ( $P < 0.001$ ),  $DM_E$  ( $P < 0.001$ ) and mass balance corrected muscle tissue (MBC) ( $P < 0.001$ ). The non-linear mathematically normalized (LN) mean  $\delta^{13}C$  was significantly higher relative to FC ( $P = 0.001$ ),  $DM_E$  ( $P < 0.001$ ) and MBC ( $P < 0.001$ ). Mean  $\delta^{15}N$  varied significantly ( $F_{2,10.4} = 4.7, P = 0.011$ ) among FC, DM and  $DM_E$  with FC being enriched relative to DM ( $P = 0.003$ ). The C:N ratio of DM  $7.8 \pm 3.4$  (mean  $\pm$

standard deviation) was significantly higher ( $F_{2, 166}=80.6$ ,  $P<0.001$ ) than the C:N ratio of either  $DM_E$  ( $3.5\pm 0.3$ ) or FC ( $3.4\pm 0.4$ ).

Among tissue and treatment types other significant  $\delta^{13}C$  linear relationships that explained between 12 and 77% of the modeled data were evident (Table 3.3). FC  $\delta^{13}C$  ratios were positively related to dorsal muscle values whether compared to the DM ( $r^2=0.32$ ,  $P<0.001$ ) or  $DM_E$  ( $r^2=0.74$ ,  $P<0.001$ , Figure 3.1), with lipid extraction significantly improving the regression model fit. Similarly FC  $\delta^{15}N$  ratios were significantly correlated with dorsal muscle tissue values whether compared to the DM ( $r^2=0.46$ , regression  $P<0.001$ ) or  $DM_E$  ( $r^2=0.62$ ,  $P<0.001$ ). The estimated  $\delta^{13}C$  and  $\delta^{15}N$  DM-FC regression slopes before extraction were not significantly different from the slope of the hypothetical 1:1 line ( $\delta^{13}C$ :  $P=0.100$ ;  $\delta^{15}N$ :  $P=0.150$ ) and the intercepts did not differ significantly from 0 for  $\delta^{15}N$  ( $P=0.420$ ) but did differ for  $\delta^{13}C$  ( $P=0.030$ ). The  $DM_E$ -FC regression slope for  $\delta^{13}C$  was not significantly different from the hypothetical 1:1 line ( $P=0.051$ ) however, the slope for  $\delta^{15}N$  did differ from 1:1 ( $P=0.001$ ) and the intercepts significantly differed from 0 ( $\delta^{13}C$ :  $P=0.040$ ;  $\delta^{15}N$ :  $P=0.040$ ). A notable relationship between FC-MBC  $\delta^{13}C$  stable isotope values was also found ( $r^2=0.77$ ,  $P<0.001$ ) which yielded a model that conformed to the hypothetical 1:1 line ( $P>0.500$ ) but does not have a zero intercept ( $P=0.020$ ).

Comparison of FC  $\delta^{13}C$  versus  $DM_E$ , MBC and LN regression via ANCOVA (Figure 3.2) indicated that  $DM_E$ , MBC and LN were best described by regression models sharing a common slope ( $t=1.95$ ,  $df=160$ ,  $P=0.971$ ), but with LN having a significantly different intercept from  $DM_E$  ( $t=9.89$   $df=57$ ,  $P<0.001$ ) and MBC ( $P<0.001$ ) indicating a consistent enrichment in the general non-linear normalization model.

### 3.4 Discussion

Stable isotope values obtained from pectoral fin clip tissue of lake sturgeon were significantly related to paired samples of dorsal muscle and provided a reasonable surrogate for muscle tissue SIA values as evidenced by the 1:1 slope of the comparative regressions. Relationships, however, explained less than 50 percent of the variation in the data given the high variation in the C:N ratio between individuals. The C:N ratio is typically used as a surrogate



measure of lipid content. While lipid extraction significantly reduced the among-individual variation in carbon stable isotope values and improved the explanatory power of the regression, when considering conformance to the hypothetical 1:1 line, the model did not have a zero intercept. Similar results were observed when using mass balance correction methods, with mass balance correction methods proving statistically superior to mathematical lipid normalization. Thus fin clips can be used in place of dorsal muscle tissue in stable isotope studies and permit the non-lethal sampling of the lake sturgeon for purposes of furthering the understanding of its trophic ecology. Fin clips may also be used where correction for lipids is required provided that mass balance approaches are used. Caution, however, should be applied when extrapolating results beyond the size range of individuals used here as juvenile lake sturgeon were not available for analysis in this study.

Previous studies have highlighted the importance of developing non-lethal sampling using fin-clip tissue as a surrogate for a variety of species at risk including: pallid sturgeon (*Scaphirhynchus albus*), Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) (Sanderson et al., 2009; Andvik et al., 2010; Hanisch et al., 2010). While a number of studies have reported FC-DM relationships conforming to the hypothetical 1:1 slope (Hanisch et al., 2010; Jardine et al., 2011), Willis et al. (2013) demonstrate a lack of 1:1 fit in many instances. Studies showing a strong 1:1 relationship are typically based on signatures with a wide range of variation (~15‰). In some cases a greater isotopic range results from use of multiple species (e.g., Jardine et al., 2011), or a single species from multiple locations (e.g., Hanisch et al., 2010), in a single regression which may result in pseudoreplication (Willis et al., 2013). Overall it has been generally concluded that fin-clips are suitable surrogates for muscle tissue in stable isotope studies of species' ecology. However, variation among species, the lack of 1:1 slope relationships in some instances, and evidence of the influence of fish length and sampling date on the strength of the obtained relationships suggest inferential errors can result in some instances (McCarthy and Waldron 2000; Hanisch et al., 2010; Jardine et al., 2011; Willis et al., 2013).

Spurious 1:1 isotopic relationships between tissues can be avoided by using migrant individuals where differences in slope are attributed to among habitat variation in isotopic

baseline, but may be induced if data from different species are combined in attempts to increase the range of the data values used in regression analysis (Willis et al., 2013). While the isotopic range seen in this study was relatively small (~5‰), the FC-DM 1:1 relationship can be attributed to potential differences in isotopic baselines. Although all individuals were captured from the same location, adult lake sturgeon are known to migrate large distances for spawning (Fisheries and Oceans Canada, 2008) and in the Rainy River system are known to consist of discrete populations likely to have originated from a number of different feeding areas, including Lake of the Woods approximately 140km downstream (Rusak and Mosindy, 1997).

Analysis of FC-DM relationships often demonstrate that fin-clip  $\delta^{13}\text{C}$  signatures are slightly enriched relative to muscle samples (McCarthy and Waldron 2000; Hanisch et al., 2010; Andvik et al., 2010), typically in the range of 0.1-0.7 ‰ (Kelly et al., 2006) which has been attributed to differential lipid content in tissue types or tissue specific turnover rates. After lipid extraction, adult lake sturgeon muscle had a non-significant enrichment in fin clip tissue relative to muscle of 0.21‰. A tissue turnover experiment by Heady and Moore (2013), suggested that fin clip tissue has a faster turnover rate than muscle for  $\delta^{15}\text{N}$ , however, Suzuki (2005) found muscle and fin clip to have comparable turnover rates for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Deviations from the hypothetical 1:1 and 0 intercept may result from differential turnover rates (Kelly et al., 2006). While there is strong evidence for the possible influence of tissue turnover rate on fin clip-muscle relationships, the effect is expected to vary according to species, life stage and season.

Mathematical lipid normalization did effectively reduce the among-individual variation in  $\delta^{13}\text{C}$  however the resulting values were significantly enriched relative to chemical lipid extraction and fin clip tissue signatures. Thus the use of general mathematical normalization is not appropriate for lake sturgeon stable isotope studies. The use of mathematical correction models has similarly been noted as deficient in the literature, with models tending to significantly underestimate lipid content (Fagan et al., 2011). In contrast, estimates from the mass balance method indicated a comparable slope and intercept to the chemical extraction method, suggesting the approach generally provides an accurate and unbiased means of

correcting bulk  $\delta^{13}\text{C}$  for lipid content. Similar results have been reported elsewhere (e.g., Hoffman and Sutton, 2010).

When coupled with results obtained here, literature studies thus favour the use of mass balance approaches for lake sturgeon when lipid correction is required. Accordingly use of fin clips in trophic studies may be adapted to accurately adjust for the effect of differential lipid accumulation in lake sturgeon that may occur as a result of age, maturation and/or feeding status (e.g., Beamish et al., 1996). Therefore, the use of stable isotope signatures obtained from fin-clips can provide a repeatable, simple, non-invasive sampling procedure that facilitates a variety of ecologically relevant comparisons (i.e., lipid, lipid extracted). As such fin clips allow changes in food web structure affecting lake sturgeon that might accompany the introduction of invasive species or other anthropogenic stressors to be accurately tracked and provide the information required for improved management and recovery of this species of concern.

### 3.5 Figures and Tables

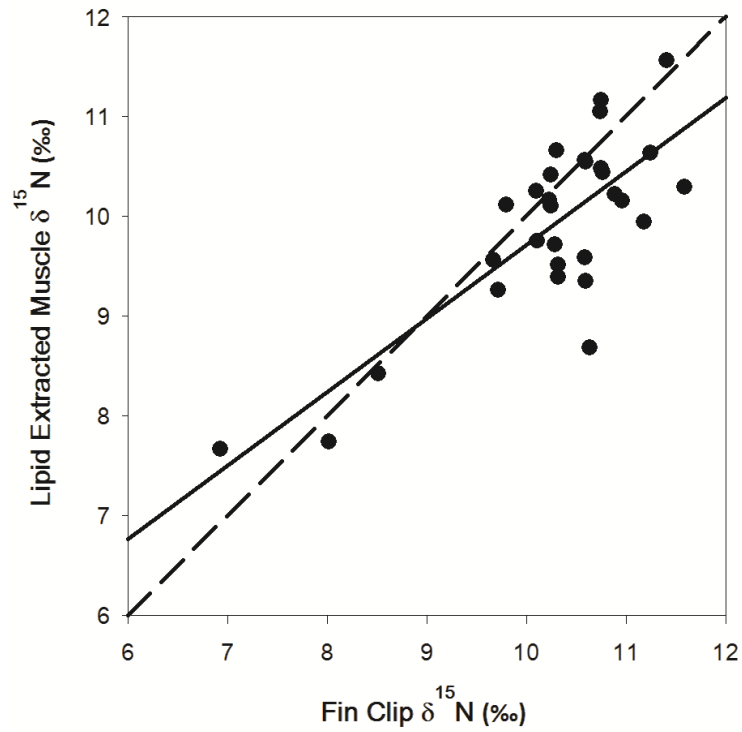


Figure 3.1 Estimated linear relationship for lipid extracted dorsal muscle and fin-clip tissue  $\delta^{15}\text{N}$  values obtained from the same individual lake sturgeon. The solid line plots the estimated regression line and the dashed line plots the hypothetical 1:1 line

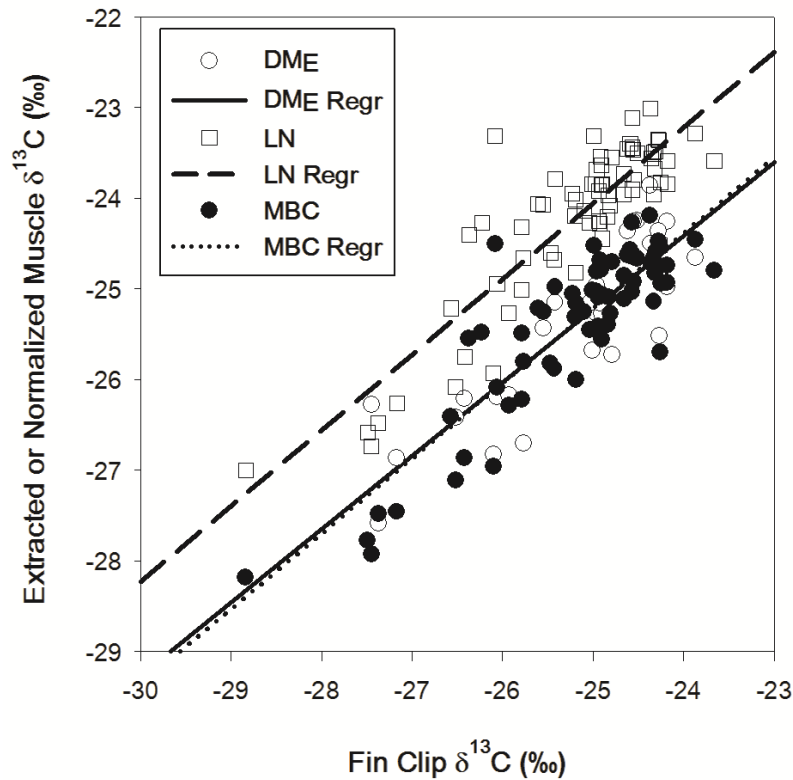


Figure 3.2 Estimated linear relationships for lipid extracted (open circles) ( $DM_E$ ), mass balance corrected dorsal muscle (black circles) (MBC) and lipid normalized (open squares) (LN) dorsal muscle against fin-clip tissue (FC)  $\delta^{13}C$  values. The models indicate a consistent but significant difference between  $DM_E$  and LN tissue values across the range of considered data.  $DM_E$ , MBC and LN regression lines, respectively are plotted as solid, dotted and dashed line.

Table 3.1 Summary statistics including mean, standard deviation (SD), minimum and maximum values for weight, total-length and age of sampled lake sturgeon.

	n	Mean	SD	Min	Max
Weight (kg)	68	15.21	6.55	0.2	38.2
Total-length (cm)	68	126.54	20.3	42.5	168.5
Age	55	18.44	6.55	3	33

Table 3.2 Summary statistics including: mean, standard deviation (SD), minimum and maximum values for  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and C:N obtained from the analyses of adult lake sturgeon dorsal muscle tissue (DM), pectoral fin-clips (FC), lipid extracted (DM<sub>E</sub>), mass balance corrected (MBC) and mathematical lipid normalized (LN) dorsal muscle tissue. Means not significantly different from one another are denoted with a common superscript (e.g., A, B).

Tissue	n	$\delta^{13}\text{C}$				$\delta^{15}\text{N}$				C:N			
		Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
DM	68	-28.17 <sup>A</sup>	1.37	-31.70	-26.13	9.67 <sup>A</sup>	1.11	6.19	12.25	7.81 <sup>A</sup>	3.36	3.3	21.80
FC	68	-25.20 <sup>B</sup>	0.97	-28.84	-23.66	10.37 <sup>B</sup>	0.90	6.32	11.59	3.43 <sup>B</sup>	0.42	2.99	3.83
DM <sub>E</sub>	30	-25.41 <sup>B</sup>	0.93	-27.59	-23.86	9.91 <sup>A</sup>	0.91	7.66	11.56	3.51 <sup>B</sup>	0.29	3.16	4.40
MBC	38	-25.38 <sup>B</sup>	0.92	-28.18	-24.19								
LN	30	-24.23 <sup>C</sup>	0.93	-27.00	-23.01								

Table 3.3 Estimated regressions for adult lake sturgeon  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data relating dorsal muscle tissue (DM), pectoral fin-clips (FC), lipid extracted dorsal (DM<sub>E</sub>), mass balance corrected (MBC) and mathematical lipid normalized (LN) muscle tissue values to one another. Where significant regressions were found slope 1:1 and intercept p-values report the results of testing significant regression slopes or intercept values, respectively, against the hypothetical 1:1 line and 0 value.

Model	N	r <sup>2</sup>	Regression P-value	Regression Equation	Slope 1:1 P-value	Intercept P-value
$\delta^{13}\text{C}$ comparisons						
DM <sub>E</sub> – DM	30	0.12	0.07	DM <sub>E</sub> = -18.3 + 0.25 (DM)	x	x
DM – FC	68	0.32	<0.01	DM = -8.13 + 0.80 (FC)	0.100	0.030*
DM – MBC	68	0.35	<0.01	DM = -5.96 + 0.88 (MBC)	0.450	0.100
DM – LN	68	0.30	<0.01	DM = -8.6 + 0.81 (LN)	0.200	0.020*
DM <sub>E</sub> -FC	30	0.74	<0.01	DM <sub>E</sub> = -4.97 + 0.81 (FC)	0.051	0.040*
DM <sub>E</sub> - MBC	30	0.72	<0.01	DM <sub>E</sub> = -6.25 + 0.75 (MBC)	0.010*	0.010*
DM <sub>E</sub> – LN	30	0.73	<0.01	DM <sub>E</sub> = -7.41 + 0.74 (LN)	0.002*	0.010*
MBC – FC	68	0.77	<0.01	MBC = -4.46 + 0.83 (FC)	0.500	0.020*
LN – FC	68	0.76	<0.01	LN = -3.20 + 0.83 (FC)	0.010*	0.003*
$\delta^{15}\text{N}$ comparisons						
DM <sub>E</sub> – DM	30	0.66	<0.01	DM <sub>E</sub> = 3.31 + 0.67 (DM)	0.001*	0.01*
DM – FC	68	0.46	<0.01	DM = 0.96 + 0.84 (FC)	0.150	0.420
DM <sub>E</sub> – FC	30	0.62	<0.01	DM <sub>E</sub> = 2.34 + 0.74 (FC)	0.020*	0.040*

## **Chapter 4 Stable Isotope Characterization of the Rainy River, ONT lake sturgeon trophic positioning and dependences.**

### **4.1 Introduction**

Lake sturgeon (*Acipenser fulvescens*) are considered threatened and many populations have been in decline since the early 1890s (Kerr et al., 2010). Overfishing, habitat alterations, poor water quality, and damming of rivers are believed to have played an important role in the decline of lake sturgeon (COSEWIC, 2006, Stelzer et al., 2008). While there has been considerable study completed on population abundances and structure (Beamish et al., 1996; Adams et al., 2006), movement and juvenile drift (Auer et al., 1996; Auer and Baker, 2002) and critical habitat (Barth et al., 2009), little is known about sturgeon diets and their role in lacustrine and riverine food webs (Stelzer et al., 2008).

Previous studies have shown that juvenile lake sturgeon feed primarily on benthic invertebrates (Jackson et al., 2002; Nilo et al., 2006) and are believed to be more benthivorous than adults (Miller, 2004; Stelzer et al., 2008). For adults, the dietary information that does exist is limited (e.g., Stelzer et al., 2008) and dated (e.g., Harkness and Dymond, 1961; Hay-Chmielewski, 1987; Mosindy and Rusak, 1991), with more recent work suggesting adult lake sturgeon undergo significant ontogenetic dietary shifts that favour greater consumption of fish as they age (Stelzer et al., 2008). The lack of current, accurate baseline information on lake sturgeon foraging has implications for our ability to predict how anthropogenic-induced changes in food web structure, e.g., increased nutrient loading (Carpenter et al., 2007), non-native species invasions (Vander Zanden et al., 1999) or climate change (IPPC, 2013), may affect lake sturgeon populations in the future.

Stable isotope analysis (SIA) has a long history of use in dietary studies because of its ability to reflect long-term dietary assimilation patterns as compared to short term dietary snapshots described via the analysis of stomach contents obtained by gastric lavage (Brosse et al., 2002). Stable carbon isotopes can provide insights into the carbon sources (e.g., benthic, pelagic) from which consumers obtain their energy (Peterson and Fry, 1987), while stable nitrogen isotopes provide a continuous measure of consumer trophic position (DeNiro and

Epstein, 1978) that may vary with size or age (e.g. Fry, 2006). Thus obtaining stable isotope data on lake sturgeon may help assess both the position of lake sturgeon within existing food webs and provide important initial information for assessing how trophic positioning and connectivity change through time (Stelzer et al., 2008).

Many analytical tools have been developed for examination and comparison of food web structures using SIA (reviewed by Layman et al., 2012), including determining resource pools (e.g., Hamilton et al., 1992; Stelzer et al., 2008), describing community and niche relationships (e.g., Vander Zanden and Rasmussen, 1999; Beaudoin et al., 2001), habitat use (e. g., Power et al., 2005; McMahon et al., 2012), intraspecific diet variability (Bearhop et al., 2004) and determining the effect of ecological perturbations on food web structure and function (Vander Zanden et al., 1999; Ives et al., 2013). SIA is now also being used to describe the spatial scales at which organisms move during feeding (Rasmussen et al., 2008) which is vital to environmental programmes assessing the scale of anthropogenic impacts on fish populations (e.g. Galloway et al., 2003; Gray and Munkittrick, 2005).

Disturbances to food webs typically impact one portion of the ecosystem more than another (i.e. benthic or pelagic portions) (Vadeboncoeur et al., 2001). Furthermore changes in the availability of the basal resources that support fish populations are generally reflected in changes in trophic position (Post et al., 2002). The organic matter sources that support aquatic food webs (i.e. allochthonous vs autochthonous), have been shown to be affected by local environmental conditions, such as substrate types, hydrologic condition and seasonal variation (Colombo et al., 2012; Goll ty et al., 2010; Lefebvre et al., 2009; Leonard et al., 1998). The resulting spatial patterning in habitat quality can yield niche variation at all levels of biological structure including: individuals, groups, and subpopulations (Cross et al., 2013). Such patterns can result in differences in the abundance and types of prey available to consumers that have implications for the diets of individuals or whole groups of individuals (e.g. a sub-population) occupying specific habitats (Johnson, 2000).

While limited food web studies have been conducted with lacustrine lake sturgeon populations (e.g. Lake Winnebago, Stelzer et al., 2008) similar studies do not exist for riverine



populations. Here we use stable isotope methods to study the dietary patterns of lake sturgeon captured in the reaches of the Rainy River downstream of the International Falls and Fort Frances dams (IFD). Specifically we use stable isotope data gathered on the resident adult lake sturgeon to: [1] examine intraspecific diet variability and ontogenetic shifts in diet; [2] determine the proportions of prey items contributing to diets; and, [3] characterize trophic position and isotopic niche space both within the river as a whole, and as a function of identified geomorphic units.

## **4.2 Methods**

All fish and food web-related samples were obtained from the Rainy River, Ontario (48.60° N, 93.40° W) in a 22km reach of the river stretching from the International Falls Dam (IFD) to its confluence with the Little Fork River. The Rainy River is located on the Minnesota-Ontario border and flows westward from Rainy Lake to the south end of Lake of the Woods over approximately 145km. It is the largest tributary of Lake of the Woods, contributing 70% of the annual flow from a drainage area of approximately 70,000km<sup>2</sup> (Eibler and Anderson, 2004). The river drains through shallow glacial lake bed deposits dominated by clays and sands interspersed with rock outcrops forming shoals and rapids (Eidler and Anderson, 2004).

Lake sturgeon were obtained during the spawning season (May-June) by night boat electrofishing and with 25.4 and 30.5 cm mesh gill nets set overnight following protocols described in Dubreuil and Cuerrier (1950), i.e., parallel or at an angle to river flow in currents and back eddies, and strategically placed to optimize capture. All relevant biological information (e.g., length, weight and girth) was obtained from freshly captured specimens and a pectoral fin ray was removed for aging purposes, with aging completed by Fisheries and Oceans Canada following protocols described in DeVries and Frie (1996).

Sampling for stable isotopes tissue involved taking one or two muscle plugs from each individual using a 3mm dermal biopsy punch from behind the third dorsal scute (Tyus et al., 1999). The resulting wounds were sealed using 3M Vetbond Tissue Adhesive™. Furthermore, fin-clips were obtained with a circular paper punch applied to the pectoral fin membrane so as to avoid sampling of the fin ray (Tyus et al., 1999). Fin tissue was used to accurately adjust for the

effect of differential lipid accumulation between individuals in adult lake sturgeon following (Smith et al., *in press*) given C:N ratios consistently  $> 3.5$  and the high inter-individual C:N variability (e.g., Post et al., 2007; Logan et al., 2008). All samples were frozen ( $-20^{\circ}\text{C}$ ) until further processing.

Aquatic macro-invertebrates, potential prey fish, periphyton, particulate organic matter (POM), macrophytes and terrestrial vegetation were sampled in the study reach. Fish other than lake sturgeon were captured by backpack electrofishing. A minimum sample of  $n=3$  was reserved and euthanized for stable isotope analysis, with carcasses being frozen at  $-20^{\circ}\text{C}$  until a sample of skinless dorsal–lateral muscle was dissected for SIA. All necessary biological information was collected from freshly captured individuals including: length (cm), weight (kg) and sex before freezing for SIA. Aquatic invertebrates were collected primarily by kick and sweep sampling with dip nets or ponar samplers. Individual invertebrates were identified to the family level where possible (Vander Zanden and Rasmussen, 1999). When necessary, multiple individuals per family per site were pooled to obtain sufficient material for stable isotope analysis (Stelzer et al., 2008). Examples of common terrestrial, aquatic vegetation and filamentous algae were collected by hand. Periphyton was sampled by scraping rocks and backwashing onto a GF/C filter. Three one-l water samples were taken at each fish sampling site and filtered onto quartz fiber filters for analysis of particulate organic matter (POM).

All fish and food web samples were kept frozen until returned to the laboratory where they were dried at  $50^{\circ}\text{C}$  for 24-48hrs. All SIA were performed at the Environmental Isotope Laboratory, University of Waterloo on a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Carlo Erba elemental analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy). Analytical precision is  $\pm 0.1\%$  and  $\pm 0.2\%$ , respectively, for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  and was determined by repeat analysis of duplicates (one in ten). All resulting measurements were expressed using standard delta notation as parts per thousand differences (‰) with respect to the international reference standards, carbonate rock from the Peedee Belemnite formation for  $\delta^{13}\text{C}$  (Craig, 1957) and nitrogen gas in the atmosphere for  $\delta^{15}\text{N}$  (Mariotti, 1983). Analytical accuracy was validated against internal laboratory standards cross-

calibrated against the International Atomic Energy Agency standards CH<sub>6</sub> for carbon and N<sup>1</sup> and N<sup>2</sup> for nitrogen.

Periphyton samples were treated with dilute HCl solution (0.1 N) to remove inorganic carbonate that would bias  $\delta^{13}\text{C}$  values (Bunn et al., 1995). Bias is possible due to the presence of accumulated sediments in the organic matter of primary interest (Connolly and Schlacher, 2013). Treating samples with acids to remove carbonates can, however, have unintended consequences for nitrogen isotopes ( $\delta^{15}\text{N}$ ) that are altered during acid treatment (Bunn et al., 1995). Therefore, half of each periphyton sample was ground and analyzed without acidifying, while the other half was acidified for at least 1h at room temperature and rinsed in distilled water before drying and grinding (Bunn et al., 1995; Connolly and Schlacher, 2013).

The proportions of prey used by consumers were determined using the Stable Isotope Analysis in R (SIAR) program and standard  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plots. Analyses were completed by applying trophic shift factors based on the literature. A value of +0.4 ‰ (Post, 2002) was assumed for  $\delta^{13}\text{C}$  following Stelzer et al. (2008). A value of +3.0 ‰ for  $\delta^{15}\text{N}$  was obtained from a re-analysis of data provided for ammonotelic freshwater stenotherms as presented in Vanderklift and Ponsard (2003). While the value is lower than the widely used trophic shift value of +3.4‰ (Post, 2002), the value specifically adjusts for the main biochemical form of nitrogenous waste in lake sturgeon known to have significant effect on the metabolic enrichment of  $\delta^{15}\text{N}$  during tissue catabolism (Vanderklift and Ponsard, 2003).

Mean isotopic signature values were used for all organisms considered as potential lake sturgeon prey with all forage fish species considered as potential prey (mean length= 42mm  $\pm$ 15mm) grouped as a single mean value. While no eggs were collected for SIA, we considered them to be potentially consumed by lake sturgeon (Scott and Crossman, 1975). In order to include them in the model we assumed isotope signatures of eggs to be equal to those of adult walleye, following results found by Vander Zanden et al. (1998) and Murchie and Power (2004) where the signatures of adult fish were not significantly different from those of the embryos (e.g. Adults:  $\delta^{13}\text{C}$ =-26.0‰;  $\delta^{15}\text{N}$ =7.0‰, Embryos:  $\delta^{13}\text{C}$ =-25.0‰;  $\delta^{15}\text{N}$ =6.3‰) (Vander Zanden et al. 1998). Priors for prey percent consumption were incorporated into the SIAR by taking the

average reported value from several lake sturgeon stomach content analyses (Harkness and Dymond, 1961; Cuerrier, 1966; Hay-Chmielewski, 1987; Stelzer et al., 2008). Prior values included: mayflies 35%, crayfish 19%, chironomids 13%, mussels 11% and snails 7%, all other prey were less than 5%. Once all prior values were incorporated from the literature (93.7%), the remaining percentage assigned to grouped forage fish (1.85%) and fish eggs (1.85%) in a 1:1 ratio.

To gain a more complete characterization of the river, habitats within the river were broadly classified on the basis of substrate and local hydraulic characteristics. Type 1 habitat consisted of bedrock outcrops with high local velocities ( $>0.8$  m/s). Type 2 habitat was comprised of boulder/cobble substrates with moderate local velocities (0.8-0.5 m/s). Type 3 habitat was dominated by sand/gravels bar substrates with low local velocities ( $<0.5$  m/s) and Type 4 habitat consisted of highly vegetated backwater regions with sand and silt substrates. SIA sampling was completed in all habitat types, with additional sampling completed in the Little Fork River tributary and the nearshore areas of Lake of the Woods proximate to the inflow of the Rainy River to the lake. Among habitat variability was assessed by comparing baseline stable isotope values for filter feeding mussels and grazing snails (Post, 2002).

Stable Isotope Bayesian Ellipses in R (SIBER) were used to generate standard ellipse areas (SEA) to describe the isotopic niche (Jackson et al., 2011). Standard ellipse areas are the bivariate equivalent to two standard deviations in univariate analysis and provide a suitable measure of the mean core population isotopic niche which is robust to variation in sample size and accounts for uncertainty (Jackson et al., 2011). Thus SEAs may be used to adequately describe the variation of any defined group within isotope space, be it samples from a single species or a community. Here we used SEAs to describe the isotopic niche for lake sturgeon, after adjustment for fractionation, and the potential isotopic niche for lake sturgeon as described by the combined isotopic niche of potential prey sampled from each of the defined habitat types within the Rainy River, the Rainy River as a whole (mean for all habitat types), Lake of the Woods and Little Fork River. Trophic niche overlap and centroid Euclidean distances were similarly calculated using SIBER to determine which habitat types were most closely associated with lake sturgeon feeding (Jackson et al., 2011).

The normality and variance homogeneity of data were assessed prior to statistical analyses using the Shapiro-Wilk W test (Shapiro and Wilk, 1965) and Levene's F test (Levene, 1960). Linear regression was used to test for significant relationships between SI signatures and the lengths of individual fish. ANOVA was used to test for differences among groups where appropriate, after checking for conformance of the data to assumptions. JMP 7.0 (SAS Institute) was used to perform reported statistical analyses, with significance level at  $\alpha=0.05$  level.

### 4.3 Results

A total of 72 lake sturgeon were captured for use in stable isotope analysis. Of those, 55 were confidently aged. A summary of the biological characteristics is presented in Table 4.1. Lake sturgeon ranged in size from 42.5 to 168.5cm and in age from three to 33. Statistical testing of differences between the sexes was not possible owing to the small number of those for whom sex could be confirmed without fatal sampling.

A  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot of the isotope data for the river as a whole (Figure 4.1) showed a range in  $\delta^{13}\text{C}$  from a low of -29.8‰ for mussels to a high of -20.9‰ for amphipods and from a low of -2.3‰ for terrestrial plants to a high of 11.8‰ for adult walleye along the  $\delta^{15}\text{N}$  axis. Within the bi-plot, vertical trophic structure consisting of four trophic levels (primary producers, primary consumers, forage and piscivorous fishes) was evident, with adult walleye sitting at the apex position. Illustrated SIA values for lake sturgeon were lipid corrected using fin clip tissue as a result of the high mean dorsal muscle tissue C:N ratios (7.81) and the high inter-individual variability in the C:N ratio (coefficient of variation =43.0%).

Regressions of lipid corrected values using fin clips against length yielded insignificant results for  $\delta^{15}\text{N}$  ( $F_{1,66}=0.02$ ,  $r^2 < 0.001$ ,  $P=0.890$ ), however,  $\delta^{13}\text{C}$  demonstrated a significant positive relationship with low explanatory power ( $F_{1,66}=12.08$ ,  $r^2=0.15$ ,  $P=0.001$ ) and reduced the intra-specific variation in  $\delta^{13}\text{C}$  (range:-23.7 to -28.8‰, coefficient of variation: 3.9%).

Median prey proportions estimated by SIAR from lipid corrected fin tissue suggested organic material derived from fish (either fish or fish eggs) formed the largest part of Rainy

River lake sturgeon diets (46%; 95% credibility interval= 38% to 54%) (Figure 4.2). Median proportions for other prey organisms contributing to the diet of lake sturgeon included: crayfish (7.5%; CI=0-25%), mussels (7.2%; CI=0-9.1%), chironomids (7.1%; CI=0-17%) and mayflies (4.3%; CI=1.1-29%), with remaining invertebrate prey median values contributing less than 0.1% with 95% CI of 0 to <20% each.

Comparison of baseline isotope signatures from the studied habitat types showed no significant differences in either  $\delta^{13}\text{C}$  (ANOVA  $F_{3, 79}=0.47$ ,  $P=0.700$ ) or  $\delta^{15}\text{N}$  (ANOVA  $F_{3, 79}=1.16$ ,  $P=0.330$ ), although greater variation among habitat types was observed in the grazing snails (coefficient of variation for all samples  $\delta^{13}\text{C}=7.9\%$ ;  $\delta^{15}\text{N}=31.6\%$ ) than filter feeding mussels (coefficient of variation for all samples  $\delta^{13}\text{C}=2.4\%$ ;  $\delta^{15}\text{N}=24.9\%$ ).

When available prey items were pooled by habitat types, the feeding opportunity ellipses calculated using SIBER showed high overlap (> 72%) and were closely clustered (Figure 4.3). The lake sturgeon standard ellipse area computed from fin tissue stable isotope data corrected for fractionation demonstrated a high degree of overlap with the feeding opportunity ellipses of all habitat types (>75%). The lowest habitat overlap for lake sturgeon was observed for Type 4 (vegetated backwaters). Centroid distances between the lake sturgeon standard ellipse and those defined by the feeding opportunity ellipses for each habitat varied little, with the closet habitat ellipse being that defined for Type 2 (moderate velocity boulder/cobble) and the furthest being that defined for Type 1 (high velocity bedrock) (Table 4.2).

When the standard ellipse analysis was repeated at larger spatial scales, using water bodies instead of habitats, large overlaps were similarly found (Figure 4.4). There was a large overlap between the feeding opportunity ellipses for the Rainy River and Lake of the Woods, with approximately 94% of the Rainy River opportunity ellipse being contained within the Lake of the Woods feeding opportunity ellipse (Table 4.3). In contrast the Rainy River showed much less overlap with the Little Fork River (<50%). The standard ellipse area for lake sturgeon fin tissue significantly overlapped with the SEAs for the Rainy River and Lake of the Woods (94% and 81%, respectively), but showed reduced overlap with Little Fork River (47%). Centroid distance computations indicated similar results, with closer proximity of the lake sturgeon

standard ellipse area to that of the Rainy River and Lake of the Woods as compared to the Little Fork River.

#### 4.4 Discussion

Rainy River lake sturgeon stable isotope data showed moderate intra-specific variability and a significant size related shift in  $\delta^{13}\text{C}$  when the data were lipid corrected. Within the food web, lake sturgeon occupy an upper trophic position dependent on their high (>50%) proportionate assimilation of fish-derived protein, with invertebrates contributing approximately 40% to the realized isotope signatures of most Rainy River lake sturgeon. At the meso-scale within the Rainy River there was no evidence to suggest lake sturgeon were preferentially connected to habitat-specific food webs (e.g. bedrock outcrops with high local velocities versus highly vegetated habitats). At the macro scale, Rainy River lake sturgeon do appear to rely more heavily on the food webs of the Rainy River and the Lake of the Woods than on available tributary systems.

Inter-individual variation in the  $\delta^{13}\text{C}$  of individual sturgeon may have been influenced by a variety of factors including: differential habitat use, differences in the turnover of carbon and nitrogen (Matthews and Mazumder, 2004) and/or differential lipid accumulation (Smith et al., *in press*). While lipid correction reduced the observed among-individual variation in  $\delta^{13}\text{C}$ , variations in feeding tactics among individuals remained. Differences in habitat use may also have contributed to the observed among-individual differences, with radio-tagging studies having indicated significant differences among groups of sturgeon that over-winter, respectively, in lentic and lotic habitats within the Rainy River-Lake of the Woods system (Rusak and Mosindy, 1997).

SIAR analysis of contributing prey proportions provided further evidence for piscivory in adult lake sturgeon populations. The reliance on fish derived protein has been similarly reported for lacustrine populations in Lake Winnebago, Wisconsin, where combined gut content and stable isotope mixing model analyses indicated high reliance on gizzard shad (*Dorosoma cepedianum*) with some 56% of gut content mass and 37% assimilated carbon being accounted for gizzard shad. Smaller forage fishes such as ninepines stickleback (*Pungitius pungitius*) and

scuplin (Cottidae sp.) have also been identified in lake sturgeon stomachs reported within the St. Lawrence River. An early study by Vladykov and Greeley (1963) reported that fish accounted for as much as 24% of sturgeon gut contents, noting that anecdotal reports suggested that hook and line capture was optimized with the use of bait fish such as American shad (*Alosa sapidissima*) or banded Killifish (*Fundulus diaphanous*).

The results contrast with previous studies suggesting adult sturgeon from Lake of the Woods relied heavily on mayfly larvae, chironomids and crayfishes, with those prey accounting for approximately 70% of the food items found in the guts of commercially harvested sturgeon (Mosindy and Rusak, 1991). This contrast may be explained by consumption of fish derived protein being temporally limited. While stomach contents provide a dietary snapshot, SIA reflects long-term dietary assimilation patterns. Stomach content studies that find high reliance on invertebrates are commonly completed in summer or fall (e.g. Mosindy and Rusak, 1991; Werner et al., 2005) while Stelzer et al. (2008) found high reliance on gizzard shad when sampled in February. The elevated signature found in lake sturgeon and use of fish derived protein may be related to availability during winter die-offs and in early spring spawning seasons. Lake sturgeon isotopic signatures consistently overlapped on the  $\delta^{15}\text{N}$  axis with adult walleye, known to rely heavily on other fish species as prey (Scott and Crossman, 1975). While the data suggest lake sturgeon are significant consumers of fish derived protein, significant contributions from invertebrate species to the diet remain, up to 40% of assimilated energy being attributable to larval invertebrates and crayfish.

Lake sturgeon are primarily opportunistic in their feeding habits and typically regarded as a benthic feeding species (Kerr et al., 2010). While historically lake sturgeon have been reported to consume fish and fish eggs (Cuerrier, 1966; Scott and Crossman, 1975), this potential dietary source has largely been ignored as anecdotal (e.g., Harkness and Dymond, 1961) and reinforced by the number of juvenile dietary studies indicating low or no incidence of fish or fish eggs in the diet (e.g., Nilo et al., 2006; Barth et al., 2013). Nevertheless, Cuerrier (1966) found evidence of heavy predation by lake sturgeon on non-identifiable eggs pre-spawn (12.5% of individuals) and lake sturgeon eggs post-spawn (100% of individuals). Recent studies in Oneida Lake, New York, have also reported dietary consumption of age-0 fish (Jackson et al., 2002). While



Harkness and Dymond (1961) suggested that egg consumption is inconsequential, they do report evidence of lake sturgeon feeding on walleye eggs. The primary species spawning in the Rainy River in the spring are known to be lake sturgeon and walleye, with survival of egg and larval stages of the latter having been shown to be low where sand substrates predominate (Ivan et al., 2010), as in the Rainy River, and related to river flow regulation (Kallemeyn, 1987). The evidence provided by the SIAR model here suggests that lake sturgeon are relying on these sources and the consumption of fish eggs or other deposited fish material would account for the elevated trophic position of lake sturgeon in the Rainy River system.

Stable isotope approaches can potentially be applied to landscape studies in any situation where spatial differences exist (Rasmussen et al., 2008). Here we proposed using feeding opportunity ellipses of habitat types as a method of identifying feeding habitat selection. Within the Rainy River feeding habitats we found a high degree of overlap making it difficult to discern which habitat type lake sturgeon are preferentially feeding within. However, the lowest reciprocal overlap and larger centroid distance were seen between lake sturgeon and highly vegetated backwater regions. Vegetated regions have been reported to be the areas of lowest lake sturgeon capture in previous feeding and movement studies of adults (Kerr et al., 2010; Choudhury et al., 1995). When individual scatter is examined in Figure 3 we also see a skewed distribution towards Type 2 habitat (moderate velocity boulder/cobble) which is reflected in both overlap (99%) and centroid distance (2.14 compared to 2.71).

Previous spatial information and feeding habitat preferences of lake sturgeon has been obtained from studies using marked and tagged individuals, and more recently with telemetry (e.g. Fortin et al., 1993; Auer, 1999; Damstra and Galarowicz, 2013). Feeding adults are routinely found in both large rivers and lakes, generally at depths of 5-10 m over a variety of substrates such as slit, mud, clay, sand or gravel (Harkness and Dymond, 1961; Rusak and Mosindy, 1997; Kerr et al., 2010). Substrate type is considered to be important for feeding habitat selection due to the prevalence of feeding on benthic organisms (Hay-Chmielewski, 1987; Damstra and Galarowicz, 2013), but in sand/gravel dominated rivers such as the Rainy (Eibler and Anderson, 2004) may not drive habitat use given the generalist feeding habits of lake

sturgeon (Kempinger, 1996; Beamish et al., 1998) and the known wide ranging movement of the species (e.g. Auer, 1999; Borkholder et al., 2002; Knights et al., 2002).

Our results suggest that lake sturgeon are potentially integrating signatures from Rainy River and Lake of the Woods sources with all individual lake sturgeon contained within the Rainy River feeding opportunity ellipse. However there is a high degree of overlap between Lake of the Woods signatures and those of Rainy River. Adult lake sturgeon have been shown to have complex movement patterns including spring migration to spawning areas, a post-spawning dispersal to feeding grounds, and a fall migration to overwintering sites (Rusak and Mosindy, 1997; Smith and King, 2005; Adams et al., 2006; Kerr et al., 2010) and would be expected to integrate feeding signatures as a result.

The stable isotope results suggest that the lake sturgeon in Rainy River obtain most of their energy from organic material derived from fish, whether that be from consumption of eggs or organic material from larval die-offs known to be high in walleye (Ivan et al., 2010). Results here parallel those recently found in lacustrine environments such as Lake Winnebago (Stelzer et al., 2008) and are important in the context of contributing modern technical verification to earlier historical reports of lake sturgeon dietary use of fish and fish eggs (e.g., Cuerrier, 1966). While very little variation was seen between habitat types in the Rainy River, resulting variation in lake sturgeon carbon signatures may be related to variability in either feeding tactics or location. In addition to providing insight on the role of lake sturgeon in riverine food webs, estimates of isotopic niches for the Rainy River establish an important baseline for future comparisons of changes in food web structure that might accompany the introduction of invasive species or the addition of anthropogenic stressors that could have implications for lake sturgeon conservation.

## 4.5 Figures and Tables

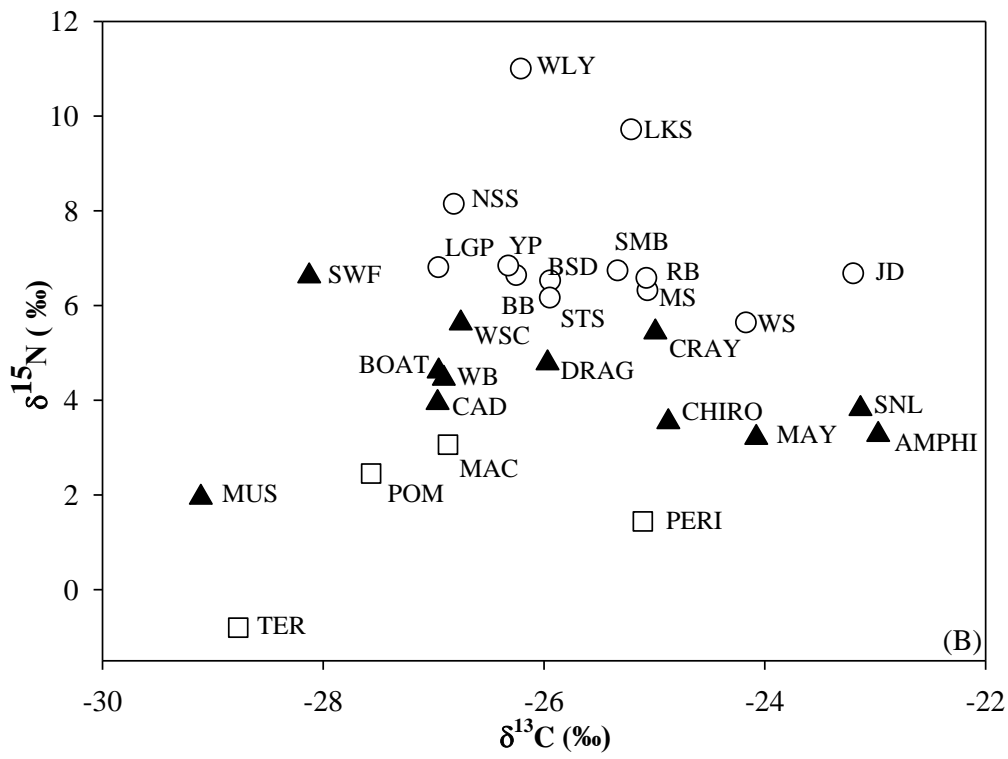
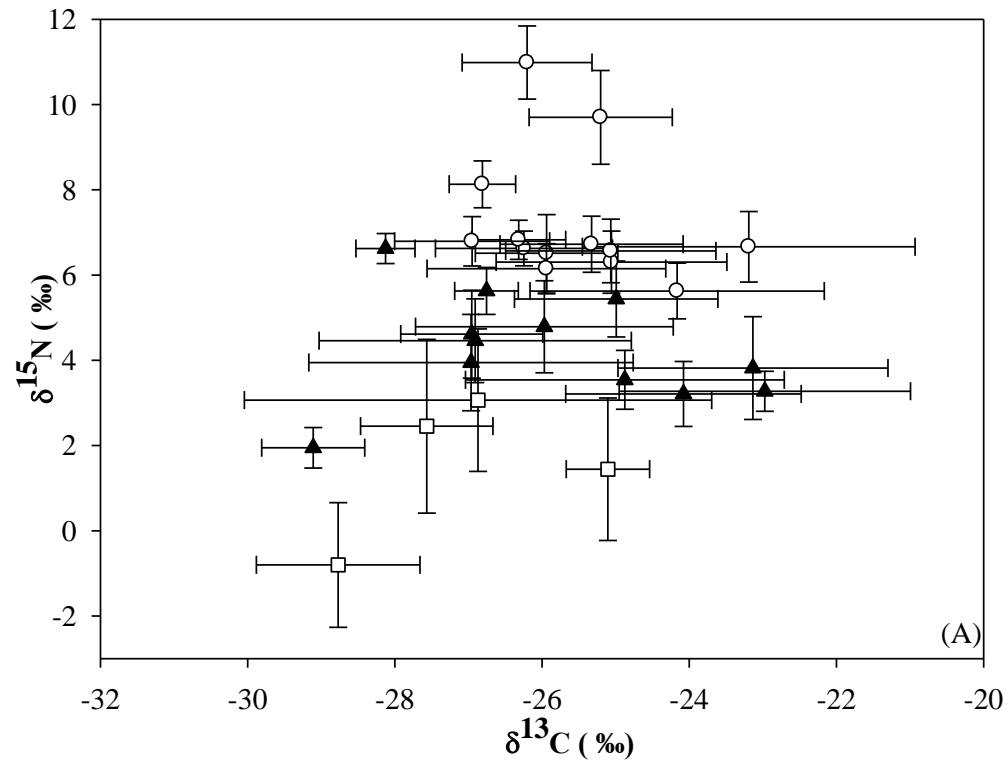


Figure 4.1 Mean values and standard deviations (A) and mean values with labels (B) of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (‰) of organisms found in the Rainy River. Basal food web carbon sources are plotted with open squares and include: TER=terrestrial vegetation, PERI=periphyton, POM=particulate organic matter and MAC=macrophytes. Macroinvertebrates are plotted as black triangles and include: AMPHI=amphipod, CAD=caddisfly, CHIRO=chironomid, CRAY=crayfish, DRAG=dragonfly, MAY=mayfly, MUS=mussel, SNL=snail, SWF=spiny water flea, WB=water beetle, BOAT=water boatman and WSC=water scorpion. Fish are plotted with open circles and include: BSD=blackside darter, BB=brown bullhead, JD=johnny darter, RB=rock bass, LKS=lake sturgeon (lipid corrected), LGP=logperch, MS=mottled sculpin, NSS=ninepsine stickleback, SMB=smallmouth bass, STS=spottail shiner, WS=white sucker, YP=yellow perch and WLY=walleye.

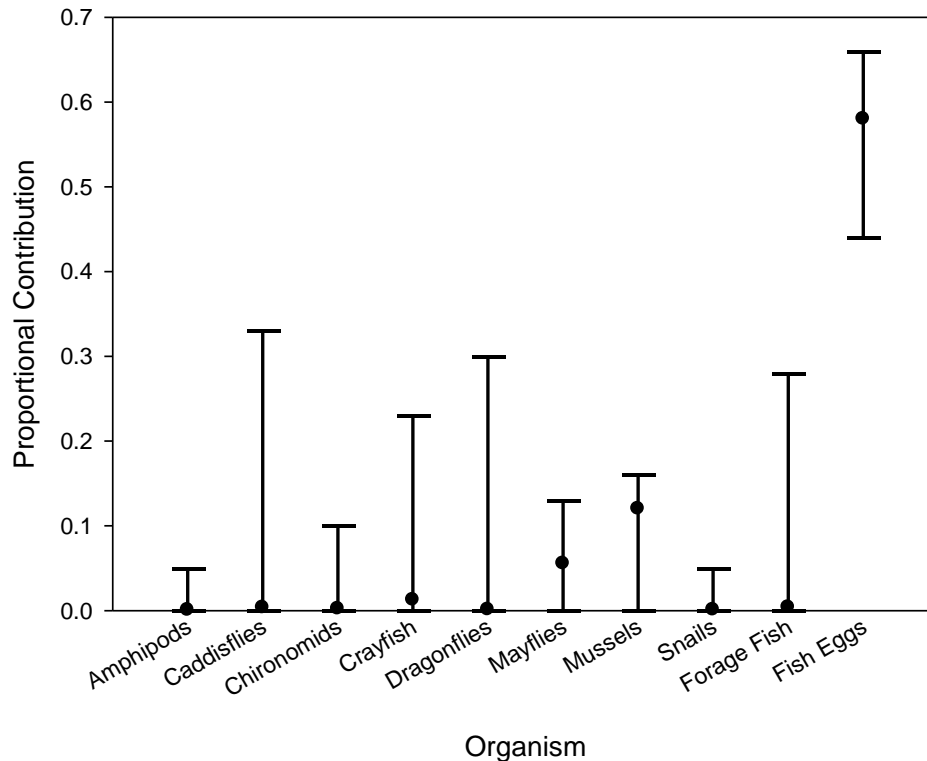


Figure 4.2 Proportion of prey contributing to the diet of lake sturgeon in the Rainy River as determined using Stable Isotope Analysis in R (SIAR). Black circles and solid lines represent median and 95% confidence intervals (CI) respectively obtained using fin tissue stable isotope signatures for lake sturgeon.

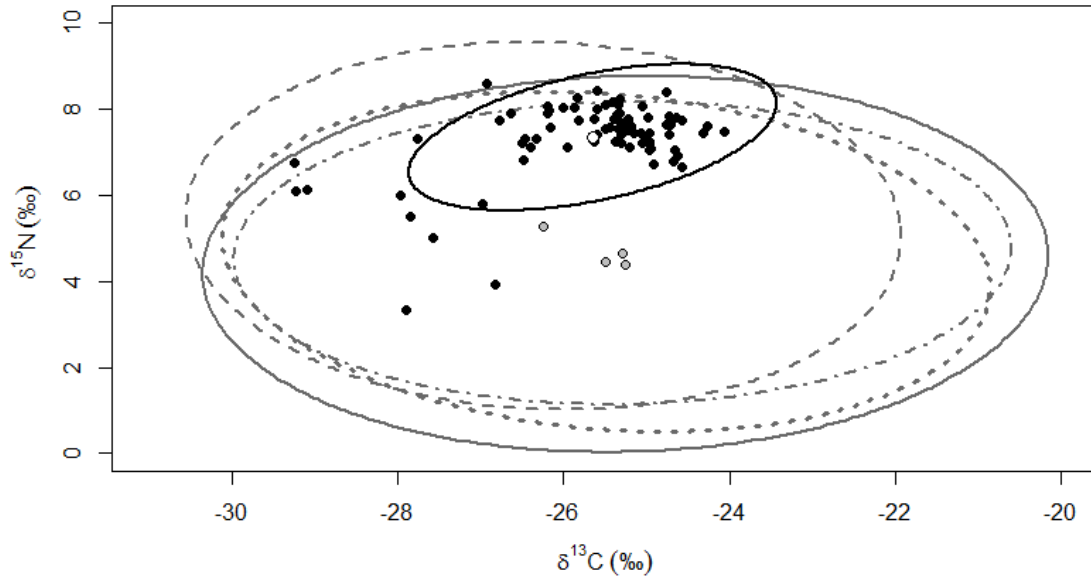


Figure 4.3 Standard ellipse areas for available prey items sampled in the four Rainy River habitat types: Type 1 (solid grey line) = high velocity bedrock, Type 2 (dashed grey line) = moderate velocity boulder/cobble, Type 3 (dotted grey line) = low velocity sand/gravel beds, and, Type 4 (dash-dot grey line) = vegetated backwaters. Centroids of the habitat ellipse are plotted as grey circles. Lake sturgeon muscle SEA is plotted as a solid black line, with individuals shown as black circles and the corresponding centroids as a white circle.

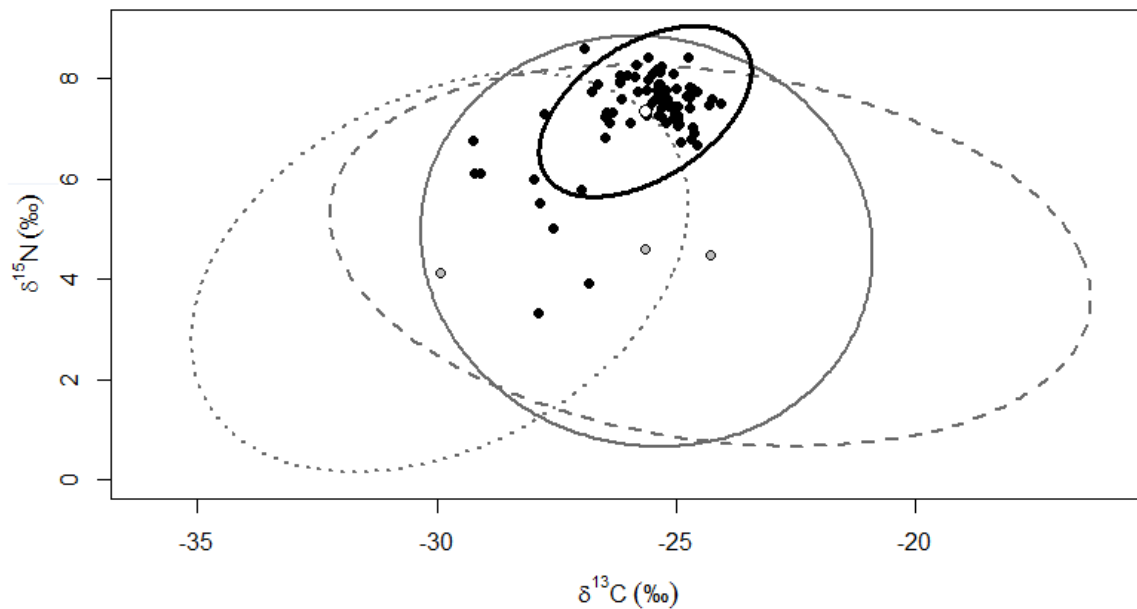


Figure 4.4 Community niche ellipses (grey lines) for all available prey items found in Rainy River (solid line), Lake of the Woods (dashed line) and Little Fork River (dotted line) with centroids for each ellipse plotted as a grey circle. Lake sturgeon muscle SEA is plotted as a solid black line, with individuals shown as black circles and the corresponding centroids as a white circle.

Table 4.1 Mean values and standard deviations (STDEV) of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (‰) of organisms found in the Rainy River.

Species	<i>n</i>	$\delta^{13}\text{C}$	STDEV	$\delta^{15}\text{N}$	STDEV
Amphipods	15	-22.97	1.98	3.27	0.47
Blackside darter	31	-25.94	0.97	6.51	0.91
Brown bullhead	6	-26.25	1.20	6.62	0.41
Caddisflies	60	-26.96	2.20	3.95	1.13
Chironomids	35	-24.87	2.17	3.54	0.69
Crayfish	39	-24.99	1.38	5.44	0.89
Dragonflies	30	-25.97	1.75	4.79	1.08
Johnny darter	42	-23.19	2.26	6.66	0.83
Macrophytes	40	-26.87	3.18	3.06	1.67
Mayflies	78	-24.08	1.60	3.21	0.76
Mottled sculpin	59	-25.06	1.57	6.30	0.73
Mussels	47	-29.11	0.70	1.95	0.48
Ninespine stickleback	28	-26.81	0.45	8.13	0.55
Acidified periphyton	16	-25.10	0.57	1.44	1.67
POM	33	-27.57	0.90	2.45	2.04
Rock bass	26	-25.07	1.43	6.57	0.75
Smallmouth bass	21	-25.33	1.24	6.72	0.66
Snails	31	-23.13	1.83	3.82	1.21
Spiny water flea	23	-28.13	0.40	6.62	0.35
Spottail shiner	42	-25.94	1.62	6.15	0.59
Terrestrial plants	99	-28.77	1.11	-0.80	1.46
Water beetles	44	-26.91	2.12	4.46	0.98
Water boatmans	22	-26.95	0.97	4.61	1.03
Water scorpions	14	-26.75	0.43	5.63	0.55
White sucker	28	-24.16	2.00	5.63	0.65
Yellow perch	66	-26.32	0.64	6.83	0.46
Lake sturgeon	68	-25.20	0.97	9.70	1.10
Walleye	55	-26.20	0.88	10.99	0.86
Logperch	15	-26.95	1.05	6.79	0.58

Table 4.2 Summary statistics for sampled lake sturgeon including: mean, standard deviation (SD), minimum and maximum values for weight, total length, age, and lipid corrected (fin) stable isotope values.

	n	Mean	SD	Min	Max
Weight (kg)	72	15.2	6.4	0.15	38.2
Total length (cm)	72	127.3	20.0	42.5	168.5
Age	55	18.4	5.7	3.0	33.0
$\delta^{15}\text{N}$ (‰)	68	9.7	1.1	6.2	12.3
$\delta^{13}\text{C}$ (‰) (lipid corrected)	30	-25.2	1.0	-28.8	-23.7

Table 4.3 Ellipse overlap and centroid distances for standard ellipse areas determined from available prey items sampled in the four Rainy River habitat types: Type 1= high velocity bedrock, Type 2= moderate velocity boulder/cobble, Type 3= low velocity sand/gravel beds, and, Type 4= vegetated backwaters. Overlaps in proportionate terms are computed using each habitat type, respectively, in the denominator indicated by the row heading. Centroid distances define the distance between the standard ellipse area for each habitat type and the standard ellipse area for lake sturgeon computed from muscle tissue biopsy values corrected for fractionation.

Ellipse Overlap	Type 1	Type 2	Type 3	Type 4	Lake sturgeon
Type 1	-	0.87	0.99	1.00	0.95
Type 2	0.72	-	0.85	0.87	0.99
Type 3	0.81	0.83	-	0.96	0.81
Type 4	0.74	0.78	0.87	-	0.77
Lake sturgeon	0.14	0.18	0.15	0.15	-
Centroid Distances					
Type 1	-	-	-	-	2.97
Type 2	1.33	-	-	-	2.14
Type 3	0.24	1.11	-	-	2.89
Type 4	0.26	1.14	0.28	-	2.71

Table 4.4 Standard ellipse area overlap and centroid distances determined from available prey items sampled in the Rainy River, Lake of the Woods and Little Fork River. Overlaps in proportionate terms are computed using each location in the comparative pairing in the denominator indicated by the row heading.

Ellipse Overall	Rainy River	Lake of the Woods	Little Fork River	Lake sturgeon
Rainy River	-	0.62	0.46	0.94
Lake of the woods	0.94	-	0.59	0.81
Little Fork River	0.46	0.39	-	0.47
Lake sturgeon	0.16	0.09	0.08	-
Centroid distances				
Rainy River	-	-	-	2.78
Lake of the Woods	1.36	-	-	3.39
Little Fork River	4.32	5.66	-	5.41



## Chapter 5 Spawning Habitat Characterization for Walleye and Lake Sturgeon in the Rainy River, Ontario.

### 5.1 Introduction

The harmful alteration or destruction of habitat is one of the major stresses affecting aquatic biodiversity and species in Ontario. For example, walleye (*Sander vitreus*), which is one of the most sought-after sportfish and commercially valuable harvest species in the United States and Canada (Scott and Crossman, 1973; Werner, 1980; Smith, 1985; Sternberg, 1986; Gilbert, 1999), and lake sturgeon (*Acipenser fulvescens*) populations have been in decline and are currently considered at risk because of habitat alteration or destruction. Both species have been subjected to similar habitat threats including: poor water quality (Leach et al. 1977), flow alterations (Colby et al. 1979) and movement restrictions (Kerr et al. 2010). Mitigating these impacts requires characterization of critical habitat, and management of human activities so as to maintain the quality and quantity of habitat needed for persistence of these species (Haxton et al. 2008).

The availability of suitable spawning habitat is critical to reproductive success and, therefore, conservation of both species (Bemis and Kynard 1997). Walleye and lake sturgeon overlap in their spawning requirements as they are both broadcast spawners. Walleye eggs are adhesive for a short period of time before they become water-hardened and lose their stickiness, and later may sink into crevices (Hartman 2009). In contrast, lake sturgeon eggs are adhesive and bond to stable substrate (Peterson et al. 2007). Once the eggs hatch, the larvae of both species drift into the crevices between rocks and plant matter where they remain until yolks are absorbed (Lyttle 2008). As a result of the broadcast nature of spawning, substrate, water velocities, depth and temperatures have been identified as important in the spawning success for each species (Scott and Crossman 1973, Werner 1980, Smith 1985).

Walleye spawn in the spring (April-June), just after ice-out, at temperatures ranging between 4.0-11.1<sup>o</sup>C in lakes, rivers and small tributaries (Scott and Crossman, 1973; Kerr et al. 1997; Lyttle 2008) on substrates that include coarse gravel mixed with cobble, gravel, and sometimes sand (Scott and Crossman, 1973; Werner, 1980; Smith, 1985). Walleye generally

spawn in water between 0.3- 1.5 m deep in moderate water velocities of between 0.4-1.0m/s (Sternberg, 1986) .

Lake sturgeon also spawn in the spring, but at slightly warmer temperatures, between 6.6 - 16<sup>0</sup>C (Scott and Crossman, 1973; Bruch and Binkowski, 2002; Lyttle 2008), typically over a mixture of coarse substrates including: bedrock, boulders and cobbles (Bruch and Binkowski, 2002; Seyler, 1997). While spawning typically occurs in shallower waters at depths above 0.5m, spawning has been reported as deep as 15m (Scott and Crossman, 1973; Kerr et al. 2010). Similar to walleye, lake sturgeon appear to prefer to spawn in water with moderate velocities.

Depending on water temperature, walleye usually hatch in about three weeks. Newly hatched larvae concentrate near the bottom for approximately 3-5 days before downstream dispersal (Kerr et al. 1997). For lake sturgeon hatching eggs takes 8-14 days and larval drift begins 13-19 days after hatch (Randal 2008). Therefore, utilization of benthic spawning habitat by both species for the egg incubation, hatching and larval stages can last up to 30 days (Randall 2008). During incubation eggs are vulnerable to changes in water levels. Impacts can range from total dewatering of eggs to eggs being dislodged from the substrate if flows are too high (Kerr et al. 1997).

The Rainy River has been identified as providing critical spawning habitat for river resident and Lake of the Woods populations of walleye and lake sturgeon (Mosindy and Rusak 1991; O'Shea 2005; Kallemeyn et al. 2009). As a result of the importance of the Rainy River for spawning in both species, Rainy River spawning populations of walleye and lake sturgeon are currently part of an ongoing critical review of the impacts of water level regulation on Rainy Lake. Water levels on the Rainy River are currently regulated by the International Falls Dam (IFD) which controls discharges from Rainy Lake to the river. Operators of the IFD use rule curves to control the outflow of Rainy Lake and, correspondingly, the discharge rate into the Rainy River. Under the current operation plan, Rainy Lake elevation must be maintained between a prescribed minimum and maximum elevation. If the water level of Rainy Lake falls below the lower limit of the rule curve, the instantaneous outflow into Rainy River must be reduced to 3,530 cfs and further lowered to 2,300 cfs if the lake water level falls below the

drought line (IJC, 2001). As long as the water level of Rainy Lake is maintained within the maximum and minimum, the discharge rate into, and the water level of, Rainy River is unrestricted. As such, the Canadian side of the IFD continues to practice hydro peaking i.e., fluctuating discharge rates about the daily mean discharge. Accordingly, the resulting hydrograph of the Rainy River is thought to have lost its natural seasonal flow pattern (O'Shea, 2005).

Given the apparent narrow range of spawning habitat preferences described for both walleye and lake sturgeon in the scientific literature, and the identified importance of the Rainy River as a source of critical spawning habitat for both species (Kallemeyn et al., 2009), this study sought to: [1] identify and characterize the spawning locations of lake sturgeon and walleye in the upper Rainy River based on temperature, velocity, depth and substrate; [2] determine if spawning substrate preferences were evident for either species; and, [3] determine if substrate, velocity and depth characteristics of the spawning sites were affected by changes in water surface elevation.

## **5.2 Methods**

The Rainy River (48°36' N, 93°24' W) is located on the Minnesota-Ontario border and flows westward from Rainy Lake over 145km to the south end of Lake of the Woods. Here the study reach was bounded by the International Falls Dam (IFD) in Fort Frances, ON, and the downstream confluence of the Rainy River with the Little Fork River, MN, an approximate 21 km length (Figure 5.1). Above the IFD, the Rainy River watershed is dominated by Canadian Shield. Below the IFD the river flows through a clay lake bed with occasional bedrock outcrop (Eibler and Anderson, 2004; O'Shea, 2005). Flow rates in the study reach are governed by IFD discharges ( $Q_{IFD}$ ) with mean annual and seasonal ranges in flows of 290 m<sup>3</sup>/s and 100 – 1000 m<sup>3</sup>/s, respectively, that result in seasonal water surface elevation (WSE) fluctuations in the river channel of up to 4 m.

All sampling was completed during the peak spawning times for the respective target species in 2012 and 2013; April – early May for walleye, and early May – early June for lake sturgeon. The fish were collected by Fisheries and Oceans Canada (DFO) by boat electrofishing

at night and additionally for lake sturgeon with 25.4 and 30.5 cm mesh overnight gillnet sets. Nets were set following protocols described in Dubreuil and Cuerrier (1950), i.e., parallel, or at an angle, to river flow in currents and back eddies and strategically placed to optimize lake sturgeon capture. Where congregations of ripe individuals were collected by fishing, the location was considered to an active spawning site. To verify spawning activity, egg mats were deployed immediately following fish capture and removed after approximately 24hrs for examination. Larval drift nets were also set at downstream locations 5 – 14 days following spawning events (Nichols et al., 2003; Wei et al., 2009).

Parallel hydraulic surveys were conducted using a SonTek® M9 Acoustic Doppler Current Profiler (ADCP). Forty-seven cross-section locations were established at approximate 500 m intervals throughout the study reach with increased spatial resolution in areas of river substrate morphological heterogeneity (Figure 5.1). ADCP transects were obtained at 7 – 10 discrete water surface elevations over the seasonal range (327-331m) at each cross-section using the moving-vessel method (Muste et. al., 2004). Elevation data throughout the study reach were collected to create detailed maps of the bathymetry of the channel using three sources: SonTek M9 RiverSurveyor Real-Time Kinetic (RTK) Acoustic Doppler Current Profiler (ADCP), Sokkia GRX1 RTK Differential GPS (DGPS), and a Digital Elevation Model (DEM).

Pressure and temperature transducers (PTT) were installed at 3 cross-section locations (7, 18 and 44) to quantify water level and temperature throughout the study reach. Temperatures from the three sites were compared to determine if temperature profiles changed with river length.

Sediment sampling was performed during low flow conditions in October 2012, allowing for sampling of the vast majority of littoral areas deemed important for spawning habitat. Sampling consisted of representative bulk samples (RBSs) and pebble counts (PCs). RBSs consisted of representative collection of pavement layer sediments at the sampling locations and associated grain size distributions were obtained through drying and mechanical sieving. PCs were conducted over the sampling locations of interest following methods described in Leopold (1970). RBSs and PCs were obtained at regular intervals throughout the study reach (at least

every 3 cross sections), as well as at locations where significant variation in substrate characteristics were noted. Sample classification was adapted from the Unified Soil Classification System as outlined in Das (2005) where the diameter for fines/sand is  $< 2$  mm, the diameter for gravels is  $2 - 64$  mm, and the diameter for cobbles/boulders is  $> 64$  mm. Substrate zones were classified based on the dominate substrate type and delineated using the coordinates obtained during field survey. Portions of the river that were not surveyed in detail were considered to be composed of fines/sand on the basis of Eckman and Ponar dredge substrate sampling completed in June of 2012. For simplification of analysis, substrate types were grouped according to the dominate substrate material as follows: coarse (boulder, bedrock with cobble/boulder, or cobble/boulder), gravels (gravel, predominantly gravel with fines/sands, or predominantly gravel with cobble/boulder) and sands (fines/sand, predominantly fines/sand with gravel or predominantly fines/sand with cobble/boulder).

Bathymetry, sediment maps, velocity and depth profiles were combined with egg mat placement locations to identify spawning habitat characteristics. Areas where deployed egg mats were found to contain eggs were identified as spawning areas. Spawning areas were considered to be discrete if they were spaced at least 500m apart and were identified by the hydraulic cross-sections nearest their location. Substrate maps described above were used to determine wetted substrate availability based on water surface elevations (WSE), with the relative availability of substrate types at varying WSEs compared using a Chi-square test for independence (Zar 2010). The distributions of velocity and depths were also examined for the range of WSEs seen throughout the spawning periods (from first egg deposition to confirmation of larval drift).

Spawning substrate preferences were tested following Manly et al. (1993), using the simplified substrate distribution data to describe habitat availability and egg mat location data within the defined substrate types to define substrate usage. Estimated substrate type areas relevant for the spawning period WSEs were converted to proportions and compared to proportionate substrate use values determined from egg mat counts through the computation of standardized selection indices,  $B_i$  (Manly et al., 1993). The resulting substrate selection ratios vary from 0 to 1 and indicate substrate preference when they exceed  $1/n$ , where  $n$  defines the number of substrate types (Manly et al., 1993). Selection ratios were further tested for statistical

significance using the  $\chi^2$  based statistic described in Manly et al. (1993) under the null hypothesis that spawning in available substrates occurred at random.

The normality and variance homogeneity of data were assessed prior to statistical analyses using the Shapiro-Wilk  $W$  test (Shapiro and Wilk 1965) and Levene's  $F$  test (Levene 1960). Linear regression was used to test for significant relationships between mean velocity, mean depth and WSEs. Non-linear regression was used to estimate relationships between identified preferred spawning substrate type and WSE following methods described in Bates and Watts (2007). ANOVA was used to test for differences among groups where appropriate, after checking for conformance of the data to test assumptions. JMP 7.0 (SAS Institute) was used to perform all reported statistical analyses, with significance judged at the  $\alpha=0.05$  level.

### **5.3 Results**

Walleye spawning occurred at temperatures ranging from 2 to 9.7 C<sup>0</sup> (Figure 5.2), with eggs found from mid-April to mid-May in 2012 and throughout May in 2013. The first instances of walleye larval drift were confirmed by sampling in mid-May in 2012 and mid-June in 2013, with eggs remaining at the spawning sites for approximately 29 days in 2012 and 39 days in 2013 (Figure 5.3). Lake sturgeon spawning occurred at temperatures ranging from 8.5-16.5 C<sup>0</sup>, with eggs found from mid to late May in 2012 and late May to mid-June in 2013. The first instances of lake sturgeon drift were seen in early June in 2012 and mid-June in 2013, with eggs remaining at the spawning sites for approximately 27 days in 2012 and 24 days in 2013 (Figure 5.3).

While the vast majority of the study reach (~75%) was characterized by substrates with fine sands and gravel or other substrate variants involving fines, sands, and gravels, spawning site substrates selected by walleye and lake sturgeon, consisted of the coarse substrates including: bedrock, bedrock with cobble/boulder, or cobble/boulder. The resulting spawning substrate preferences examined using Manly's preference indices indicated that walleye (cross sections 1-3, 15-17, and 18-19) and lake sturgeon (cross sections 1-3 and 4-6) in the Rainy River both preferred coarse substrates (Table 5.1) ranging from bedrock to cobble. No significant

differences were observed for selection preferences at different WSEs (all comparative  $\chi^2$   $P>0.5$ ).

Spawning adults, eggs or larvae were present in the spawning areas at a wide range of water surface elevations. Over the two spawning seasons both walleye and lake sturgeon experienced WSEs from 327.3m to 330.8m (Figure 5.3). ANOVA indicated significant differences in depth and velocity between water surface elevations in all cases (all  $P<0.001$ ). While the expected hydrological relationship predicting positive correlation between WSE and mean depth and mean velocity generally prevailed (all  $r^2>0.65$ ;  $P\leq 0.03$ ), notable exceptions were found for depth at cross-sections 4-6 ( $r^2=0.05$ ,  $P=0.90$ ) and for velocity at cross-sections 18-19 ( $r^2<0.01$ ,  $P=0.10$ ).

A wide range of depths and velocities were evident at the walleye (Figure 5.4) and lake sturgeon (Figure 5.5) spawning sites based on the temporally integrated ADCP profiles taken over the relevant spawning season for all identified spawning sites. For walleye spawning site associated depths ranged from 0.3 to 6.7m (C of V=51%) and velocities from 0 to 1.9m/s (C of V=60%). For lake sturgeon spawning site associated depths ranged from 0.3 to 10.5m (C of V=45%) and velocities from 0 to 1.9m/s (C of V=70%). The range of depths and velocities reported here compared favourably with those reported in the literature, although were more variable, covering almost the entire range of literature reported values (Figure 5.4, 5.5).

Comparisons of the relative proportions of substrate types available at identified spawning locations during low (327m), moderate (329m) and high (331m) water surface elevations indicated no significant differences at any of the spawning sites (cross-sections 1-3:  $\chi^2=8.83$ ,  $df=7$ ,  $P=0.16$ ; cross-sections 4-6:  $\chi^2=13.6$ ,  $df=9$ ,  $P=0.25$ ; cross-sections 15-17:  $\chi^2=14.4$ ,  $df=8$ ,  $P=0.43$ ; cross-sections 18-19:  $\chi^2=12.1$ ,  $df=4$ ,  $P=0.85$ ).

Although the proportion of available substrate types did not change as WSE increased, the total quantity of the preferred substrate type increased. Significant non-linear regression models were estimated for both walleye ( $r^2=0.99$ ;  $P<0.01$ ; Figure 5.6) and lake sturgeon ( $r^2=0.96$ ;  $P=0.03$ ; Figure 5.7) that related preferred spawning substrate availability to WSE.

Availability for walleye increased from approximately 11 453m<sup>2</sup> at a WSE of 327 to 26 629 m<sup>2</sup> at 331m. Similarly for lake sturgeon, preferred spawning habitat availability increased from 4 600m<sup>2</sup> at 327m to 13 542m<sup>2</sup> at 331m. In both cases, availability of preferred spawning substrate type only increased marginally after 329.5m (Figures 5.6, 5.7).

#### **5.4 Discussion**

Walleye and lake sturgeon spawning sites on the Rainy River were generally spatially and temporally separated, with only a single location overlap at the site immediately below the International Falls Dam. Both species showed preference for similar substrates, preferring coarse substrates dominated by bedrock, bedrock with cobble/boulder, or cobble/boulder. Spawning adults, eggs or larvae of both species were present in the spawning areas for over a month and experienced a wide range of water surface elevations. The resulting depths and velocities experienced varied significantly and encompassed reported values from previous scientific studies on spawning preferences for each species. Although the proportions of preferred spawning habitats did not change significantly as WSE rose, the total availability of preferred spawning substrate types increased, more than doubling for both species over the 4m range observed in this study.

The range of depths and velocities measured in this study were comparable to those reported from multiple literature sources including: agency reviews (Kerr et al. 1997; Dick et al. 2006; Randall 2008; Hartman 2009; Kerr et al. 2010;), classic reference literature (Scott and Crossman 1973), habitat assessments (Liaw 1991; LaHaye et al. 1992; Chalupnicki et al. 2010), habitat restoration papers (Manny et al. 2004; Dumont et al. 2009; McGrath 2009) and habitat suitability indices (Lyttle 2008). The ranges seen here incorporated all those reported suggesting that spawning experienced depths and velocities are much larger than described by previously reported point values. Differences in mean depth and velocity were found in this study compared with the overall means implied by available literature studies. For example, walleye mean velocity and depth values (0.53 m/s, 2.82m) in this study differed significantly ( $P \geq 0.005$ ) from the mean of literature reported values (0.74m/s, 1.19m). For lake sturgeon mean velocity (0.47 m/s) differed significantly ( $P=0.053$ ) from the mean literature value (0.76 m/s), whereas mean depth (3.39 m) in this study did not differ significantly ( $P=0.079$ ) from the mean of literature



value (4.01m). Thus while a wider range of flow-related metrics are experienced by walleye generally and in the case of velocity by lake sturgeon, lake sturgeon appear to favour a narrow range of water depths for spawning.

Typically field surveys have collected variables, such as velocity and depth, as point-measurements only at the time of egg deposition (eg; LaHaye et al. 1992; Chalupnicki et al. 2010). To improve available analytical data sets, many approaches have resorted to use of hydraulic simulation models, such as IFIM (Jowett and Duncan 1990), River2D (Waddle 2010; O'Shea 2005), and other 2-dimensional simulators to synthesize detailed sets of velocity data over several different discharge scenarios for predictive biological habitat applications. While useful, simulation methods potentially fail to characterize the spatial and temporal habitat heterogeneity that can influence habitat quality. Even relatively simple natural channels can display complex flow patterns that are not adequately accounted for by typical measurement field techniques or derivative simulation models (Gillenwater et al. 2006). Unless the reported values for depth and velocity collected from point measurements or 1-D hydraulic simulation models suitably cover the range of dates over which spawning-related activities (e.g., egg deposition, incubation, larval development and drift), analyses will not appropriately incorporate the variability experienced over the spawning with the result that incorrect inferences about "preferred" or "optimal" conditions are likely to be made.

Previous studies have noted that of the flow and substrate characteristics used to characterize spawning site suitability, depth is the least strongly related with walleye egg densities and thus that substrate and velocity are more likely used in spawning habitat selection (Liaw 1991; Gillenwater et al. 2006). Here we found that both lake sturgeon and walleye preferentially selected habitats based on substrate, with preferred coarse substrates of bedrock, boulders or cobbles (size class > 64 mm) being utilized over a wide range of velocities and depths. Strong relationships have also been observed between egg density and substrate type, with larger substrates being positively associated with egg density (Ivan et al. 2010; Manny et al. 2010). For example, walleye have been found to preferentially select for substrate as illustrated by the proportionally greater egg deposition that occurs on rock and gravel substrates when available (Manny et al 2010). Selection may be fitness related given that walleye egg survival in

terms of percentage survival and the numbers of eggs surviving has been shown to be greater on substrates dominated by gravel-rubble with occasional boulder (Johnson 1961). Nevertheless, when preferred substrates are removed or limited, spawning will shift to less preferred substrate types when other environmental conditions (e.g. water velocity) are suitable for spawning (Manny et al. 2010).

While literature studies of lake sturgeon egg densities and survival are generally lacking, studies reporting on observed spawning locations have noted a range of similar requirements. For example, reports suggest that for lake sturgeon substrate diameter of at least 30mm is significant (Manny and Kennedy 2002; Caswell et al. 2004; Randall 2008). Habitat suitability curves, developed by Threader et al. (1998), report that optimal substrates include cobbles (81- 250mm) and boulders (>250mm). And Shaw et al (2013) used the habitat suitability curves developed by Threader et al. (1998) to demonstrate that only tributaries with high-quality spawning habitat were associated with the capture of reproductive lake sturgeon. Collectively the literature reports accord with the Rainy River findings reported here and elsewhere where spawning sites identified at Long Sault and Manitou rapids, were also characterized by 72% boulders, 18% cobbles and 10% silt/sands (Mosindy and Rusak, 1991).

Considerable effort has been devoted to the development of fish Habitat Suitability Models (HSMs) (Haxton et al. 2008). HSMs are a common method for evaluating the quality and quantity of critical habitat under a range of discharges and HSMs have been developed for a number of species (Aadland et al. 1991). The strength of HSMs is their potential to represent limiting habitats that can then be used as targets for management initiatives and protection (Boisclair 2001). While HSMs have been successfully applied to assess the impact of management activities, (O'Neil et al. 1988; Bray 1996; Brooks 1997; Roloff and Kernohan 1999), few HSMs have been independently validated (e.g. Haxton et al. 2008). Many fail to record habitat resource use in relation to availability (Heggenes 1989) and thus yield data not suited to statistical significance testing (Manly et al., 1993). For example, Lowie et al. 2001 found that none of the habitat parameters used in walleye HSM (velocity, depth, temperature, dissolved oxygen, pH) were distinguishable between the microhabitats where walleye were observed to spawn and randomly chosen sites surveyed on the same dates. If, as noted here,

preference for substrate dominates the other variables (e.g. velocity and depth), HSMs may not accurately represent spawning habitat suitability by failing to account for the hierarchical selection of habitat implied by the strong preferences for substrate and the use of substrate over a wide range of depth and velocity values.

The range of WSEs over which spawning adults, eggs or larvae are present poses a potential risk as quickly changing water levels can lead to total dewatering of eggs or eggs being dislodged from the substrate (Kerr et al. 1997). For example, in 2012 walleye began spawning at WSE of approximately 327.4m and eggs remained on the sites while the water level was raised to 329m. A similar change was observed during lake sturgeon spawning in 2012 (327 to 329.3m), while in 2013 the change was much less significant (328.7 to 329.8m). Although the addition of spawning habitat is unlikely to have significant negative impacts on spawning fish, subsequent lowering of the WSE will have negative impacts if newly added habitats are used, or if lowering WSEs impinge on the apparent spawning depth requirement (3-4m) noted here and implied elsewhere in the literature (Scott and Crossman 1973, LaHaye et al. 1992, Manny et al. 2004, Dick et al. 2006, Lyttle 2008, Randall 2008, , Dumont et al. 2009, McGrath 2009, Kerr et al. 2010). Furthermore, while the availability of spawning habitat varies with WSE, models suggest that a WSE greater than 329m does not result in appreciable increases in habitat availability. For example, a 69% and 52% increase in available preferred spawning habitat area, respectively, for walleye and lake sturgeon results from a 1m increase in WSE from 327 to 328, whereas the changes resulting from a similar 1m increase in WSE at 329m are 6% and 11%, respectively, for walleye and lake sturgeon.

Variations in flow have been shown to directly affect spawning lake sturgeon populations. Auer (1996) noted a greater number of females, better reproductive success and a reduction in both the spawning densities and time spent on the spawning grounds when natural or 'run-of-river' flow regimes prevailed. Results suggest spawning ground densities may inhibit reproductive success. While current population estimates are not available, data from 1991 estimated a Rainy River/Lake of the Woods population of 7 549-42 275 mature size (>100 cm) lake sturgeon evenly split between males and females (Mosindy and Rusak 1991). With a spawning periodicity of 4-8 years, between 629 and 5 284 females will potentially spawn in any

given year. If each female requires between 13-48m<sup>2</sup> of spawning habitat (e.g., Fortin et al. 2002), the data imply a spawning habitat requirement between 8178 and 253 632 m<sup>2</sup>. Thus, in addition to maintaining a constant WSE to prevent dewatering of spawning sites, or a reduction of water depths over selected lake sturgeon spawning sites as noted above, a minimum WSE of 328m must be maintained to optimize lake sturgeon spawning if the lower spawning habitat requirements and population numbers prevail. And the spawning habitat requirements cannot be met in the upper reaches of the Rainy River below the IFD if the larger individual spawning habitat requirements and population numbers prevail without direct consideration of the ability of the spawning habitat identified at Long Sault and Manitou rapids to provide supplementary spawning habitat.

While similar estimates of Lake of the Woods/Rainy River walleye population and area requirements for females have not been estimated to date, potential spawning-related egg deposition based on the availability of preferred coarse substrate habitats can be estimated. Walleye egg densities have been estimated to range from approximately 350 eggs/m<sup>2</sup> (Johnson 1961) to 750 eggs/m<sup>2</sup> (Ivan et al. 2010), implying that in the Rainy River between 6 million eggs at WSE of 327 and 13 million at a WSE of 329m can be produced each year if a suitable resident population exists. With estimated survival rates of between 33-91% on the coarse rubble, cobble and boulder substrates (Jones et al. 2003), between 0.19 and 1.18 million larvae will emerge. Thus while the quantity of available spawning habitat will vary as WSE varies, spawning habitat and associated larval recruitment does not appear limited in the Rainy River system

## 5.5 Figures and tables



Figure 5.1 The Rainy River is located at the border of Fort Frances, ON, and International Falls, MN. The International Falls dam and the confluence with Little Fork River represent the boundaries of the study reach. Grey bars indicate locations where hydraulic cross-sections (1 to 44). Cross-sections 1-3 and 4-6 denote lake sturgeon spawning locations. Cross-sections 1-3, 15-17 and 18-19 denote walleye spawning locations.

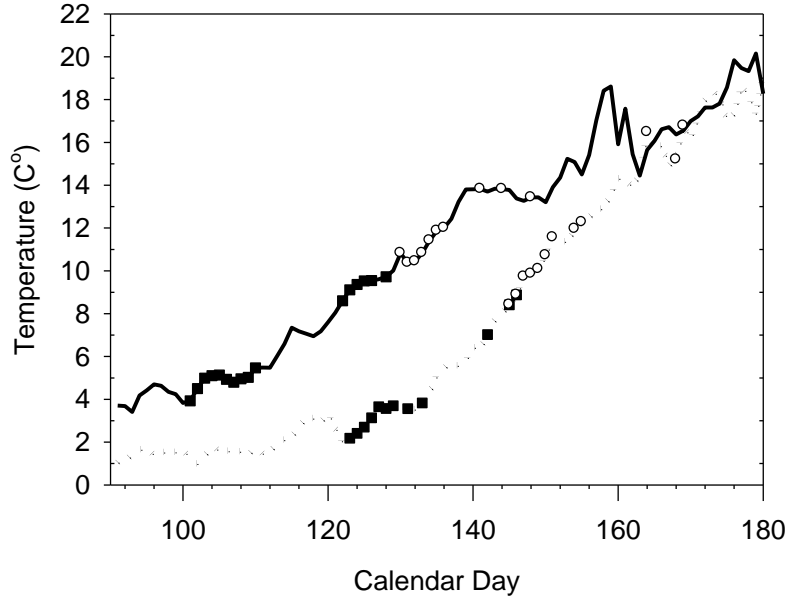


Figure 5.2 Average Rainy River temperature ( $^{\circ}\text{C}$ ) taken from pressure and temperature transducers (PTT) at cross-section 7, 18 and 44 within the study reach. Solid line indicates temperatures for 2012 and dotted line temperatures for 2013. Instances of walleye spawning are indicated by black squares and lake sturgeon spawning by white circles.

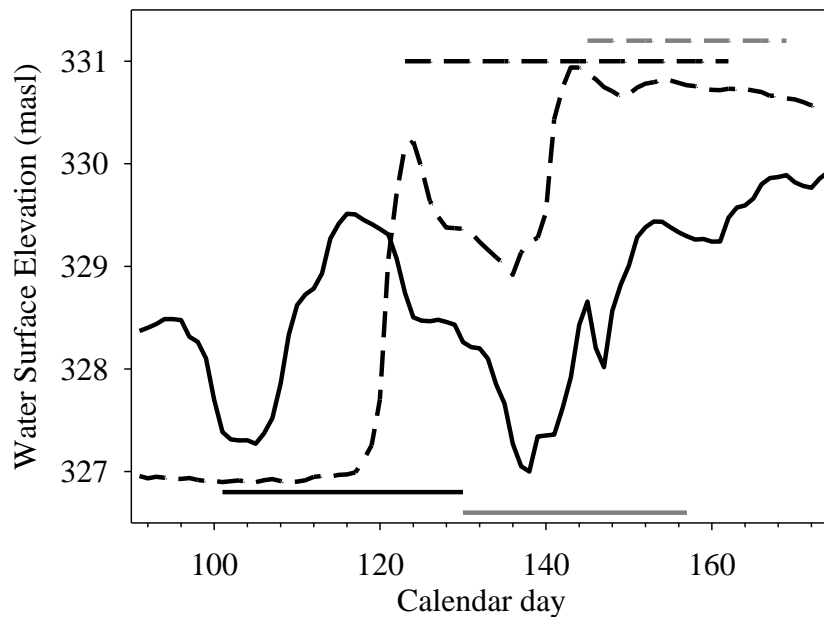


Figure 5.3 Rainy River water surface elevations (WSE) taken from pressure and temperature transducer (PTT) placed immediately below the International Falls Dam (IFD). Solid line indicates WSEs for 2012 and dotted line WSEs for 2013. Periods where walleye adults, eggs or larvae were present at spawning sites are indicated in black for 2012 (solid) and 2013 (dashed) while lake sturgeon is indicated by grey lines for 2012 (solid) and 2013 (dashed).

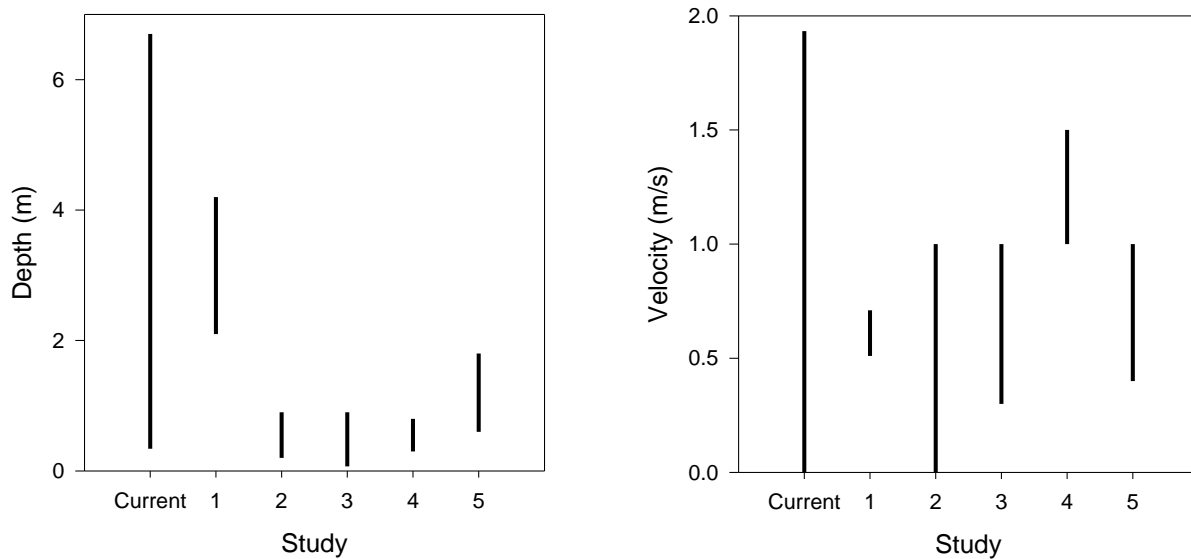


Figure 5.4 Range of depths (m) and velocities (m/s) measured at walleye spawning sites in the current Rainy River study (current) over the range of WSEs observed when spawning adults, eggs or larvae were present on site. Ranges are compared to those found in a variety of literature studies for depth and velocity including: [1] Chalupnicki et al. 2010, [2] Hartman 2009, [3] Liaw 1991, [4] Kerr et al. 1997 and [5] Lyttle 2008.

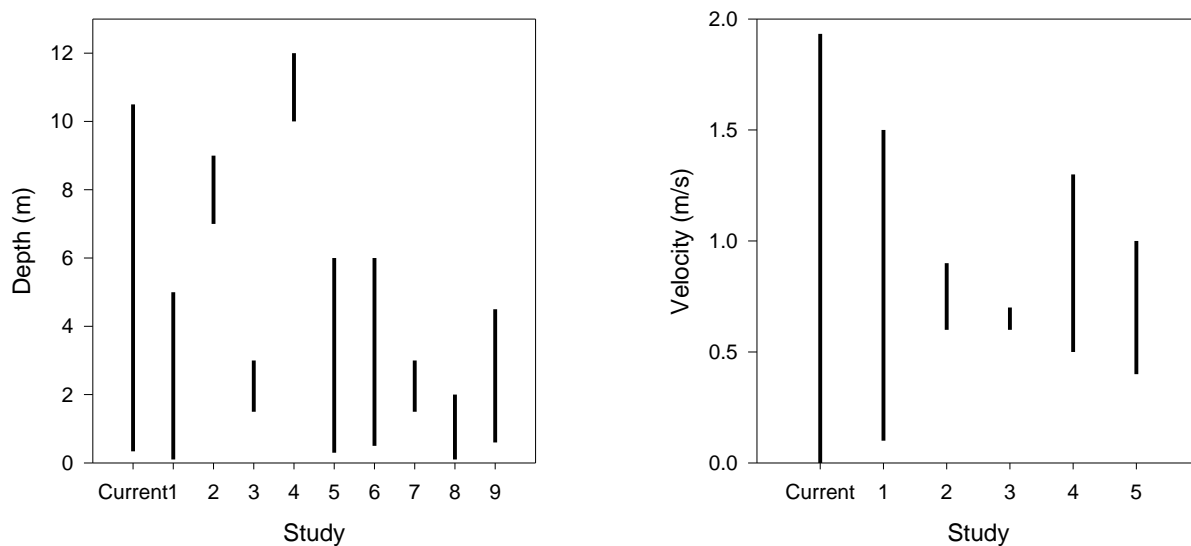


Figure 5.5 Range of depths (m) and velocities (m/s) measured at lake sturgeon spawning sites in the Rainy River study (current) over the range of WSEs observed when spawning adults, eggs or larvae were present on site. Ranges are compared to those found in a variety of literature studies for depth including: [1] Kerr et al. 2010, [2] Manny et al. 2004, [3] Dumont et al. 2009, [4] McGrath 2009, [5] Scott and Crossman (1973), [6] Dick et al. 2006, [7] LaHaye et al. 1992, [8]

Randall 2008, and [9] Lyttle 2008 and for velocity including: [1] Kerr et al. 2010, [2] Manny et al. 2004, [3] McGrath 2009, [4] Randall 2008 and [5] Lyttle 2008.

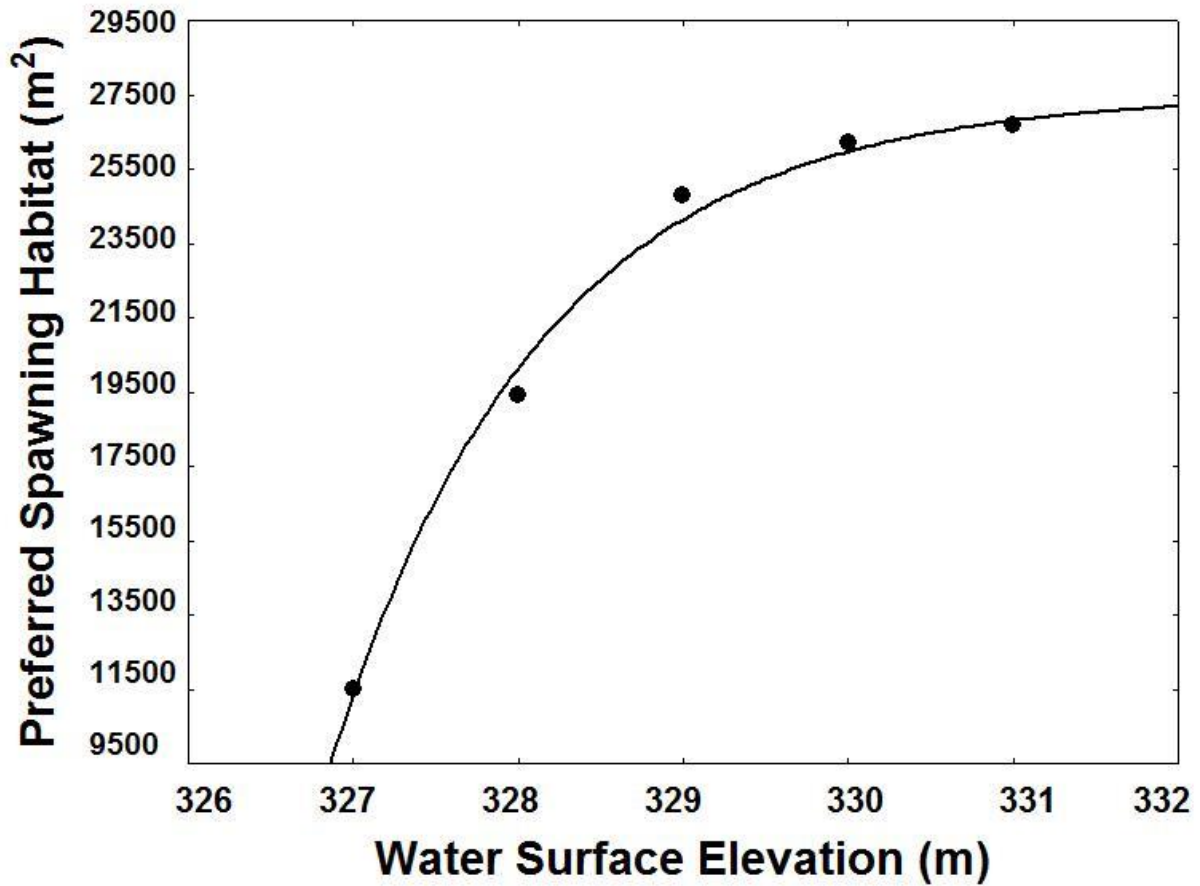


Figure 5.6 Spawning substrate areas by water surface elevation for walleye. Significant non-linear regression designated by the solid line.



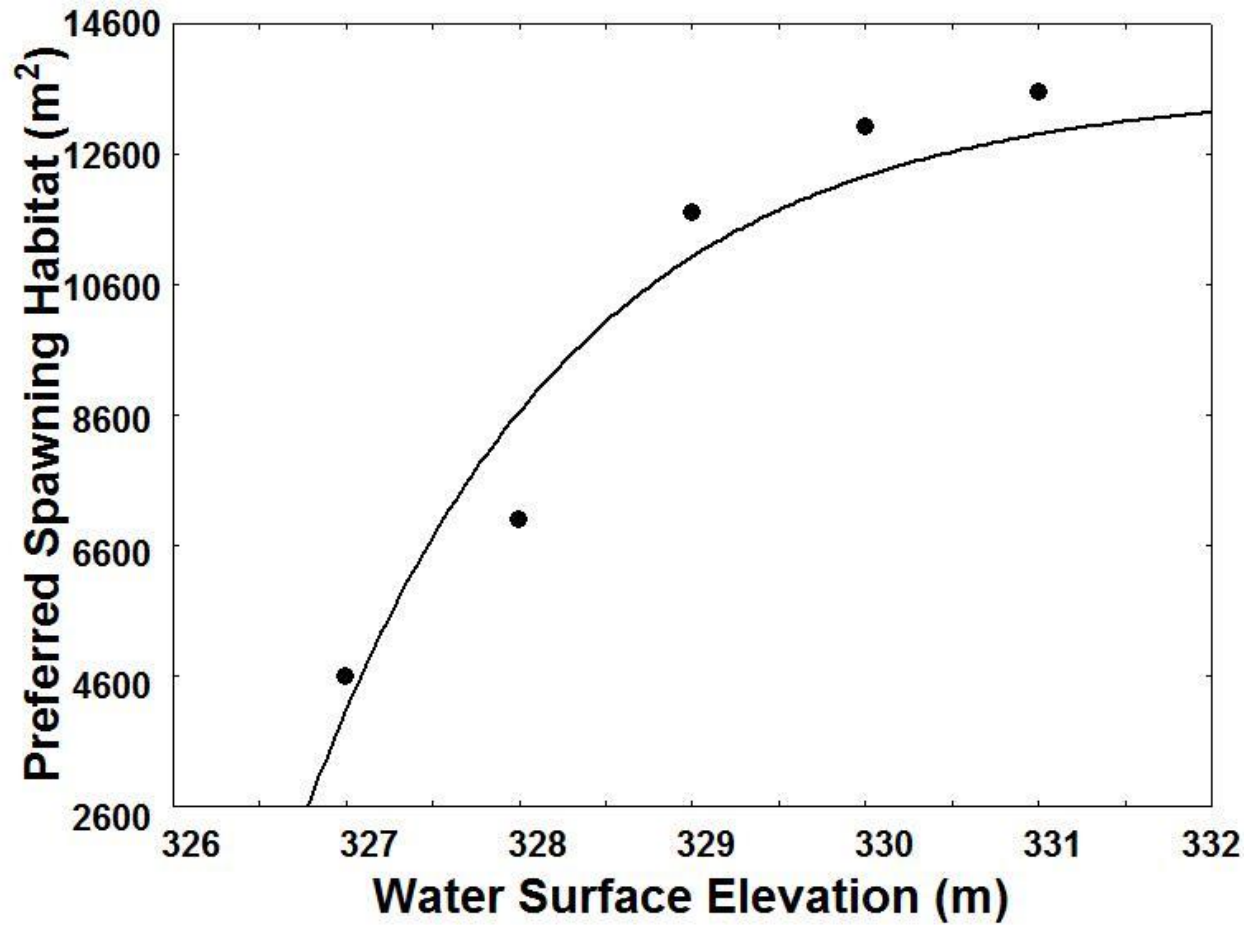


Figure 5.7 Spawning substrate areas by water surface elevation for lake sturgeon. Significant non-linear regression designated by the solid line.

Table 5.1 Walleye and lake sturgeon spawning sites, positive egg mats found at each substrate type and substrate preferences ( $B_i$ ). Preferences were determined at a range of water surface elevations (WSEs), substrates were considered preferred (\*) when  $B_i > 0.33$  (e.g. Manly et al. 1993).

Site	Substrate	# of egg mats	Walleye			Lake sturgeon			
			327 $B_i$	329 $B_i$	331 $B_i$	# of egg mats	327 $B_i$	329 $B_i$	331 $B_i$
1-3	Coarse*	6	0.77*	0.77*	0.77*	19	0.91*	0.91*	0.90*
	Gravels	2	0.22	0.22	0.23	2	0.08	0.08	0.08
	Sands	1	0.00	0.00	0.01	4	0.01	0.01	0.01
4-6	Coarse*					2	1.00*	1.00*	1.00*
	Gravels					0	0.00	0.00	0.00
	Sands					0	0.00	0.00	0.00
15-17	Coarse*	18	1.00	0.99*	0.99*				
	Gravels	0	0.00	0.00	0.00				
	Sands	2	0.00	0.01	0.01				
18-19	Coarse*	2	1.00*	1.00*	1.00*				
	Gravels	0	0.00	0.00	0.00				
	Sands	0	0.00	0.00	0.00				

## Chapter 6 General Conclusions and Future Considerations

### 6.1 Summary

The research presented in this thesis addressed several knowledge gaps identified by the International Joint Commission concerning the evaluation of the existing rule curves and discharge practices on the Rainy River. Chapter 2 reported on the current population condition of the target species. Study findings included weight-length relationships (WLRs) for walleye, lake sturgeon and logperch from the Rainy River system, captured from April-June, 2012 and 2013. From the WLRs, a relative condition factor equation was developed. Weight models and condition indices for Rainy River target species were near the upper limits of values reported previously in the literature for the species. Growth models indicated no significant changes in growth patterns for walleye in comparison to the Rainy River/Lake of the Woods population in the late 1990s. In contrast, lake sturgeon population exhibited faster growth and condition than estimated for 2002 Rainy Lake populations. In addition to the limitations of body condition as a measure of fish health commonly noted in the literature, it should be noted that samples used here were taken during spawning season and condition may be biased by the use of reproductively developed individuals.

In Chapter 3, application of stable isotope analysis provided us with the opportunity to explore the feasibility of using pectoral fin clips as an alternative to dorsal muscle tissue in feeding studies for adult lake sturgeon. While strong relationships between the isotope signatures of fin clip and muscle tissues available in the literature demonstrate that fin clips can be used in place of muscle tissue, evidence of conversion biases and the dominance of salmonid studies highlighted the need for further studies on non-salmonid species (Willis et al., 2013). One species for which tissue comparisons have not been made is lake sturgeon (*Acipenser fulvescens*). Furthermore, lake sturgeon represent a unique fin-muscle comparison given the high muscle tissue C:N ratios as compared to previously examined species (e.g., Tronquart et al., 2012). Because lipid content can affect stable isotope ratios, we also determined whether lipid extraction and mathematical normalization affected the relationship between fin-clip and muscle tissue signatures. The strong relationships between fin-clip and muscle tissue signatures demonstrated that fin-clips should be considered as good surrogates for muscle tissue. The result

will allow trophic studies to accurately adjust for the effect of differential lipid accumulation and for adult lake sturgeon will effectively limit the need for lethal sampling to complete additional trophic studies.

Chapter 4 applied the method described in Chapter 3 to the study the dietary patterns of lake sturgeon inhabiting the upper reach of the Rainy River. In general the Rainy River supports a healthy food web with typical vertical trophic structure consisting of four trophic levels, with adult walleye sitting at the apex position. Rainy River lake sturgeon stable isotope data showed moderate intra-specific variability and a significant size related shift in  $\delta^{13}\text{C}$  when the data were lipid corrected. Within the food web, lake sturgeon occupied an upper trophic position dependent on their high proportionate assimilation of fish-derived protein. The study findings provide further evidence for reliance on consumption of fish eggs or other substrate deposited fish material. The reliance on fish derived protein has been similarly reported for lacustrine populations in Lake Winnebago, Wisconsin (Stelzer et al., 2008), while historical studies report consumption of fish eggs (e.g. Cuerrier, 1966). At the meso-scale within the Rainy River there was no evidence to suggest lake sturgeon were preferentially connected to habitat-specific food webs (e.g. bedrock outcrops with high local velocities versus highly vegetated habitats). At the macro scale, Rainy River lake sturgeon appear to rely more heavily on the food webs of the Rainy River and the Lake of the Woods than on available tributary systems.

Chapter 5 identified and characterized the critical spawning habitat for both walleye and lake sturgeon in the Rainy River. Walleye and lake sturgeon overlap in their spawning requirements as they are both broadcast spawners, as a result, substrate, water velocities, depth and temperatures have been identified in the past as important in the spawning success for each species (Scott and Crossman, 1973; Werner, 1980; Smith, 1985). Walleye and lake sturgeon spawning sites on the Rainy River were generally separated, with only a single location overlap. Both species showed preference for coarse substrates dominated by bedrock, bedrock with cobble/boulder, or cobble/boulder. The depths and velocities experienced by adults, eggs or larvae present at spawning grounds, however, varied significantly and encompassed reported values from previous studies. Although the proportions of preferred spawning habitats did not change significantly as WSE rose, the total availability of preferred spawning substrate types

increased, more than doubling for both species. Walleye and lake sturgeon spawning does not appear to be limited within the Rainy River system (Mosindy and Rusak, 1991), although the quality and quantity of these areas is directly affected by fluctuations in river flow.

## **6.2 Study significance**

This thesis is one of the first studies conducted on the Rainy River to be considered in the evaluation of the 2000 rule curve change. The primary focus of past studies has been on monitoring the aquatic and riparian ecosystems of the upstream Rainy Lake and Namakan Reservoir, both of which influence Rainy River water levels. The only past study of fish habitat of which we are aware was spatially limited to Manitou Rapids and Long Sault Rapids and did not address concerns for areas immediately below IFD (O'Shea, 2005). The area immediately below the dam had been identified as an important site for lake sturgeon and walleye spawning. Furthermore, flows and water levels in this area are likely to be more responsive to releases from the IFD than the sites studied by O'Shea (2005). In a system such as the Rainy River, where water levels and discharge on the river are regulated by upstream management practices, gathering evidence for the evaluation of potential impacts is significant.

This study has also confirmed a novel method for examining dietary trends for lake sturgeon. The use of fin clips had yet to be applied for the study of lake sturgeon trophic studies. Past studies on lake sturgeon diet have relied on the gastric lavage method or collection of stomach and muscle tissue from corpses (Haley, 1998; Stelzer et al., 2008). Stomach content analysis provides only a snap-shot of the diet for lake sturgeon, while stable isotope analysis provides an integrated long-term signature. Use of stable isotope analysis provided evidence for heavier reliance on fish-derived protein in the diet of lake sturgeon than, in the past, where fish consumption was regarded as largely insignificant (e.g. Harkness and Dymond, 1961). The use of fin tissue for stable isotope analysis provided a simple, non-invasive and repeatable method for improved understanding of lake sturgeon diets.

### 6.3 Future research

This thesis has provided valuable information for the evaluation of the existing rule curves and discharge practices on the Rainy River, knowledge gaps remain for examination of flow regime impacts. While lake sturgeon and walleye are very valuable species in the Rainy River system, they are similar in their spawning behaviours and habitats and not necessarily representative of other resident fish species. The Rainy River provides spawning habitat to a variety of other species including other popular sport fish such as smallmouth bass (*Micropterus dolomieu*) and northern pike (*Esox lucius*). The impacts of the 2000 rule curves on northern pike and smallmouth bass spawning habitat quality has been examined for Rainy Lake and Namakan Reservoirs, but similar studies have not been completed on the Rainy River (Kallemeyn et al., 2009).

The development of the use of fin clip tissues provides a method for simple, non-lethal and repeated sampling for stable isotope analysis. This study and only one other have applied the use of stable isotopes to study the diets of adult lake sturgeon. Both studies have found evidence for the increased reliance on fish derived protein. Our study found that lake sturgeon are potentially feeding on fish eggs. Rainy River has an abundant population of spring or summer spawning species with very few fall spawners, including lake whitefish (*Coregonus clupeaformis*) and cisco (*Coregonus artedi*). This reliance of lake sturgeon on eggs may be linked to availability of eggs in the spring and summer. Stable isotope analysis could be used to determine if lake sturgeon in systems dominated by fall spawners demonstrate the same reliance on fish derived protein.

## 7.0 References

### 7.1 Chapter 1

- Adams, W.E., Kallemeyn, L.E. & Willis, D.W. 2006. Lake sturgeon population characteristics in Rainy Lake, Minnesota and Ontario. *J. Appl. Ichthyol.* 22: 97–102.
- Auer, N.A. 1996. Response of Spawning Lake Sturgeons to Change in Hydroelectric Facility Operation. *Trans. Am. Fish. Soc.* 125(1): 66-77.
- Berland, G., Nickelsen, T., Heggenes, J., Økland, F., Thorstad, E. B. & Halleraker, J. 2004. Movements of Wild Atlantic Salmon Parr in Relation to Peaking Flows Below a Hydropower Station. *River Res. and Appl.* 20: 957–966.
- Bunn, S. E. and Arthington, A. H.: 2002. Basic Principles and Ecological Consequences of Altered Flow Regimes for Aquatic Biodiversity. *Environ. Manage.* 30: 492–507.
- Cholmondeley, R. 1985. Assessment of walleye spawning grounds in the Raisin River and Hoople Creek. File Report, Ontario Ministry of Natural Resources, Cornwall, Ontario.
- COSEWIC. 2006. COSEWIC assessment and update status report on the lake sturgeon *Acipenser fulvescens* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 107 pp. ([www.sararegistry.gc.ca/status/status\\_e.cfm](http://www.sararegistry.gc.ca/status/status_e.cfm)).
- Coutant, C.C. 2000. Fish Behavior in Relation to Passage through Hydropower Turbines: A Review. *Trans. Am. Fish. Soc.* 129: 351–380.
- DePhilip, M.M., Diana, J.S. & Smith, D. 2005. Movement of Walleye in an Impounded Reach of the Au Sable River, Michigan, USA. *Environ. Biol. Fish.* 72: 455–463.
- Eidler J. & Anderson, A. 2004. Upper Rainy River 2002-03. Division of Fisheries, Minnesota Department of Natural Resources.
- Fisk, J.M., Kwak, T. J., Heise, R. J. & Sessions, F. W. 2012. Redd Dewatering Effects on Hatching and Larval Survival of the Robust Redhorse. *River Res. and Appl.* 29 (5): 574-581.
- Finlay, J. C., Power, M. E. & Cabana, G. 1999. Effects of water velocity on algal carbon isotope ratios: Implications for river food web studies. *ASLO.* 44: 1198–1203.
- Geiling, W.D., Kelso, J.R.M. & Iwachewski, I. 1996. Benefits from Incremental Additions to Walleye Spawning Habitat in the Current River, With Reference to Habitat Modification as a Walleye Management Tool in Ontario. *Canadian. J. Fish. Aquat. Sci.* 53 (Suppl. 1): 79–87.
- Halleraker, J. H., Saltveit, S. J., Harby, A., Arnekleiv, J. V., Fjeldstad, H.-P. & Kohler, B. 2003. Factors Influencing Stranding of Wild Juvenile Brown Trout (*Salmo trutta*) During Rapid and Frequent Flow Decreases in an Artificial Stream. *River Res. and Appl.* 19: 589–603.

- Hart, D.D. and Finelli, C.M. 1999. Physical-Biological Coupling in Streams: The Pervasive Effects of Flow on Benthic Organisms. *Annu. Rev. Ecol. Syst.* 30: 363-395.
- Holm, E., Mandrak, N.E. & Burrige, M.E. 2009. *The ROM field guide to Freshwater fishes of Ontario*. ROM science publication, Toronto, Ontario.
- Houston, J.J.P., 1986. COSEWIC Status Report on the Lake Sturgeon *Acipenser fulvescens* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. 1-32 pp.
- Humphries, P., King, A.J., Koehn, J.D. 1999 Fish, flows and flood plains: Links between freshwater fishes and their environment in the Murray-Darling River system, Australia *Environ. Biol.Fish.* 56 (1-2): 129-151.
- Humphries P. & Lake, P.S. 2000. Fish larvae and the management of regulated rivers. *Regul. Rivers Res. Manage.* 16:421–432.
- International Joint Commission (IJC). 2001. In the Matter of Emergency Regulation of the Level of Rainy Lake and of other Boundary Waters in the Rainy Lake Watershed. Order Prescribing Method of Regulating the Levels of Boundary Waters. 16pp.
- Ivan, L.N., Rutherford, E.S. Riseng, C. & Tyler, J.A. 2010. Density, Production, and Survival of Walleye (*Sander vitreus*) Eggs in the Muskegon River, Michigan. *J. Gt. Lakes Res.* 36: 328–337.
- Johnson, J. H. LaPan, S. R. Klindt, R. M. & Schiavone A. 2006. Lake sturgeon spawning on artificial habitat in the St Lawrence River. *J. Appl. Ichthyol.* 22: 465–470.
- Kerr, S.J., Corbett, B.W., Hutchinson, N.J., Kinsman, D., Leah, J.H., Puddister, D., Standfield, L. & Ward, N. 1997. Walleye Habitat: A Synthesis of Current Knowledge with Guidelines for Conservation. Percid Community Synthesis Walleye Habitat Working Group.
- King, J., Cambray, J.A. & Impson, N.D. 1998. Linked effects of dam-released floods and water temperature on spawning of the Clanwilliam yellowfish *Barbus capensis*. *Hydrobiologia* 384: 245–265.
- Marty, J., Smokorowski, K. and Power, M. 2009. The influence of fluctuating ramping rates on the food web of boreal rivers. *River Res. and Appl.* 25: 962-974.
- Milton, D. A. & Arthington, A. H. 1983. Reproduction and growth of *Craterocephalus marjoriae* and *C. stercusmuscarum* (Pisces: Atherinidae) in south-eastern Queensland, Australia. *Freshw. Biol.* 13: 589-597.
- Milton, D. A. & Arthington, A. H. 1984. Reproductive Strategy and Growth of the Crimson-spotted Rainbowfish, *Melanotaenia splendida jluviatilis* (Castelnau) (Pisces : Melanotaeniidae) in South-eastern Queensland. *Aust. J. Mar. Freshw. Res.* 35: 75-83.



Mosindy, T., and J. Rusak. 1991. An assessment of lake sturgeon populations in Lake of the Woods and the Rainy River 1987-1990. Ontario Ministry of Natural Resources, Lake of the Woods Fisheries Assessment Unit Report 1991:01, Toronto.

O'Shea, D. 2005. Water management recommendations for the Rainy River. Stream Habitat Program Final Advisory Report; Rainy River Peaking Group. Division of Ecological Services, Minnesota Department of Natural Resources, St. Paul, MN. 62 pp.

Payer, R.D., Pereira, D.L., Larson, M.L., Younk, J.A., Frie, R.V., Schupp, D.H. & Osborn, T.C. 1987. Status and Simulation Model of Lake of the Woods, Minnesota, Walleye Fishery Minnesota Department of Natural Resources Section of Fisheries. Investigation Report #389.

Poff, N.L., Allan, J.D., Bain, M.B., Karr, J.R., Prestegard, K.L., Richter, B.D., Sparks, R.E., and Stromberg, J.C. 1997. The Natural Flow Regime. *BioScience* 47(11): 769 – 784.

Poff, N.L., Richter, B.D., Arthington, A.H., Bunn, S.E., Naiman, R.J., Kendy, E., Acreman, M., Apse, C., Bledsoe, B.P, Freeman, M.C., Henriksen, J., Jacobson, R.B, Kennen, J.G., Merritt, D.M., O'Keefe, J.H., Olden, J.D, Rogers, K., Tharme, R.E & Warner, A. 2010. The ecological limits of hydrologic alteration (ELOHA): a new framework for developing regional environmental flow standards. *Freshw. Biol.* 55: 147–170.

Sabater, S. 2008. Alterations of the global water cycle and their effects on river structure, function and services. *Freshwater Reviews* 1: 75-88.

Tuhtan, J.A., Noack, M., and Wieprecht, S. 2012. Estimating Stranding Risk due to Hydropeaking for Juvenile European Grayling Considering River Morphology. *J. Civ. Eng.* 16(2): 197-206.

Zhong, Y., and G. Power. 1996. Environmental impacts of hydroelectric projects on fish resources in China. *Regul. Rivers Res. Manage.* 12: 81–98.

## **7.2 Chapter 2**

Aadland, L.P., Cook, C.M., Negus, M.T., Drewes, H.G. & Anderson, C.S. 1991. Microhabitat preferences of selected stream fishes and a community-oriented approach to instream flow assessments. Minnesota Department of Natural Resources: Section of Fisheries Investigational Report #406

Adams, W.E., Kallemeyn, L. W. & Willis, D. W. 2006. Lake sturgeon population characteristics in Rainy Lake, Minnesota and Ontario. *J. Appl. Ichthyol.* 22: 97–102.

Balshine, S., Verma, A., Chant, V., & Theysmeyer, T. 2005. Competitive Interactions between Round Gobies and Logperch. *J. Gt. Lakes Res.* 31: 68–77.

- Bates, D.M. and Watts, D.G. 1988. Nonlinear regression analysis and its applications. New York: John Wiley & Sons. Xiv + 365 pp.
- Beamish, F.W.H, Jebbink, J., Rossiter, A., & Noakes, D.L.G. 1996. Growth strategy of juvenile lake sturgeon (*Acipenser fulvescens*) in a northern river. *Can. J. Fish. Aquat. Sci.* 53: 481–489.
- Bergstrom, M.A. and Mensinger, A.F. 2009. Interspecific Resource Competition between the Invasive Round Goby and Three Native Species: Logperch, Slimy Sculpin, and Spoonhead Sculpin. *Trans. Am. Fish. Soc.* 138: 1009–1017
- von Bertalanffy, L. 1938. A quantitative theory of organic growth (inquiries on growth laws. II). *Human Biology.* 10: 181–213.
- Booth, D.J. and Keast, J.A. 1986. Growth energy partitioning by juvenile bluegill sunfish, *Leponis macrochirus* Rafinesque *J. Fish Biol.* 28: 37-45
- Bruch, R. M. 1999. Management of lake sturgeon on the Winnebago System – long term impacts of harvest and regulations on population structure. *J. Appl. Ichthyol.* 15: 142–152.
- Bruch, R. M. and Binkowski, F. P. 2002. Spawning behavior of lake sturgeon (*Acipenser fulvescens*). *J. Appl. Ichthyol.* 18: 570–579.
- Bruch, M., Kamke, K.K. and Haxton, T. 2011. Use of a modified form factor to compare condition among North American lake sturgeon stocks. *J. Appl. Ichthyol.* 27 (Suppl. 2): 34–40
- Casselman, J.M. 1976. Age and growth of northern pike (*Esox Lucius*) of the upper St Lawrence River M.Sc. thesis Univ. Guelph 219pp
- Casselman, J. M. 1987. Determination of age and growth. in A. H. Weatherley and H. S. Gill, editors. *The biology of fish growth.* Academic Press, London. 209–242pp
- Chen, Y., Jackson, D. A. and Harvey, H. H. 1982. A comparison of von Bertanaffy and polynomial functions in modelling fish growth data. *Can. J. Fish. Aquat. Sci.* 49: 1228-1235.
- COSEWIC. 2006. Committee on the Status of Endangered Wildlife in Canada assessment and update status report on the lake sturgeon (*Acipenser fulvescens*) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. xi + 107 pp.
- Craig, J.M., Thomas, M. V. & Nichols, S. J. 2005. Length–weight relationship and a relative condition factor equation for lake sturgeon (*Acipenser fulvescens*) from the St Clair River system (Michigan, USA) *J. Appl. Ichthyol.* 21: 81–85.
- Craig, J.F., Babaluk, J.A., Stevenson, S.G., & Williams, P.C. 1995. Variation in growth and reproduction of walleye (*Stizostedion vitreum*) in three Manitoba lakes. *Can. J. Zool.* 73: 367-372

- Csirke, J.F., Caddy, S., & Garcia, J. 1987. Methods of size frequency analysis and their incorporation in programs for fish stock assessment. *Length-Based methods in fisheries research*, Eds: D.Pauly and GR. Morgan
- De Robertis, A. and Williams, K. 2008. Weight-Length Relationships in Fisheries Studies: The Standard Allometric Model Should Be Applied with Caution. *Trans. Am. Fish. Soc.* 137 (3): 707-719
- de Veen, F. 1970. On some aspects of maturation in the common sole (*Solea solea* (L)). *Verichte der Deutschen Wissenschaftlichen Kommission fur Meeresforschung*, 21: 78-91.
- DeVries, D. R. and Frie, R. V. 1996. Chapter 16: Determination of Age and Growth. *Fisheries Techniques 2nd edition* Eds: B. R. Murphy and D. W. Willis. American Fisheries Society.
- Dubreuil, R. and Cuerrier, J.P. 1950. Cycle du maturation des glands génitales chez l'esturgeon de lac (*Acipenser fulvescens*, Raf.). Institut de Biologie général et de Zoologie, Université de Montréal.
- Eibler, J. and Anderson, A. 2004. Major river survey: Upper rainy river, 2002-03, Completion Report F-29-R-23, Study 3, Job 4, Minnesota Department of Natural Resources Division of Fisheries, St. Paul, Minnesota.
- Froese, R. 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. *J. Appl. Ichthyol.* 22: 241-253
- Fortin, R., Dumont, P., and Guénette, S. 1996. Determinants of growth and body condition of lake sturgeon (*Acipenser fulvescens*). *Can. J. Fish. Aquat. Sci.* 53: 1150-1156.
- Hails, A.J 1983. Temporal changes in fat and protein levels in the tropical anabantid, (*Trichogaster pectoralis*) (Rgen) *J. Fish Biol.* 22: 203-213.
- Hall, S.R. and Rudstam, L.G. 1999. Habitat use and recruitment: a comparison of long-term recruitment patterns among fish species in a shallow eutrophic lake, Oneida Lake, NY, U.S.A. *Hydrobiologia* 408/409: 101-113.
- Hansen, M.J. and Nate, N.A. 2005. A method for correcting the relative weight ( $W_r$ ) index for seasonal patterns in relative condition (Kn) with length as applied to walleye in Wisconsin. *North Am. J. Fish Manage.* 25: 1256-62.
- Hayes, J., Brodziak, K.T., and O'Gorman, J.B. 1995. Efficiency and bias of estimators and sampling designs for determining length-weight relationships of fish. *Can. J. Fish. Aqua. Sci.* 52: 84-92.
- He, J. X., Rudstam, L. G., Forney, J. L., VanDeValk, A. J., & Stewart, D. J. 2005. Long-term patterns in growth of Oneida Lake walleye: a multivariate and stage-explicit approach for applying the von Bertalanffy growth function. *J. Fish Biol.* 66: 1459-1470.

- Htun-han, M. 1978. The reproductive biology of the dab (*Limanda limanda* (L)) in the North Sea; gonadosomatic Index; Hepatosomatic Index and condition factor. *J. Fish Biol.* 13: 369-378.
- Kaufman, S. D., Johnston, T. A., Leggett, W. C., Moles, M. D., Casselman, J. M. & Schulte-Hostedde, A. I. 2007. Relationships Between Body Condition Indices and Proximate Composition in Adult Walleyes. *Trans. Am. Fish. Soc.* 136: 1566–1576.
- King, M. 1995. Fisheries Biology Assessment and Management. *Fishing News Books* Blackwell Science Ltd., London, pp: 341.
- Le Cren, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). *J. Anim. Ecol.* 20: 201–219.
- Lester, N. P., Shuter, B. J., Kushneriuk, R. S. & Marshall, T. R. 2000. Life History Variation in Ontario Walleye Populations: Implications for Safe Rates of Fishing. Percid Community Synthesis Population and Yield Characteristics Working Group, Ontario Ministry of Natural Resources.
- Levene, H. 1960. Robust tests for equality of variances in: I. Olkin et al. (eds). *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, Stanford University Press. pp: 279-292.
- Mosindy, T. and Rusak, J. 1991. An assessment of lake sturgeon populations in Lake of the Woods and the Rainy River, 1987– 90. Ontario Ministry of Natural Resources, Lake of the Woods Fisheries Assessment Unit Report 1991:01, Toronto, ON, p. 67.
- Mosindy, T. and Mucha, J. 2005. Fall walleye index netting of South Sector 5, Lake of the Woods, Ontario (1997 and 2002). Ont. Min. Natur. Resour., Northwest Sci. and Info. NWSI Technical Report TR-137. 23 pp. + append.
- Murphy, B.R., Brown, M.L. & Springer, T.A. 1990. Evaluation of the Relative Weight ( $W_r$ ) Index, with New Applications to Walleye. *North Am. J. Fish. Manage.* 10 (1): 85-97.
- Overman, N.C. and Parrish, D.L. 2001. Stable isotope composition of walleye:  $\delta^{15}\text{N}$  accumulation with age and area-specific differences in  $\delta^{13}\text{C}$ . *Can. J. Fish. Aquat. Sci.* 58: 1253–1260.
- Payer, R.D., Pereira, D.L., Larson, M.L., Younk, J.A., Frie, R.V., Schupp, D.H. & Osborn, T.C. 1987. Status and simulation model of Lake of the Woods, Minnesota, walleye fishery. Minnesota Department of Natural Resources, Division of Fish and Wildlife, Investigational Report 389, 128pp.
- Power, M. and McKinley, R.S. 1997. Latitudinal Variation in Lake Sturgeon Size as Related to the Thermal Opportunity for Growth. *Trans. Am. Fish. Soc.* 126 (4): 549-558.

- Quist, M.C., Guy, C.S., Schultz, T.D., & Stephen, J.L. 2003. Latitudinal Comparisons of Walleye Growth in North America and Factors Influencing Growth of Walleyes in Kansas Reservoirs *North Am. J. Fish. Manage.* 23: 677–692.
- Ratkowsky. 1983. *Nonlinear Regression Modelling*. Marcel Dekker Inc., New York, NY. 276 p.
- Rossiter, A., Noakes, D.L.G. & Beamish, F.W.H. 1995. Validation of age estimation for the lake sturgeon. *Trans. Am. Fish. Soc.* 124: 777–781.
- Sass, G.G. and Kitchell, J.F. 2005. Can growth be used as a surrogate measure of walleye (*Sander vitreus*) abundance change? *Can. J. Fish. Aquat. Sci.* 62: 2159–2168
- Schmitt, C.C. and Skud, B.E. 1978. Relation of Fecundity to Long-Term Changes in Growth, Abundance and Recruitment. Scientific Report No. 66 International Pacific Halibut Commission.
- Schneider, J.C., Laarman, P. W. & Gowing, H. 2000. Length-weight relationships. Chapter 17 in Schneider, James C. (ed.). *Manual of fisheries survey methods II: with periodic updates*. Michigan Department of Natural Resources, Fisheries Special Report 25, Ann Arbor.
- Shapiro, S. S. and Wilk, M. B. 1965. An analysis of variance test for normality (complete samples). *Biometrika.* 52: 591-611.
- Treasurer, J.W. 1976. Age, growth and length-weight relationship of brown trout (*Salmo trutta*) in the Loch of Strathbeg, Aberdeenshire. *J. Fish Biol.* 8: 241-253.
- Trested, D. G. and Isely, J. J. 2011. Age, growth, mortality, and abundance of lake sturgeon in the Grasse River, New York, USA. *J. Appl. Ichthyol.* 27: 13–19.
- VanDeValk, A.J., Forney, J.L., & Jackson, J.R. 2008. Relationships between Relative Weight, Prey Availability, and Growth of Walleyes in Oneida Lake, New York. *North Am. J. Fish. Manage.* 28: 1868–1875.
- Winn, H.E. 1958. Comparative Reproductive Behavior and Ecology of Fourteen Species of Darters (Pisces-Percidae). *Ecol. Monogr.* 28(2): 155-191
- Wootton, R.J. 2003. *Ecology of teleost fishes*. Chapman and Hall, New York, NY
- Zar, J.T. 2010. Biostatistical analysis 5<sup>th</sup> edition. Person Education Inc. Editor: Deirdre Lynch. Upper Saddle River, New jersey 07458.

### 7.3 Chapter 3

Alexander, S.A., Hobson, K.A., Gratto-Trevor, C.L., & Diamond, A.W., 1996. Conventional and isotopic determinations of shorebird diets at an inland stopover: the importance of invertebrates and *Potomageton pectinatus* tubers. *Can. J. Zool.* 74: 1057–1068.

Andvik, R.T., VanDeHey, J.A., Fincel, M.J., French, W.E., Bertrand, K.N., Chipps, S.R., Kulmb, R.A., & Graeb, B.D.S., 2010. Application of non-lethal stable isotope analysis to assess feeding patterns of juvenile pallid sturgeon *Scaphirhynchus albus*: a comparison of tissue types and sample preservation methods. *J. Appl. Ichthyol.* 26: 831–835.

Arrington, D.A., Davidson, B.K., Winemiller, K.O., & Layman, C.A., 2006. Influence of life history and seasonal hydrology on lipid storage in three Venezuelan fish species. *J. Fish Biol.* 68: 1–16.

Beamish, F.W.H., Jebbink, J.A., Rossiter, A., & Noakes, D.L.G., 1996. Growth strategy of juvenile lake sturgeon (*Acipenser fulvescens*) in a northern river. *Can. J. Fish. Aquat. Sci.* 53: 481-489.

Brosse, L., Dumont, P., Lepage, M., & Rochard, E., 2002. Evaluation of a gastric lavage method for sturgeons. *N. Amer. J. Fish. Manage.* 22 (3): 955-960.

Carlisle, A.B., Kim, S.L., Semmens, B.X., Madigan, D.J., & Jorgensen, S.J., 2012. Using stable isotope analysis to understand the migration and trophic ecology of Northeastern Pacific white sharks (*Carcharodon carcharias*). *PLoS one* 7 (2), e30492.

Church, M.R., Ebersole, J.L., Rensmeyer, K.M., Couture, R.B., Barrows, F.T., & Noakes, D.L.G., 2009. Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. *Can. J. Fish Aquat.Sci.* 66: 1-5.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada), 2006. COSEWIC assessment and update status report on the lake sturgeon (*Acipenser fulvescens*) in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa.xi + 107 pp.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada), 2007. COSEWIC Annual Report. Available: [www.sararegistry.gc.ca/](http://www.sararegistry.gc.ca/).

Craig, H., 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. *Geochim.Cosmochim. Ac.* 12: 133–149.

DeNiro, M.J., and Epstein S., 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim.Cosmochim. Ac.* 14: 465-506.

Dubreuil, R., and Cuerrier, J.P., 1950. Cycle du maturation des glands génitales chez l'esturgeon de lac (*Acipenser fulvescens*, Raf.). Institut de Biologie général et de Zoologie, Université de Montréal.

Fagan, K.A., Koops, M.A., Arts, M.T., & Power, M., 2011. Assessing the utility of C:N ratios for predicting lipid content in fishes. *Can. J. Fish. Aquat. Sci.* 68: 374–385.

Finlay J.C., and Kendall C., 2007. Chapter 10: Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In: Michener, R., Lajtha, K., (Eds.) *Stable Isotopes in Ecology and Environmental Science* 2<sup>nd</sup> edition, Blackwell Publishing Ltd.

Fisheries and Oceans Canada, 2008. Recovery potential assessment of Great Lakes and St. Lawrence River watersheds (Designatable Unit 8) lake sturgeon (*Acipenser fulvescens*) population. *DFO Can. Sci. Advis. Sec. Sci. Advis. Rep.* 2008/042.

Folch J., Lees M., and Sloane Stanley G.H. 1957. A Simple Method for the Isolation and Purification of Total Lipides from Animal Tissues. *J. Biol. Chem.* 226: 497-509.

Fry, B., Baltz, D.B., Benfield, M.C., Fleeger, J.W., Gace, A., Haas, H.L., & Quiñones-Rivera, Z.J., 2003. Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marsh scapes. *Estuaries* 26: 82–97.

Griffiths, H., 1991. Applications of stable isotope technology in physiological ecology. *Funct. Ecol.* 5 (2): 254-269.

Haley, N., 1998. A gastric lavage technique for characterizing the diets of sturgeons. *N. Amer. J. Fish. Manage.* 18: 978–981.

Hanisch, J.R., Tonn, W.M., Paszkowski, C.A., Scrimgeour, G.J., 2010.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures in muscle and fin tissues: nonlethal sampling methods for stable isotope analysis of salmonids, *N. Amer. J. Fish. Manage.* 30 (1): 1-11.

Haxton T. J., Findlay S., 2008. Variation in lake sturgeon (*Acipenser fulvescens*) abundance and growth among river reaches in a large regulated river. *Can. J. Fish Aquat. Sci.* 65: 645–657.

Heady, W.N., Moore, J.M., 2013 Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. *Oecol.* 172 (1): 21-34.

Hoffman, J.C., Sutton, T.T., 2010. Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep-Sea Res. I.* 57: 956–964.

Jardine, T.D., Hunt, R.J., Pusey, B.J., Bunn, S.E., 2011. A non-lethal sampling method for stable carbon and nitrogen isotope studies of tropical fishes. *Mar. Freshw. Res.* 62: 83–90.

Kauffman, S.D., Johnston, T.A., Leggett, W.C., Moles, M.D., Casselman, J.M., Schulte-Hostedde, A.I., 2007. Relationships between body condition indices and proximate composition in adult walleyes. *Trans. Am. Fish. Soc.* 136: 1566-1576.

- Kelly, M.H., Hagar, W.G., Jardine, T.D., Cunjak, R.A., 2006. Nonlethal sampling of sunfish and slimy sculpin for stable isotope analysis: How scale and fin tissue compare with muscle tissue. *N. Amer. J. Fish. Manage.* 26: 921–925.
- Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., Jones, R.I., 2006. A revised model for lipid normalizing  $\delta^{13}\text{C}$  values from aquatic organisms, with implications for isotope mixing models. *J. Appl. Ecol.* 43: 1213–1222.
- Levene, H., 1960. Robust tests for equality of variances. In: Olkin, I., Ghurye, S.G., Hoeffding, W., Madow, W.G., Mann, H.B., (eds). *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, Stanford University Press. pp: 279-292.
- Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., Lutcavage, M.E., 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J. Anim. Ecol.* 77: 838–846.
- Mariotti, A., 1983. Atmospheric nitrogen is a reliable standard for natural  $^{15}\text{N}$  abundance measurements. *Nature* 303: 685–687.
- McCabe, D. J., Beekey, M. A., Mazloff, A., Marsden, J. E., 2006. Negative effect of zebra mussels on foraging and habitat use by lake sturgeon (*Acipenser fulvescens*). *Aquatic Conserv: Mar. Freshw. Ecosyst.* 16: 493–500.
- McCarthy, I. D., Waldron, S., 2000. Identifying migratory (*Salmo trutta*) using carbon and nitrogen stable isotope ratios. *Rapid Commun. Mass Spectrom.* 14: 1325–1331.
- McConnaughey, T., McRoy, C.P., 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar. Biol.* 53: 257–262.
- Nelson, J., Chanton, J., Coleman, F., Koeing, C., 2011. Patterns of stable carbon isotope turnover in gag, *Mycteroperca microlepis*, an economically important marine piscivore determined with a non-lethal surgical biopsy procedure. *Environ. Biol. Fish.* 90: 243–252.
- Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. *Ann. Rev. Ecol. Syst.* 18: 293-320.
- Pinnegar, J.K., Polunin, N.V.C., 1999. Differential fractionation of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among fish tissues: implications for the study of trophic interactions. *Funct. Ecol.* 13: 225-231.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., Montana, C.G., 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecol.* 152; 179–189.
- Rusak, J. A., Mosindy, T., 1997. Seasonal movements of the lake sturgeon in Lake of the Woods and the Rainy River, Ontario. *Can. J. Zool.* 74: 383-395.



Sanderson, B.L., Tran, C.D., Coe, H.J., Pelekis, V., Steel, E.A., Reichert, W.L., 2009. Non-lethal sampling of fish caudal fins yields valuable stable isotope data for threatened and endangered fishes. *Trans. Am. Fish. Soc.* 138 (5): 1166-1177.

Sinnatamby, R. N., Dempson, J.B., Power, M., 2008. A comparison of muscle and scale-derived  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  across three life-history stages of Atlantic salmon, *Salmo salar*. *Rapid Commun. Mass Spectrom.* 22: 2773–2778.

Shapiro, S. S., Wilk, M. B., 1965. An analysis of variance test for normality (complete samples). *Biometrika.* 52: 591-611.

Suzuki, K.W., Kasai, A., Nakayama, K., Tanaka, M., 2005. Differential isotopic enrichment and half-life among tissues in Japanese temperate bass (*Lateolabrax japonicus*) juveniles: implications for analyzing migration. *Can. J. Fish. Aquat. Sci.* 62: 671–678

Sweeting, C.J., Polunin, N.V.C., Jennings, S., 2006. Effects of chemical lipid extraction and arithmetic lipid correction on stable isotope ratios of fish tissues. *Rapid Commun. Mass Spectrom.* 20: 595–601.

Tronquart, N.H., Mazeas, L., Reuilly-Manenti, L., Zahm, A., Belliard, J., 2012. Fish fins as non-lethal surrogates for muscle tissues in freshwater food web studies using stable isotopes. *Rapid Commun. Mass Spectrom.* 26: 1603–1608.

Tyus H. M., Starnes W. C., Karp C. A., Saunders J. F. 1999. Effects of invasive tissue collection on rainbow trout, razorback sucker, and bonytail chub. *N. Amer. J. Fish. Manage.* 19: 848–855.

Willis, T.J., Sweeting, C.J., Bury, S.J., Handley, S.J., Brown, J.C.S., Freeman, D.J., Cairney, D.G., Page, M.J., 2013. Matching and mismatching stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) ratios in fin and muscle tissue among fish species: a critical review. *Mar. Biol.* 160: 1633–1644.

Zar, J.T., 2010 Biostatistical analysis 5<sup>th</sup> edition. Lynch, D., (Eds) Person Education Inc. Upper Saddle River, New jersey 07458.

## 7.4 Chapter 4

Adams W.E., Kallemeyn L. W. & Willis D. W. 2006. Lake sturgeon population characteristics in Rainy Lake, Minnesota and Ontario. *J. Appl. Ichthyol.* 22: 97–102.

Auer N. A., 1996. Response of spawning lake sturgeon to change in hydroelectric facility operation. *Trans. Am. Fish. Soc.* 125: 66–77.

Auer N. A. 1999. Population characteristics and movements of Lake Sturgeon in the Sturgeon River and Lake Superior. *J. Gt. Lakes Res.* 25: 282–293.

- Auer N. A. and Baker E. A. 2002. Duration and drift of larval lake sturgeon in the Sturgeon River, Michigan. *J. Appl. Ichthyol.* 18: 557–564.
- Barth C. C., Peake S. J., Allen P. J. & Anderson W. G. 2009. Habitat utilization of juvenile lake sturgeon, *Acipenser fulvescens*, in a large Canadian river. *J. Appl. Ichthyol.* 25 (Suppl. 2): 18–26.
- Beamish F.W.H., Jebbink J., Rossiter A., & Noakes D.L.G. 1996. Growth strategy of juvenile lake sturgeon (*Acipenser fulvescens*) in a northern river. *Can. J. Fish. Aquat. Sci.* 53: 481–489.
- Beamish, F. W. H.; Noakes, D. L. G.; Rossiter, A., 1998. Feeding ecology of juvenile lake sturgeon, *Acipenser fulvescens*, in northern Ontario. *Can. Field-Natur.* 112: 459–468.
- Bearhop S., Adams C.E., Waldron S., Fuller R.A. & Macleod H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.* 73: 1007–1012.
- Beaudoin C.P., Prepas E.E., Tonn W.M., Wassenaar L.I., Kotak B.G. 2001. A stable carbon and nitrogen isotope study of lake food webs in Canada's Boreal Plain. *Freshw. Biol.* 46: 465–477.
- Borkholder B. D., Morse S. D., Weaver H. T., Hugill R., Linder A. T., Schwarzkopf L. M., Perrault T. E., Zacher M. J., & Frank J. A. 2002. Evidence of a year-round resident population of lake sturgeon in the Kettle River, Minnesota, based on radiotelemetry and tagging. *N. Amer. J. Fish. Manage.* 22: 888–894.
- Brosse L., Dumont P., Lepage M., & Rochard, E. 2002. Evaluation of a gastric lavage method for sturgeons. *N. Amer. J. Fish. Manage.* 22 (3): 955–960.
- Bunn S. E., Loneragan N. R. & Kempster M. A. 1995. Effects of Acid Washing on Stable Isotope Ratios of C and N in Penaeid Shrimp and Seagrass: Implications for Food-Web Studies Using Multiple Stable Isotopes. *Limnol. Oceanogr.* 40 (3): 622–625.
- Carpenter S. R., Cole J. J., Hodgson J. R., Kitchell J. F., Pace M. L., Bade D., Cottingham K. L., Essington T. E., Houser J. N., & Schindler D. E. 2001. Trophic cascades, nutrients, and lake productivity: whole-lake experiments. *Ecol. Monogr.* 71: 163–186.
- Choudhury A. and Dick T.A. 1993. Parasites of lake sturgeon, (*Acipenser fulvescens*) (Chondrostei: Acipenseridae), from central Canada. *J. Fish Biol.* 42: 571–584.
- Colombo F., Costa V., Dubois S.F., Gianguzza P., Mazzola A., & Vizzini S. 2012. Trophic structure of vermetid reef community: High trophic diversity at small spatial scales. *J. Sea Res.* 77: 93–99.
- Connolly R.M. and Schlacher T.A. 2013. Sample acidification significantly alters stable isotope ratios of sulfur in aquatic plants and animals. *Mar. Ecol. Prog. Ser.* 493: 1–8.

COSEWIC (Committee on the Status of Endangered Wildlife in Canada), 2006. COSEWIC assessment and update status report on the lake sturgeon *Acipenser fulvescens* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa.xi + 107 pp.

Craig H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass spectrometric analysis of carbon dioxide. *Geochim.Cosmochim. Ac.* 12: 133–149.

Cross W.F., Baxter C.V., Rosi-Marshall E.J., Hall Jr. R.O., Kennedy T.A., Donner K.C., Wellard-Kelly H.A., Seegert S.Z., Behn K.E. & Yard M.D. 2013. Food-web dynamics in a large river discontinuum. *Ecol. Monogr.* 83(3): 311–337.

Cunjak R.A., Roussel J.M., Gray M.A., Dietrich J.P., Cartwright D.F., Munkittrick K.R., and Jardine T.D. 2005. Using stable isotope analysis with telemetry or mark-recapture data to identify fish movement and foraging. *Oecol.* 144: 636-646.

Cuerrier, J.P. 1996. The lake sturgeon (*Acipenser fulvescens*) of the Lake St. Pierre region during the spawning period. O.T. Observations sur l'esturgeon de lac (*Acipenser fulvescens*) dans le region du lac St-Pierre au course de la periode du frai. *Naturaliste Canadien.* 93 (4): 279-334

Damstra R.A. and Galarowicz T.L. 2013. Summer Habitat Use by Lake Sturgeon in Manistee Lake, Michigan. *Trans. Am. Fish. Soc.* 142: 931–941.

DeNiro M.J. and Epstein S. 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Ac.* 14: 465-506.

DeVries D. R. and Frie R. V. 1996. Chapter 16: Determination of Age and Growth. *Fisheries Techniques 2nd edition* Eds: B. R. Murphy and D. W. Willis. American Fisheries Society.

Dick T. A., Campbell R. R., Mandrak N. E., Cudmore B., Reist J. D., Rice J., Bentzen J., & Dumont P. 2006. COSEWIC assessment and update status report on the lake sturgeon (*Acipenser fulvescens*) in Canada. Ottawa, Ontario. 107 p.

Dubreuil R. and Cuerrier J.P. 1950. Cycle du maturation des glands génitales chez l'esturgeon de lac (*Acipenser fulvescens*, Raf.). Institut de Biologie général et de Zoologie, Université de Montréal.

Eibler J. and Anderson A. 2004. Major river survey: Upper rainy river, 2002-03, Completion Report F-29-R-23, Study 3, Job 4, Minnesota Department of Natural Resources Division of Fisheries, St. Paul, Minnesota.

Fry B. 2006 *Stable Isotope Ecology*. Springer Science LLC, New York, New York, 295 pp.  
Fortin R., Mongeau J.-R., DesJardins G., & Dumont P. 1993. Movements and biological statistics of lake sturgeon (*Acipenser fulvescens*) populations from the St. Lawrence and Ottawa River system, Quebec. *Can. J. Zool.* 71: 638–650.

- Galloway J.N., Aber J.D., Erisman J.W., Seitzinger S.P., Howarth R.W., Cowling E.B, Cosby B.J. 2003. The Nitrogen Cascade. *BioScience*. 53 (4): 341-356.
- Gerrity P.C, Guy C.S. & Gardner W.M. 2006. Juvenile Pallid Sturgeon are Piscivorous: A Call for Conserving Native Cyprinids. *Trans. Am. Fish. Soc.* 135 (3): 604-609.
- Golléty C., Riera P., & Davoult D. 2010. Complexity of the food web structure of the *Ascophyllum nodosum* zone evidenced by a  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  study. *J. Sea Res.* 64: 304–312.
- Gray M.A. and K.R. Munkittrick. 2005. An effects-based assessment of slimy sculpin (*Cottus cognatus*) populations in potato agriculture regions of northwestern New Brunswick. *Water Qual Res J Can.* 40: 16-27.
- Hamilton S.K., Lewis Jr W.M. & Sippel S.J. 1992. Energy sources for aquatic animals in Orinoco river floodplain: evidence from stable isotopes. *Oecol.* 89: 324-330.
- Harkness, W.J.K. and Dymond J.R. 1961. The lake sturgeon: The history of its fisheries and problems of conservation. Ontario Department of Lands and Forests, Fish and Wildlife Branch, Toronto, ON. 121 pp.
- Hay-Chmielewski E. M. 1987. Habitat preferences and movement patterns of the Lake Sturgeon (*Acipenser fulvescens*) in Black Lake, Michigan. Michigan Department of Natural Resources, Fisheries Research Report 1949, Lansing.
- IPCC. 2013. Summary for Policymakers. In: *Climate Change 2013: The Physical Science Basis*. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Ivan, L.N., Rutherford, E. S., Riseng, C., Tyler, J. A. 2010. Density, production, and survival of walleye (*Sander vitreus*) eggs in the Muskegon, River, Michigan. *Journal of Great Lakes Research.* 36: 328-337.
- Ives J. T., Marty J., de Lafontaine Y., Johnson T. B., Koops M. A. & Power M. 2013. Spatial variability in trophic position and food sources of *Hemimysis anomala* in lentic and lotic ecosystems within the Great Lakes basin. *J. Plankton Res.* 35: 772-784.
- Jackson A.L., Inger R., Parnell A.C. & Bearhop S. 2011. Comparing Isotopic Niche Widths Among and Within Communities: SIBER- Stable Isotope Bayesian Ellipses in R. *J. Anim. Ecol.* 80: 595-602.
- Jackson J. R., VanDeValk A. J., Brooking T. E., VanKeeken O. A., & Rudstam L. G. 2002. Growth and feeding dynamics of Lake Sturgeon (*Acipenser fulvescens*) in Oneida Lake, New York: results from the first five years of a restoration program. *J. Appl. Ichthyol.* 18: 439–443.

- Johnson K.H. 2000. Trophic-dynamic considerations in relating species diversity to ecosystem resilience. *Biol. Rev.* 75: 347-376.
- Kallemeyn, L. W. 1987. Correlations of regulated lake levels and climatic factors with abundance of young-of-the-year walleye and yellow perch in four lakes in Voyageurs National Park. *N. Amer. J.Fish. Manage.* 7: 513-521.
- Kempinger, J. J., 1996: Habitat, growth, and food of young lake sturgeons in the Lake Winnebago system, Wisconsin. *N. Amer. J.Fish. Manage.* 16, 102–114.
- Kerr S. J., Davison M. J., & Funnell E. 2010. A review of lake sturgeon habitat requirements and strategies to protect and enhance sturgeon habitat. Fisheries Policy Section, Biodiversity Branch. Ontario Ministry of Natural Resources. Peterborough, Ontario. 58 p. + appendices.
- Knights B. C., Vallazza J.M., Zigler S. J., & Dewey M. R. 2002. Habitat and movement of Lake Sturgeon in the upper Mississippi River system, USA. *Trans. Am. Fish. Soc.* 131: 507–522.
- Layman C.A., Araujo M.S., Boucek R., Hammerschlag-Peyer C.M. Harrison E., Jud Z.R. Matich P. Rosenblatt A.E. Vaudo J.J., Yeager L.A. Post D.M. & Bearhop S. 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol. Rev.* 87: 545–562.
- Lefebvre S., Marin Leal J.C., Dubois S., Orvain F., Blin J.L., Bataille M.P., Ourry A., & Galois R. 2009. Seasonal dynamics of trophic relationships among co-occurring suspensionfeeders in two shellfish culture dominated ecosystems. *Est. Coast. Shelf Sci.* 82: 415–425.
- Leonard G.H., Levine J.M., Schmidt P.R. & Bertness M.D. 1998. Flow-Driven Variation in Intertidal Community Structure in a Maine Estuary. *Ecology.* 79 (4): 1395-1411.
- Levene H. 1960. Robust tests for equality of variances in: I. Olkin et al. (eds). *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, Stanford University Press. pp: 279-292.
- Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., and Lutcavage, M.E. 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J. Anim. Ecol.* 77: 838-846.
- Matthews B. and Mazumder A. 2004. A critical evaluation of intrapopulation variation of  $\delta^{13}\text{C}$  and isotopic evidence of individual specialization. *Oecol.* 140: 361–371.
- Mariotti A. 1983. Atmospheric nitrogen is a reliable standard for natural  $^{15}\text{N}$  abundance measurements. *Nature* 303: 685–687.
- McMahon K. W., Berumen M. L., & Thorrold S. R. 2012. Linking habitat mosaics and connectivity in a coral reef seascape. *Proc. Natl. Acad. Sci. U. S. A.* 109: 15372-15376.

- Miller M. J. 2004. The ecology and functional morphology of feeding of North American sturgeon and paddlefish. Pages 87–102 in G. T. O. LeBreton, F. W. H. Beamish and R. S. McKinley, editors. *Sturgeons and paddlefish of North America*. Kluwer, Boston.
- Mosindy, T. and J. Rusak. 1991. An assessment of the lake sturgeon populations in Lake of the Woods and the Rainy River 1987-90. Lake of the Woods Fisheries Assessment Unit Report. Ontario Ministry of Natural Resources, Kenora, ON. 59 pp.
- Murchie, K.J. and Power, M. 2004. Growth- and feeding-related isotopic dilution and enrichment patterns in young-of-the-year yellow perch (*Perca flavescens*). *Freshw. Biol.* 49: 41–54.
- Nilo P., Bolon A., Dodson J., Dumont P., Fortin R. 2006: Feeding ecology of juvenile lake sturgeon in the St. Lawrence River system. *Trans. Am. Fish. Soc.* 135: 1044–1055.
- Petersen B. J. and Fry B. 1987. Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Syst.* 18: 293-320.
- Post D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology.* 83: 703–718.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., and Montana, C.G. 2007. Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecol.* 152: 179-189.
- Power M., O’Connell M.F. & Dempson J.B. 2005. Ecological segregation within and among Arctic char morphotypes in Gander Lake, Newfoundland. *Environ. Biol. Fishes.* 73: 263–274.
- Rasmussen J.B., Trudeau V., & Morinville G. 2009. Estimating the scale of fish feeding movements in rivers using  $\delta^{13}C$  signature gradients. *J. Anim. Ecol.* 78: 674–685.
- Rusak J. A. and Mosindy T. 1997. Seasonal movements of the lake sturgeon in Lake of the Woods and the Rainy River, Ontario. *Can. J. Zool.* 74: 383-395.
- Shapiro S. S. and Wilk M. B. 1965. An analysis of variance test for normality (complete samples). *Biometrika.* 52: 591-611.
- Smith A., Marty J., & Power M. *in press*. Non-lethal sampling of lake sturgeon for stable isotope analysis: comparing pectoral fin-clip and dorsal muscle for use in trophic studies. *J. Gt. Lakes Res.*
- Smith K. M. and King D. K. 2005. Movement and habitat use of yearling and juvenile Lake Sturgeon in Black Lake, Michigan. *Trans. Am. Fish. Soc.* 134: 1159–1172.
- Stelzer R.S., Drecktrah H.G., & Shuppyt M.P. 2008. Carbon sources for lake sturgeon in Lake Winnebago, Wisconsin. *Trans. Am. Fish. Soc.* 137: 1018–1028.

Tyus H. M., Starnes W. C., Karp C. A. & Saunders J. F. 1999. Effects of invasive tissue collection on rainbow trout, razorback sucker, and bonytail chub. *N. Amer. J. Fish. Manage.* 19: 848–855.

Vadeboncoeur Y., Lodge D. M., & Carpenter S. R. 2001. Whole-lake fertilization effects on distribution of primary production between benthic and pelagic habitats. *Ecology* 82: 1065–1077.

Vanderklift M. A., and S. Ponsard. 2003. Sources of variation in consumer-diet  $\delta^{15}\text{N}$  enrichment: a meta-analysis. *Oecol.* 136: 169–182.

Vander Zanden J.M., Hulshof M., Ridgway M.S., & Rasmussen J.B. 1998. Application of Stable Isotope Techniques to Trophic Studies of Age-0 Smallmouth Bass. *Trans. Am. Fish. Soc.* 127: 729–739.

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

Vander Zanden, J.M. & Rasmussen, J.B. 1999. Primary Consumer  $\text{d}^{13}\text{C}$  and  $\text{d}^{15}\text{N}$  and the Trophic Position of Aquatic Consumers. *Ecology*, 80(4): 1395–1404.

Wanner G.A., Shuman D.A. & Willis D.W. 2007. Food Habits of Juvenile Pallid Sturgeon and Adult Shovelnose Sturgeon in the Missouri River Downstream of Fort Randall Dam, South Dakota, *Journal of Freshw. Ecol.* 22 (1): 81-92.

Werner R.G. 2005. Contributing Factors in Habitat Selection by Lake Sturgeon (*Acipenser fulvescens*). United States Environmental Protection Agency- Great Lakes National Program Office. Albany, New York.

## 7.5 Chapter 5

Aadland, L.P., Cook, C.M., Negus, M.T., Drewes, H.G. & Anderson, C.S. 1991. Microhabitat preferences of selected stream fishes and a community-oriented approach to instream flow assessments. Minnesota Department of Natural Resources: Section of Fisheries Investigational Report #406.

Auer, N.A. 1996. Response of Spawning Lake Sturgeons to Change in Hydroelectric Facility Operation. *Trans. Am. Fish. Soc.* 125 (1), 66-77.

Bates, D.M. and Watts, D.G. 2007. *Non-linear regression analysis and its applications*. John Wiley & Sons, New York, NY. 329pp

Bemis, W. E. and Kynard, B. 1997. Sturgeon rivers: an introduction to Acipenseriform biogeography and life history. *Environ. Biol. Fish.* 48, 167–183.

Boisclair, D. 2001. Fish habitat modeling: from conceptual framework to functional tools. *Can. J. Fish. Aquat. Sci.* 58, 1–9.

- Bray, K. E. 1996. Habitat models as tools for evaluating historic change in the St. Marys River. *Can. J. Fish. Aquat. Sci.* 53(Supplement 1), 88–98.
- Brooks, R. P. 1997. Improving habitat suitability index models. *Wildlife Soc. B.* 125, 163–167.
- Bruch, R. M. and Binkowski, F. P. 2002. Spawning behavior of lake sturgeon (*Acipenser fulvescens*). *J. Appl. Ichthyol.* 18, 570–579.
- Caswell, N. M., Peterson, D. L., Manny, B. A. & Kennedy, G. W. 2004. Spawning by lake sturgeon (*Acipenser fulvescens*) in the Detroit River. *J. Appl. Ichthyol.* 20, 1–6.
- Chalupnicki M.A., Johnson J.H., McKenna Jr. J.E., & Dittman D. 2010. Habitat Selection and Spawning Success of Walleyes in a Tributary to Owasco Lake, New York. *North Am. J. Fish. Manage.* 30, 170–178.
- Colby, P. J., McNicol, R. E., & Ryder R. A. 1979. Synopsis of biological data on the walleye. Fisheries Synopsis No. 119, Food and Agriculture Organization, Rome, Italy. 139 p.
- Corbett, B.W., and Powles, P.M. 1986. Spawning and larval drift of sympatric walleyes and white suckers in an Ontario stream. *Trans. Am. Fish. Soc.* 115, 41–46.
- Das, B.M. 2005. Fundamentals of Geotechnical Engineering, Second Edition. Thompson Canada Ltd.: Toronto, ON, 50 – 56.
- Dick T. A., Campbell R. R., Mandrak N. E., Cudmore B., Reist J. D., Rice J., Bentzen J., & Dumont P. 2006. COSEWIC assessment and update status report on the lake sturgeon (*Acipenser fulvescens*) in Canada. Ottawa, Ontario. 107 p.
- Dubreuil R. and Cuerrier J.P. 1950. Cycle du maturation des glands génitales chez l'esturgeon de lac (*Acipenser fulvescens*, Raf.). Institut de Biologie général et de Zoologie, Université de Montréal.
- Dumont, P., D'Amours, J. D., Thibodeau, S., Verdon, R., Garceau, S., Bilodeau, P. Malhiot, Y. & Fortin, R. 2009. Effects of the implementation of a newly created spawning ground in the Des Prairies River on the reproductive success of lake sturgeon (*Acipenser fulvescens*). Presentation at the 6<sup>th</sup> International Symposium on Sturgeon. October 25-31, 2009. Wuhan, China.
- Eibler J. and Anderson A. 2004. Major river survey: Upper rainy river, 2002-03, Completion Report F-29-R-23, Study 3, Job 4, Minnesota Department of Natural Resources Division of Fisheries, St. Paul, Minnesota.
- Fortin, R., D'Amours, J.D., & Thibodeau, S. 2002. Effets de l'aménagement d'un nouveau secteur de frayere sur l'utilisation du milieu en periode de fraie et sur le success de reproduction de l'esturgeon jaune (*Acipenser fulvescens*) a la frayere de la riviere des Prairies. Rapport synthese 1995-1999. Pur l'Unite Hydraulique et Environment, Hydro-Quebec et la Societe de la



fuane du Quebec, Direction de l'aménagement de la faune de Montreal de Laval et de la Monteregie Department des Sciences biologiques, Universite de Quebec a Montreal.

Gilbert, A.H. 1999. Lake Champlain Angler Survey 1997. Federal Aid Job Performance Report. Final Report. Revised 2000. F-23-R, Job5. VTDFW, Waterbury, VT. 87pp.

Gillenwater, D., Granata, T., & Zikab, U. 2006. GIS-based modeling of spawning habitat suitability for walleye in the Sandusky River, Ohio, and implications for dam removal and river restoration. *Ecol. Eng.* 28, 311–323.

Hartman, G.F. 2009. A Biological Synopsis of Walleye (*Sander vitreus*). *Can. Manuscr. Rep. Fish. Aquat. Sci.* 2888: v + 48p.

Haxton, T.J., Findlay, C.S. & Threader, R.W. 2008. Predictive Value of a Lake Sturgeon Habitat Suitability Model. *North Am. J. Fish. Manage.* 28:5, 1373-1383,

Heggenes, J. 1989. Physical habitat selection by brown trout (*Safmo trutta*) in riverine systems. *Nord. J. Freshw. Res.* 64, 74-90.

Ivan, L.N., Rutherford, E.S., Riseng, C. and Tyler, J.A. 2010. Density, production, and survival of walleye (*Sander vitreus*) eggs in the Muskegon River, Michigan. *J. Gt. Lakes Res.* 36, 328–337.

Johnson, F.H. 1961. Walleye Egg Survival during Incubation on Several Types of Bottom in Lake Winnibigoshish, Minnesota, and Connecting Waters. *Trans. Am. Fish. Soc.* 90 (3), 312-322.

Jones, M.L., Netto, J.K., Stockwell, J.D., Mion, J.B., 2003. Does the value of newly accessible spawning habitat for walleye (*Stizostedion vitreum*) depend on its location relative to nursery habitats? *Can. J. Fish. Aquat. Sci.* 60, 1527–1538.

Jowett IG, and Duncan MJ. 1990. Flow variability in New Zealand rivers and its relationship to in-stream habitat and biota. *N. Z. J. Mar. Freshw. Res.* 24(3), 305–17.

Kallemeyn, L., W.R. Darby, E. Eaton, K. Peterson, K. Smokorowski and J. Van den Broeck. 2009. Plan of study for the evaluation of the IJC 2000 Order for Rainy and Namakan lakes and Rainy River. Unpublished report prepared for the International Joint Commission by the 2000 Rule Curve Assessment Workgroup, 55+ pp.

Kerr, S.J., Corbett, B.W., Hutchinson, N.J., Kinsman, D., Leah, J.H., Puddister, D., Standfield, L. & Ward, N. 1997. Walleye Habitat: A Synthesis of Current Knowledge with Guidelines for Conservation. Percid Community Synthesis Walleye Habitat Working Group.

Kerr S. J., Davison M. J., & Funnell E. 2010. A review of lake sturgeon habitat requirements and strategies to protect and enhance sturgeon habitat. Fisheries Policy Section, Biodiversity Branch. Ontario Ministry of Natural Resources. Peterborough, Ontario. 58 p. + appendices.

- LaHaye, M., A. Branchaud, M. Gendron, R. Verdon and R. Fortin. 1992. Reproduction, early life history, and characteristics of the spawning grounds of the lake sturgeon (*Acipenser fulvescens*) in Des Prairies and L'Assomption rivers, near Montréal, Québec. *Can. J. Zool.* 70, 1681-1689.
- Leach, J. H., Johnson, M. G., Kelso, J. R. M., Hartmann, J., Nomann, W., & Entz, B. 1977. Responses of percid fishes and their habitats to eutrophication. *J. Fish. Res. Board Can.* 34, 1964-1971.
- Leopold, L.B. 1970. An Improved Method for Size Distribution of Stream Bed Gravel. *Water Resour. Res.* 6, 1357-1366.
- Levene H. 1960. Robust tests for equality of variances in: I. Olkin et al. (eds). *Contributions to Probability and Statistics: Essays in Honor of Harold Hotelling*, Stanford University Press. pp: 279-292.
- Liaw, W.K. 1991. Habitat suitability criteria for walleye spawning and egg incubation in Saskatchewan. *Sask. Env. & Resour. Manag., Fish. Br., Fish. Tech. Rept.* 91-1. 24 p.
- Lowie, C.E., J.M. Haynes, and R.P. Walter. 2001. Comparison of walleye habitat suitability index (HSI) information with habitat features of a walleye spawning stream. *J. Freshw. Ecol.* 16, 621-631.
- Lyttle, M. 2008. Spawning Habitat Suitability for Walleye and Lake Sturgeon in the Missisquoi River. U.S. Fish and Wildlife Service, Lake Champlain Fish and Wildlife Resources Office.
- Manly, B.F.J., McDonald, L.L. & Thomas, D.L. 1993. Resource selection by animals: statistical design and analysis for field studies. London: Chapman and Hall. 353 pp.
- Manny, B.A. and Kennedy, G. W. 2002. Known lake sturgeon (*Acipenser fulvescens*) spawning habitat in the channel between lakes Huron and Erie in the Laurentian Great Lakes. *J. Appl. Ichthyol.* 18, 486-490.
- Manny, B. A., Read, J., Kennedy, G. W., Denison, D., Caswell, N. M., Boase, J., Kaproski, S., McLain, J. R., Reider, R. H. & Taylor W. 2004. Creation of lake sturgeon spawning habitat in the Detroit River. In Program and Abstracts of the 134<sup>th</sup> Annual Meeting of the American Fisheries Society. August 21-26, 2004. Madison, Wisconsin.
- Manny, B.A., Kennedy, G.W., Boase, J.C., Allen, J.D, & Roseman, E.F. 2010. Spawning by walleye (*Sander vitreus*) and white sucker (*Catostomus commersoni*) in the Detroit River: Implications for spawning habitat enhancement. *J. Gt. Lakes Res.* 36, 490-496.
- McGrath, K. 2009. Development of lake sturgeon spawning beds on the St. Lawrence River near Waddington, New York. Presentation at the 52<sup>nd</sup> Annual Conference on Great Lakes Research. May 18-22, 2009, Toledo, Ohio.

- Mosindy, T. and Rusak, J. 1991. An assessment of lake sturgeon populations in Lake of the Woods and the Rainy River, 1987– 90. Ontario Ministry of Natural Resources, Lake of the Woods Fisheries Assessment Unit Report 1991:01, Toronto, ON, p. 67.
- Muste, M., Yu, K., and Spasojevic, M. 2004. Practical Aspects of ADCP Data for Quantification of Mean River Flow Characteristics; Part 1: Moving-Vessel Measurements. *Flow Meas. & Instr. Man.* 15, 1–16.
- Nichols, S.J., Kennedy, G., Crawford, E., Allen, J., French, J., Black, G., Blouin, M., Hickey, J., Chernyák, S., Haas, R., & Thomas, M. 2003 Assessment of Lake Sturgeon (*Acipenser fulvescens*) Spawning Efforts in the Lower St. Clair River, Michigan. *J. Great Lakes Res.* 29 (3), 383–391.
- O’Neil, L.J., Roberts, T. H., Wakeley, J. S., and Teaford, J. W. 1988. A procedure to modify habitat suitability index models. *Wildlife Soc. B.* 16, 33–36.
- O’Shea, D. 2005. Water management recommendations for the Rainy River. Stream Habitat Program Final Advisory Report; Rainy River Peaking Group. Division of Ecological Services, Minnesota Department of Natural Resources, St. Paul, MN. 62 pp.
- Peterson, D.L., Vecsei, P., & Jennings, C.A. 2007. Ecology and biology of the lake sturgeon: a synthesis of current knowledge of a threatened North American Acipenseridae. *Rev Fish Biol Fisheries.* 17, 59–76.
- Randall, R.G., 2008. Narrative description and quantification of the habitat requirements of lake sturgeon (*Acipenser fulvescens*) in the Great Lakes and upper St. Lawrence River. Research Document. Fisheries and Oceans Canada. Ottawa, Ontario.
- Roloff, G. J., and Kernohan, B. J. 1999. Evaluating reliability of habitat suitability index models. *Wildlife Soc. B.* 27, 973–985.
- Scott, W.B. and E.J. Crossman. 1973. *Freshwater fishes of Canada*. Bull. Fish. Res. Board Can. 184:1-966.
- Seyler, J. 1997. Adult lake sturgeon (*Acipenser fulvescens*) habitat use, Groundhog River, Ontario. Ministry of Natural Resources, TR-035, Timmins, Ontario.
- Shapiro S. S. and Wilk M. B. 1965. An analysis of variance test for normality (complete samples). *Biometrika.* 52, 591-611.
- Shaw, S.L., Chipps, S.R., Windels, S.K., Webb, M.A.H. & McLeod, D.T. 2013. Influence of Sex and Reproductive Status on Seasonal Movement of Lake Sturgeon in Namakan Reservoir, Minnesota–Ontario. *Trans. Am. Fish. Soc.* 142 (1), 10-20.
- Smith, L.C. 1985. *The inland fishes of New York State*. The New York State Department of Environmental Conservation, Albany, NY, pp. 348-349.

Sternberg, D. 1986. Walleye. Cy DeCosse Incorporation, Minnetonka, Minnesota, pp. 5-35.

Threader, R.W. Pope, R.J. & Schaap, P. R. H. 1998. Development of a habitat suitability index model for lake sturgeon (*Acipenser fulvescens*). Ontario Hydro report number H-07015.01-0012, Toronto, Canada.

Waddle, T. 2010. Field evaluation of a two-dimensional hydrodynamic model near boulders for habitat calculation. *River Res. Appl.* 26 (6), 730-741.

Wei, Q. W., Kynard, B., Yang, D. G., Chen, X., Du, H., Shen, L., & Zhang, H. 2009. Using drift nets to capture early life stages and monitor spawning of the Yangtze River Chinese sturgeon (*Acipenser sinensis*) *J. Appl. Ichthyol.* 25 (Suppl. 2), 100–106.

Werner, R. G. 1980. Freshwater Fishes of New York State: A field guide. Syracuse University Press, Syracuse, NY, pp. 169-171.

Zar J.T. 2010. Biostatistical analysis 5<sup>th</sup> edition. Person Education Inc. Editor: Deirdre Lynch. Upper Saddle River, New jersey 07458.

## 7.6 Chapter 6

Cuerrier, J.P. 1996. The lake sturgeon (*Acipenser fulvescens*) of the Lake St. Pierre region during the spawning period. O.T. Observations sur l'esturgeon de lac (*Acipenser fulvescens*) dans le region du lac St-Pierre au course de la periode du frai. *Naturaliste Canadien.* 93 (4): 279-334

Haley, N., 1998. A gastric lavage technique for characterizing the diets of sturgeons. *N. Amer. J. Fish. Manage.* 18: 978–981.

Harkness, W.J.K. and Dymond J.R. 1961. The lake sturgeon: The history of its fisheries and problems of conservation. Ontario Department of Lands and Forests, Fish and Wildlife Branch, Toronto, ON. 121 pp.

Mosindy, T. and Rusak, J. 1991. An assessment of lake sturgeon populations in Lake of the Woods and the Rainy River, 1987– 90. Ontario Ministry of Natural Resources, Lake of the Woods Fisheries Assessment Unit Report 1991:01, Toronto, ON, p. 67.

Scott, W.B. and E.J. Crossman. 1973. *Freshwater fishes of Canada.* Bull. Fish. Res. Board Can. 184:1-966.

Smith, L.C. 1985. *The inland fishes of New York State.* The New York State Department of Environmental Conservation, Albany, NY, pp. 348-349.

Stelzer R.S., Drecktrah H.G., & Shuppyt M.P. 2008. Carbon sources for lake sturgeon in Lake Winnebago, Wisconsin. *Trans. Am. Fish. Soc.* 137: 1018–1028.

Tronquart, N.H., Mazeas, L., Reuilly-Manenti, L., Zahm, A., Belliard, J., 2012. Fish fins as non-lethal surrogates for muscle tissues in freshwater food web studies using stable isotopes. *Rapid Commun. Mass Spectrom.* 26: 1603–1608.

Werner, R. G. 1980. *Freshwater Fishes of New York State: A field guide*. Syracuse University Press, Syracuse, NY, pp. 169-171.

Willis, T.J., Sweeting, C.J., Bury, S.J., Handley, S.J., Brown, J.C.S., Freeman, D.J., Cairney, D.G., Page, M.J., 2013. Matching and mismatching stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) ratios in fin and muscle tissue among fish species: a critical review. *Mar. Biol.* 160: 1633–1644.

## 8.0 Appendix A

### 8.1 Walleye Stable Isotope Analysis

Within the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot vertical trophic structure adult walleye are located at the apex position of top predator. Intra-specific variation among sampled walleye was lower on the  $\delta^{13}\text{C}$  axis (range: -27.5 to -22.8‰; coefficient of variation: 3.4%) than on the  $\delta^{15}\text{N}$  axis (range: 8.2 to 12.5‰; coefficient of variation: 7.8%). There was no evidence of size-related changes in  $\delta^{13}\text{C}$ , with regressions of dorsal muscle stable isotope values against length yielding insignificant results for walleye ( $r^2 < 0.001$ ,  $P = 0.97$ ). However regressions of  $\delta^{15}\text{N}$  demonstrated a significant positive relationship with low explanatory power ( $r^2 = 0.26$ ,  $P < 0.001$ ).

Median prey proportions estimated by SIAR from muscle tissue suggested organic material derived from fish formed the largest part of Rainy River walleye diets (59%; 95% credibility interval = 30% to 81%) (Figure 8.1). Median proportions for other prey organisms contributing to the diet of walleye included: mussels (19%, 95% CI = 4.4-29%), dragonflies (1.3%; 95% CI = 0-19%), and caddisflies (2.0%; 95% CI = 0-26%), with remaining invertebrate prey median values contributing less than 0.1% with 95% CI of 0 to <15% each.

The walleye standard ellipse area (Figure 8.2) computed from stable isotope data corrected for fractionation demonstrated a high degree of overlap with the feeding opportunity ellipses of Type 2 (moderate velocity boulder/cobble) habitats (~98%). The lowest habitat overlap for walleye was observed for Type 4 (vegetated backwaters) with Types 2 and 3 demonstrating moderate overlap (~60%). Centroid distances between the walleye standard ellipse and those defined by the feeding opportunity ellipses for each habitat varied little, with the closest habitat ellipse being that defined for Type 2 and the furthest being that defined for Type 1 (high velocity bedrock) (Table 8.1).

The standard ellipse area for walleye significantly overlapped with the SEAs for the Rainy River (83%) (Figure 8.3), but showed reduced overlap with Lake of the Woods (58%) and Little Fork River (41%). Centroid distance computations indicated similar results, with closer

proximity of the Rainy River and Lake of the Woods as compared to the Little Fork River (Table 8.2).

## 8.2 Logperch Stable Isotope Analysis

Within the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot vertical trophic structure logperch are located among the forage fishes and slightly negative compared to other forage fishes. Logperch demonstrated similar variation on the  $\delta^{13}\text{C}$  axis (range: -28.7 to -24.33‰; coefficient of variation: 3.9%) and  $\delta^{15}\text{N}$  axis was also higher (range: 5.5 to 7.7‰; coefficient of variation: 8.5%). There was no evidence of size-related changes in diets, with regressions of dorsal muscle stable isotope values against length yielding insignificant results for logperch ( $\delta^{13}\text{C}$ :  $r^2 < 0.001$ ,  $P=0.94$ ;  $\delta^{15}\text{N}$ :  $r^2=0.18$ ,  $P=0.13$ ).

Median prey proportions estimated by SIAR from muscle tissue suggested organic material derived from mussels formed the largest part of Rainy River logperch diets (24%; 95% credibility interval= 10% to 39%) (Figure 8.4). Median proportions for other prey organisms contributing to the diet of logperch included: caddisflies (18%, 95% CI=0-39%), dragonflies (15%; 95% CI= 0-31%), and crayfish (9.0%; 95% CI=0-26%), with remaining invertebrate prey median values contributing less than 1% with 95% CI of 0 to <11% each.

The logperch standard ellipse area (Figure 8.2) computed from stable isotope data corrected for fractionation demonstrated a complete overlap with all habitat types. While centroid distances for each habitat varied little, with the closest habitat ellipse being that defined for Type 2 and the furthest being that defined for Type 1 (high velocity bedrock) (Table 8.1).

### 8.3 Figures and Tables

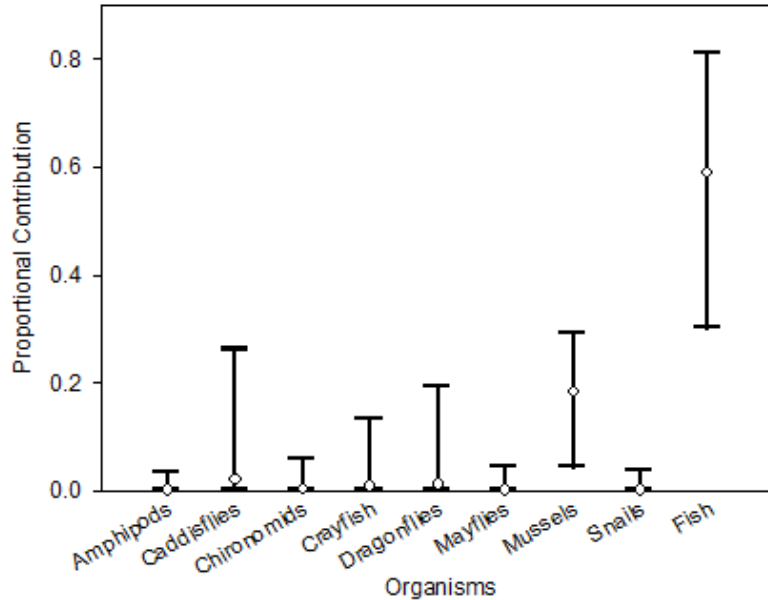


Figure 8.1 Proportion of prey contributing to the diet of walleye in the Rainy River as determined using Stable Isotope Analysis in R (SIAR). White circles and solid lines represent median and 95% confidence intervals (CI) respectively obtained using muscle stable isotope signatures for walleye.

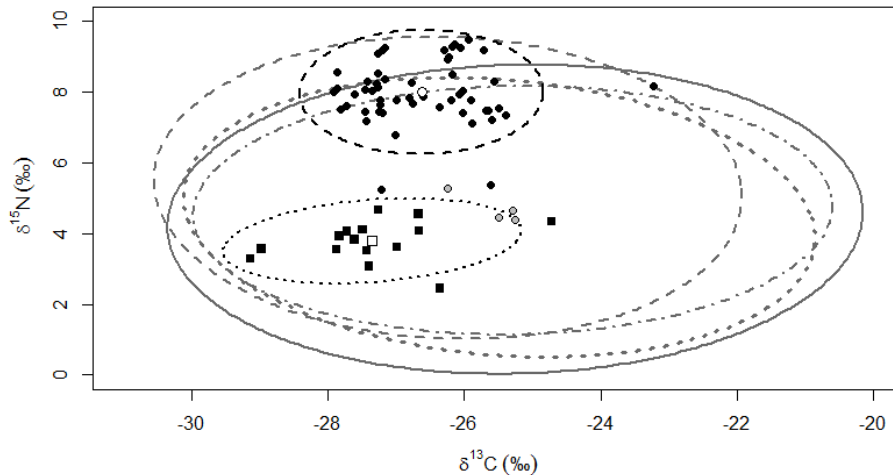


Figure 8.2 Standard ellipse areas for available prey items sampled in the four Rainy River habitat types: Type 1 (solid grey line) = high velocity bedrock, Type 2 (dashed grey line) = moderate velocity boulder/cobble, Type 3 (dotted grey line) = low velocity sand/gravel beds, and, Type 4 (dash-dot grey line) = vegetated backwaters. Centroids of the habitat ellipse are plotted as grey circles. Walleye muscle SEA is plotted as a dashed black line, with individuals shown as black circles and the corresponding centroids as a white circle. Logperch muscle SEA is plotted as a dotted black line, with individuals shown as black squares and the corresponding centroids as a white square.



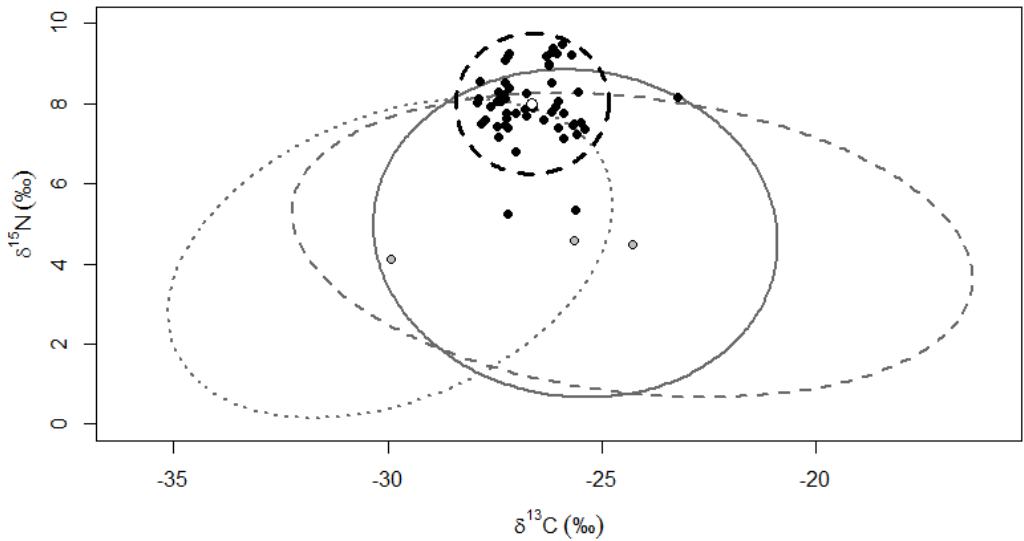


Figure 8.3 Community niche ellipses (grey lines) for all available prey items found in Rainy River (solid line), Lake of the Woods (dashed line) and Little Fork River (dotted line) with centroids for each ellipse plotted as a grey circle. Walleye muscle SEA is plotted as a dashed black line, with individuals shown as black circles and the corresponding centroids as a white circle.

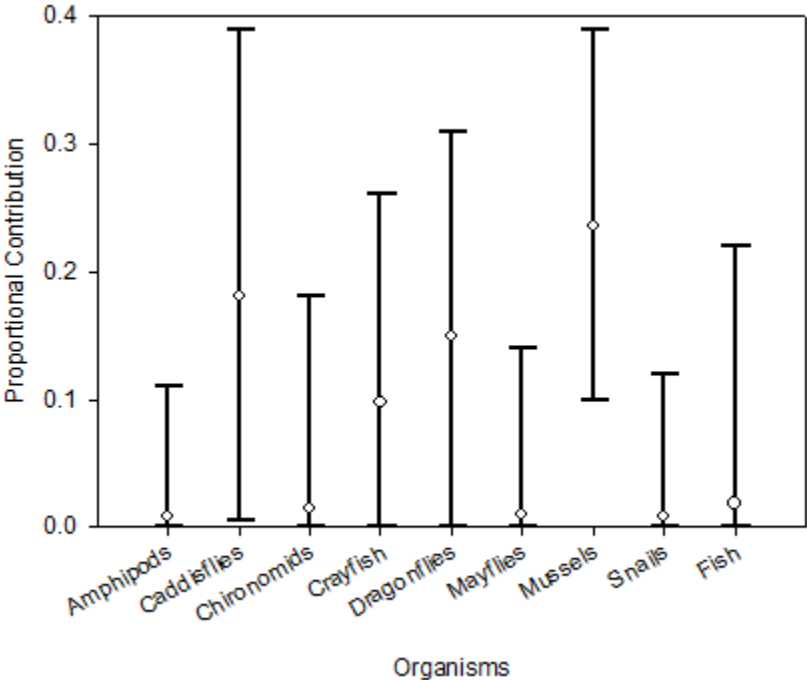


Figure 8.4 Proportion of prey contributing to the diet of logperch in the Rainy River as determined using Stable Isotope Analysis in R (SIAR). White circles and solid lines represent median and 95% confidence intervals (CI) respectively obtained using muscle stable isotope signatures for logperch.

Table 8.1 Ellipse overlap and centroid distances for standard ellipse areas determined from available prey items sampled in the four Rainy River habitat types: Type 1= high velocity bedrock, Type 2= moderate velocity boulder/cobble, Type 3= low velocity sand/gravel beds, and, Type 4= vegetated backwaters. Overlaps in proportionate terms are computed using each habitat type, respectively, in the denominator indicated by the row heading. Centroid distances define the distance between the standard ellipse area for each habitat type and the standard ellipse area for walleye and logperch computed from muscle tissue biopsy values corrected for fractionation.

Ellipse Overlap	Type 1	Type 2	Type 3	Type 4	Walleye	Logperch
Type 1	-	0.87	0.99	1.00	0.67	1
Type 2	0.72	-	0.85	0.87	0.98	1
Type 3	0.81	0.83	-	0.96	0.61	1
Type 4	0.74	0.78	0.87	-	0.47	1
Walleye	0.09	0.17	0.10	0.09	-	-
Logperch	0.11	0.14	0.14	0.15	-	-
Centroid Distances						
Type 1	-	-	-	-	3.83	2.18
Type 2	1.33	-	-	-	2.72	1.86
Type 3	0.24	1.11	-	-	3.69	1.98
Type 4	0.26	1.14	0.28	-	3.58	2.24

Table 8.2 Standard ellipse area overlap and centroid distances determined from available prey items sampled in the Rainy River, Lake of the Woods and Little Fork River. Overlaps in proportionate terms are computed using each location in the comparative pairing in the denominator indicated by the row heading.

Ellipse Overall	Rainy River	Lake of the Woods	Little Fork River	Walleye
Rainy River	-	0.62	0.46	0.83
Lake of the Woods	0.94	-	0.59	0.58
Little Fork River	0.46	0.39	-	0.41
Walleye	0.12	0.06	0.07	-
Centroid distances				
Rainy River	-	-	-	3.52
Lake of the Woods	1.36	-	-	3.60
Little Fork River	4.32	5.66	-	5.09