

# **Effects of the 2000 Rule Curves on Upper Rainy River Spawning Critical Habitats and Characterization of the Food Web**

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## **1.0 Introduction**

### **1.1 Scope of Work**

In 2000, the International Joint Commission (IJC) issued an Order prescribing a revised method of regulating the levels of the boundary waters of Rainy and Namakan lakes. The 2000 rule curves will be subject to review in 2015, which will consider monitoring information that may indicate the ecosystem effects of flow changes (IJC, 2001). Since 2001, a number of resource agencies have been conducting studies to evaluate effects of the 2000 rule curve change. The primary focus 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. In 2007, the IJC formed a Rule Curve Assessment Workgroup to develop a plan of study (POS) in which the Workgroup would prioritize the monitoring and analyses required to review the IJC Order in 2015. Specifically, the POS was written to identify priority studies and describe information/data that remained to be collected, identify what entities might collect the data and perform the studies, and provide an estimate for the cost to accomplish this work by 2015. The Plan of Study (POS) for the Evaluation of the International Joint Commission (IJC) 2000 Order for Rainy and Namakan Lakes and Rainy River was completed in 2009 (Kallemeyn et al., 2009).

The operators of the International Falls Dam (IFD) at Fort Frances, ON/International Falls, MN (the start of the Rainy River) 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. As such, the Canadian side of the IFD continues to practice hydro-peaking (i.e., fluctuating rates about the daily mean discharge). The American dam has not peaked since 2001 (O'Shea, 2005). 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).

To date, only limited monitoring has been conducted on the Rainy River (Kallemeyn et al., 2009), and one of the priority information gaps identified was to measure critical spawning and nursery habitats of key fish species and assess how they have been affected by the rule curve. 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 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.

## 1.2 Site Location and Study Reach

The Rainy River is located in northwestern Ontario and flows westward from Fort Frances, Ontario / International Falls, Minnesota to Lake of the Woods (LOW) over an approximate 145 kilometer length. The Rainy River is the largest tributary of LOW, contributing 70% of the annual flow and having a drainage area of approximately 70,000km<sup>2</sup>. The watershed can be divided into two portions. Upstream from the dam, the watershed is almost entirely on the Canadian Shield made up primarily of bedrock and lake basins. The secondary portion below the dam is an ancient lake bed dominated by heavy clays and occasional rock outcrop.

O'Shea (2005) identified biologically significant flows and the potential impacts of flow alterations in the lower Rainy River, focusing on Manitou and Long Sault rapids, which were identified as important fish spawning and food production areas. O'Shea (2005) used bathymetric and river stage vs discharge data to assess habitat conditions at different discharges for walleye, lake sturgeon, and log perch. The POS prioritized that a study similar to O'Shea (2005) was needed for the upper river where flows and levels are likely to be more responsive to releases from the IFD. Thus, the study reach described in this report is bound by IFD and the downstream confluence with the Little Fork River, constituting approximately 22km of river length (Figure1).

## 1.3 Target Species

The POS identified three key species: lake sturgeon (*Acipenser fulvascens*), 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 & Anderson, 2004).

The Lake of the Woods/Rainy River lake sturgeon population has demonstrated some recovery since its decline in the late 1890s and is still 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 to late June when water temperatures range from 8.5 to 18°C, although optimum temperatures range between 14 and 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 (Auer, 1996; Adams et al., 2006; COSEWIC, 2006).

Walleye make up the largest component of the Rainy River fishery. Payer (1987) tagged over 10,000 walleye in the LOW and found that a large portion of LOW walleye spawn in Rainy River. Similar to lake sturgeon, many walleye populations are known to migrate to rivers or tributary streams characterized by fast flowing water and gravel-coarse rock substrates to spawn (Geiling et al., 1996; DePhilip, 2005; Ivan, 2010). Spawning occurs in early spring (late April to late May) when temperatures range from 4 to 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.

## **1.4 Objectives**

Three main research topics on the Rainy River were identified to provide data for evaluation of the rule curves. The first objective was to characterize and catalogue 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 will be made with published literature on Lake of the Woods/Rainy River populations prior to the 2000 rule curve change.

Secondly, we sought to characterize the Rainy River food web using stable isotope analysis to provide a baseline for assessment of the health of the Rainy River food web and trophic structure. Specifically, we 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.

The third overall objective was to identify and characterize the critical spawning habitat of the target species. Specifically, we 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.

## **2.0 Background**

### **2.1 Population Condition**

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 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-hun, 1978; King, 1995; Kaufman et al., 2007), and calorific analysis (Hails, 1983; Booth & 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; Frosse, 2006; Kaufman et al., 2007; De Robertis & 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 & Nate, 2005), stage of development or sex (Bruch et al., 2011), latitude and longitude of the population (Power & 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 aging is required (Casselman, 1987). Fisheries scientists have developed several methods for aging 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, 1958; de Veen, 1970; Schimt & Skud, 1978).

## **2.2 Stable Isotope Analysis**

Stable isotopes (SI) have been increasingly used in environmental research as tracers and for cycling processes (Peterson & Fry, 1987). Several elements, including carbon, nitrogen, sulfur, hydrogen, and oxygen have been used for a variety of applications, including geochemistry and ecology (Peterson & Fry, 1987). 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. Stable carbon isotopes can provide insights into the ecosystem portions (e.g., benthic, pelagic) from which consumers obtain their energy (Peterson & Fry, 1987) and typically display low fractionation between prey and consumer (0-1‰). While stable nitrogen isotopes become enriched with each trophic transfer (typically 3.4‰, but can vary from 1.4 to 5.4‰) and provide a continuous measure of consumer trophic position (DeNiro and Epstein, 1978) that may vary with size or age (e.g., Fry, 2006)

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 & 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., 2009), which is vital to environmental programmes assessing the scale of anthropogenic impacts on fish populations (e.g., Galloway et al., 2003; Gray & 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 or derived from outside the water vs autochthonous or derived from within the water) have been shown to be affected by local environmental conditions, such as substrate types, hydrologic condition, and seasonal variation (Leonard et al., 1998; Lefebvre et al., 2009; Goll  ty et al., 2010; Colombo et al., 2012). 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).

Predicting how assemblages will respond to perturbations from a food web perspective will benefit from a method like SIA that quantifies the identities, magnitudes, and strengths of trophic interactions across space and time (Polis & Strong, 1996; Vander Zanden et al., 2006). Conservation efforts are often focused on a target species rather than whole ecosystems because the species are threatened or endangered, have commercial value, or are strong interaction species in food webs (Paine, 1980; Power et al., 1996). However, because populations of any species depend critically on their resources and trophic interactions that respond to environmental change, a community or food web perspective may be more effective (Power et al., 1996).

### **2.3 Critical Spawning Habitat**

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*), one of the most sought-after sportfish and commercially valuable harvest species in the United States and Canada (Scott and Crossman, 1979; 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 & 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), while 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 et al., 2008).

Substrate, water velocities, depth, and temperatures have been identified as important in the spawning success for each species (Scott & Crossman, 1979; Werner, 1980; Smith, 1985). Walleye spawn in the spring (April-June) just after ice-out at temperatures ranging between 4.0<sup>o</sup> and 11.1<sup>o</sup>C in lakes, rivers, and small tributaries (Scott & Crossman, 1979; Kerr et al., 1997; Lyttle et al., 2008). Lake sturgeon also spawn in the spring but at slightly warmer temperatures, between 6.6<sup>o</sup> and 16<sup>o</sup>C (Scott & Crossman, 1979; Bruch & Binkowski, 2002; Lyttle et al., 2008). The spawning substrate of walleye includes coarse gravel mixed with cobble, gravel, and sometimes sand in streams and shoals of lakes with good water flow (Scott & Crossman, 1979; Werner, 1980; Smith, 1985). Walleye generally spawn in water between 0.3 and 1.5 m deep (Sternberg, 1986). Lake sturgeon spawn in depths greater than 0.5m and have been reported as deep as 15m (Scott & Crossman, 1979; Kerr et al., 2010) over a mixture of coarse substrates, including bedrock, boulders, and cobbles (Seyler, 1997; Bruch & Binkowski, 2002). Both walleye and lake sturgeon spawn in moderate velocities between 0.4 and 1.0 m/s.

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 et al., 2008). Therefore, use of benthic spawning habitat by both species for the egg incubation, hatching and larval stages can last up to 30 days (Randal et al., 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).

## **3.0 Methods**

### **3.1 Population Condition**

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 greatest 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 demonstrates reliable sexual dimorphism (Craig et al., 2005). Logperch do not demonstrate reliable sexual dimorphism, and sex was determined via dissection (Winn, 1958). For aging purposes, the first dorsal fin ray (Overman & 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). 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  $t$ . Estimated parameter  $L_{MAX}$  is the maximum length,  $K$  is the growth coefficient defining how fast the maximum length is reached (growth rate), and  $t_0$  is theoretical length at age zero. Model parameters were estimated using non-linear methods (Bates & Watts, 1988) using the Levenberg-Marquardt algorithm in Statistica, version 8 (Statsoft Inc., Tulsa, OK). Where data on ages  $<5$  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 & Watts, 1988).

### 3.2 Stable Isotope Analysis

Sampling for stable isotopes tissue involved taking muscle plugs from walleye and sturgeon, as well as fin clips from sturgeon. Lake sturgeon 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 for sturgeon and in the dorsal-lateral region of walleye to obtain at least 1mg of muscle tissue (Tyus et al., 1999). The resulting wounds were sealed using 3M Vetbond Tissue Adhesive™. Samples of dorsal-lateral muscle were collected from logperch when returned to the lab. All samples were frozen 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, before freezing for SIA, from freshly captured individuals, including length (cm), weight (kg), and sex. 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 & Rasmussen 1999). When necessary, multiple individuals per family per site were pooled to obtain sufficient material for stable isotope analysis (Stelzer, 2008). Examples of common terrestrial, aquatic vegetation, and filamentous algae were collected by hand. Periphyton was sampled by scraping rocks and backwashing onto a filter when necessary. Three 1-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 to 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). Machine analytical precision was  $\pm 0.1\%$  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  $\text{CH}_6$  for carbon and  $\text{N}^1$  and  $\text{N}^2$  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 & 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 & 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 known to have significant effect on the metabolic enrichment of  $\delta^{15}\text{N}$  during tissue catabolism (Vanderklift & 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= $42\text{mm} \pm 15\text{mm}$ ) grouped as a single mean value. While no eggs were collected for SIA, we considered them to be potentially consumed by lake sturgeon (Scott & Crossman, 1975). 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), where the signatures of adult smallmouth bass (*Micropterus dolomieu*) ( $\delta^{13}\text{C}=-26.0\%$ ;  $\delta^{15}\text{N}=7.0\%$ ) were not significantly different from those of the embryos ( $\delta^{13}\text{C}=-25.0\%$ ;  $\delta^{15}\text{N}=6.3\%$ ). Priors for prey percent

consumption were incorporated into the SIAR by taking the average reported value from several lake sturgeon stomach content analyses (Harkness & Dymond, 1961; Cuerrier, 1966; Hay-Chmielewski, 1987; Stelzer, 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 was assigned to grouped forage fish (1.85%) and fish eggs (1.85%) in a 1:1 ratio. Prior values were not included in the models for walleye or logperch.

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/gravel 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 describe the variation of any defined group within isotope space adequately, 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). In other words, calculating the SEA for our target species was used to compare available prey from different habitat types and different potential geographic sources to help identify where they are obtaining the bulk of their food sources.

### 3.3 Critical Spawning Habitat

All sampling was completed during the peak spawning times for the respective target species in both 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 by boat electrofishing at night or with 25.4 and 30.5 cm mesh gill nets, for lake sturgeon only, set overnight. 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). Congregations of ripe individuals were identified as indicative of spawning. To verify successful spawning had occurred, egg mats were deployed immediately following fish capture and removed after approximately 24hrs for examination. Based on fish captures, a total of 218 egg mats were set throughout the study reach over the two field seasons. Two independent spawning areas were identified for lake sturgeon through 31 positive egg mats at XS 1-3 and XS 4-6. While three independent spawning areas were identified for walleye through 27 positive egg mats. Walleye spawning was seen at cross-sections 1-3, 15-17, and 18-19. Walleye and lake sturgeon spawning were generally spatially separated, with only a single location overlap. Larval drift nets were set at downstream locations 5 to 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 500m intervals throughout the study reach with increased spatial resolution in areas of river substrate morphological heterogeneity (Figure 1). ADCP transects were obtained at 7 – 10 discrete discharges over the range of seasonal flows (100 – 900 m<sup>3</sup>/s) at each cross-section using the moving-vessel method (Muste et. al., 2004). Elevation data through 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 three cross-section (XS) locations (XS 7, XS 18, and XS 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 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 three cross sections), as well as at locations where significant variation in substrate characteristics was noted. Sample classification was adapted from the Unified Soil Classification System as outlined in Das (2005) where: fines/sand diameter < 2 mm, gravel diameter 2 – 64 mm, cobbles/boulders diameter > 64 mm. Substrate zones were classified based on Table 1 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 2012. For simplification of analysis, substrate types were grouped according to the dominant substrate type: Coarse (B, B-CB, CB), Gravels (G, G-FS, G-CB), and Sands (FS, FS-G, FS-CB).

Bathymetry, sediment maps, velocity, and depth profiles were combined with egg mat locations to identify spawning habitat characteristics. Areas where deployed egg mats contained eggs were identified as spawning areas. Spawning areas were considered to be discrete if they were found in different 500m river length segments; the discrete segments are named by the hydraulic cross-sections that bound the areas. Substrate distributions, based on water surface elevations, were compared using a Chi-square test for independence (Zar, 2010). Velocity and depths were examined for the range of WSEs seen throughout the spawning periods (from first egg deposition to confirmation of larval drift). Finally, Manly's forage preference indices (Manly et al., 1993) were determined for all substrate types found within spawning areas to determine the substrate preferences of lake sturgeon and walleye.

Habitat preference indices were computed following Manly et al. (1993) using substrate simplified distribution data to describe habitat availability and egg mat location data within the defined habitat types to define habitat usage. To test for selection preferences among substrate types, area estimates were converted to proportions and compared to proportionate use values as determined from egg mat counts through the computation of standardized selection indices ( $B_i$ ) (Manly et al., 1993), where  $B_i = W_i (\sum_{i=1}^n W_i)^{-1}$  and  $W_i = o_i P_i^{-1}$  and  $W_i$  is the selection ratio for the  $i^{\text{th}}$  habitat type,  $n$  is the number of habitat types and  $P_i$  and  $o_i$ , express the proportional availability of the habitat type and its use, respectively. Selection ratios may vary from 0 to 1, but when expressed as standardized values will sum to 1. Standardized ratios >  $n^{-1}$  indicate relative preference (Manly et al., 1993). Selection ratios were tested for statistical significance using the  $X^2$  based statistic described in Manly et al. (1993) under the null hypothesis that egg mat placement in available substrates occurred at random.



## 4.0 Results and Discussion

### 4.1 Lake Sturgeon

#### 4.1.1 Population Condition

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 although some males were identifiable through release of gametes at time of capture. 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 1.

WLRs explained 90% of the variation in the overall data (Figure 2A). When compared to an assumed slope of 3, lake sturgeon demonstrated allometric growth ( $df=1$ ,  $t=6.83$ ,  $P<0.050$ ) (Table 1). 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. Lake sturgeon evidenced a significant but weak positive relationship between relative condition and age ( $F_{1, 261}=6.0$ ,  $r^2=0.02$ ,  $P=0.020$ , Figure 3) 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  $F_{3, 342}=1018.8$ ,  $r^2=0.83$ ,  $P=0.770$ ); therefore, data from both years were used in the plots presented in Figure 2A. A significant difference was observed between the slope of the overall equation and that for males ( $F_{3, 342}=1038.9$ ,  $r^2=0.90$ ,  $P=0.005$ ).

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 & McKinley, 1997). The WLR (slope= 3.38; 95% CI (3.50, 3.26)) 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 & Isely, 2011). A previous study of the 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 less positive

slope from the overall equation (2.68; 95% CI (3.03, 2.34)), but no significant difference was seen in the condition of males. 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.

Table 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 to 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 to 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 4. For lake sturgeon, the 2012 Rainy River model differed significantly from the 2002 Rainy Lake 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 were not available.

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 & McKinley, 1997; Adams et al., 2006; Trested & 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.

#### **4.1.2 Stable Isotopes**

A total of 72 lake sturgeon were captured for use in stable isotope analysis. Of those, 55 were confidently aged. Lake sturgeon ranged in size from 42.5 to 168.5cm and in age from three to 33 years. 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 6) demonstrated vertical trophic structure consisting of four trophic levels (primary producers, primary consumers, forage, and

piscivorous fishes) with lake sturgeon occupying an upper level trophic position. The bi-plot showed a range in standard deviation from a low of -29.8‰ for mussels to a high of -20.9‰ for amphipods along the  $\delta^{13}\text{C}$  axis 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.

Reported 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 against length yielded insignificant results for  $\delta^{15}\text{N}$  ( $r^2 < 0.001$ ,  $P=0.89$ ); however,  $\delta^{13}\text{C}$  demonstrated a significant positive relationship with low explanatory power ( $r^2=0.15$ ,  $P=0.001$ ).

Median prey proportions estimated by SIAR from lipid-corrected fin tissue suggested organic material derived from fish (either forage fish or fish eggs) formed the largest part of Rainy River lake sturgeon diets (46%; 95% credibility interval= 38% to 54%) (Figure 7). 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  $F_{3, 79}=0.47$ ,  $P=0.70$ ) or  $\delta^{15}\text{N}$  (ANOVA  $F_{3, 79}=1.16$ ,  $P=0.33$ ), 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 8). 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 closest 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 3).

When the standard ellipse analysis was repeated at larger spatial scales using water bodies instead of habitats, large overlaps were similarly found (Figure 9). 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). In contrast, the Rainy River showed much less overlap with the 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.

#### **4.1.3 Critical Spawning Habitat**

The vast majority of the study reach was characterized by substrates with fine sands with gravel or another substrate variant involving fines, sands, and gravels. Sporadic outcroppings of bedrock with cobbles or boulders were evident, specifically in the upper 7 km of the study reach where all spawning sites were located.

Lake sturgeon spawning took place at temperatures ranging from 8.5 to 16.5 °C, with eggs found from 10 May to 28 May 2012 and 25 May to 18 June 2013 (Figure 14). The first instances of lake sturgeon drift were seen 6 June 2012 and 18 June 2013, with eggs remaining at the spawning site for approximately 27 days in 2012 and 24 days in 2013.

Spawning adults, eggs, or larvae were present in the spawning area through a wide range of water surface elevations (WSE). Over the two spawning seasons, lake sturgeon experienced WSEs from 327.3m to 330.8m. Distributions of depths and velocities based on temporally integrated profiles were taken over the relevant spawning season at each site. ANOVA indicated significant differences between water surface elevations in all cases (all p-values <0.001). While the expected hydrological relationship predicting positive correlation WSE and mean depth and mean velocity generally prevailed (all  $R^2 > 0.65$ ;  $P > 0.03$ ), notable exceptions were found for depth at XS 4-6 ( $r^2 = 0.05$ ,  $P = 0.90$ ) and for velocity at XS\_18-19 ( $R^2 = 0.001$ ,  $P = 0.1$ ). This means that as WSE increases, generally, depth and velocity also increase; deviations from the expected relationships could indicate areas with unique local morphological conditions and/or hydraulic routing.

WSE does not result in significant changes to the relative proportions of each substrate type available, likely resulting from the relatively high proportion of fine sands with gravel substrate throughout the study reach. Comparison of low (327m), moderate (329m) and high (331m) water surface elevations for differences in substrate distribution relative to WSE indicated no significant differences for

any of the spawning sites (XS 1-3:  $df=7$ ,  $X^2=8.83$ ,  $p\text{-value}=0.16$ ; XS 4-6:  $df=9$ ,  $X^2=13.6$ ,  $p\text{-value}=0.25$ ; XS\_15-17:  $df=8$ ,  $X^2=14.4$ ,  $p\text{-value}=0.43$ ; XS18-19:  $df=4$ ,  $X^2=12.1$ ,  $p\text{-value}=0.85$ ) (Table 5).

When spawning substrate preferences were examined using Manly's preference indices, it was found that lake sturgeon prefer coarse substrates (Table 6); coarse substrates ranged from bedrock to cobbles. No significant differences were observed for selection preferences at different WSEs. For sites where preference values were obtained for multiple substrate types (XS 1-3), significant differences in selection preference were found between coarse substrates and gravel ( $X^2$ : XS 1-3,  $df=2$ ,  $P < 0.005$ ) and sand ( $X^2$ : XS 1-3,  $df=2$ ,  $P < 0.005$ ). However, no significant differences were found between gravel and sand substrate ( $X^2$ : XS 1-3,  $df=2$ ,  $P = 0.71$ ).

Although the proportions of substrates available did not change as WSE increased, the total quantity of the preferred substrate type did. Non-linear regression models were estimated for relationships between preferred substrate and WSE. A significant model for lake sturgeon ( $\text{Area} = 9000 - 35477.61 \times e^{-0.79 \times \text{WSE}}$ ;  $p=0.03$ ; Figure 17) demonstrated high explanatory power at 96%. Lake sturgeon preferred spawning habitat availability increased from 4 600m<sup>2</sup> to 13 542m<sup>2</sup> when water surface elevation is raised from 327m to 331m.

Variations in flow have been shown to affect spawning lake sturgeon populations directly. 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. Our results suggest that spawning ground densities may inhibit reproductive success. Data from a 2014 mark-recapture fishing effort estimated a Rainy River/Lake of the Woods population of 92,286 mature size (>100 cm) lake sturgeon evenly split between males and females (Heinrich MDNR, 2014). With a spawning periodicity of 3-5 years, between approximately 9,229 and 15,381 females will potentially spawn in any given year. If each female requires between 13 and 48m<sup>2</sup> of spawning habitat (e.g., Fortin et al. 2002), the data imply a spawning habitat requirement between approximately 119,971 and 738,288 m<sup>2</sup>. Although the habitat available in the upper Rainy River does not provide enough preferred habitat to maintain total population numbers, based on the large number of spawning adults captured during our sampling efforts, the area does make an important contribution to the total supply of spawning habitat. Supplementary spawning habitats have been previously identified at Long Sault rapids, Manitou rapids, and Little Fork River.

## 4.2 Walleye

### 4.2.1 Population Condition

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, therefore, we relied on the release of gametes at time of capture. Mean and standard error of biologically relevant variables are presented in Table 1. Sex-specific comparisons were made for walleye, with significant differences 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 ( $F_{1, 235}=146.7$ ,  $r^2=0.18$ ) (all tests:  $P<0.001$ ). While female walleye were found to be generally larger, they were also significantly older. The significant differences identified between males and females, are consistent with evidence that walleye growth is sexually dimorphic (Sass & Kitchell, 2005).

The overall WLR explained 97% of the variation in the data (Figure 2B). When compared to an assumed slope of 3, walleye demonstrate allometric growth ( $df=1$ ,  $t=6.38$ ,  $P<0.001$ , Table 1). 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  $F_{3,417}=5314.1$ ,  $r^2=0.97$ ,  $P=0.220$ ); therefore, data from both years were used in the plots presented in Figure 2B. Using ANCOVA analysis, the regression for males and females were shown to be significantly different in slope ( $F_{3,240}=610.2$ ,  $r^2=0.88$ ,  $P=0.280$ ). Results were, therefore, consistent with evidence that walleye growth is sexually dimorphic (Sass and Kitchell 2005).

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.

Table 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 was significant and 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 5 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 ( $F_{6,20}=1.27$ ,  $P=0.330$ ).

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.

#### 4.2.2 Stable Isotopes

Adult walleye are located at the apex position of top predator as shown by the  $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$  bi-plot of vertical trophic structure, (Figure 6). 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 ( $r^2=0.26$ ,  $P < 0.001$ ). Walleye processed for stable isotope analysis ranged in length from 7 to 67cm, with a coefficient of variation of 33%. 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%).

Median prey proportions estimated by SIAR from muscle tissue suggested that organic material derived from fish formed the largest part of Rainy River walleye diets (59%; 95% credibility interval= 30% to 81%) (Figure 10). 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 11) 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 3).

The standard ellipse area for walleye significantly overlapped with the SEAs for the Rainy River (83%) (Figure 12) 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 4).

#### **4.2.3 Critical Spawning Habitat**

Walleye spawning took place at temperatures ranging from 2 to 9.7 °C (Figure 14), with eggs found from 11 April to 8 May 2012, and 3 May to 26 May 2013. The first instances of walleye drift was seen 1 May 2012 and 11 June 2013, with eggs remaining at the spawning site for approximately 20 days in 2012 and 16 days in 2013.

When spawning substrate preferences are examined using Manly's preference indices, it was found that walleye prefer coarse substrates (Table 7); coarse substrates ranged from bedrock to cobbles. No significant differences were observed for selection preferences at different WSEs. For sites where preference values were obtained for multiple substrate types (XS 1-3 and XS 15-17), significant differences in selection preference were found between coarse substrates and gravel ( $X^2$ : XS 1-3,  $df=2$ ,  $P < 0.005$ ; XS 15-17,  $df=2$ ,  $P < 0.005$ ) and sand ( $X^2$ : XS 1-3,  $df=2$ ,  $P < 0.005$ ; XS 15-17,  $df=2$ ,  $P < 0.005$ ). However, no significant differences were found between gravel and sand substrate ( $X^2$ : XS 1-3,  $df=2$ ,  $P = 0.71$ ; XS 15-17,  $df=2$ ,  $P = 0.67$ ).

Although the proportions of substrates available did not change as WSE increased, the total quantity of the preferred substrate type did. Non-linear regression models were estimated for relationships between preferred substrate and WSE. A significant model for walleye ( $\text{Area} = 16000 - 35477.61 \times e^{-0.79 \times \text{WSE}}$ ;  $p < 0.001$ ; Figure 18) demonstrated high explanatory power at 99%. Preferred habitat availability



for walleye increased from approximately 11 453m<sup>2</sup> to 26 629 m<sup>2</sup> when water surface elevation is raised from 327m to 331m.

Estimates of Lake of the Woods/Rainy River walleye population and area requirements for females have not been estimated to date; however, 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 our upper Rainy River study reach only, 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 and 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 upper Rainy River.

## **4.3 Logperch**

### **4.3.1 Population Condition**

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 1.

WLRs for logperch explained >90% of the variation in the data (Figure 2C). When compared to an assumed slope of 3, logperch demonstrated isometric growth (df=1, t=1.64, *P*=0.150, Table 1). 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.

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 & Rudstam, 1999) or species interaction studies (e.g., Balshine et al., 2005; Bergstrom & 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 that the use of the WLR for blackside darter (*Percina maculata*) could be applied to logperch. The relationship for blackside darter ( $\log_{10}(W) = -5.49 +$

$3.24 \cdot \log_{10}(L)$ ) was developed for fish in Illinois waters; while both species demonstrate similar slopes, the intercepts of the regressions are 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.

### **4.3.2 Stable Isotopes**

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 11). 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 13) 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 3).

## **5.0 Conclusions**

### **5.1 Population Condition**

This study reported the weight-length relationship for walleye, lake sturgeon, and logperch from the Rainy River system captured from April to June 2012 and 2013. From the WLRs, a relative condition factor equation was developed. Weight models and condition indices for Rainy River target species are near the upper limits of values reported previously for these species. Growth models indicate no

significant changes in growth for walleye since the late 1990s in the Rainy River/Lake of the Woods, while lake sturgeon population exhibit faster growth than 2002 Rainy Lake populations. However, in addition to the stated limitations of body condition as a measure of fish health commonly found in the literature, our samples were taken solely during spawning season and, thus, likely include predominantly reproductively developed individuals.

## 5.2 Stable Isotope Analysis

The Rainy River supports a large healthy food web with typical vertical trophic structure consisting of four trophic levels (primary producers, primary consumers, forage, and piscivorous fishes), with adult walleye sitting at the apex position and a great deal of overlap and redundancy within the system. 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, meaning that a shift in basal resource use occurs as lake sturgeon grow larger. 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 the remaining 50% 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 that 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.

Walleye stable isotope data showed moderate intra-specific variability and a significant size-related shift in  $\delta^{15}\text{N}$ , indicating higher trophic level feeding at greater sizes (i.e., they consumed more fish as they got bigger). Within the food web, walleye occupy the apex trophic position dependent on their high (60%) proportionate assimilation of fish protein, with invertebrates contributing approximately 40%. At the meso-scale within the Rainy River, there was some evidence to suggest that walleye were preferentially connected to habitat-specific food webs, with boulder/cobble habitats being preferred over highly vegetated areas. At the macro scale, Rainy walleye do appear to rely more heavily on the food web of the Rainy River than on Lake of the Woods or available tributary systems. Within the food web, logperch was found among the forage fish, and stable isotope data showed moderate intra-specific variability, with no size related changes in diets. Logperch were found to feed on a variety of macro-invertebrates, including mussels, caddisflies, dragonflies, and mayflies, with mussels contributing the greatest proportion (24%).

### 5.3 Critical Spawning Habitat

Walleye and lake sturgeon spawning on the Rainy River were generally spatially separated, with only a single location overlap at the site immediately below the International Falls Dam. Three independent sites were identified for walleye, while only two sites were identified for lake sturgeon. Spawning occurred at commonly reported temperatures (walleye 2-10°C and lake sturgeon 8-16°C), and sites were generally characterized by coarse substrates (bedrock, boulders, or cobbles). Spawning adults, eggs, or larvae of both species were present in the spawning area 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 studies on spawning preferences for each species (Figures 15 and 16).

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 smaller (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 & 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 results in a lower rate of increase. 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 to 329m are 6% and 11%, respectively, for walleye and lake sturgeon. Therefore, as WSE is increased, the rate of increase of available habitat is reduced, although the incremental additions could still be of value. Moreover, we feel it is important that the WSE provided during spawning be maintained within a narrow range throughout the incubation period to avoid desiccation or flushing of eggs and larvae. Although the habitat available in the upper Rainy River does not provide enough preferred habitat to maintain total system population numbers, we feel that it provides a vital contribution to the total supply and thus

towards maintaining healthy populations. Supplementary spawning habitats have been previously identified at Long Sault rapids, Manitou rapids, and Little Fork River.

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## 7.0 Tables

Table 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 are reported.

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

\*p-value <0.001

Table 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<br>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<br>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<br>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<br>Sturgeon | Rainy River<br>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<br>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<br>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  $TL = 1.06 FL + 38.9$

Table 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, walleye and logperch computed from muscle tissue biopsy values corrected for fractionation.

| Ellipse Overlap    | Type 1 | Type 2 | Type 3 | Type 4 | Lake sturgeon | Walleye | Logperch |
|--------------------|--------|--------|--------|--------|---------------|---------|----------|
| Type 1             | -      | 0.87   | 0.99   | 1.00   | 0.95          | 0.67    | 1        |
| Type 2             | 0.72   | -      | 0.85   | 0.87   | 0.99          | 0.98    | 1        |
| Type 3             | 0.81   | 0.83   | -      | 0.96   | 0.81          | 0.61    | 1        |
| Type 4             | 0.74   | 0.78   | 0.87   | -      | 0.77          | 0.47    | 1        |
| Lake sturgeon      | 0.14   | 0.18   | 0.15   | 0.15   | -             |         |          |
| Walleye            | 0.09   | 0.17   | 0.10   | 0.09   | -             | -       | -        |
| Logperch           | 0.11   | 0.14   | 0.14   | 0.15   | -             | -       | -        |
| Centroid Distances |        |        |        |        |               |         |          |
| Type 1             | -      | -      | -      | -      | 2.97          | 3.83    | 2.18     |
| Type 2             | 1.33   | -      | -      | -      | 2.14          | 2.72    | 1.86     |
| Type 3             | 0.24   | 1.11   | -      | -      | 2.89          | 3.69    | 1.98     |
| Type 4             | 0.26   | 1.14   | 0.28   | -      | 2.71          | 3.58    | 2.24     |

Table 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 | Walleye |
|--------------------|-------------|-------------------|-------------------|---------------|---------|
| Rainy River        | -           | 0.62              | 0.46              | 0.94          | 0.83    |
| Lake of the Woods  | 0.94        | -                 | 0.59              | 0.81          | 0.58    |
| Little Fork River  | 0.46        | 0.39              | -                 | 0.47          | 0.41    |
| Lake sturgeon      | 0.16        | 0.09              | 0.08              | -             | -       |
| Walleye            | 0.12        | 0.06              | 0.07              | -             | -       |
| Centroid distances |             |                   |                   |               |         |
| Rainy River        | -           | -                 | -                 | 2.78          | 3.52    |
| Lake of the Woods  | 1.36        | -                 | -                 | 3.39          | 3.60    |
| Little Fork River  | 4.32        | 5.66              | -                 | 5.41          | 5.09    |

Table 5. Comparison of low (327m), moderate (329m), and high (331m) water surface elevations for differences in substrate distribution relative to WSE. Substrate types include B= Bedrock, CB=cobbles, G=gravels, FS=fine sand, and V=vegetation. Significance level at  $\alpha=0.05$

| Site     | Substrate type | Water Surface Elevation (masl) |      |      |
|----------|----------------|--------------------------------|------|------|
|          |                | 327                            | 329  | 331  |
| 1-3      | B              | 1.8                            | 2.6  | 2.6  |
|          | CB             | 0.7                            | 2.3  | 2.4  |
|          | FS             | 0.1                            | 0.5  | 0.8  |
|          | FS-CB          | 0.2                            | 0.6  | 1.0  |
|          | FS-G           | 77.7                           | 81.2 | 82.2 |
|          | G-CB           | 0                              | 0.2  | 0.2  |
|          | G-FS           | 2.9                            | 5.4  | 5.4  |
|          | V              | 0.1                            | 1.3  | 5.4  |
| $\chi^2$ | 8.83           |                                |      |      |
| p-value  | 0.16           |                                |      |      |
| 4-6      | B              | 0.6                            | 1.2  | 1.8  |
|          | B-CB           | 0                              | 1.5  | 2.2  |
|          | CB             | 0                              | 0.7  | 0.7  |
|          | FS             | 1.9                            | 3.2  | 3.7  |
|          | FS-CB          | 0                              | 0.3  | 0.3  |
|          | FS-G           | 56.1                           | 62.4 | 63.7 |
|          | G-CB           | 2.9                            | 2.9  | 2.9  |
|          | G-FS           | 9.9                            | 16.4 | 18.6 |
|          | T              | 0                              | 0.3  | 4.3  |
|          | V              | 0                              | 0    | 1.9  |
| $\chi^2$ | 13.6           |                                |      |      |
| p-value  | 0.25           |                                |      |      |
| 15-17    | B              | 0.1                            | 2.6  | 3.6  |
|          | B-CB           | 0.5                            | 1.4  | 2.1  |
|          | CB             | 0                              | 1.5  | 1.6  |
|          | FS             | 0.2                            | 2.0  | 3.1  |
|          | FS-CB          | 0                              | 0.2  | 0.3  |
|          | FS-G           | 70.6                           | 74.3 | 75.4 |
|          | G-CB           | 0.2                            | 0.4  | 0.4  |
|          | G-FS           | 3.5                            | 6.4  | 6.4  |
|          | V              | 0.1                            | 2.5  | 7.1  |
| $\chi^2$ | 14.4           |                                |      |      |
| p-value  | 0.43           |                                |      |      |
| 18-19    | B              | 3.9                            | 6.7  | 6.7  |
|          | FS             | 12.0                           | 14.0 | 14.0 |
|          | FS-G           | 62.3                           | 67.3 | 68.0 |
|          | G-FS           | 0                              | 1.1  | 2.7  |
|          | V              | 0.1                            | 2.4  | 8.6  |
| $\chi^2$ | 12.1           |                                |      |      |

Table 6. Lake sturgeon spawning sites, substrate areas, and positive egg mats found at each substrate type. Substrate preferences ( $B_i$ ) were determined at a range of water surface elevations (WSEs), substrates were considered preferred (\*) when  $B_i > n^{-1}$ , where  $n^{-1}=0.33$ .

| Site   | Substrate | # of egg mats | 327                    |       | 329                    |       | 331                    |       |
|--------|-----------|---------------|------------------------|-------|------------------------|-------|------------------------|-------|
|        |           |               | Area (m <sup>2</sup> ) | $B_i$ | Area (m <sup>2</sup> ) | $B_i$ | Area (m <sup>2</sup> ) | $B_i$ |
| XS 1-3 | Coarse    | 19            | 3869.80                | 0.91* | 7325.39                | 0.91* | 7537.95                | 0.90* |
|        | Gravels   | 2             | 4449.43                | 0.08  | 8423.59                | 0.08  | 8508.24                | 0.08  |
|        | Sands     | 4             | 117968.02              | 0.01  | 124537.02              | 0.01  | 127093.45              | 0.01  |
| XS 4-6 | Coarse    | 2             | 731.12                 | 1*    | 4366.42                | 1*    | 6004.42                | 1*    |
|        | Gravels   | 0             | 16483.90               | 0     | 24884.89               | 0     | 27617.58               | 0     |
|        | Sands     | 0             | 74670.66               | 0     | 84766.32               | 0     | 86995.59               | 0     |

Table 7. Walleye spawning sites, substrate areas, and positive egg mats found at each substrate type. Substrate preferences ( $B_i$ ) were determined at a range of water surface elevations (WSEs), substrates were considered preferred (\*) when  $B_i > n^{-1}$ , where  $n^{-1}=0.33$ .

| Site     | Substrate | # of egg mats | 327                    |       | 329                    |       | 331                    |       |
|----------|-----------|---------------|------------------------|-------|------------------------|-------|------------------------|-------|
|          |           |               | Area (m <sup>2</sup> ) | $B_i$ | Area (m <sup>2</sup> ) | $B_i$ | Area (m <sup>2</sup> ) | $B_i$ |
| XS 1-3   | Coarse    | 6             | 3869.80                | 0.77* | 7325.39                | 0.77* | 7537.95                | 0.77* |
|          | Gravels   | 2             | 4449.43                | 0.22  | 8423.59                | 0.22  | 8508.24                | 0.23  |
|          | Sands     | 1             | 117968.02              | 0     | 124537.02              | 0     | 127093.45              | 0.01  |
| XS 15-17 | Coarse    | 18            | 590.57                 | 1     | 5377.55                | 0.99  | 7030.91                | 0.99  |
|          | Gravels   | 0             | 3567.52                | 0     | 6606.53                | 0.00  | 6606.53                | 0.00  |
|          | Sands     | 2             | 68664.51               | 0     | 74200.91               | 0.01  | 76408.49               | 0.01  |
| XS 18-19 | Coarse    | 2             | 6992.73                | 1*    | 12060.50               | 1*    | 12060.50               | 1*    |
|          | Gravels   | 0             | 0                      | 0     | 2012.71                | 0     | 4952.87                | 0     |
|          | Sands     | 0             | 134186.85              | 0     | 146866.74              | 0     | 148084.73              | 0     |



## 8.0 Figures

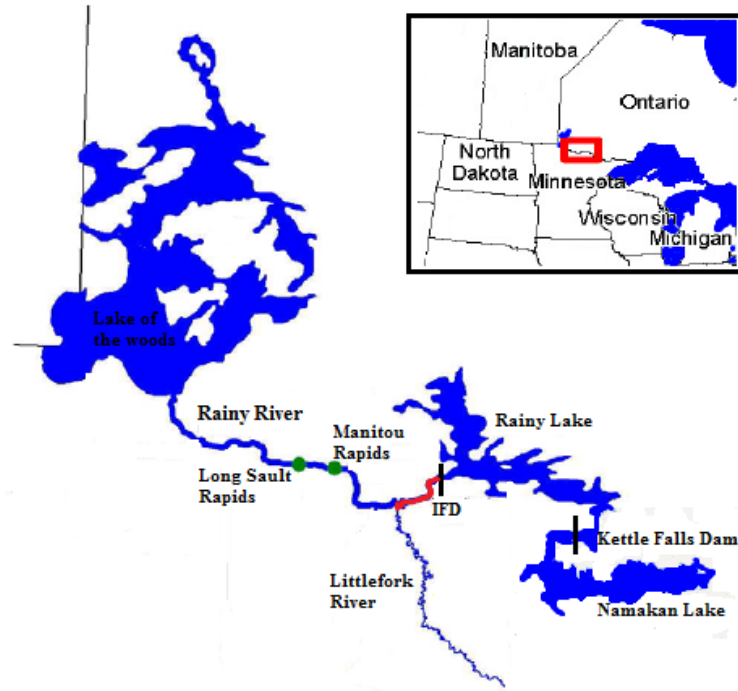


Figure 1. Rainy River stretches approximately 145 km from International Falls dam (IFD) to Lake of the Woods. The upper Rainy River study reach (red) extends from IFD to the confluence with Little Fork River an approximately 22km reach. Discharge into Rainy River is regulated by the IFD.

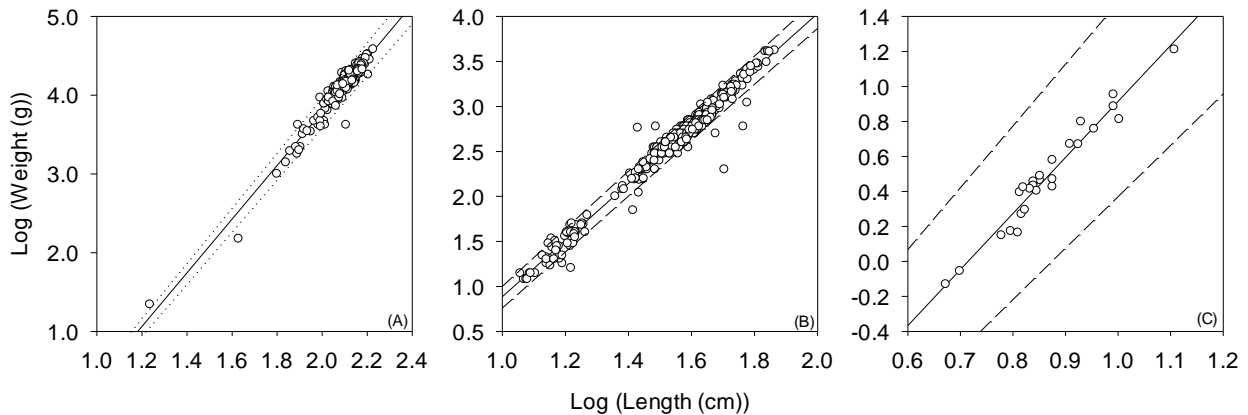


Figure 2. Weight-length relationships for lake sturgeon (A), walleye (B), and logperch (C) captured on the Rainy River during the spawning season. Data combines all years (2012 and 2013) and all sexes.

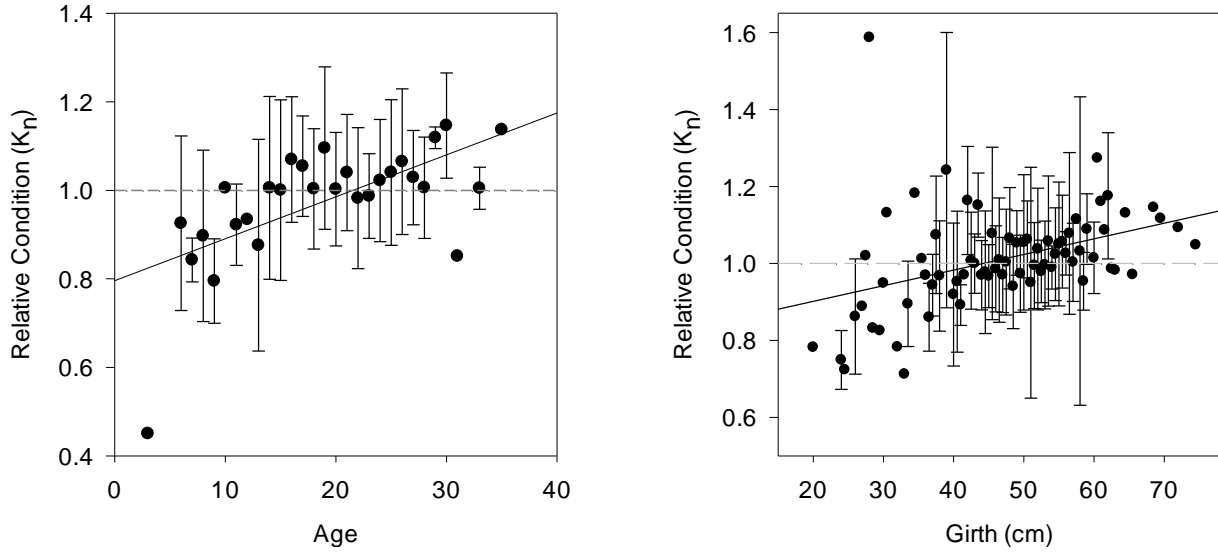


Figure 3. Relationships between age and relative condition for lake sturgeon captured on the Rainy River during spawning seasons of 2012 and 2013. Points represent mean condition at age or girth with STDEV bars. Significant regression lines indicated by black line, and relative condition of 1.0 indicated by grey dashed line

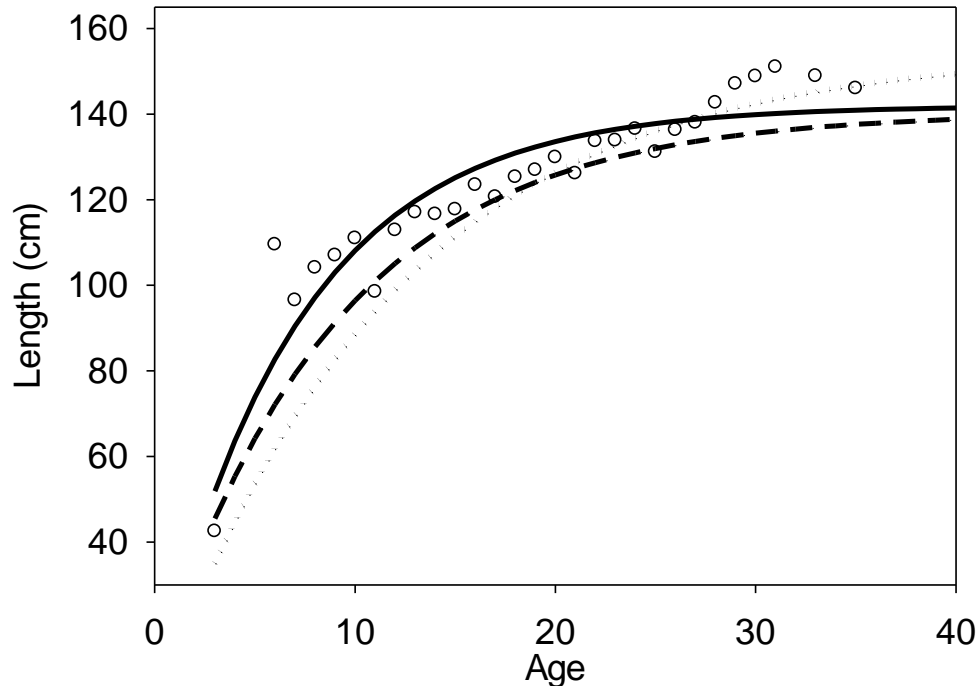


Figure 4. Mean length at age and von Bertalanffy growth model (solid line) developed for lake sturgeon caught on the Rainy River from 2012-2013. The dotted line indicates model previously reported by Mosindy and Rusak (1991) for lake sturgeon on the Rainy River caught from 1987-1989. The dashed line 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.

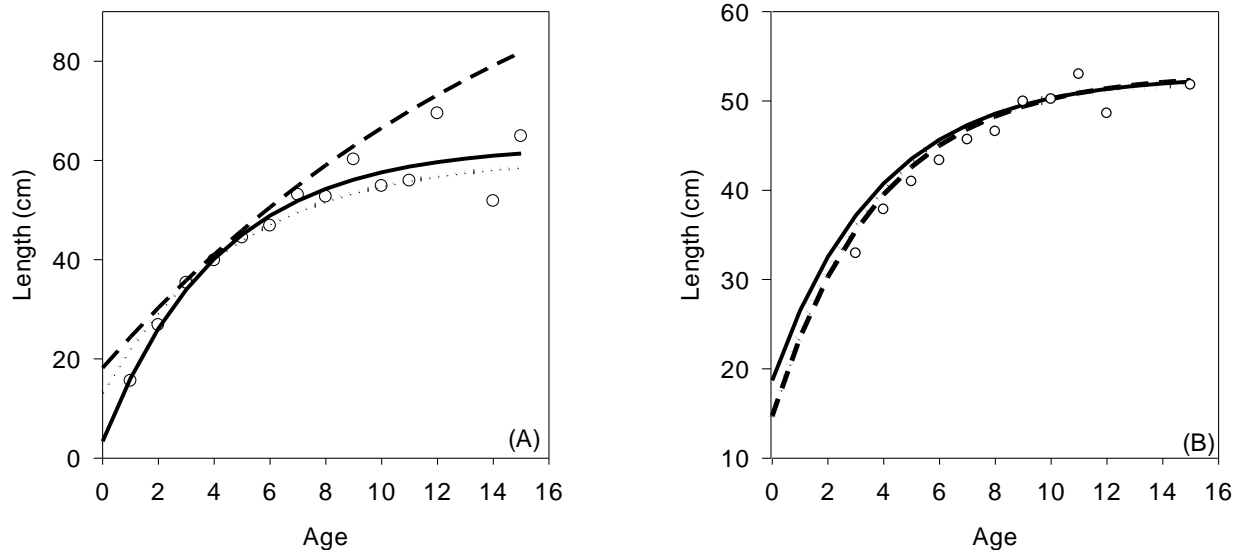


Figure 5. Mean length at age (open circles) and von Bertalanffy growth model (solid line) developed for all walleye (A) and male walleye (B) caught on the Rainy River from 2012-2013. The dotted and dashed lines indicate models previously reported by Mosindy and Mucha (2006) from 2002 and 1997, respectively, for all walleye (A) and male walleye (B) caught in Lake-of-the-Woods.

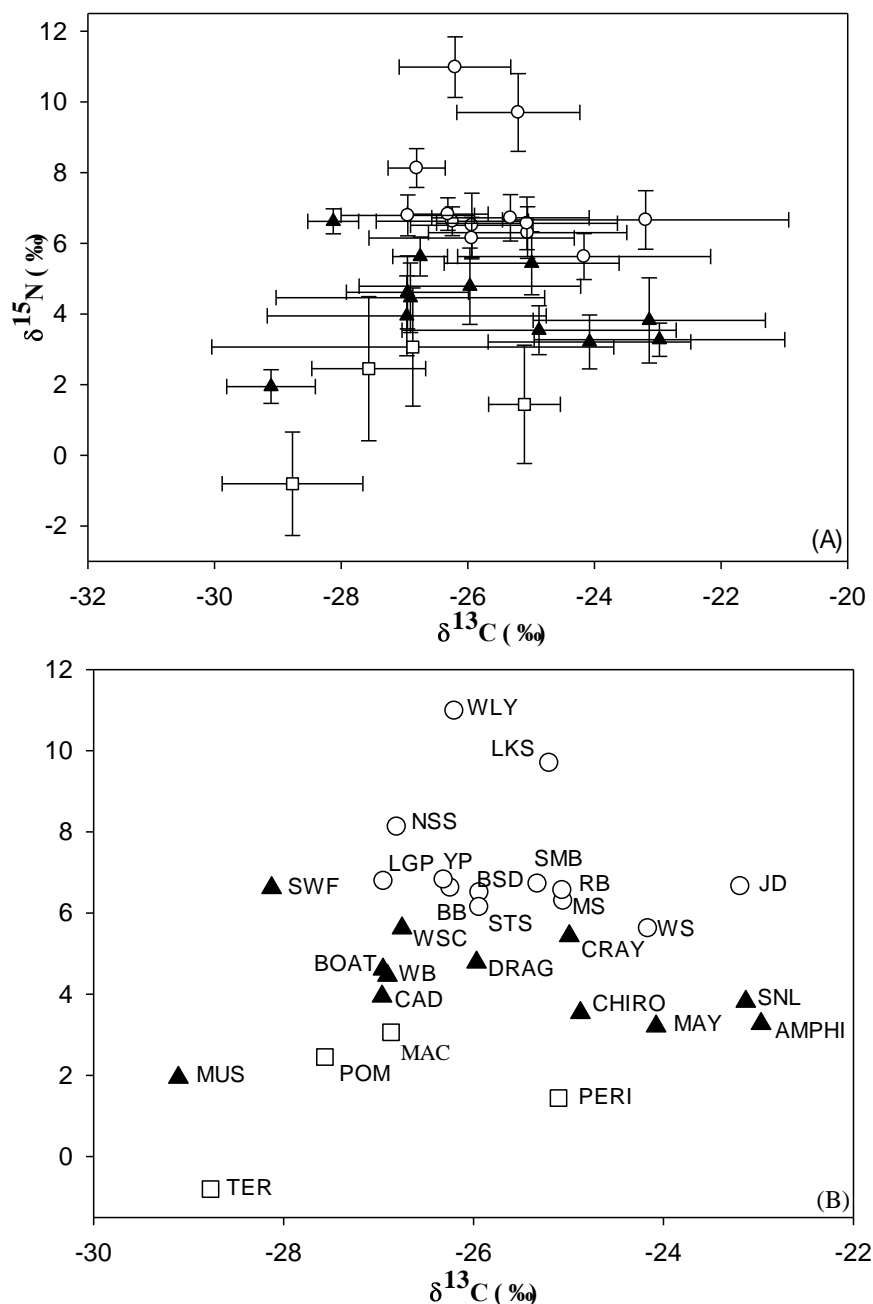


Figure 6. Mean values and standard deviations (A) and mean values with labels (B) of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (‰) of organisms found in the Rainy River. Basal food web carbon sources are plotted with open squares and include: TER=terrestrial vegetation, PERI=periphyton, POM=particulate 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=ninespine stickleback, SMB=smallmouth bass, STS=spottail shiner, WS=white sucker, YP=yellow perch and WLY=walleye

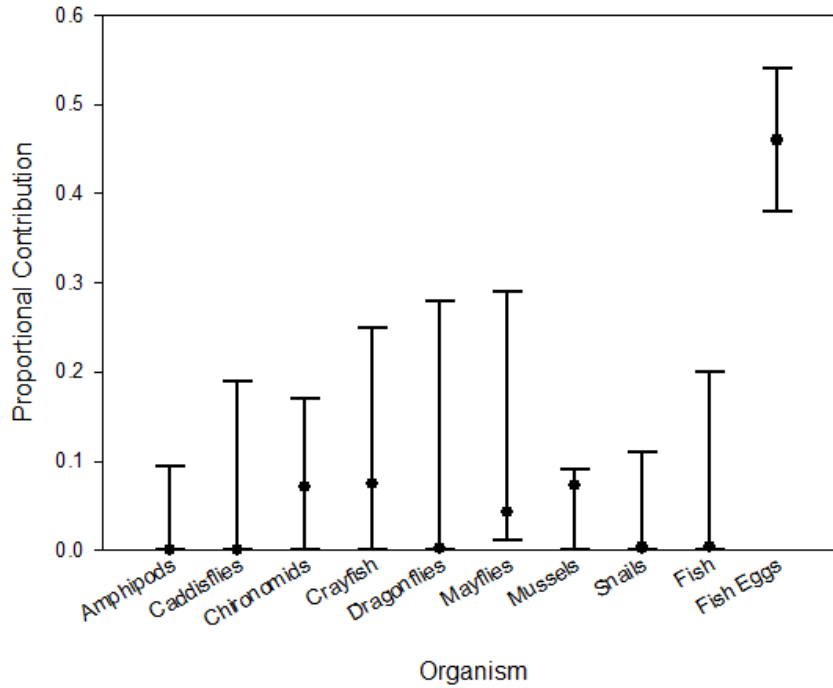


Figure 7. Proportion of prey contributing to the diet of lake sturgeon in the Rainy River as determined using Stable Isotope Analysis in R (SIAR). Black circles and solid lines represent median and 95% confidence intervals (CI), respectively, obtained using fin tissue stable isotope signatures for lake sturgeon.

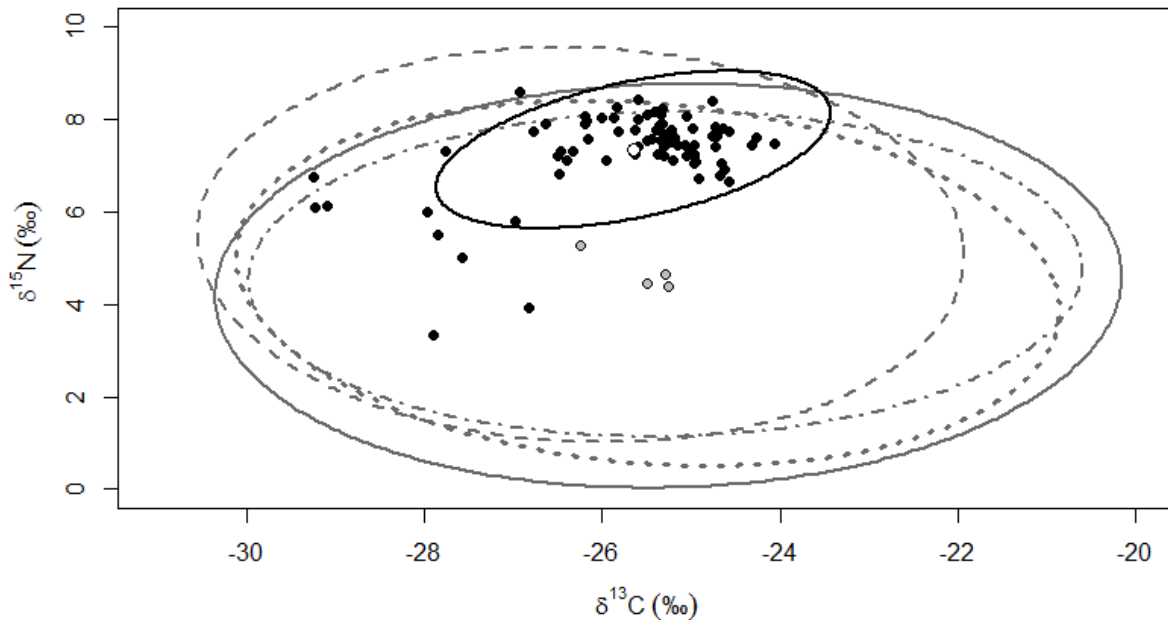


Figure 8. Standard ellipse areas for available prey items sampled in the four Rainy River habitat types: Type 1 (solid grey line) = high velocity bedrock, Type 2 (dashed grey line) = moderate velocity boulder/cobble, Type 3 (dotted grey line) = low velocity sand/gravel beds, and, Type 4 (dash-dot grey 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.

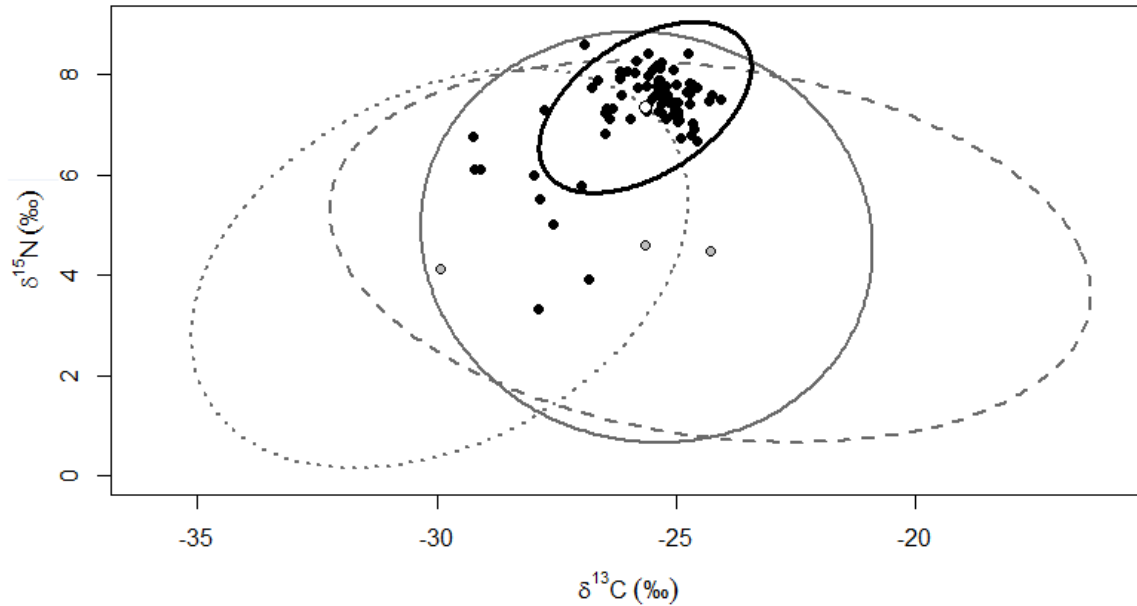


Figure 9. Community niche ellipses (grey lines) for all available prey items found in Rainy River (solid line), Lake of the Woods (dashed line), and Little Fork River (dotted line) with centroids for each ellipse plotted as a grey circle. Lake sturgeon muscle SEA is plotted as a solid black line, with individuals shown as black circles and the corresponding centroids as a white circle.

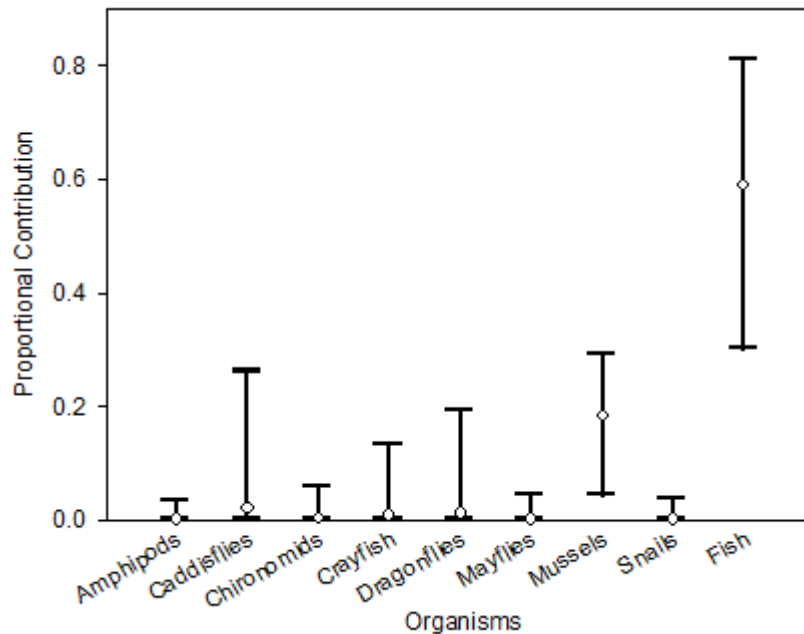


Figure 10. Proportion of prey contributing to the diet of walleye in the Rainy River as determined using Stable Isotope Analysis in R (SIAR). Open circles and solid lines represent median and 95% confidence intervals (CI), respectively, obtained using muscle stable isotope signatures for walleye.

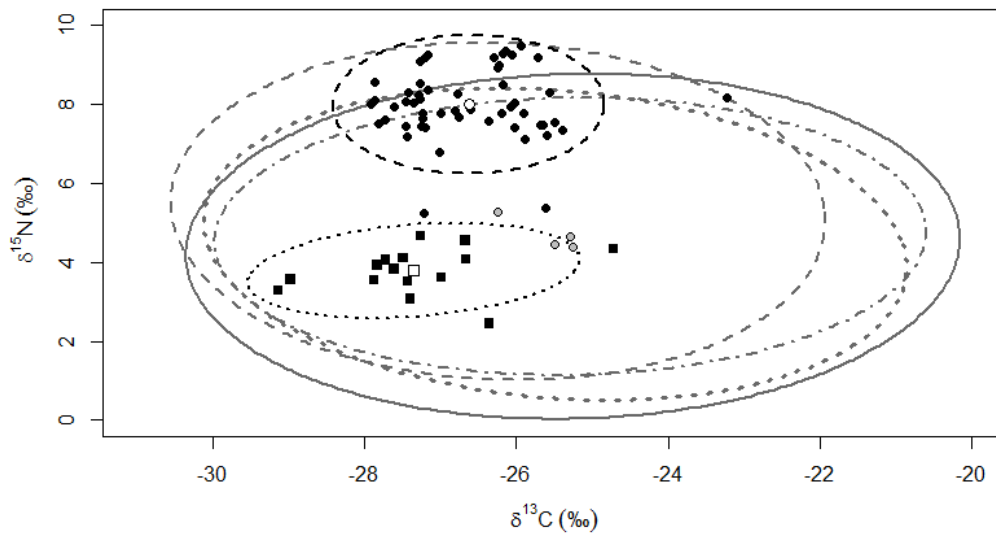


Figure 11. Standard ellipse areas for available prey items sampled in the four Rainy River habitat types: Type 1 (solid grey line) = high velocity bedrock, Type 2 (dashed grey line) = moderate velocity boulder/cobble, Type 3 (dotted grey line) = low velocity sand/gravel beds, and Type 4 (dash-dot grey 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 open circle. Logperch muscle SEA is plotted as a dotted black line, with individuals shown as black squares and the corresponding centroids as an open square.

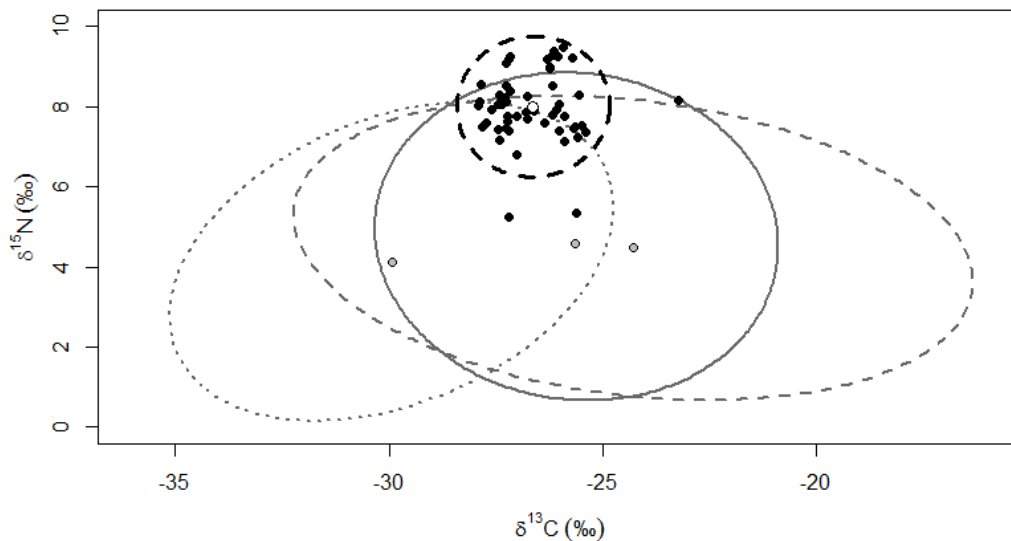


Figure 12. Community niche ellipses (grey lines) for all available prey items found in Rainy River (solid line), Lake of the Woods (dashed line) and Little Fork River (dotted line), with centroids for each ellipse plotted as a grey circle. Walleye muscle SEA is plotted as a dashed black line, with individuals shown as black circles and the corresponding centroids as a open circle.

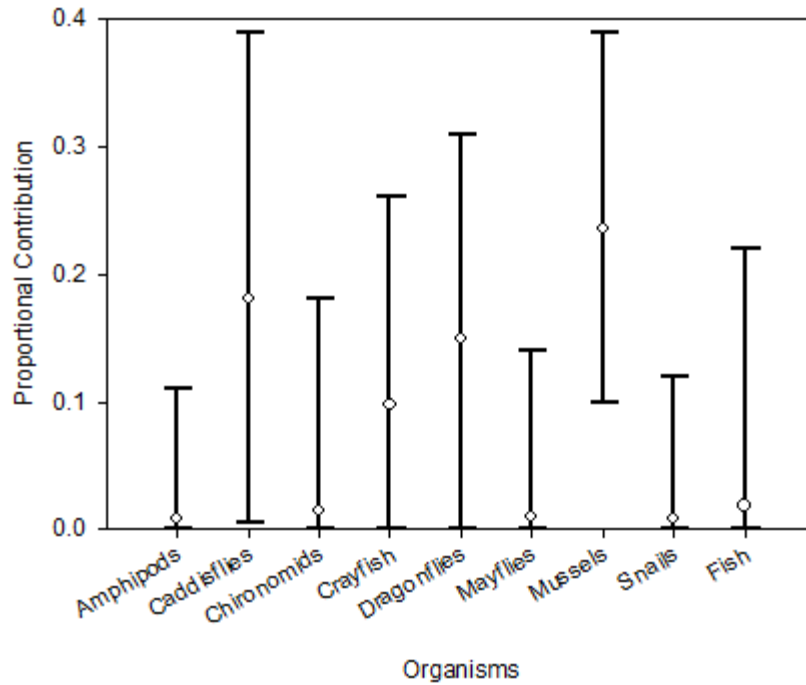


Figure 13. Proportion of prey contributing to the diet of logperch in the Rainy River as determined using Stable Isotope Analysis in R (SIAR). Open circles and solid lines represent median and 95% confidence intervals (CI), respectively, obtained using muscle stable isotope signatures for logperch.

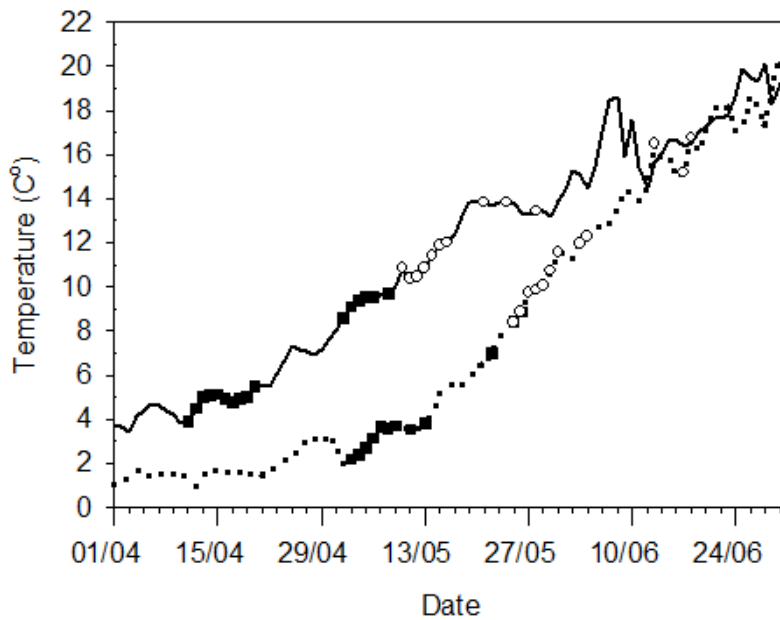


Figure 14. Average Rainy River temperature taken from pressure and temperature transducers (PTT) at three cross-section locations within the study reach. Solid line indicates temperatures for 2012 and dotted line temperatures for 2013. Instances of walleye spawning are indicated by black squares and lake sturgeon spawning by open circles.



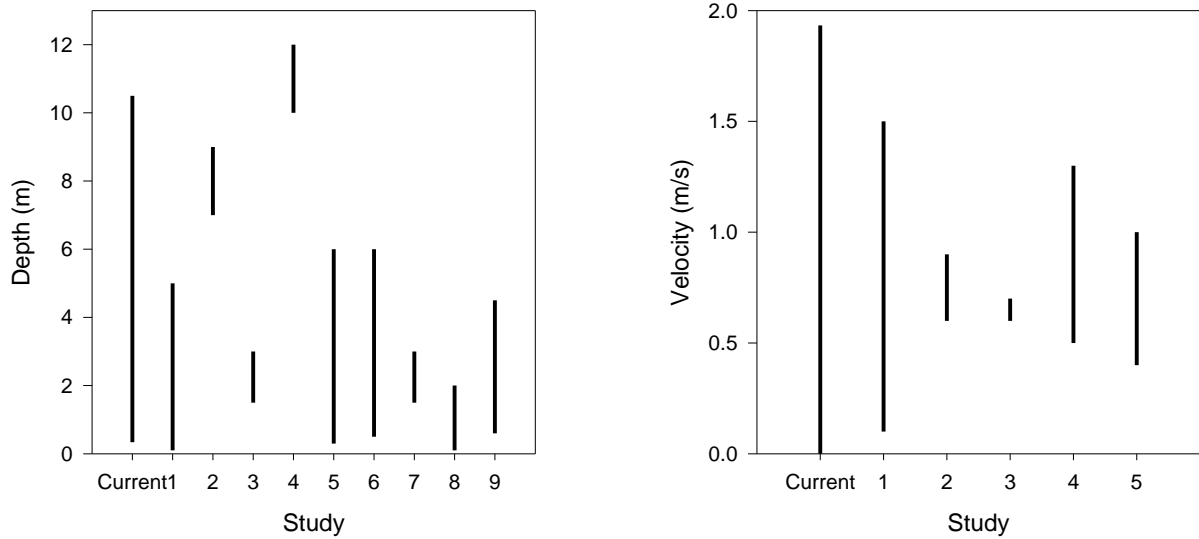


Figure 15. Range of depths (m) and velocities (m/s) measured at lake sturgeon spawning sites in the current Rainy River study (current) over the range of WSEs when spawning adults, eggs, or larvae were present on site. Ranges are compared to those found in a variety of studies for depth [1] Kerr et al., 2010, [2] Manny et al., 2004, [3] Dumont et al., 2009, [4] McGarth et al., 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 [1] Kerr et al., 2010, [2] Manny et al., 2004, [3] McGarth et al., 2009, [4] Randall, 2008 and [5] Lyttle, 2008.

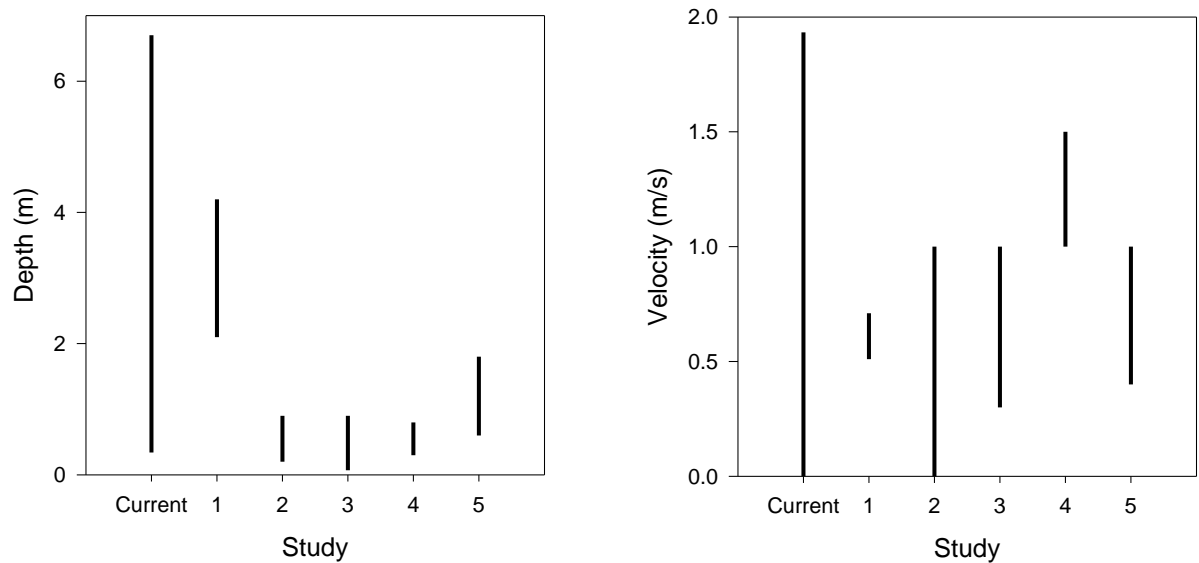


Figure 16. Range of depths (m) and velocities (m/s) measured at walleye spawning sites in the current Rainy River study (current) over the range of WSEs when spawning adults, eggs, or larvae were present on site. Ranges are compared to those found in a variety of studies for depth and velocity [1] Chalupnicki et al., 2010, [2] Hartman, 2009, [3] Liaw, 1991, [4] Kerr et al., 1997 and [5] Lyttle, 2008.

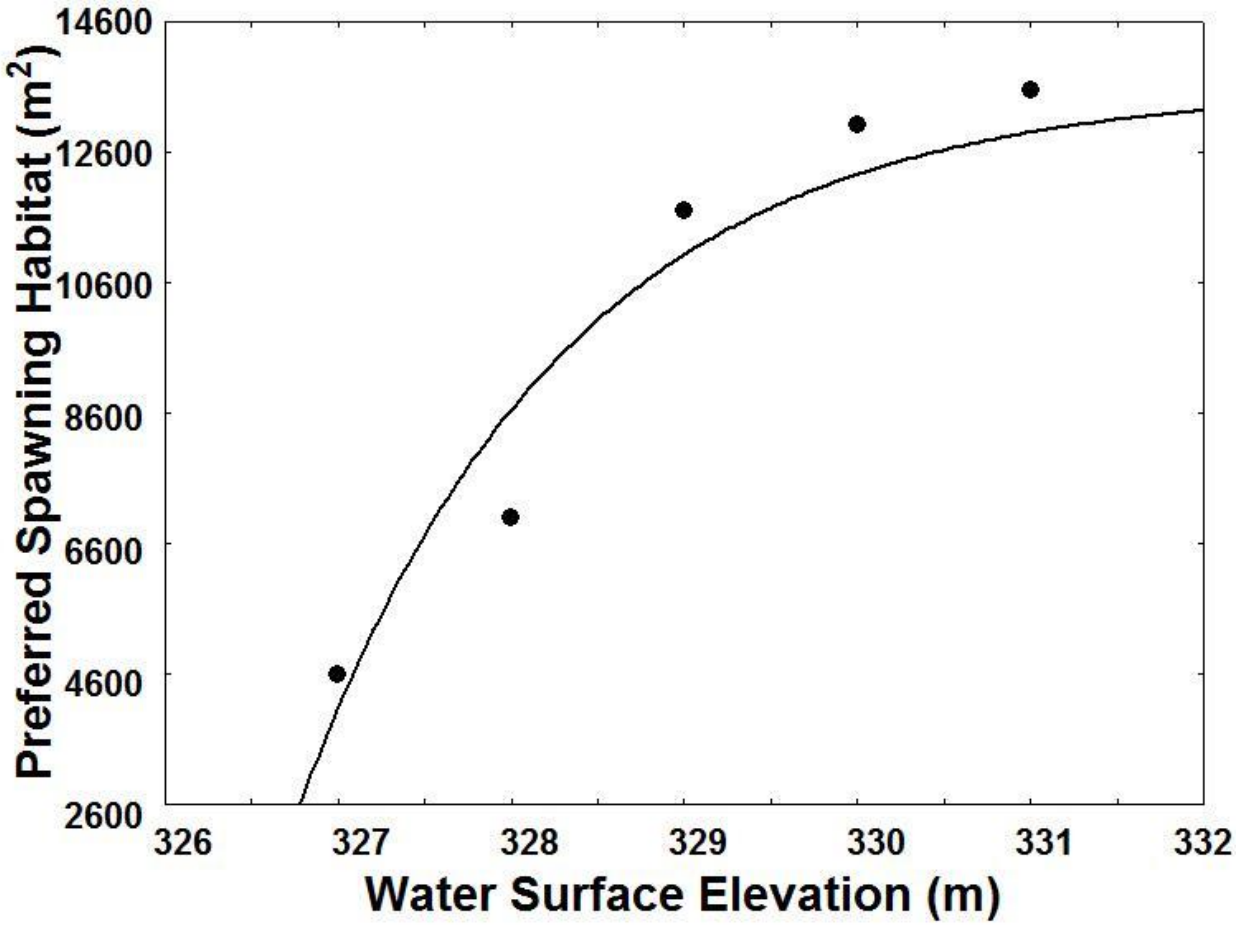


Figure 17. Spawning substrate areas by water surface elevation for lake sturgeon. Significant non-linear regression designated by the solid line.

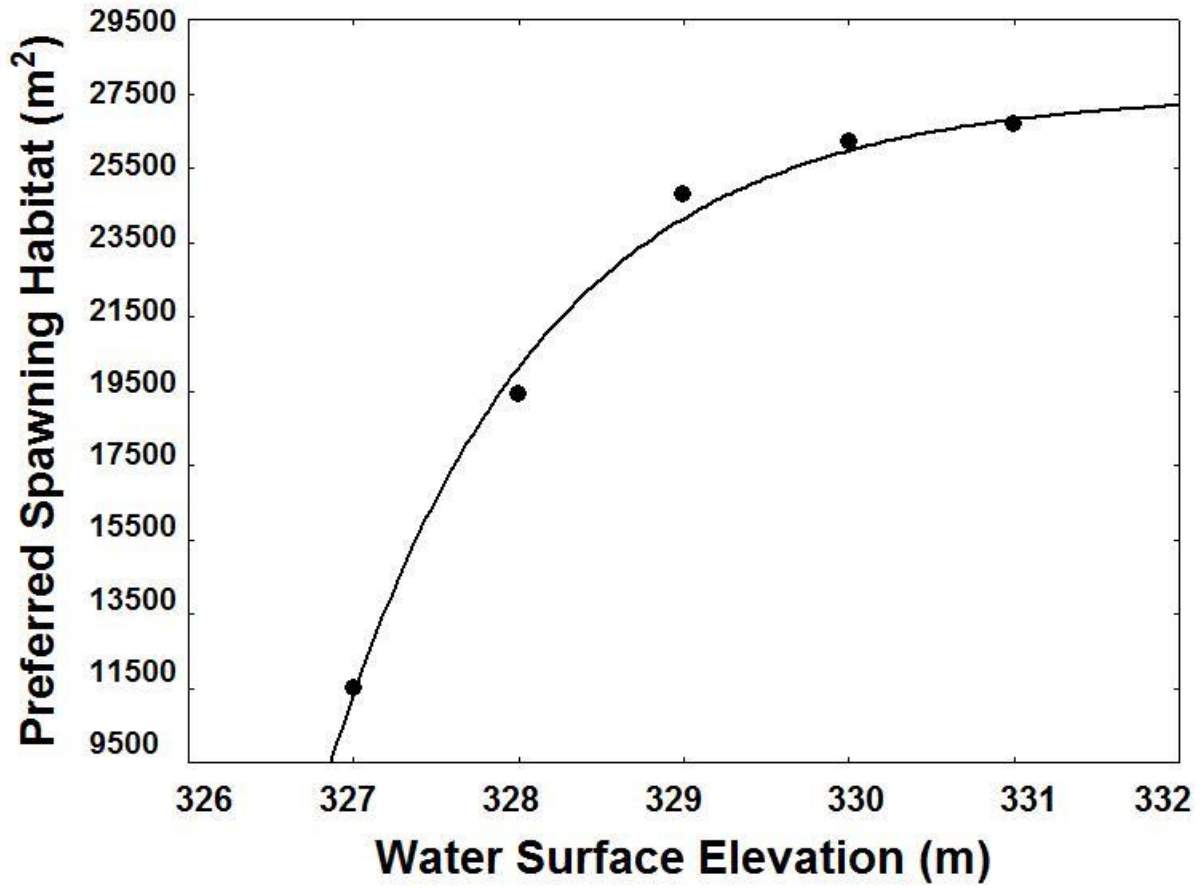


Figure 18. Spawning substrate areas by water surface elevation for walleye. Significant non-linear regression designated by the solid line.